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Animal Food GRAS Notice

Use of Mannanase 19287 Enzyme Preparation in Poultry Diets

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PART 1: SIGNED STATEMENTS AND CERTIFICATION
(21 CFR §570.225)

A. Claim Regarding GRAS Status

This GRAS Notice is submitted in accordance with 21 CFR Part 570, Subpart E – Generally Recognized as Safe (GRAS) Notice. BASF Corporation hereby notifies the FDA of the determination by BASF Corporation that mannanase 19287 enzyme preparations produced by *Thermothelomyces thermophilus* strain DSM 33149*,* expressing a gene encoding the mannanase 19287 enzyme (marketed as Natupulse® TS and Natupulse® TS L), when used as intended to reduce the viscosity of intestinal digesta in poultry diets, are generally recognized as safe (GRAS), based on scientific procedures.

B. Name and Address of Notifier

BASF Corporation
100 Park Avenue
Florham Park, NJ 07932
USA

Contact for this dossier:
Andreas Kern, PhD
Sr. Reg. Affairs Manager Animal Nutrition
BASF Corporation
3350 John Hopkins Court
San Diego, CA 92121

C. Name of Notified Substance

The notified substance being addressed in this submission is the mannanase 19287 enzyme preparation which is marketed as the final dry product Natupulse® TS and the final liquid product Natupulse® TS L.

D. Intended Conditions of Use

a. Natupulse® TS L will be added in a post-pelleting application to complete pelleted feeds; Natupulse® TS will be added to animal feeds in non-pelleted compound feed, pelleted compound feed, and for use in premix.

b. Proposed levels of use: The recommended level of supplementation in a complete feed is 800 TMU / kg of feed. One TMU is defined as the amount of enzyme that liberates 1 µmol reducing sugars determined as mannose per minute under the conditions of the test method (pH 3.5 and 50 °C).

c. Animal species intended: Natupulse® TS and Natupulse® TS L will be added to poultry feed.

d. Natupulse® TS and Natupulse® TS L are intended for use in animal feed to reduce the viscosity of intestinal digesta by increasing the digestibility of β-mannans in poultry diets.

E. Basis for Conclusion of GRAS Status

The statutory basis for the conclusion of GRAS status for mannanase 19287 enzyme preparation, which is marketed as Natupulse® TS and Natupulse® TS L, is based upon scientific procedures, as described in this submission.

F. Premarket Approval Exemption

It is the notifier's view that the notified substance is not subject to the premarket approval requirements of Federal Food, Drug, and Cosmetic Act, based on the notifier's conclusion that mannanase 19287 enzyme products, which are marketed as Natupulse® TS and Natupulse® TS L, are GRAS under the conditions of intended use to reduce the viscosity of intestinal digesta in poultry diets.

G. Statement of Availability of Data and Information

The data and information that are the basis for BASF Corporation's conclusion of GRAS status are available for FDA's review, and copies will be sent to FDA upon request, in either electronic format or by paper copy. Requests for copies and arrangements for review of materials

cited may be directed to the contact for this dossier listed above, Andreas Kern, PhD, Sr. Regulatory Affairs Manager, Animal Nutrition.

H. Statement of Exemption from FOIA Disclosure

The following information is exempt from FOIA Disclosure (5 USC §582(b)(4)):

Information	**Reason for FOIA Exemption**
Mannanase 19287 amino acid sequence	The synthetic amino acid sequence of mannanase 19287 protein was created to optimize the endo-1,4-β-mannanase for the intended use to reduce the viscosity of intestinal digesta in poultry diets. The specific amino acid sequence is considered confidential business information.
Mannanase 19287 amino acid sequence alignments with GH5-7 fungal endo-1,4-β-mannanases	The specific amino acid sequence of the mannanase 19287 protein is considered confidential business information; therefore, the amino acid sequence alignment of the mannanase 19287 protein with GH5-7 fungal endo-1,4-β-mannanases and any protein characterization analysis is also considered confidential business information.
Mannanase 19287 gene nucleotide sequences	The synthetic amino acid sequence of mannanase 19287 protein was created to optimize the endo-1,4-β-mannanase for the intended use. The specific amino acid sequence is considered confidential business information; therefore, the specific nucleotide sequences are considered confidential business information.
Construction of genetic elements and of production organism	The generation of the genetic elements and their use during transformation of the (b) (4) host

	and the specific nucleotide sequences are considered confidential business information.
Methods of mannanase activity determinations	Proprietary modifications to commercially available test kits ensure specificity for the mannanase 19287 activity determination and are therefore considered confidential business information.
Certificates of Analysis for mannanase 19287 enzyme preparations and commercial products	These certificates contain information related to the activity levels that are included in the final products and are therefore considered confidential business information.
List of raw materials used in the manufacturing of the mannanase 19287 enzyme preparation	The nature and quantities of raw materials used in fermentation, recovery and formulation processes of the mannanase 19287 enzyme preparation, and its final products are considered confidential business information.
Detailed manufacturing information: fermentation, recovery, and formulation	The detailed information on the manufacturing process of the mannanase 19287 enzyme preparations is considered confidential business information.
Product compositions	The quantities of raw materials used to formulate the products of commerce are considered confidential business information.
Stability and homogeneity study data	The details of activity changes of the commercial enzyme products may create competitive disadvantages and are therefore considered confidential business information.
Animal study reports	The details of enzyme products and specific performance data in animal studies may create competitive disadvantages and are considered confidential business information.

Certification

On behalf of BASF Corporation, I certify to the best of my knowledge, the GRAS Notice is a complete, representative, and balanced submission that includes unfavorable information, known to me, and BASF Corporation, and pertinent to the evaluation of safety and GRAS status of mannanase 19287 enzyme, which is marketed as Natupulse® TS and Natupulse® TS L, under the conditions of intended use.

Signed,

(b) (6)

January 23, 2024

Gisele Santos Bin
Head of Sales NA, Animal Nutrition
BASF Corporation

Date

Please Note: The following definitions have been aligned with multiple terms throughout the R&D process. To provide the necessary clarity, this GRAS Notice will refer only to the dossier terms (**Table 1**). However, supplemental materials may contain dossier terms or the in-house identifiers, respectively.

Table 1: Naming Convention

Definition	**Dossier Term**	**In-house identifiers**
Dry final product	Natupulse® TS	(b) (4)
Liquid final product	Natupulse® TS L	
Liquid mannanase concentrate	Mannanase CC	
Spray-dried mannanase concentrate	(b) (4)	
Production strain name	*Thermothelomyces (T.) thermophilus* DSM 33149	
Host strain name	*Thermothelomyces thermophilus* (b) (4)	
Mannanase enzymes in general	endo-β-1,4-D-mannanase, β-mannanase	
Synthetic mannanase gene expressed in DSM 33149	manF gene	
Mannanase protein produced with DSM 33149	mannanase 19287	
Mannanase 19287 purified protein	purified mannanase 19287	

Table 2: List of Acronyms

Acronym	Definition
AAFCO	Association of American Feed Control Officials
ADFI	Average daily feed intake
ADJ FCR	Adjusted feed conversion ratio
AGRN	Animal GRAS Notice
AOAC	Association of Official Analytical Chemists
APO	Absence of Production Organism
ATCC	American Tissue Culture Collection
BLAST	Basic Local Alignment Search Tool
CAS	Chemical Abstracts Service
cDNA	complementary DNA
CFR	Code of Federal Regulations
CFSAN	Center for Food Safety and Applied Nutrition
CFU	Colony-forming units
cGMPs	Current Good Manufacturing Practices
CONF	Confidential
cP	Centipoise
CV	Coefficient of Variation
DSM	German Collection of Microorganisms and Cell Cultures
DSP	Downstream Processing
ECHA – REACH	European Chemicals Agency – Registration, Evaluation, Authorisation & Restriction of Chemicals
EDI	Estimated Daily Intake
EU	European Union
FAMI-QS	Quality and Safety System for Specialty Feed Ingredients
FAO	Food and Agriculture Organization of the United Nations
FCC	Food Chemicals Codex
FDA	Food and Drug Administration
FSMA	Food Safety Modernization Act
GCP	Good Clinical Practice
gDNA	genomic DNA
GRAS	Generally Recognized as Safe
HPLC	High Performance Liquid Chromatography
ISO	International Organization for Standardization
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC	Liquid Chromatography
MALDI-TOF MS	Matrix-assisted Laser Desorption/Ionization-time of Flight Mass Spectrometry
MCB	Master Cell Bank
MMC	Mitomycin C
MOS	Mannan Oligosaccharide
MS	Mass Spectrometry
MT	Myceliothermophin

NOAEL	No Observed Adverse Effect Levels
NSP	Non-Starch Polysaccharide
OECD	Organization for Economic Co-operation and Development
Pas	Pascal Second
PBL	Peripheral Blood Lymphocytes
PCR	Polymerase Chain Reaction
PO	Production Organism
PPLA	Post Pellet Liquid Application
ppb	parts per billion
ppm	parts per million
rDNA	recombinant DNA
SAS	Statistical Analysis System
SD	Standard Deviation
SOP	Standard Operating Procedure
TA	Test Article
TOS	Total Organic Solids
UF	Ultra-Filtration
USP	US Pharmacopeia
VICH	Veterinary International Cooperation on Harmonization
VKM	(b) (6)
WCB	Working Cell Bank
WHO	World Health Organization

PART 2: IDENTITY, MANUFACTURE, SPECIFICATIONS, TECHNICAL EFFECT

A. Scientific Data that Identifies the Notified Substance

1. Enzyme identity

a. Identity

The mannanase 19287 enzyme in the notified substance is classified as a β-mannanase according to the IUBMB enzyme nomenclature recommendations (**Table 3**) (Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB), 1972).

Table 3: IUBMB Enzyme Classification

Enzyme Name	β-mannanase
Official / Accepted Name	mannan endo-1,4-β-mannosidase
Alternative Names	endo-1,4-β-mannanase; endo-β-1,4-mannanase; β-mannanase B; β-1,4-mannan 4-mannanohydrolase; endo-β-mannanase; β-D-mannanase; 1,4-β-D-mannan mannanohydrolase
Systematic Name	1,4-β-D-mannan mannanohydrolase
IUB Number	3.2.1.78
CAS Registry Number	37288-54-3
Reaction	β-(1,4)-D-mannan + H_2O → 2 β-(1,4)-mannan oligosaccharides

b. Amino acid sequence

The endo-β-1,4-D-mannanase protein in the notified substance (mannanase 19287 enzyme preparation) is encoded by a synthetic β-mannanase gene (b) (4)(Appendix 1 and Appendix 3). The identity of mannanase 19287 protein expressed in the production organism *T. thermophilus* DSM 33149 has been confirmed by mass spectrometry (MS) protein analysis and gene sequence analysis.

The mannanase 19287 protein sequence shows >90 % homology to a (b) (4) enzyme from *Trichoderma reesei* (*T. reesei,* GenBank Q99036), and both (b) (4) belong to Family

5 Subfamily 7 of glycosyl hydrolases (Davies, G. and Henrissat, B., 1995; (b) (4) See Appendix 2 and Part 2.B.1.a for further information.

c. Enzyme substrate

Beta-mannans (β-mannans) are important soluble constituents of the hemicellulose component of the hull elements of cereals and other seeds. Linear β-mannans and galactomannans contain a linear β-D-mannose polysaccharide chain and galactose side chains (galactomannans). Both insoluble and soluble β-mannans are present in food ingredients from plant sources (Knudsen, K.E.B., 1997; (b) (4)) and β-mannans are able to tightly bind water (b) (4)).

The presence of soluble β-mannans in animal feed for non-ruminants can result in digesta of high viscosity with associated negative effects on food digestion and nutrient uptake (Choct, M. *et al.*, 2010; (b) (6) Lee, J.T. *et al.*, 2003a). The supplementation of β-mannan-degrading enzymes to animal feed results in reduction of digesta viscosity and has shown positive effects on animal performance (Balasubramanian, B. *et al.*, 2018; Daskiran, M. *et al.*, 2004; Hosseindoust, A. *et al.*, 2019; Jackson, M.E. *et al.*, 2004; Latham, R.E. *et al.*, 2015; Sundu, B. *et al.*, 2006). Like all endo-1,4-β-mannanases (E.C. 3.2.1.78), mannanase 19287 catalyzes the hydrolysis of β-mannans and releases mannan oligosaccharides (MOS):

$$\beta\text{-(1,4)-D-mannan} + H_2O \rightarrow 2\ \beta\text{-(1,4)-mannan oligosaccharides}$$

d. Product characteristic properties

Two commercial mannanase 19287 products are available:

(1) Natupulse® TS is a dry powder product which consists of heterogeneously formed particles of yellowish to brown color.

(2) Natupulse® TS L is a clear, brownish liquid. Its viscosity at 20°C was determined to be 3.5 mPas.

e. Product compositions

Table 4: Natupulse® TS composition

Ingredient	AAFCO Section Reference
Mannanase (b) (4)	N/A
(b) (6)	93.1
	57.88
	33.2

Table 5: Natupulse® TS L composition

Ingredient	FDA Reference
Mannanase (Mannanase CC)	N/A
Glycerin	21 CFR 582.1320
Sodium benzoate	21 CFR 582.3733
Water	N/A

f. Characteristic properties

See Appendix 4 - Biochemical Characterization for more details.

Specific Activity

A purified mannanase 19287 sample was lyophilized and analyzed for water, sodium benzoate, and ash content. The endo-β-mannanase activity was determined using the proprietary validated Method of Analysis FEN/0004/01 - Photometric assay of mannanase activity in feed additives. The assay procedure is specific for endo-1,4-β-D-mannanase activity and uses dyed carob galactomannan to generate soluble β-mannan oligosaccharides. One TMU is defined as the amount of enzyme that liberates 1 μmol reducing sugars determined as mannose per minute under the conditions of the test method (pH 3.5 and 50 °C).

Hydrolase Activity of Mannanase 19287

One TMU is defined as the amount of enzyme that liberates 1 µmol reducing sugars determined as Mannose per minute under the conditions of the test method (pH 3.5 and 50 °C). The mannanase 19287 enzyme (EC 3.2.1.78) catalyzes the hydrolysis of beta-1,4 glycosidic bonds within the linear chain of β-D-mannose polysaccharides:

$$\beta\text{-(1,4)-D-mannan} + H_2O \rightarrow 2\ \beta\text{-(1,4)-mannan oligosaccharides}$$

To confirm the β-mannanase functionality of mannanase 19287, a lab study evaluated the presence of mannan oligosaccharides after incubation of (b) (4) with the notified substance, the mannanase 19287 enzyme preparation. The presence of mannan oligosaccharides was tested for by MS. (b) (4) Therefore, the mannanase 19287 enzyme preparation demonstrated the endo-β-mannanase activity, generating β-mannan oligosaccharide fragments of various lengths.

Side activities

The mannanase 19287 enzyme preparation produced with *T. thermophilus* DSM 33149, the notified substance, was tested for potential glycolytic or proteolytic side activities using validated enzyme activity assays. The results demonstrated the absence of significant xylanase, cellulase, α-amylase, β-glucanase, or acidic protease activities. These results agree with published data ((b) (4)) showing that endogenous cellulase, protease and chitinase activities were reduced to background levels during the development of the host strain.

pH-dependent mannanase 19287 activity

The effect of pH on enzyme activity was determined by modifying the pH of the citrate assay buffer. Preparations of the citrate-phosphate buffer were adjusted to the pH range from 2.0 to 11.9, and these buffers were then used to prepare enzyme-, substrate-, and mannan standard solutions. To compare activity levels throughout the pH range from 2.65 to 12.0, enzyme activity (TMU/g) at pH 3.5 was set as 100 %. Activity remained stable (100 % ± 15%) up to pH 5.3. As

the pH dropped into neutral and alkaline conditions, enzyme activity reached background levels (< 20%) around pH 7.5.

Temperature dependence of the β-mannanase activity assay

The effect of temperature on the mannanase 19287 activity assay was determined over a temperature range from 40°C to 75°C. Mannanase 19287 was diluted in assay dilution buffer (sodium citrate buffer, pH 3.5, Tween 20 10%) and the assay was performed at pH 3.5 and temperatures of (b) (4) respectively.

Enzyme activity increased with assay temperature in the range of (b) (4) The optimum enzyme activity was observed at (b) (4). Performing the activity assay at higher temperatures resulted in a drop of activity with a complete loss of mannanase hydrolysis at (b) (4). The increased temperature at pH 3.5 may impact the folding of the enzyme, including the catalytic domain, as well as the binding of the substrate to the enzyme. **Please note**: this study evaluated the temperature impact on the enzyme activity assay at standard pH 3.5, not the thermotolerance of the enzyme to withstand exposure to high temperatures prior to performing the β-mannanase assay under standard conditions.

Thermotolerance of mannanase 19287 activity

The temperature effect on the enzyme activity assay described above differs from the tolerance of the enzyme preparation to elevated temperatures before digesting β-mannans in feed during passage through the digestive tract. This characteristic is important regarding temperatures encountered in typical animal feed production processes, including pelleting, where conditions can reach < 90 °C for short periods of time.

Initial analyses of short exposures of the mannanase 19287 enzyme preparation to temperatures between 75°C to 90°C demonstrated minimal loss of enzyme activity after incubation at 75 or 80 °C, and higher losses at 85 or 90°C. Additional studies at 88°C and 90°C demonstrated that mannanase 19287 retains 50% of initial activity for approx. 10 minutes at 88°C. The enzyme maintains half of its initial activity up to 5 minutes at 90°C.

2. Source organism

a. Taxonomic source:

The mannanase 19287 enzyme in Natupulse® TS and Natupulse® TS L is produced with *Thermothelomyces thermophilus* strain DSM 33149. The official taxonomic classification of *T. thermophilus* is described in the Mycobank database and listed in **Table 6**:

Table 6: *T. thermophilus* taxonomic classification per Mycobank (MB) database

Thermothelomyces thermophilus (Apinis) Y. Marín, Stchigel, Guarro & Cano, [redacted]	
Kingdom	Fungi
Strain	Ascomycota
Substrain	Pezizomycotina
Class	Sordariomycetes
Order	Sordariales
Family	Chaetomiaceae
Genus	*Thermothelomyces*
Species	*Thermothelomyces thermophilus*

The taxonomic classification of the fungal organism has been changed several times and the current *T. thermophilus* species has been characterized in 2015 (Marin-Felix, Y. *et al.*, 2015). *T. thermophilus* and the previous identification *Myceliophthora thermophila* are being used interchangeably in scientific literature, regulatory documents, established cell culture collections and in commercial production. Relevant *T. thermophilus* strain names (underlined text) in the lineage include ATCC 42464, the source of the published genome sequence of the organism (Berka, R.M. *et al.*, 2011), CBS 117.65^T, the *T. thermophilus* Type Strain (MycobankID MB 823054), [redacted] a natural isolate, and C1-HC, a [redacted]-derived mutagenic strain with deregulated cellulase production (Visser, H. *et al.*, 2011). Strain *T. thermophilus* C1-HC is the progenitor of the host and production strains for the mannanase 19287 enzyme preparation.

To confirm the taxonomic identity of the *T. thermophilus* DSM 33149 strain, the assembled whole genome sequence (WGS) was probed with GenBank reference sequences using the BLAST program (BLASTN 2.2.26, available at https://bioweb.pasteur.fr/packages/pack@blast@2.2.26) (Altschul, S.F. *et al.*, 1997). The following three gene sequences from type strain CBS 117.65^T were queried against the *T. thermophilus* DSM 33149 and reference strain ATCC 42464 genomes

(Marin-Felix, Y. *et al.*, 2015). Both DSM 33149 and ATCC 42464 genomes demonstrated 100% homology to the Type Strain sequences, confirming that the production strain DSM 33149 is a strain of *T. thermophilus* (Appendix 5):

(b) (4)

In addition, the DSM 33149 and ATCC 42464 genomes were probed with equivalent gene sequences (or barcodes) from three closely related *Thermothelomyces* species, *T. guttulatus*, *T. hinnuleus* and *T. heterothallicus* (Wang, X. *et al.*, 2022). The identified homologies ranged from 96% to 99% identity, demonstrating both the close taxonomic relationship and separate identities between the four *Thermothelomyces* species.

These results confirm the identity of DSM 33149 as a strain of species *T. thermophilus*.

b. Part of plant or animal used as source

The fungal organism *T. thermophilus* is not a plant or an animal.

c. Any known toxicants

Experimental results demonstrate the absence of mycotoxins (aflatoxins, deoxynivalenol/vomitoxin, fuminosins, zearalenone, ochratoxin A) from DSM 33149 fermentation supernatants, Mannanase CC and (b) (4) Two Natupulse® TS test articles demonstrated the presence of Deoxynivalenol/Vomitoxin (DON) and zearalenone (ZEA) at low levels, likely from the main ingredient of the product form, the feed material wheat bran. Three Natupulse® TS production batches have been analyzed and all were found to be below the LOD/LOQ of these mycotoxins (Appendix 16).

In addition, no publications have been identified that would indicate the production of mycotoxins (aflatoxins, deoxynivalenol/vomitoxin, fuminosins, zearalenone, ochratoxin A) by *T. thermophilus/M. thermophila*. Lastly, a GRAS Notification to CFSAN evaluated a cellulase preparation produced by a recombinant production strain of the progenitor C1-HC lineage for the intended use as an enzyme in the production of wine, beer and fruit juices. The notice demonstrated the absence of mycotoxins in the enzyme preparation (Dyadic International (USA) Inc, 2009; FDA

Center for Food Safety and Applied Nutrition (CFSAN), 2009). **Please note:** the *T. thermophilus* production strain DSM 33149 is also derived from the progenitor strain *T. thermophilus* C1-HC.

Tenuazonic Acid (TeA) is a secondary metabolite produced primarily by *Alternaria ssp.* and a few other ascomycetes, including *Epicoccum sorghinum* and *Pyricularia oryzae* (previously identified as *Magnaporthe oryzae*). TeA biosynthesis is catalyzed by the enzyme TeA synthetase (TAS1), which in turn is regulated by transcription factor TAS2. The *T. thermophilus* genome contains both TAS1 and TAS2 homologs (Yun, C.-S. *et al.*, 2017). Mannanase CC, Natupulse® TS and TS L final products were analyzed by an external laboratory holding an Accreditation Certificate per ISO/IEC 17025:2018 for the analysis of food, including TeA, issued by the German Accreditation Institute DAkkS (German acronym for Deutsche Akkreditierungsstelle GmbH). No significant quantities of TeA were identified in Mannanase CC or final Natupulse® TS or TS L products (Appendix 6).

A systematic literature search identified two references reporting the isolation of a class of secondary metabolites from *T. thermophilus*, named myceliothermophins (MTs) A-F, in organic extracts of the organism. A subset of these polyketides, MTs A, C, E and F show cytotoxic activity in some cancer cell lines (Gao, Y.-L. *et al.*, 2019; Yang, Y.L. *et al.*, 2007). Using commercially available MT standards, a total of five Mannanase CC production batches were tested by LC-MS after methanol extraction, either in the absence or the presence of spiked MTs. The absence of myceliothermophins in non-spiked samples was confirmed in LC/MS analyses of Mannanase CC batches (Appendix 6).

Test article and commercial production batches of Mannanase CC were analyzed for mycotoxins and the results confirmed the absence of these metabolites. The safety evaluation of the mannanase 19287 preparation produced with *T. thermophilus* DSM 33149 has been published and demonstrates the absence of genotoxicity, subchronic oral toxicity and acute inhalation toxicity (Kern, A. *et al.*, 2020). See Part 2.B.1.c below for the confirmed absence in the genome of DSM 33149 of fungal toxins and virulence factors listed in relevant databases.

B. Method of Manufacture

2. Production organism

a. Gene source

As discussed previously in Part 2.A.1.c, the presence of β-mannans in animal feed for non-ruminants can result in digesta of high viscosity with associated negative effects on food digestion and nutrient uptake (Choct, M. *et al.*, 2010; (b) (4); Lee, J.T. *et al.*, 2003a). The supplementation of mannan-degrading enzymes to animal feed can result in viscosity reduction and has shown positive effects on animal performance (Balasubramanian, B. *et al.*, 2018; Daskiran, M. *et al.*, 2004; Hosseindoust, A. *et al.*, 2019; Jackson, M.E. *et al.*, 2004; Latham, R.E. *et al.*, 2015; Sundu, B. *et al.*, 2006).

Many bacterial and fungal species express enzymes that degrade hemicellulose components, including mannanases and hemicellulases (b) (4)). Several β-mannanase enzymes and their source organisms are listed in Table 30.1 of the 2023 Official Publication from the Association of American Feed Control Officials (AAFCO OP) for their respective intended uses in poultry and swine (Association of American Feed Control Officials (AAFCO), 2023b):

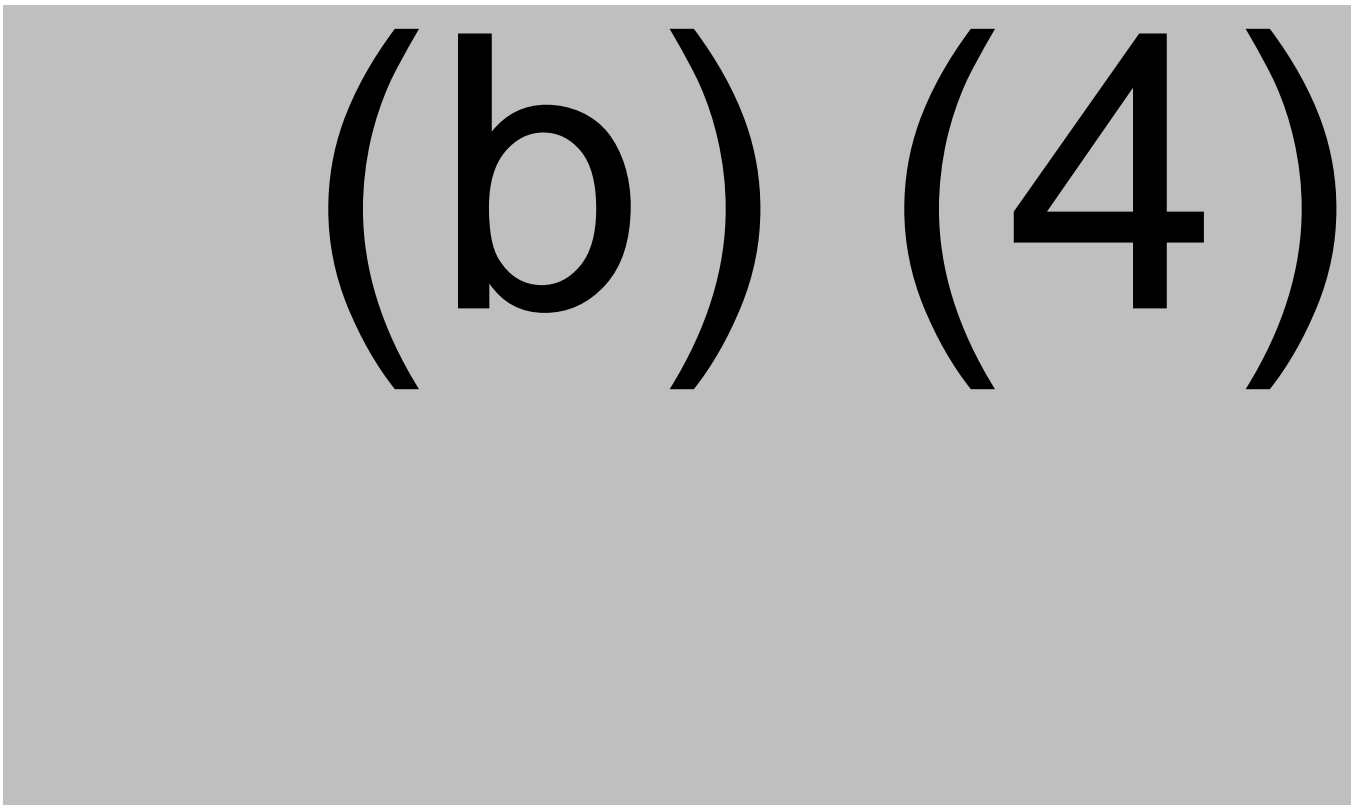
- *Aspergillus niger*, var.
- *Trichoderma longibrachiatum* (a.k.a. *T. reesei* or *T. viride*)
- *Bacillus lentus*
- *Paenibacillus lentus*
- *B. subtilis* (swine diets)

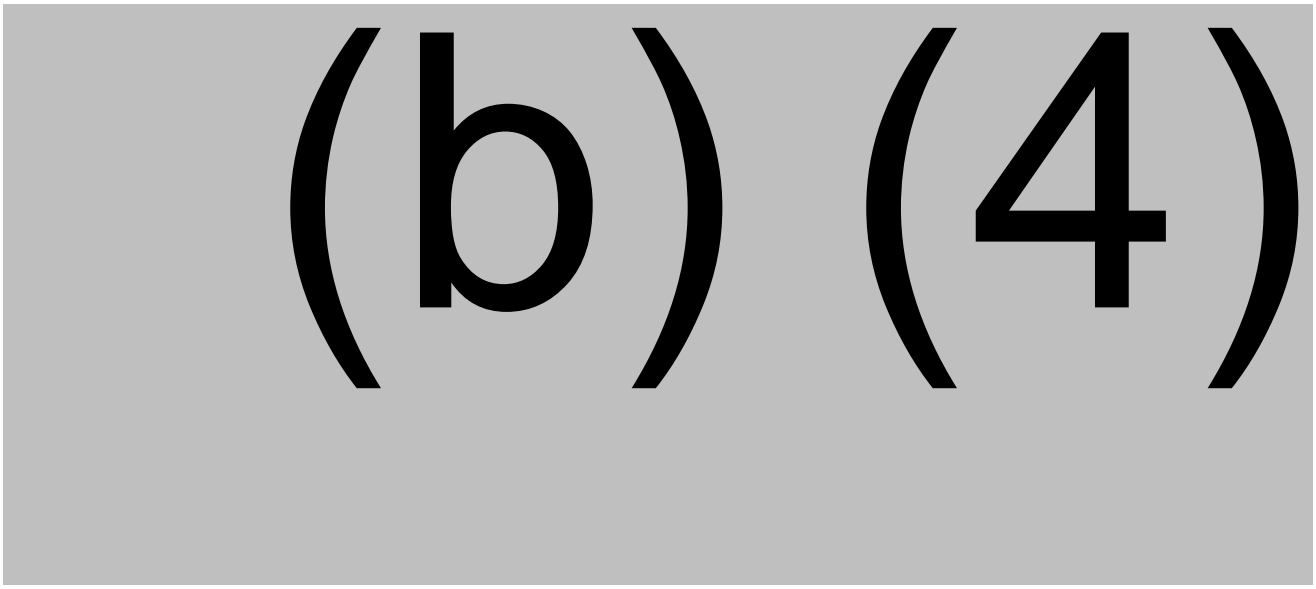
Mannanase 19287 shows high levels of amino acid identities and similarities with the *A. niger* and *T. reesei* β-mannanases and belongs to the same GH-family 5-7. Mannanases in the GH 5-7 family share a highly conserved beta/alpha fold structure with identical amino acids in strictly conserved positions (Hilge, M. *et al.*, 1998). These amino acids are preserved in mannanase 19287 derived from gene(b) (6)(Appendix 7). The β-mannanases from *B. lentus, P. lentus* and *B. subtilis* are members of the separate GH26 family and are examples of mannanases approved for feed even though they do not share the highly conserved positions of identical amino acids in GH 5-7.

The biotech company Direvo developed synthetic endo-β-1,4-D-mannanase enzyme variants based on the endogenous *T. reesei* (b) (4)), which is encoded by the wild-type *T. reesei* (b) (4)). Two patents describe the impact of modifications to the *T. reesei* GH5-7 mannanase, (b) (4), after heterologous expression of the variants in *S. cerevisiae* (Kensch, O., 2015; Kensch, O. *et al.*, 2010). A total of 85 preferred amino acid changes have been listed in Kensch *et al* 2010 that demonstrate beneficial outcomes in thermostability, either as single substitutions and/or in combination (Kensch, O. *et al.*, 2010). The characteristics of a large number of variants, including thermostability and activity under pH and pepsin conditions encountered during digestion of animal food, are disclosed in both patents.

The synthetic mannanase 19287 protein is one of the Man5A protein variants and is encoded by the synthetic (b) (4) derived from the wild-type *T. reesei* (b) (4). The modifications of the *T. reesei* (b) (4) allow the mannanase 19287 enzyme to withstand high temperatures encountered during the production of pelleted feeds and to maintain enzyme activity at low pH conditions found in the gizzard of the target animals. Details of the amino acid sequence variations are described in Appendix 7.

b. Construction of the expression cassette





c. Production strain

Host strain development

The naturally occurring *T. thermophilus* (current species name) strain (b) (4) was isolated first from (b) (6), (b) (4) and was deposited in the (b) (4) Originally classified as *Chrysosporium lucknowense* based on the morphology of the isolate (Emalfarb, M.A. *et al.*, 1998), it was subsequently identified as a strain of *M. thermophila* (Apinis), (Visser, H. *et al.*, 2011). The (b) (4) strain grows within a temperature range of 25 - 44°C and pH range of 4.5 – 7.0. This wild-type strain has been developed into a family of versatile proprietary host strains for industrial production of enzymes using both mutagenic and recombinant DNA modification approaches (Visser, H. *et al.*, 2011).

The initial mutagenic exposures resulted in a line of parental strains with useful phenotypes for industrial production of enzyme preparations. The strain development has been published (Visser, H. *et al.*, 2011) and is illustrated in **Figure 1**. The strain was derived from the *T. thermophilus* (b) (4) isolate and its descendant strains, using random mutagenic or recombinant DNA techniques. (b) (4)

h

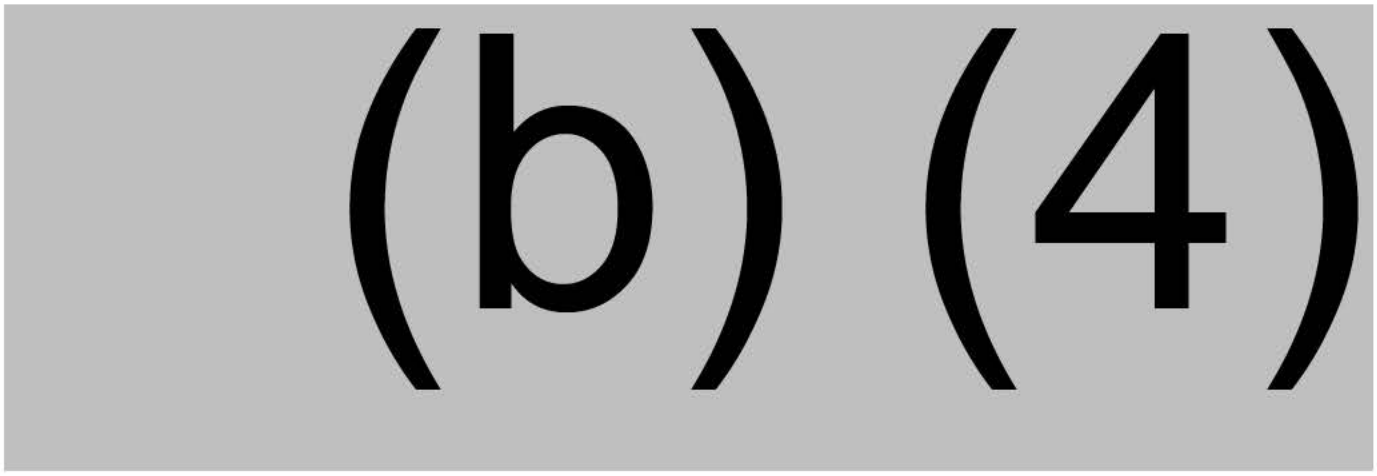
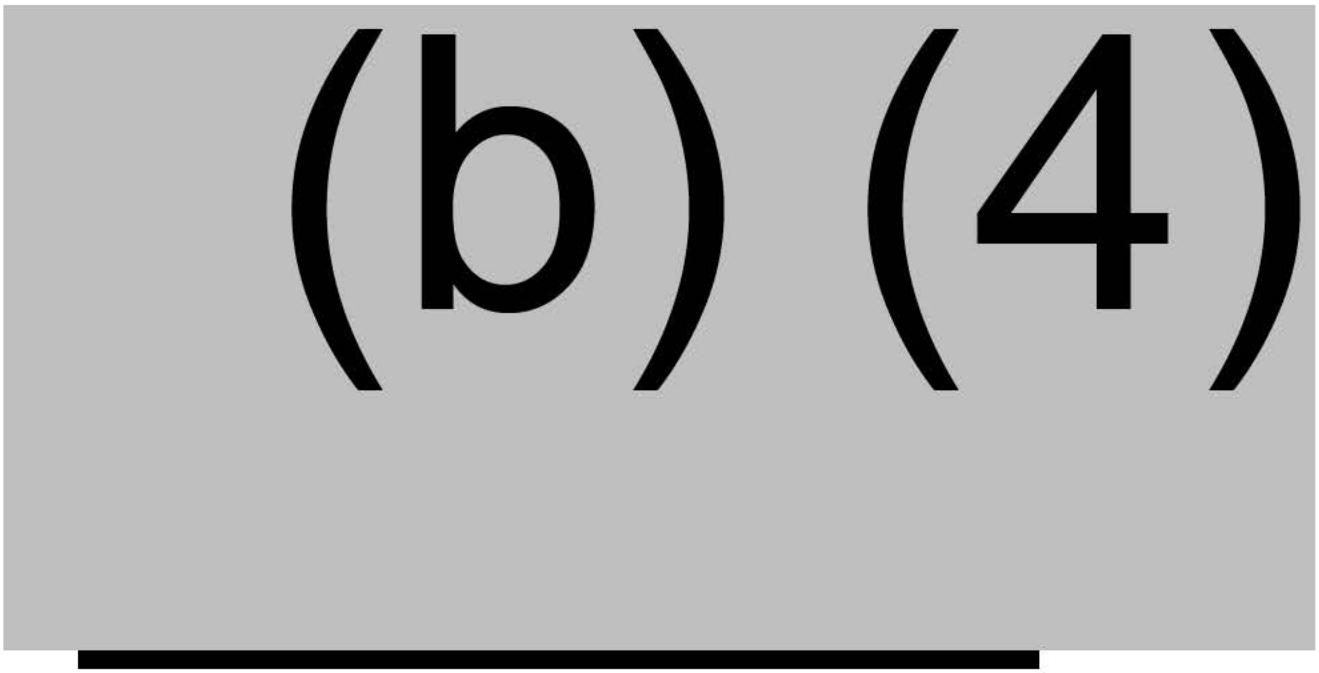
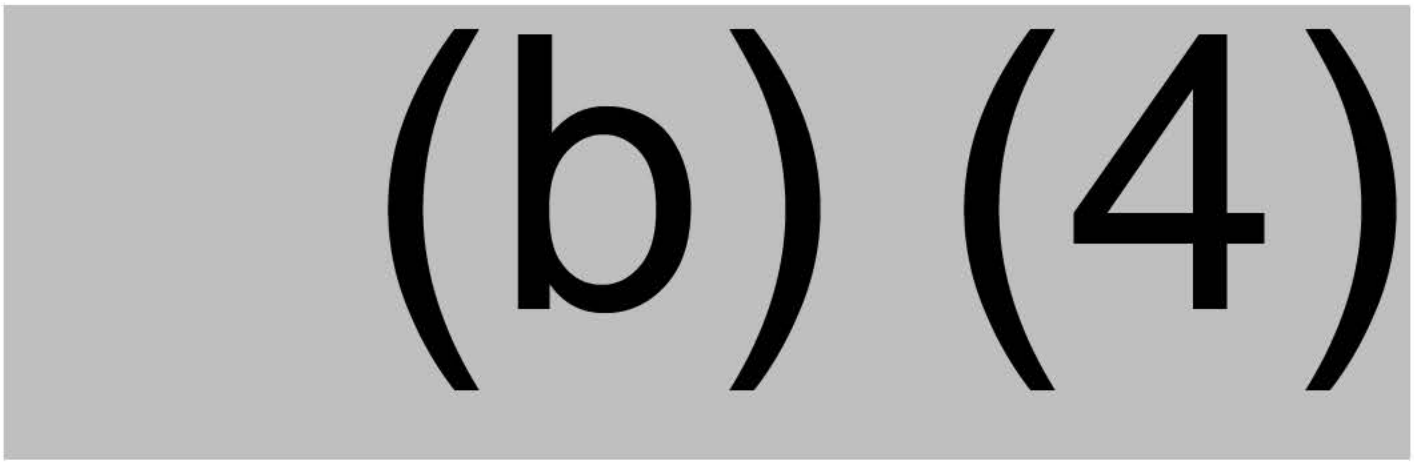


Figure 1. Strain lineage of mannanase 19287 production strain *T. thermophilus* DSM





(b) (4)

Two selection markers were used in the development of host- and production strains (b) (4) and DSM 33149, (a), the endogenous orotate (b) (4) *T. thermophilus* strain (b) (4) and (b), the *Aspergillus nidulans* (b) (4) Both (b) (6), (b) (4) homologs and (b) (6) have been used as selective markers to develop fungal production organisms for over 30 years (Fincham, J.R.S., 1989). See the Visser 2011 publication for further details of the homologous recombination process in (b) (4) (Visser, H. *et al.*, 2011). The sequences of the knock-out constructs are provided in Appendix 8.

To generate the host strain for the mannanase 19287 enzyme preparation, Dyadic targeted

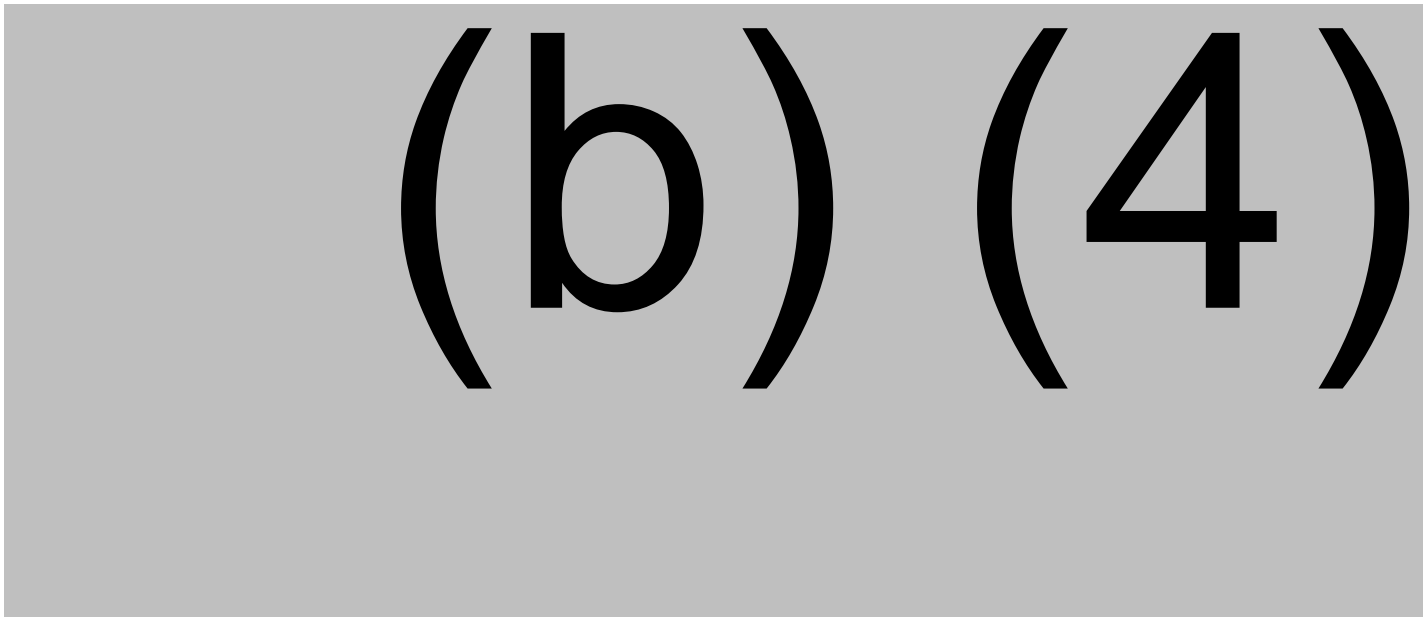
(b) (4)

(b) (4)

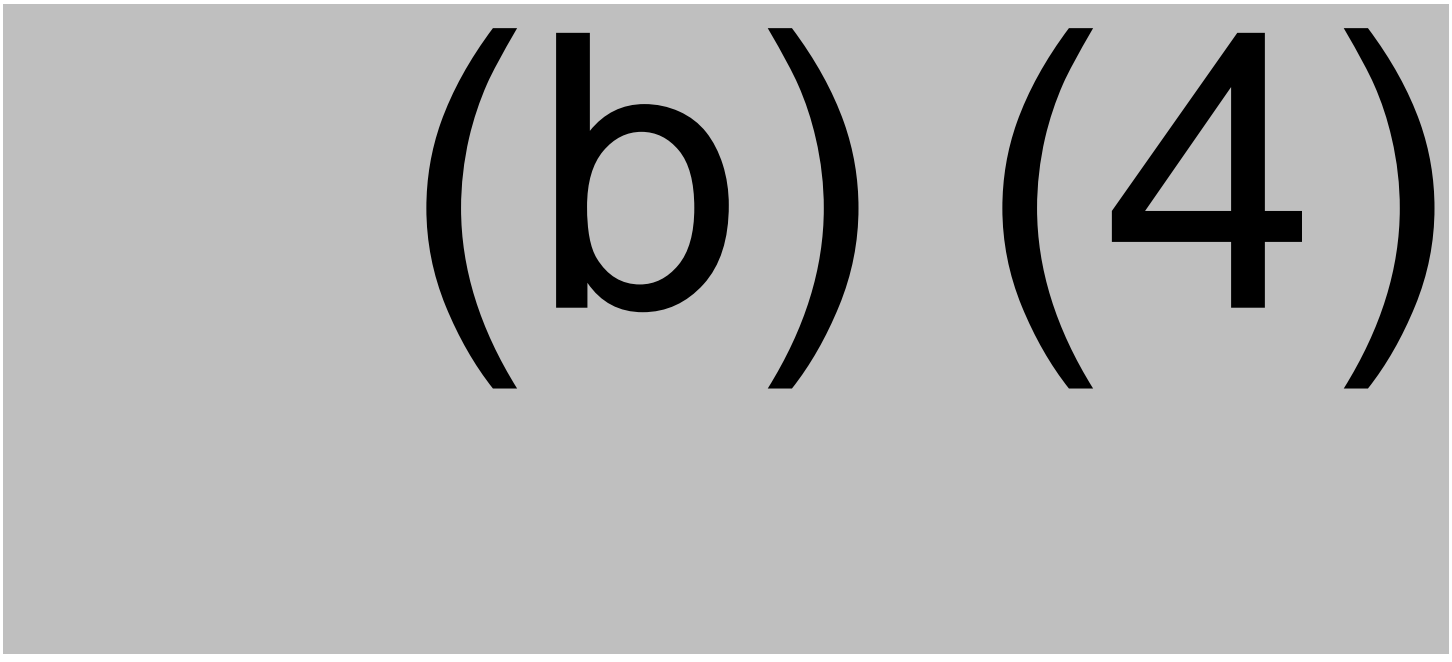
Production strain DSM 33149 construction

In contrast to the homologous recombination approach that was used for the targeted gene inactivation during the development of the host organism (b) (4) integration of (b) (4) . The NHEJ mechanism is used by organisms to repair double strand DNA breaks (DSB). In the case of integrating linear double-stranded DNA, the NHEJ system connects the linear expression cassette

into an existing DSB, thereby incorporating the recombinant DNA randomly into the host strain genome (Fincham, J.R.S., 1989; Ruiz-Díez, B., 2002).



d. Gene copy numbers



e. Genetic Stability

(b) (4)

f. Absence of transformable rDNA

The mannanase 19287 enzyme is expressed by *T. thermophilus* DSM 33149. The well-characterized recombinant DNA (rDNA) is free of attributes that would render it unsafe for constructing microorganisms and has been integrated into the host genome. (b) (4)

No rDNA was detected in samples of Mannanase CC liquid concentrates. The analyses of

(b) (4)

g. Absence of antibiotic resistance markers

(b) (4)

(b) (4)

h. Absence of production organism (APO)

(b) (6)

3. Manufacturing process

The production organism *T. thermophilus* DSM 33149 meets the criteria for a safe production microorganism as described by Pariza and Cook (Pariza, M.W. and Cook, M., 2010). The non-pathogenic DSM 33149 strain is considered appropriate for manufacturing in accordance with both current Good Manufacturing Practices (cGMPs) for animal foods and the OECD's criteria for Good Industrial Large Scale Practice (GILSP) (OECD, 1992) given the long history of safe industrial use of the (b) (4) production strain system for over 20 years and the previous GRAS determination for the parental (b) (4) strain UV18-25 evaluated in GRN 292 (Dyadic International (USA) Inc, 2009; FDA Center for Food Safety and Applied Nutrition (CFSAN), 2009).

The notifier has implemented and maintains a Feed Safety Management System in compliance with the Food Safety Modernization Act (FSMA) Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventive Controls for Food for Animals rule. The notifier also complies with the FAMI-QS Code (Version 6.0) for the production and trade of feed additives and maintains a Quality Management System that fulfills the requirements of ISO 9001:2015 (Appendix 11).

(b) (4)

The manufacturing processes for Mannanase CC and the final Natupulse® TS and TS L products proceed through several phases. (b) (4)

a. Raw materials

Raw materials used in the fermentation, recovery, and formulation process for the mannanase 19287 enzyme preparation are standard ingredients used in the animal food/enzyme industry (Appendix 6). The raw materials are of a purity and quality suitable for their intended use. They are Food Chemicals Codex (FCC) grade (US Pharmacopeia, 2020) or high-quality chemical or pharmaceutical grades (USP, NF, ACS, or reagent grades). All raw materials are sourced from approved suppliers, have internal specifications, and are quality inspected before use (Appendix 13).

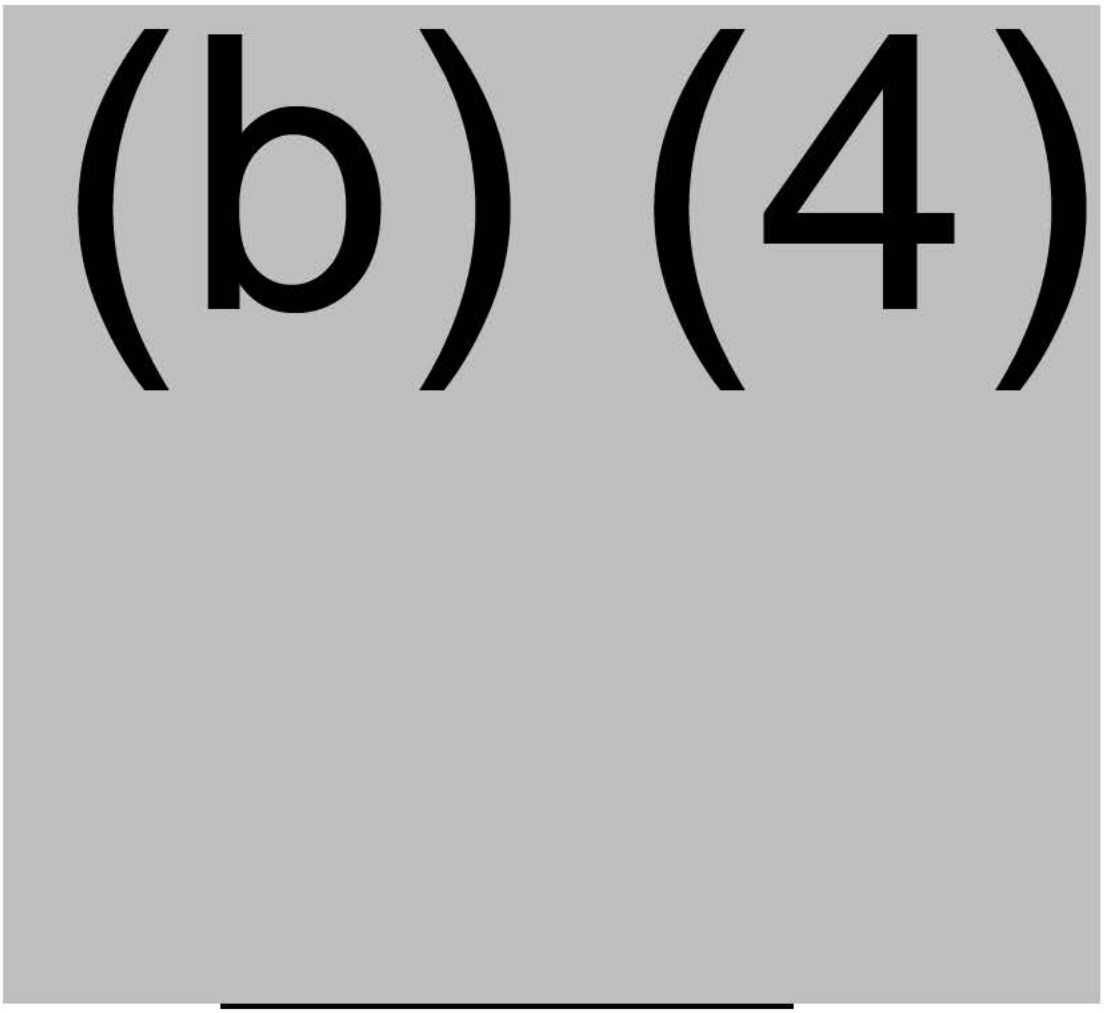
b. Master and working cell banks

(b) (4)

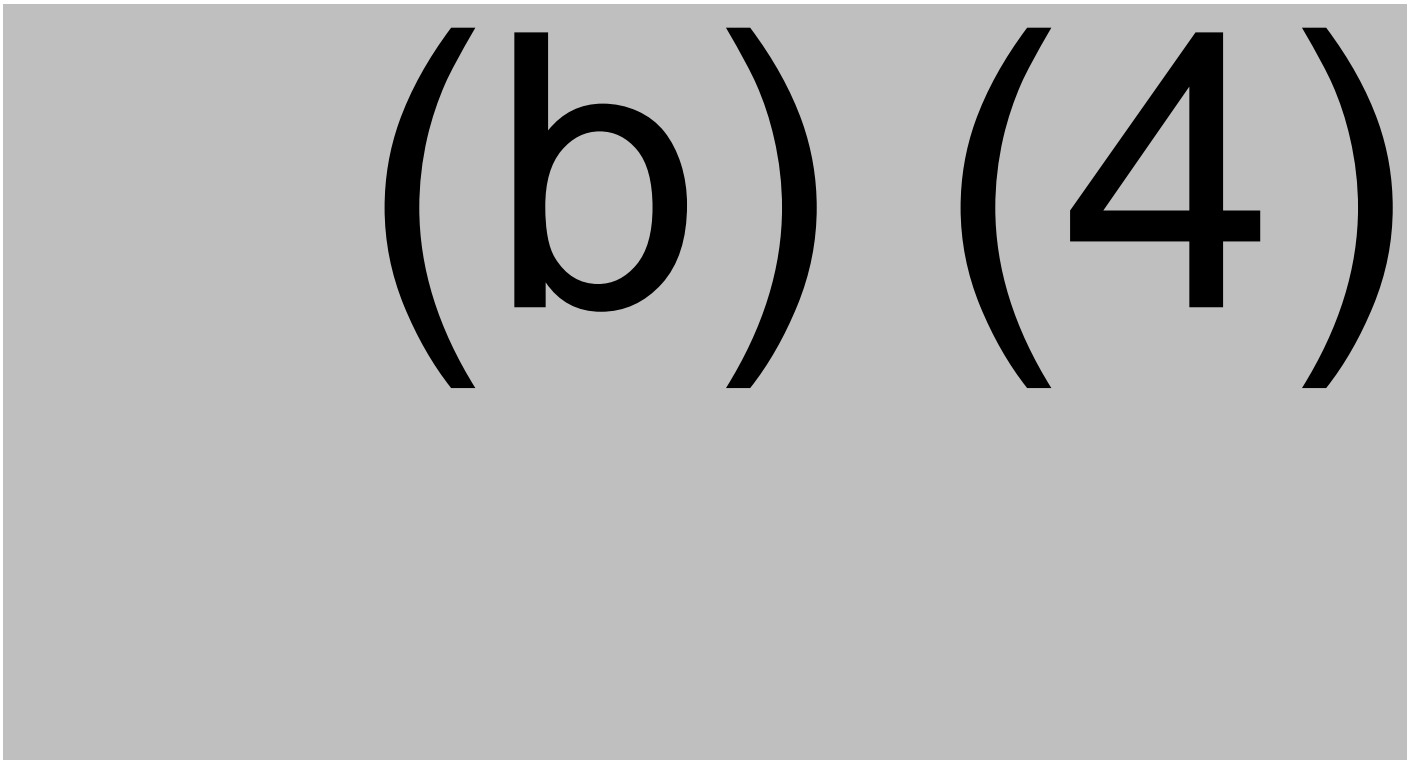
c. Fermentation

(b) (4)

Figure 2. Production process of Mannanase CC, the mannanase 19287 enzyme concentrate.



(b) (4)



d. Recovery

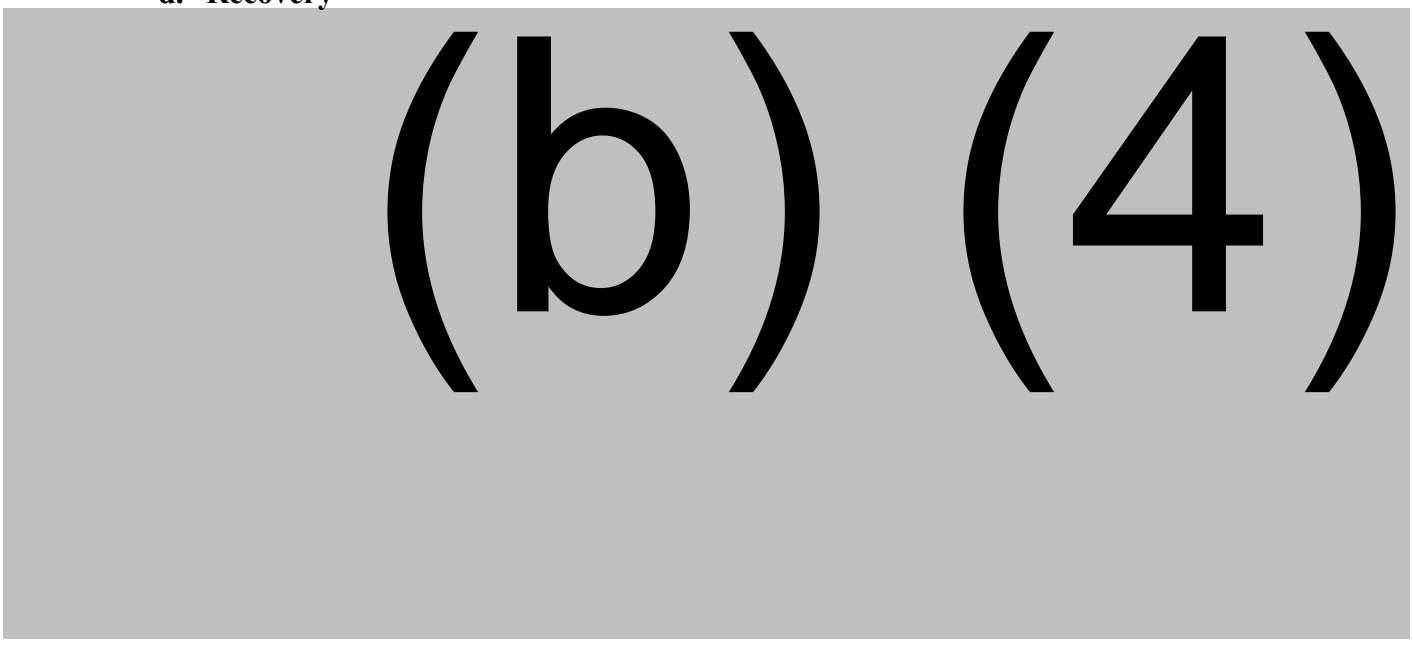
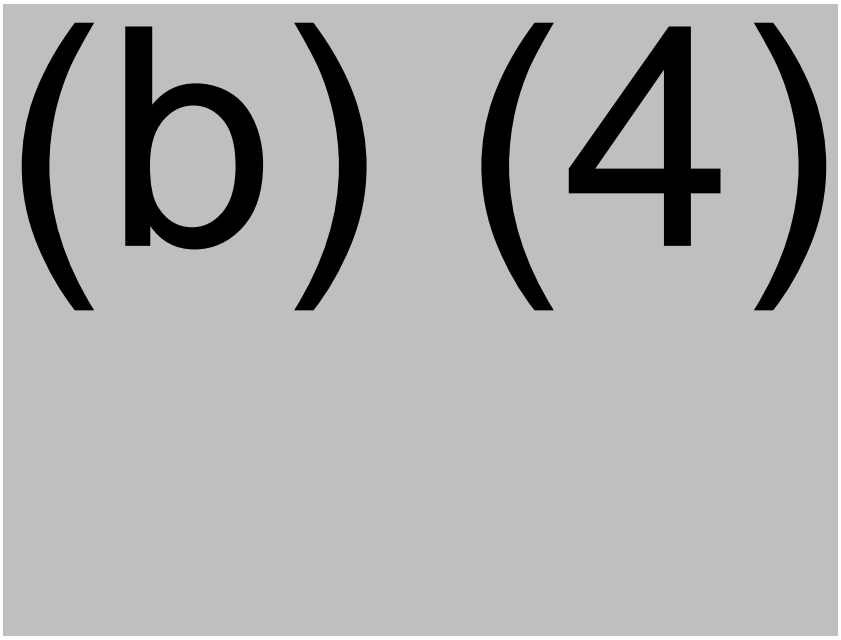


Figure 3: Recovery Overview



e. Formulation

The mannanase 19287 enzyme preparation is provided in two different final products:

1. Natupulse® TS, a dry beige to brown powder formulation
2. Natupulse® TS L, a light brown liquid formulation

For more detailed information, see Appendix 12.

Manufacturing process - Natupulse® TS

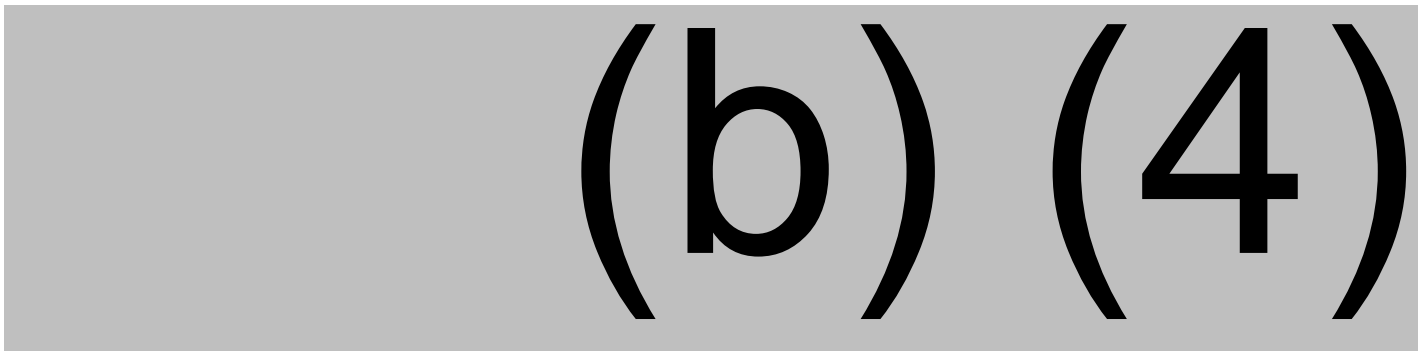
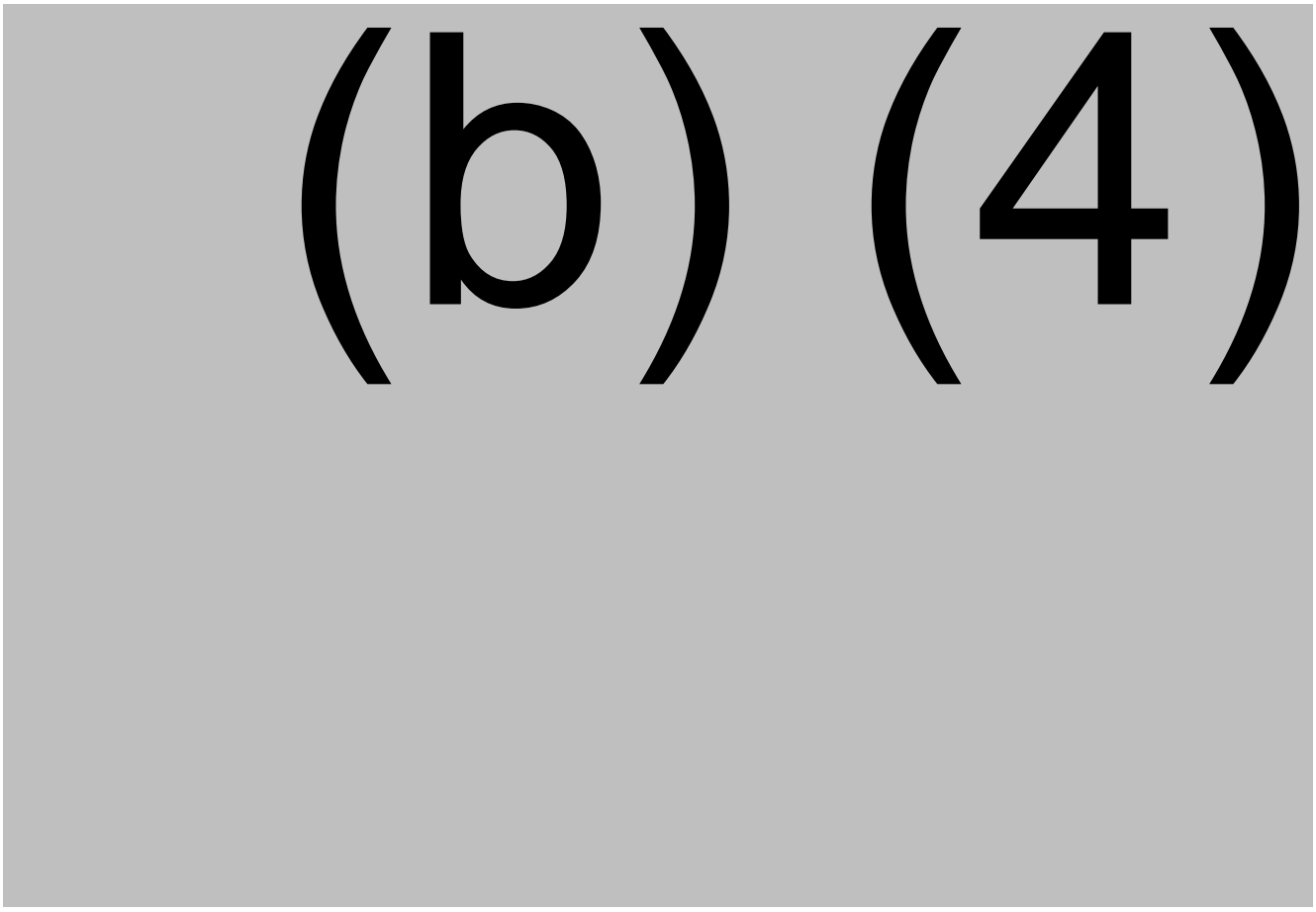


Figure 4. Production process of Natupulse® TS



Manufacturing process - Natupulse® TS L

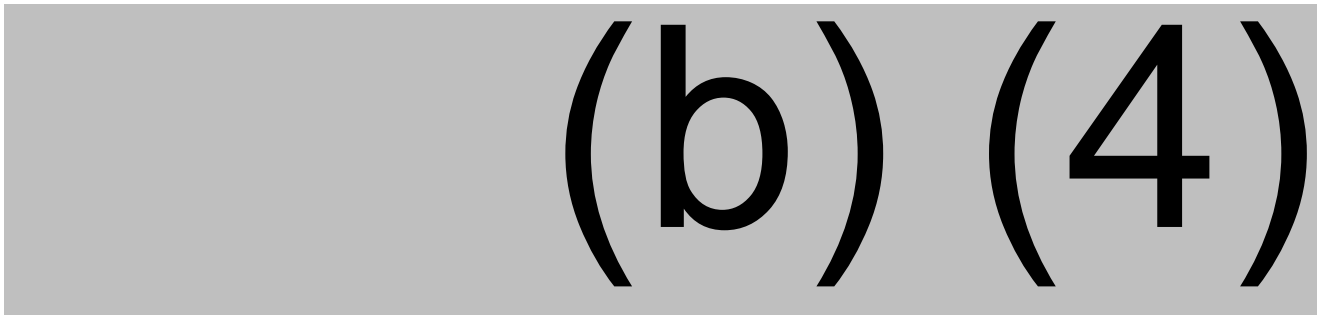
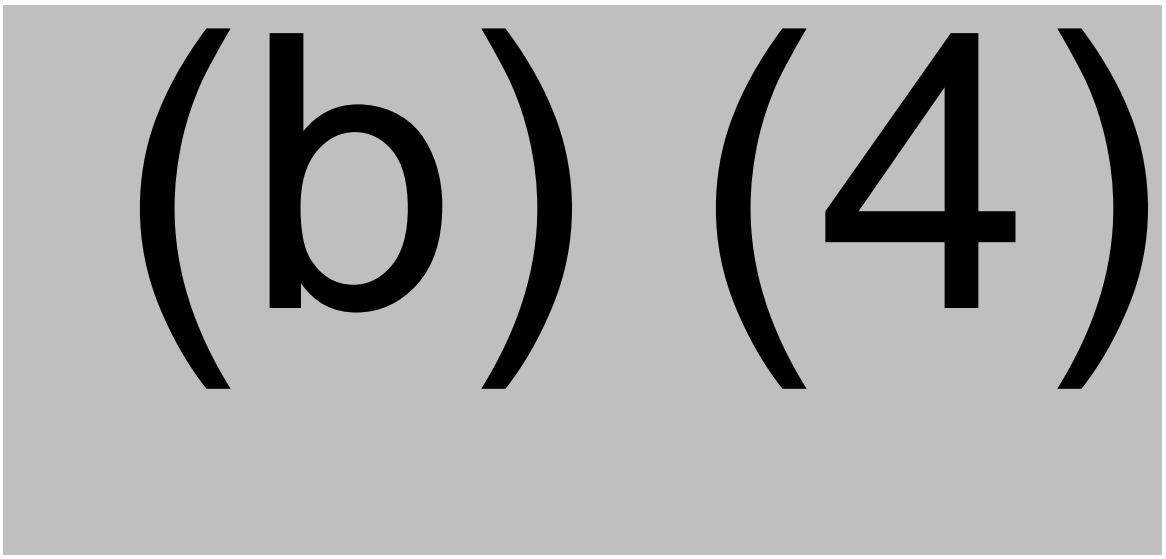


Figure 5. Production process of Natupulse® TS L



C. Composition and Specifications

1. Enzyme concentrate

The liquid stabilized Mannanase CC is the source for the liquid **Natupulse® TS L** and dry **Natupulse® TS** final products. The composition is characterized by its enzyme activity in TMU/g and its Total Organic Solids (TOS).

One mannanase unit (TMU) is defined as the amount of enzyme that liberates 1 µmol reducing sugars determined as mannose per minute under the conditions of the test method (pH 3.5 and 50 °C).

TOS is the sum of all organic compounds present in the enzyme preparation derived from the enzyme source and manufacturing process. TOS (in % or g/100g) is calculated by subtracting the water content, the ash residue, and any diluents, including formulation ingredients:

TOS (%) = 100 - A - W – D, where:
A = % ash
W = % water
D = % diluents and other formulation ingredients

2. Final product compositions

The liquid **Natupulse® TS L** and dry **Natupulse® TS** enzyme products are the two commercial product forms available for the notified mannanase 19287 enzyme preparation. They provide a minimum of (b) (4) The recommended dose for broiler is 800 TMU/kg feed, which is (b) (4) Natupulse® TS or TS L per (b) (4)

See Part 2 A.1.(e) above for their formulation ingredients and Appendix 13 for their composition. All raw materials used in the final formulations are either approved animal food additives published in 21 CFR 573, substances that are Generally Recognized as Safe (GRAS) for the intended use in 21 CFR 582 or are otherwise acceptable ingredients for use in animal food, such as those defined in the most recent AAFCO Official Publication (AAFCO OP) and comply with prescribed limits.

3. Final product specifications

Specifications have been established to confirm the physical, chemical and microbial composition, as well as the enzyme activity (**Table 7)**. These specifications conform to the specifications for enzyme preparations, as outlined in the FCC, 12th Edition (US Pharmacopeia, 2020), and to the general specifications and considerations for enzyme preparations used in food processing by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), as well as identified in the AAFCO OP, Enzyme Marketing Coordination Section (Association of American Feed Control Officials (AAFCO), 2023a; FAO/WHO, 2006).

Table 7: JECFA/FCC purity requirements for mannanase 19287 enzyme preparations

Test parameter	Limits
Chemical Composition	
Lead [mg/kg]	≤ 5
Antimicrobial activity	absent
Microbial Composition	
Coliform bacteria [CFU/g]	<30 CFU/g
Salmonella [CFU]	absent in 25g
Escherichia coli [CFU]	absent in 25g

Three Test Article (TA) batches and three production batches of Mannanase CC, Natupulse® TS L, and Natupulse® TS, respectively, were formulated and were tested against FCC/JECFA requirements, and other internal specifications (Appendices 16); the TA names may refer to in-house identifiers as listed in **Table 1**.

4. Analytical methods

Mannanase 19287 enzyme activity

One TMU is defined as the amount of enzyme that liberates 1 µmol reducing sugars determined as Mannose per minute under the conditions of the test method (pH 3.5 and 50 °C).

Enzyme activity is measured by three validated assay procedures specific for endo-1,4-β-D-mannanase activity:

1. calibration standards, FEN/0003/01 - Determination of β-Mannanase activity in reference materials using dinitrosalicylic acid (DNS),
2. feed additives, FEN/0004/01 – Photometric assay of mannanase activity in feed additives, and
3. feeds and mineral premixes FEN/0005/01 - Photometric assay of mannanase activity in feed and mineral premix.

See Appendices 15 for the respective validation reports of Standard Operating Procedures (SOPs) FEN/003 - 005 and their evaluations by the European Union Reference Laboratory (EURL) for Feed Additives(European Union Reference Laboratory (EURL), 2021).

Antimicrobial Activity

Antimicrobial activity analyses of test article and production batches were performed in accordance with JECFA requirements (FAO/WHO, 2006) using Novobiocin as the antibiotic positive control substance (see Appendix 16, JECFA results). No antimicrobial activities were detected with any of the six bacterial organisms required by JECFA (*Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Bacillus circulans*, *Streptococcus pyrogenes* and *Serratia marcescens*).

5. Stability

a. Final product stability

Shelf life- Natupulse® TS L (Appendix 17)

To establish the shelf-life of Natupulse® TS L, a 24-month stability study has been conducted to examine the stability behavior of three different batches of the additive when stored for 24 months at temperatures of 6°C, 20°C and 30°C/75% relative humidity (r.h.). The retention of mannanase 19287 enzyme activity was calculated using the formula below:

$$\text{retention (\%)} = \frac{\text{analyzed activity of Natupulse® TS L after x months (TMU/kg)}}{\text{analyzed activity of Natupulse® TS L at } t_0 \text{ (TMU/kg)}} \times 100$$

Natupulse® TS L showed good stability for all three batches at temperatures of 6 °C, 20 °C and 30 °C/75% r.h.. After storage for 24 months at the respective storage conditions, all three Natupulse® TS L test article batches showed enzyme activities at (b) (4) of timepoint t_0 values. The data support the shelf life of 24 month for Natupulse® TS L, when stored in a cool, dry place (below 20ºC) in unopened original packing.

Shelf life - Natupulse® TS (Appendix 17)

To establish the shelf-life of the additive, an 18-month stability study has been conducted to investigate the impact of temperature on the storage stability of the dry product. Three batches each of Natupulse® TS were packaged in paper bags with PE-inner lining and stored at temperatures of 20°C or at 30°C/75% relative humidity (r.h.), respectively. Samples were removed from storage at regular intervals and tested. The retention of mannanase 19287 enzyme activity was calculated according to the following formula:

$$\text{retention (\%)} = \frac{\text{analyzed activity of Natupulse® TS after x months (TMU/kg)}}{\text{analyzed activity of Natupulse® TS at } t_0 \text{ (TMU/kg)}} \times 100$$

During storage for 18 months, Natupulse® TS experienced activity losses of about (b) (4) % during storage at 20 °C, and of about (b) (4) during storage at 30 °C/75% r.h.

The data support the shelf life of Natupulse® TS for 18 months when stored at 20°C and for 12 months when stored at 30 °C/75% r.h.

b. In-feed stability

To determine the stability of mannanase 19287 enzyme activity in feed, three test article batches of Natupulse® TS were mixed with a typical poultry basal diet mash, respectively. See Appendix 11 for feed ingredients, their common names and AAFCO ID/21 CFR references.

Stability in poultry feed - Natupulse® TS (Appendix 18)

Mash samples were collected and stored for three months at 20°C and at 30°C/75% r.h., respectively. In order to normalize the activity results, mash samples containing the Natupulse® TS batches were analyzed to obtain the mannanase 19287 enzyme activity at t_0 (b) (4) recovery). The retention of mannanase 19287 enzyme activity was calculated by the following formula:

$$\text{retention (\%)} = \frac{\text{analyzed activity of feed sample after x months (TMU/kg)}}{\text{analyzed activity of feed sample at } t_0 \text{ (TMU/kg)}} \times 100$$

After storage for three months, (b) (4) of enzyme activity was retained when stored at 20 °C. Storage at 30°C/75% r.h. resulted in (b) (4) retention of mannanase 19287 enzyme activity.

In addition, a portion of each mash preparation containing Natupulse® TS underwent pelleting according to standard conditions used in the feed industry. At t_0, pellets retained (b) (4)% enzyme activity of the mash batches; after storage for three months at 20 °C, (b) (4) of mannanase 19287 enzyme activity remained in the pelleted feed samples. A similar result was found after storage for three months at 30°C/75% r.h.; (b) (4) of enzyme activity were retained under these conditions.

In summary, the data support good Natupulse® TS stability in feed mash or pellets when stored for three months at 20 °C or at 30°C/75% r.h..

Enzyme stability in poultry feed - Natupulse® TS L (Appendix 17)

Three lots of Natupulse® TS L were sprayed onto pellets of a typical poultry basal diet using a Post Pelleting Liquid Application (PPLA) system, respectively. Natupulse TS® L was added with a dose rate of 0.1 g/kg via PPLA to reach the targeted activity of 800 TMU/kg. Samples were collected and stored for three months at 20°C or at 30°C/75% r.h., respectively. In order to normalize the activity results, pellet samples sprayed with Natupulse® TS L were analyzed to obtain the mannanase 19287 enzyme activity at t_0. The retention of mannanase 19287 enzyme activity was calculated as follows:

$$\text{retention (\%)} = \frac{\text{analyzed activity of feed sample after x months (TMU/kg)}}{\text{analyzed activity of feed sample at } t_0 \text{ (TMU/kg)}} \times 100$$

At t_0, pellets demonstrated (b) (4) enzyme activity retention; after storage for three months at 20 °C, (b) (4) of mannanase 19287 enzyme activity remained in the feed pellet samples. A similar result was found after storage for three months at 30°C/75% r.h.; 7(b) (4) of enzyme activity was retained under these conditions.

In summary, the data support good Natupulse® TS L stability when applied to feed pellets using a PLLA system.

c. Premix stability

Stability in mineral premix - Natupulse® SD Powder (Appendix 18)

The stability of mannanase 19287 enzyme activity was evaluated in a blend of Natupulse® SD Powder with a typical broiler mineral premix. The mineral premix carrier contained vitamins and minerals, equal to or exceeding current National Research Council (NRC) requirements (National Research Council (U.S.), 1994). See Appendix 21 for feed ingredients, their common names and AAFCO ID/21 CFR references.

Three different lots of Mannanase Natupulse® SD Powder were blended with the mineral premix and then stored for up to 6 months in a refrigerator (6°C) or in climate chambers at 20°C or 30°C / 75% r.h., respectively. In order to normalize the activity results, blended samples were analyzed to obtain the mannanase 19287 enzyme activity at t_0. The retention of mannanase 19287 enzyme activity was calculated using the following formula:

$$\text{retention (\%)} = \frac{\text{analyzed activity of sample after x months (TMU/kg premix)}}{\text{analyzed activity of sample at } t_0 \text{ (TMU/kg premix)}} \times 100$$

After 6 months of storage, the premix blend retained (b) (4) enzyme activity at 6°C, (b) (4) activity at 20°C and (b) (4) % activity at 30°C / 75% r.h., respectively.

In summary, the data support good stability of mannanase 19287 enzyme activity from Natupulse® SD Powder when mixed into a typical mineral broiler premix.

d. Pelleting stability in poultry feed

Thermostability in poultry feed - Natupulse® TS (Appendix 19)

To determine the effects of increasing pelleting temperatures on mannanase 19287 enzyme activity in poultry feed, three test article batches of Natupulse® TS were mixed with a typical poultry basal diet to prepare three separate mash batches. See Appendix 21 for feed ingredients, their common names and AAFCO ID/21 CFR references. Mash samples were analyzed for enzyme activity and contained (b) (4)% of the targeted TMU/kg values.

Subsequently, each of the three mash batches were pelleted according to standard industry practices at temperatures of 80, 85, or 90 °C, respectively. The pelleting settings demonstrated low

temperature variations at each condition. The retention of mannanase 19287 enzyme activity was calculated by the following formula:

$$\text{retention (\%)} = \frac{\text{analyzed activity of pelleted feed (TMU/kg)}}{\text{analyzed activity of mash feed (TMU/kg)}} \times 100$$

Enzyme activity decreased when exposed to increasing pelleting temperatures. Feed samples pelleted at 80 °C retained (b) (4)% enzyme activity. Pelleting at a temperature of 85 °C resulted in activity retention of (b) (4)% in comparison to the mash samples. Finally, after pelleting temperatures of 90 °C, poultry feed pellets showed (b) (4)% mannanase 19287 activity remaining from the initial mash batches.

In summary, the data support good stability of mannanase 19287 enzyme activity from Natupulse® TS under pelleting conditions commonly practiced in the feed industry.

e. Homogeneous mixing

Homogeneity in poultry feed (mash and pellets) - Natupulse® TS (Appendix 20)

To determine the homogeneity of mannanase 19287 enzyme activity in feed, three test article batches of Natupulse® TS were mixed with a typical poultry basal diet to prepare feed mash. See Appendix 21 for feed ingredients, their common names and AAFCO ID/21 CFR references.

Mash samples were subsequently pelleted according to standard conditions used in the feed industry. Twelve samples each were collected after mixing (mash) and after pelleting for each of the three feed batches (pellets), respectively.

In order to normalize the activity results, the 12 samples from mash and pellet steps of each respective batch were analyzed to measure the mannanase 19287 enzyme activity. Homogeneity was expressed as % coefficient of variation (CV) in 12 samples of each blend batch, and recovery of mannanase 19287 enzyme activity was calculated using the following formula:

$$\text{recovery (\%)} = \frac{\text{analyzed activity of premix sample (TMU/kg premix)}}{\text{analyzed activity of product (TMU/g SD) x dosage (g/kg premix)}} \times 100$$

The CV values for 12 mash samples of the three Natupulse® TS/poultry feed blends ranged from (b) (4) respectively. The average mannanase 19287 enzyme activity values of mash samples in the respective blends varied from (b) (4)

After pelleting, the CV values for 12 pellet samples from each respective Natupulse® TS batch ranged from (b) (4) and the average enzyme activity values for the pellets from each blend varied from (b) (4)

In summary, the data support the homogeneous distribution of mannanase 19287 enzyme activity from Natupulse® TS in broiler feed.

Confirmation of Mannanase 19287 Enzyme Activity and Homogeneity in Feed

In order to confirm homogeneity of feed samples in a published broiler study (Fickler, A. *et al.*, 2023), 12 samples each were collected from T-4 starter and T-4 grower diets containing the target activity of 800 TMU/kg (Appendix 20) Feed samples used in the published study demonstrated an average mannanase 19287 activity of (b) (4) with a Coefficient of Variation (CV) of 7% compared to the target value. The homogeneity analyses of T4 starter and grower feeds (target rate 800 TMU/kg) showed recoveries of (b) (4) . The data confirmed the *in vitro* homogeneous distribution of Natupulse® TS in broiler feed shown above.

Homogeneity in poultry feed - Natupulse® TS L (Appendix 20)

The homogeneous distribution of Natupulse® TS L in pelleted feed was examined after applying three separate lots of the final product via a PPLA system. A typical poultry feed mash (see Appendix 11 for the ingredients) was conditioned and pelleted according to standard conditions used in the feed industry. Natupulse® TS L was then added to the pellets at the target activity of (b) (4).

After mixing, twelve samples were collected from each batch and analyzed for enzyme activity, in order to normalize the activity results. Homogeneity was expressed as % coefficient of variation (CV) in 12 samples of each feed batch, and recovery of mannanase 19287 enzyme activity was calculated using the following formula:

$$\text{recovery (\%)} = \frac{\text{analyzed activity of feed sample (TMU/kg)}}{\text{analyzed activity of product (TMU/g TS L) x dosage (g/kg feed)}} \text{ x } 100$$

The recovered mannanase 19287 enzyme activities ranged from (b) (4) in pellets from the three feed batches, respectively; the associated CV values within each batch varied between 4.9% to 6.7%.

In summary, the data support the homogeneous distribution of mannanase 19287 enzyme activity in broiler feed after PLLA system use of Natupulse® TS L.

Homogeneity in premixtures - (b) (4) Appendix 20)

The homogeneous distribution of mannanase 19287 enzyme activity was evaluated in a blend of (b) (4) with a typical broiler mineral premix. The mineral premix carrier contained vitamins and minerals, equal to or exceeding current National Research Council (NRC) requirements (National Research Council (U.S.), 1994). See Appendix 21 for feed ingredients, their common names and AAFCO ID/21 CFR references.

Three different lots of (b) (4) Powder were blended with the mineral premix and 12 samples were collected from each blend batch directly after production. In order to normalize the activity results, the 12 samples from the respective batch were analyzed to obtain the mannanase 19287 enzyme activity. Homogeneity was expressed as percent (%) coefficient of variation (CV) in 12 samples of each blend batch, and recovery of mannanase 19287 enzyme activity was calculated using the following formula:

$$\text{recovery (\%)} = \frac{\text{analyzed activity of premix sample (TMU/kg premix)}}{\text{analyzed activity of product (TMU/g SD) x dosage (g/kg premix)}} \text{ x } 100$$

The CV values per 12 samples of the three blends, respectively, ranged from (b) (4) Recoveries of enzyme activity varied from (b) (4) in the respective blends.

In summary, the data support the homogeneous distribution of mannanase 19287 enzyme activity from (b) (4) when mixed into a typical broiler mineral premix.

D. Technical Effect

1. Technical effect Natupulse® TS

Economic pressure on the poultry industry has increased, since demand for meat and eggs as affordable protein sources in human nutrition has increased significantly. On the other hand, prices for the main poultry feed ingredients have risen due to various underlying factors, including increased demand, raw material availabilities, transportation and the impact of fast-spreading diseases on current production processes cannot be underestimated (https://www.fao.org/poultry-production-products/en/).

The main ingredients of animal feeds, grains and seeds contain cellulose, hemicellulose and lignin as structural plant components. Depending on the plant sources, hemicelluloses contain varying amounts of non-starch polysaccharides (NSPs), with xylans and mannans making up the bulk of these substances. While grain sources are generally low in NSPs, the protein sources used in animal feed, including soybean, guar or copra meal, contain varying levels of soluble and insoluble xylans (Dierick, N.A., 1989; Knudsen, K.E.B., 1997). Poultry are not able to digest NSPs in the stomach and the small intestine due to the lack of adequate endogenous enzymes.

In particular, the presence of soluble NSPs can result in higher viscosity of the content in the digestive tract (Choct, M. *et al.*, 2010; Lee, J.T. *et al.*, 2003a; McNab, J.M. and Smithard, R.R., 1992). This in turn may slow the transport of the ileal content, reduce nutrient digestibility and lead to activation of an immune response that diverts energy (Shastak, Y. *et al.*, 2015).

Enzymes that belong to the class of NSP-degrading hydrolases (NSPases), including xylanases and β-mannanases, have demonstrated improvements in digesta viscosity of poultry target animals. Several publications demonstrated digesta viscosity reductions in animals consuming food supplemented with β-mannanases (Choct, M., 1999; Daskiran, M. *et al.*, 2004; Fickler, A. *et al.*, 2023; Jackson, M.E. *et al.*, 2004). These results support the intended use of the mannanase 19287 enzyme preparation, the β-mannanase enzyme (E.C. 3.2.1.78) present in Natupulse® TS by demonstrating the decrease in NSPs.

2. Use levels required to produce desired effect

As formalized in 21 CFR 570.230, use levels and technical effect are required to address safety of a notified substance:

"In Part 2 of your GRAS notice, you must include: (…)

(d) When necessary to demonstrate safety, relevant data and information bearing on the physical or other technical effect the notified substance is intended to produce, including the quantity of the notified substance required to produce such effect."

In the GRAS Final Rule ("Substances Generally Recognized as Safe" *Federal Register* 81, (August 17, 2016): 54960) FDA provided further clarifications in responses to comments received to the 2010 Pilot Program Notice ("Substances Generally Recognized as Safe Added to Food for Animals; Notice of Pilot Program" *Federal Register* 75, (June 4, 2010): 54960). Regarding GRAS substances in animal feed, CVM stated in response to comment 144: "We agree that data and information bearing on the physical or other technical effect the notified substance is intended to produce are only necessary when they bear on safety."

CVM provides examples where the utility of an animal feed enzyme is considered important for the safety of the target animals: Phytases are added to animal feed to increase the bioavailability of phosphorous for the target animal. Such animal diets usually are formulated with reduced amounts of available phosphorous. In case the notified substance phytase does not demonstrate its utility, the target animals may develop adverse conditions due to the lack of sufficient nutrients, which in turn would detrimentally impact animal welfare and thus safety. Another example mentioned in the Final Rule relates to the supplementation of animal feed with a protease. In the absence of protease utility, the animals may not receive the necessary amounts of amino acids and thus may experience adverse effects similar to the phytase example above.

In contrast, a potential lack of utility of β-mannanases or similar NSPases, e.g., xylanases or other carbohydrases, has been demonstrated repeatedly not to have adverse effects. Control diets, either standard diets in the absence of enzyme supplementation, or diets where soybean meal has been substituted with other protein sources containing elevated levels of soluble NSPs can be regarded as examples for the lack of enzyme functionality (no enzyme supplemented activity). In a recently published broiler study (Fickler, A. *et al.*, 2023), the negative control diet contained 7.5% guar meal, a protein source with significant levels of soluble (galacto-)mannans. No

significant differences were observed in safety-related outcomes for the target animals as consequence of the guar meal present in feed.

Furthermore, standard corn/soybean-based feed served as the control diet in a recently published tolerance study ((b) (4)) where the control diet was supplemented with increasing amounts of the mannanase 19287 enzyme preparation. The control diet can be regarded as a worst-case model of non-functional endo-β-mannanase being present in broiler feed. As shown in the publication, no adverse effects or significant differences in target animal safety outcomes (average body weight, average daily gain or number of dead or culled birds) were found between control and Natupulse® TS-containing diets. In addition, the presence of the endo-β-mannanase indicated small, statistically non-significant increases in gross energy and apparent ileal digestible energy levels. Similar results were discussed in a publication (Latham, R.E. *et al.*, 2018), where broiler chicken were fed negative diets containing lower apparent metabolizable energy levels. The authors concluded that the presence of β-mannanase improved the ileal digestible energy in the animals.

We have determined that our tolerance study and the scientific literature clearly demonstrate that the intended use of endo-β-mannanases (E.C. 3.2.1.78) to reduce viscosity in digesta has no bearing on target animal safety and that therefore no utility data are required in this section.

PART 3: TARGET ANIMALS AND HUMAN EXPOSURE

A. Target Animal Exposure

1. Amount of the notified substance during intended use

The Natupulse® TS/TS L final commercial products are formulated to contain (b) (4) TMU/g, and the mannanase 19287 enzyme preparation is recommended to deliver a dose of the product at 800 TMU/kg feed (0.8 TMU/g feed). This corresponds to 100 g Natupulse® TS per metric ton of feed. One TMU is defined as the amount of enzyme that liberates 1 µmol reducing sugars determined as Mannose per minute under the conditions of the test method (pH 3.5 and 50 °C).

As described in more detail in the safety publication (Kern, A. *et al.*, 2020), the final Natupulse® products are formulated from Mannanase CC (for Natupulse® TS L) or (b) (4) (for Natupulse® TS). Using well-established Cobb-500 Performance Objectives as an example (Cobb Vantress, 2022), the following maximal Estimated Daily Intake (EDI_{max}) can be calculated for mannanase 19287 enzyme preparation at the target rate of 800 TMU/kg feed. The average Cobb 500 broiler takes in 220 g feed on day 42 at a weight of 3.3 kg and the EDI_{max} for this broiler breed feed equals:

220 g feed ÷ 3.3 kg body weight (BW) = 66.7 g feed/kg body weight (BW)

At 0.8 TMU/g feed, the EDI_{max} (TMU) for mannanase 19287 is:

66.7 g feed/kg BW /day x 0.8 TMU/g feed = 53.1 TMU/kg BW/day

Using the TOS value of 67.5 g/100 g (see Kern *et al.*, 2020), this results in an EDI_{max} of 0.18 mg mannanase 19287 preparation/ kg BW on day 42. This calculation also confirms the EDI_{max} determination of 0.19 mg/ kg BW on day 42 discussed in (Kern, A. *et al.*, 2020).

2. Amount of other substances formed in feed because of notified substance

As outlined in Part 2.A.1.a, the mannanase 19287 enzyme, like all β-mannanase enzymes, catalyzes the hydrolysis of beta-mannan components of hemicellulose (Association of American Feed Control Officials (AAFCO), 2023a). Therefore, the mannanase 19287 enzyme will liberate

mannan oligomers from the hemicellulose polymer constituents (Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB), 1972). The resulting β-(1,4)-mannan oligosaccharides can be hydrolyzed further by endogenous glycosidases, including β-mannosidases (EC 3.2.1.25) that act on the terminal β-glycosidic bond, and α-galactosidases (EC 3.2.1.22) removing the side chains (Dhawan, S. and Kaur, J., 2007). The monosaccharides generated by these enzymatic activities will be metabolized as nutrients and will not remain in the edible animal tissues. The endo-β-mannanase enzyme will be inactivated and degraded during the passage through the digestive tract. The food-grade processing aids are not expected to affect target animal safety.

3. Amount of other substances present from the notified substance

As noted in the batch analyses from four final product batches and numerous test article materials, the mannanase 19287 enzyme preparations meet the established specifications for enzymes. The maximal broiler exposure is estimated at 0.18 mg/kg body weight /day, demonstrating a safe product. Therefore, the use of Natupulse® TS and TS L as a feed additive will not contribute to undesirable residues in animal products.

4. Data for notified substance and other substances consumed by target animal

The mannanase 19287 enzyme preparation is manufactured by fermentation of the production organism *T. thermophilus* DSM 33149 and subsequent DSP steps using raw materials suitable for food and feed production. The resulting commercial products formulated from Mannanase CC will contain very small levels of any residual fermentation products (e.g., salts, carbohydrates, and proteins).

Enzyme preparations are used in small quantities during feed production, and it is expected that the target animals will digest any residual salts, carbohydrates, and proteins to use their constituents (sugars, amino acids and minerals) as nutrients during metabolism.

B. Human Exposure

1. Potential quantities consumed in humans from edible animal tissues

a. Residues of notified substance

Mannanase 19287 enzyme is a protein and, like any protein, is expected to be digested into its amino acid constituents in the animal's gastro-intestinal (GI) tract. When the enzyme is digested in the GI tract, it will be broken down into its amino acid constituents, making it indistinguishable from other food molecules and, therefore, the potential for residues in edible animal tissue appears minimal (Association of American Feed Control Officials (AAFCO), 2023a).

b. Residues of substances formed in animal food from the notified substance

As outlined in Part 3.A.2 above, the mannanase 19287 enzyme, like all β-mannanase enzymes, catalyzes the hydrolysis of beta-mannan components of hemicellulose (Association of American Feed Control Officials (AAFCO), 2023b). Therefore, the mannanase 19287 enzyme will liberate mannan oligomers from the hemicellulose polymer constituents (Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB), 1972). The resulting carbohydrates will be metabolized as nutrients and not remain in the edible animal tissues.

c. Residues of substances present with or generated by the notified substance

The soluble low molecular substances (salts, etc.) present in the mannanase 19287 enzyme preparation will be removed by the target animal's digestive system and will therefore not remain in the edible animal tissues.

2. Data for notified substance and other substances consumed by humans

As discussed above, mannanase 19287 is a protein and will be digested into amino acids in the animal's digestive tract. The Enzyme Marketing Coordination document of the AAFCO OP confirms in its Safety section that enzymes as protein catalysts are expected to be digested by the target animals(Association of American Feed Control Officials (AAFCO), 2023a). Enzymes evaluated by the safety assessment process developed by Pariza and Foster (Pariza, M.W. and

Foster, E.M., 1983) should raise no human safety concerns. See Part 6.C.4 below for the assessment of the production organism according to Pariza and Cook, which was updated for microbial enzyme preparations in animal feed (Pariza, M.W. and Cook, M., 2010).

PART 4: SELF- LIMITING LEVELS OF USE

This part is not applicable. There are no self-limiting levels of use associated with Natupulse® TS or Natupulse® TS L products that would result in the animal food being unpalatable or technologically impractical.

PART 5: EXPERIENCE BASED ON COMMON USE IN FOOD BEFORE 1958

This part is not applicable. The statutory basis for the notifier's conclusion of GRAS status is based on scientific procedures in accordance with §570.30(a).

PART 6: NARRATIVE ON THE CONCLUSION OF GRAS STATUS

A. Introduction

Enzymes proteins are ubiquitous in nature and play a critical role in the life of all pro- and eukaryotic organisms. In addition, enzymes have always been present as natural components of human and non-human food. Lastly, the proteinaceous nature of enzymes presents a high degree of safety assurance, since they are digested into amino acids during the metabolism of nutrients contained in the food source (Association of American Feed Control Officials (AAFCO), 2023a).

Several publications from Dr. Pariza and co-authors extensively addressed the issue of food and feed enzyme safety. The authors have concluded that enzymes *per se* used in food or feed are inherently nontoxic. Therefore, a safety evaluation should focus on possible contaminants which could be present in the final enzyme preparation. Within this framework, the safety of the production strain should be the primary consideration when evaluating enzyme safety (Pariza, M.W. and Cook, M., 2010; Pariza, M.W. and Foster, E.M., 1983; Pariza, M.W. and Johnson, E.A., 2001). Previous parts of this AGRN have detailed the data to evaluate the safety aspects of the notified substance. Part 6.C. below will review the safety of the production organism following the decision flow chart developed by Dr. Pariza and colleagues.

Overall, the following subsections discuss the safety aspects of the notified substance in this AGRN, the mannanase 19287 enzyme preparation produced with *T. thermophilus* DSM 33149. Each section reviews information provided in the previous parts of this GRAS notice. The intent of the final summary is to bring together the arguments that mannanase 19287 preparations are safe for their intended use in animal feed to reduce the viscosity of intestinal digesta in poultry diets.

B. Safety of Endo-β-1,4-D-Mannanase

1. History of safe use

Microbial endo-β-1,4-D-mannanases have been produced at industry-scale for the paper/pulp-, food-, feed-, energy- and detergent applications. Manufacturing processes for the enzyme preparations involve both bacterial and fungal organisms. For technical applications, β-mannanases are listed on the US EPA TSCA Chemical Substance Inventory (ACTIVE, exempt per 40 CFR 711); in addition, they are registered under ECHA REACH Regulation in the EU (Dawood, A. and Ma, K., 2020; Dhawan, S. and Kaur, J., 2007; Environmental Protection Agency (EPA); European Chemicals Agency (ECHA), 2013).

For applications in poultry feed, β-mannanases from *Bacillus lentus, Paenibacillus lentus*, *Aspergillus niger* or *Trichoderma longibrachiatum* (also known as *T. reesei*) are listed in Table 30.1 of the AAFCO Official Publication for reduction of digesta viscosity in poultry diets (Association of American Feed Control Officials (AAFCO), 2023b). Numerous animal studies demonstrating the utility of β-mannanases in poultry have been published over the last 30+ years (Balasubramanian, B. *et al.*, 2018; Choct, M. *et al.*, 2010; Daskiran, M. *et al.*, 2004; Hosseindoust, A. *et al.*, 2019; Jackson, M.E. *et al.*, 2004; Latham, R.E. *et al.*, 2015; Lee, J.T. *et al.*, 2003b; Sundu, B. *et al.*, 2006).

Regulatory authorizations in the European Union (EU) for endo-β-1,4-D-mannanases as feed enzymes have been granted for enzyme products produced by *P. lentus* (EU Reg 2023/2645) and by *A. niger* (EU Reg 2023/2850) following the publications of the EFSA Scientific Opinions for each β-mannanase, respectively (EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) *et al.*, 2023; EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) *et al.*, 2019).

Additional support for the safety of endo-β-1,4-D-mannanase enzyme preparations come from two GRAS notifications, GRN 566 and GRN 739, submitted to FDA-CFSAN for their intended uses as food processing aids (AB Enzymes, 2014; Novozymes, 2017). The notified substance of GRN 566 is *T. reesei* endo-β-1,4-D-mannanase enzyme Man5A, expressed in *T. reesei*; GRN 739 documents the evaluation of a β-mannanase from *Taleromyces leycettanus* expressed in *A. niger*. CFSAN responded with "No questions" letters to the respective notifiers (FDA Center for Food Safety and Applied Nutrition, 2015; FDA Center for Food Safety and Applied Nutrition (CFSAN), 2018). Both enzyme preparations have been authorized the EU after

the publication of the scientific opinions (EFSA Panel on Food Contact Materials Enzymes and Processing Aids (CEP) *et al.*, 2022a; EFSA Panel on Food Contact Materials Enzymes and Processing Aids (CEP) *et al.*, 2022b).

In summary, endo-β-1,4-D-mannanases have been safely used for decades in a variety of commercial applications, including food production and supplementation of animal feed. The long history of safe use and publications noted above support the safety of endo-β-1,4-D-mannanases.

The notified substance of this AGRN is the mannanase 19287 enzyme preparation produced with the production strain *T. thermophilus* DSM 33149. The preparation is commercialized in final products Natupulse® TS, a light-brown dry powder product, and Natupulse TS L, a clear brown liquid product, respectively. The final products are formulated with safe and suitable ingredients to contain mannanase 19287 enzyme activities (b) (4) product (Appendix 14).

Protein chemistry of mannanase 19287

The biotech company (b) (4) developed synthetic endo-β-1,4-D-mannanase enzyme variants based on the endogenous *T. reesei* Man5A protein (GenBank ID Q99036), which is encoded by the wild-type *T. reesei* (b) (4)). Two patents describe the impact of the modifications to the native Man5A protein (Kensch, O., 2015; Kensch, O. *et al.*, 2010). A total of (b) (4) have been listed that demonstrate beneficial outcomes after heterologous expression of the variants in *S. cerevisiae*, either as single substitutions and/or in combination.

The synthetic mannanase 19287 protein (aka Man F), one of the *T. reesei* Man5A variants, demonstrates high homology to the natural endo-β-1,4-D-mannanase. The mannanase 19287 protein is encoded by the synthetic (b) (4) gene, a synthetic DNA sequence derived from the native *T. reesei* (b) (4). The resulting amino acid modifications improve the mannanase 19287 enzyme for conditions encountered during animal feed production as well as during passage through the target animals' digestive tract.

To evaluate the safety of this synthetic endo-β-1,4-D-mannanase (E.C. 3.2.1.78), the enzyme preparation has been thoroughly characterized and its detailed properties have been discussed in Part 2.A. "Scientific data that identifies the notified substance":

1. The synthetic endo-β-1,4-D-mannanase is a variant of the native *T. reesei* Man5A enzyme (GenBank ID Q99036) and shows >90 % homology to the natural protein. Both β-mannanases function as glycohydrolases (GHs) and belong to GH Family 5, subfamily 7 (GH 5-7). Mannanase 19287 shows high homologies to other members of the GH 5-7 family, e.g., ManA of *A. niger*.

2. Importantly, mannanase 19287 preserves the highly conserved beta/alpha fold structure of GH 5-7 β-mannanases, including all (b) (4) in strictly conserved positions (Hilge, M. *et al.*, 1998).

3. Mannanase 19287 (E.C. 3.2.1.78) catalyzes the following reaction:

$$\beta\text{-(1,4)-D-mannan} + H_2O \rightarrow 2\ \beta\text{-(1,4)-mannan oligosaccharides}$$

resulting in the generation of randomly sized β-mannan fragments. We have confirmed this β-mannanase activity in lab studies using (b) (4). The masses of released oligosaccharides were identified by mass spectroscopy and showed the expected values of randomly hydrolyzed fragments.

4. Mannanase 19287 demonstrates enzyme activity in a commercially available endo-β-1,4-D-mannanase-specific activity assay (Appendix 4). This method is the basis of the proprietary assay methods for the characterization of purified mannanase 19287 standards, enzyme activity in premixes / feed additives and enzyme activity in feed materials. One enzyme unit (TMU) is defined as the amount of enzyme that liberates 1 µmol reducing sugars determined as mannose per minute under the conditions of the test method (pH 3.5 and 50 °C).

5. The mannanase 19287 enzyme preparation is free of xylanase, cellulase, α-amylase, β-glucanase, or acidic protease side activities, has pH and temperature optima appropriate for the intended use in target animals and demonstrates thermotolerance at temperatures typically encountered during feed production processes.

In summary, these results confirm that the notified substance of this AGRN, the mannanase 19287 enzyme preparation, is an endo-β-1,4-D-mannanase (E.C. 3.2.1.78). As such, the safety aspects of endo-β-1,4-D-mannanases discussed at the beginning of this chapter support our determination that the mannanase 19287 enzyme preparation is safe for its intended use in poultry feed.

C. Safety of the Production Organism *Thermothelomyces thermophilus*

1. History of safe use

As outlined in the publications of Pariza and colleagues and in the introduction above, the safety of the production strain should be an important consideration when evaluating enzyme safety. From a historical standpoint, the production organism has been used for decades in the industrial manufacture of enzymes. *T. thermophilus* (synonym *M. thermophila, Sporotrichum thermophile* or *Corynascus thermophilum*) is a thermophilic filamentous fungus and has been isolated globally from diverse sources, primarily from soil or self-heating vegetable matter composts (Singh, B., 2016; Visser, H. *et al.*, 2011; Zhang, Y. *et al.*, 2014). Similar to other fungi such as *T. reesei*, the organism expresses high levels of cellulolytic enzymes including xylanases, hemicellulases etc. (Karnaouri, A. *et al.*, 2014). The genome of *T. thermophilus* reference strain ATCC 42464 has been sequenced completely (Berka, R.M. *et al.*, 2011), and based on the DNA assembly, the micro-organism contains over 9000 genes located on seven chromosomes. The full genome information has allowed for the isolation and characterization of a variety of endogenous enzymes.

Taxonomy

The majority of scientific publications and the CFSAN GRAS Notification GRN 292 for an endogenous cellulase expressed in *T. thermophilus* refer to the microorganism by its previous taxonomic name, *M. thermophila* (Dyadic International (USA) Inc, 2009). Based on sequence analysis of ribosomal genes, *M. thermophila* was re-classified to the novel genus *Thermothelomyces* as species *T. thermophilus* in 2015 (Marin-Felix, Y. *et al.*, 2015). This nomenclature is also listed in the Mycobank database. **Table 8** demonstrates that the classification change is on the genus level, not any higher classification levels. More recently, the taxonomy of Chaetomiaceae, in particular its thermophilic species, has been re-evaluated (Wang, X. *et al.*, 2022). No changes were proposed for *T. thermophilus* and its closest related species, *T. guttulatus*, *T. heterothallicus* and *T. hinnuleus*.

Table 8: Taxonomic Classification of *T. thermophilus* versus *M. thermophila*.

Species	**Classification (*Genus*)**
Thermothelomyces thermophilus (Apinis)	Fungi, Ascomycota, Pezizomycotina, Sordariomycetes, Sordariomycetidae, Sordariales, Chaetomiaceae, *Thermothelomyces*
Myceliophthora thermophila (Apinis)	Fungi, Ascomycota, Pezizomycotina, Sordariomycetes, Sordariomycetidae, Sordariales, Chaetomiaceae, *Myceliophthora*

Two endogenous *T. thermophilus* enzymes have been the subjects of GRAS notifications for their intended uses in human food; FDA issued "No additional questions" letters for both submissions:

1. Dyadic International, Inc. submitted a GRAS notification GRN 292 for the above-mentioned overexpression of an endogenous cellulase enzyme preparation expressed in the C1-HC lineage of *T. thermophilus* (see below in Section 6.C.3. Production Strain Development). The submitter cited a safety evaluation of a *T. thermophilus* laccase gene expressed in *A. oryzae* (Brinch, D.S. and Pedersen, P.B., 2002) and summarized unpublished toxicology reports to demonstrate the safety of the *T. thermophilus* host and enzyme preparation for the use in wine and juice production. Based on test reports summarized by (b) (4) in GRN 292, the *T. thermophilus* host strain did not express mycotoxins of regulatory concern or listed in several publications related to the safety of microbial enzyme preparations (Blumenthal, C.Z., 2004; Olempska-Beer, Z.S. *et al.*, 2006; Pariza, M.W. and Cook, M., 2010; Pariza, M.W. and Johnson, E.A., 2001). **Please note:** the *T. thermophilus* production strain DSM 33149 is also derived from the progenitor strain *T. thermophilus* C1-HC.

2. GRN 122 informed FDA-CFSAN that an endogenous *T. thermophilus* laccase gene expressed in *A. oryzae* was GRAS for use in chewing gums and mouthwashes; the center issued a "No questions" letter (FDA Center for Food Safety and Applied Nutrition (CFSAN), 2003). The data in the GRN was published (Brinch, D.S. and Pedersen, P.B., 2002) and JECFA referenced these results in their evaluation of the laccase preparation (FAO and WHO, 2003).

Several Regulatory documents provide risk assessments of *T. thermophilus* (formerly *M. thermophila*):

- The German Committee for Biological Agents (ABAS) is responsible for the classification of biological agents into risk groups related to work activities. ABAS publishes the classifications in Technical Rules for Biological Agents (TRBA) and fungal organisms are classified in TRBA 460. The current document (updated April 14, 2020) lists *M. thermophila* (*T. thermophilus*) into Risk Group 1.
- The German Central Committee on Biological Safety (ZKBS) evaluated *T. thermophilus* and finalized its recommendation in May 2019 (Zentrale Kommission für die Biologische Sicherheit, 2019) as follows:

    1. Based on the long history of safe industrial use of production organisms derived from the *T. thermophilus* strain C1-HC, subsequent host and recipient strains in the lineage were categorized as Risk Group 1. Please note: Host strain *T. thermophilus* (b) (4) and *T. thermophilus* production strain DSM 33149 for mannanase 19287 are descendants of *T. thermophilus* strain C1-HC and fall into Risk Group 1.
    2. Wild-type *T. thermophilus* may, on rare occasions, infect healthy humans; the committee recommended to classify the wild-type organism in Risk Group 2.

- GESTIS (Hazardous substance information system of the German Social Accident Insurance) *Myceliophthora thermophila* – anamorph: Risk Group 1 (reference ABAS, TRBA 460) (Information system on hazardous substances of the German Social Accident Insurance, 2020)
- EU Directive 2000/54/EC does not list *T. thermophilus/M. thermophilus* in Annex III (COMMUNITY CLASSIFICATION for agents which are known to infect humans).

These Regulatory evaluations support the safety of *T. thermophilus* when used as a host or production organism for enzyme preparations.

(b) (4) *Strain Family*

The naturally occurring and highly productive *T. thermophilus* strain (b) (4) was isolated first from (b) (4) and was deposited in (b) (4) (accession number (b) (4)). Originally classified as *Chrysosporium lucknowese* based on the morphology of the isolate, it was subsequently re-classified to *M. thermophila* / *T. thermophilus* following DNA homology analyses of ribosomal protein genes. The (b) (4) strain was developed into versatile host strains for industrial production of enzymes (Visser, H. *et al.*, 2011) and the non-recombinant derivative strain C1-HC (UV18-25) was modified using recombinant methodologies for the generation of the production strain of a cellulase preparation for use in wine, beer and juice production ((b) (4)). As mentioned above, GRN 292 also references unpublished toxicology and pathogenicity reports to demonstrate the safety of the cellulase production organism.

Further support for the safety of the (b) (4) strain of *T. thermophilus* is provided by more than 25 years of industrial use of the microorganism with up to 50 products for food, feed, textiles and paper/pulp. (b) (4) has been manufacturing (b) (4)-produced technical enzymes since 1996 ((b) (6), (b) (4); later (b) (4) licensed the (b) (4) technology to other enzyme producers in a number of applications.

In summary, the history of safe use of the *T. thermophilus* (b) (4) strain family together with previous published assessments by Regulatory bodies support the safety of the production organism *T. thermophilus*, strain C1-HC.

2. Absence of pathogenicity and toxicity

In order to assess the role of *T. thermophilus* in human infections, a comprehensive literature search was performed for the species. Five cases were identified and compared to seven reports of previous publications (Nourrisson, C. *et al.*, 2017). Five out of twelve cases involved severely immunocompromised patients; infections were linked to injuries related to the severe trauma or immune suppression. Fatal outcomes resulted from infections in four confirmed and one suspected immunocompromised patient. All seven previous case studies listed in Nourrisson *et al*, 2017 were also identified independently in our literature review.

The search for the *T. thermophilus* species in medical literature was recently updated; one additional publication was identified reporting a case of fatal pneumonia linked to *T. thermophilus*

(Yoo, I.Y. *et al.*, 2023). Similar to cases discussed in Nourrisson *et al*, 2017, the patient would be considered immunocompromised after an unrelated peripheral blood stem cell transplantation. Taken together, the published case studies indicate that *T. thermophilus* and other filamentous fungi pose a higher risk for immunocompromised patients. The organism is susceptible to antifungal treatments in case of infection.

The SWISSPROT/UniProt Toxin-Database (https://www.uniprot.org/program/Toxins) currently identifies 114 fungal toxins (as of 12/5/2023). The DSM 33149 genome was queried against all 114 sequences of identified fungal toxins to evaluate the potential for expressing toxins found in fungal species. Six toxin proteins were identified that showed low levels of homology against three DSM 33149 coding sequences; the levels of homology were not considered significant (Appendix 6).

3. Production strain development

The host strain *T. thermophilus* (b) (4) and the production strain *T. thermophilus* DSM 33149 are derived from the parental *T. thermophilus* (b) (4) family. As discussed in Section 6.C.2 above, based on published safety evaluations by international Regulatory bodies, host and production strains derived from *T. thermophilus* C1-HC, both *T. thermophilus* (b) (4) and *T. thermophilus* DSM 33149 are considered safe as derivatives of parental strain C1-HC.

Host strain *T. thermophilus* (b) (4) and production strain DSM 33149 are derived from the parental strain C1-HC described in (Visser, H. *et al.*, 2011) and in GRAS Notice GRN 292 for the cellulase enzyme preparation with the intended use in food production (b) (4), 2009; FDA Center for Food Safety and Applied Nutrition (CFSAN), 2009).

The development process for the mannanase 19287 production strain *T. thermophilus* DSM 33149 has been described in in Part 2, Section B.1.(c) of this AGRN and in the safety publication of the mannanase 19287 enzyme preparation (Kern, A. *et al.*, 2020). The strain lineage of the production strain DSM 33149 is outlined in **Figure 1** in Part 2, Section B.1.(c).

Table 9 below recaps the stepwise description of mutagenic and recombinant methods used to develop the host strain (b) (4) and production strain DSM 33149.

Table 9: Mutagenic and Recombinant Approaches DSM 33149

ID	Description	Genetic approach	Reference
(b) (4)			

The host strain (b) (4) was derived from the non-recombinant *T. thermophilus* strains C1-HC and (b) (4) was modified further using well-established recombinant methodologies available for over 20 years. The (b) (4) knock-out sites

in the host strain were analyzed for the presence of new open reading frames (ORFs) of potential novel proteins not present prior to gene inactivation. The protein sequences of these ORFs were blasted against fungal toxin and virulence factors found in the UniProt database as well as the curated list of all proteins SWISSPROT. No significant homologies were identified during these searches (Appendix 8)

The expression cassette (b) (4)

(b) (4)

In summary, well-known and standard recombinant DNA methodologies were used to generate *T. thermophilus* host and production strains (b) (4) and DSM 33149, respectively. All methods have been established more than 20 years ago and have been used in the development of many recombinant production organisms for enzyme preparations. No significant homologies to fungal proteins of concern were identified with ORF analyses of gene knockout or expression cassette insertion sites. The long history of genetic modifications of microorganisms supports the safety of the production strain DSM 33149.

4. Safety of the production strain

Taxonomic classification

Using the established barcode sequences for *Thermothelomyces* species described for reclassification from *Myceliophthora* and analysis of *Chaetomiaceae* (Marin-Felix, Y. *et al.*, 2015; Wang, X. *et al.*, 2022), we have confirmed the taxonomic identity for the production strain of the notified mannanase 19287 enzyme preparation, *T. thermophilus* DSM 33149. As discussed previously in Part 2.A.2.a, DSM 33149 and reference strain ATCC 42464 matched all genomic sequences identified for *T. thermophilus* in both Marin-Felix 2015 and Wang 2022 at 100% identity. The genomes of DSM 33149 and ATCC 42464 demonstrated 97-99% sequence identities for the barcodes of *T. guttulatus*, *T. heterothallicus* and *T. hinnuleus*, confirming the close relationship with but separate identity from these species.

In summary, the mannanase 19287 production strain DSM 33149 has been identified as a strain of the species *T. thermophilus*.

Based on the extensive literature search, several publications have demonstrated that *T. thermophilus* has the potential to generate mycotoxins, including myceliothermophins and tenuazonic acid. *T. thermophilus* has been shown to produce a family of cytotoxic substances called myceliothermophins (Yang, Y.L. *et al.*, 2007). Internal laboratory studies of five independent production batch liquid concentrates have shown the absence of these toxins when compared to samples spiked with commercially available standards at 10 and 1 µg/kg (Appendix 6).

The *T. thermophilus* genome has been shown to contain the necessary genes to produce the mycotoxin tenuazonic acid (TeA) (Yun, C.-S. *et al.*, 2017). An accredited external test laboratory analyzed commercial batches of Mannanase CC, Natupulse® TS and Natupulse® TS L using a published LC/MS methodology (Siegel, D. *et al.*, 2009). The results identify trace levels of TeA in some samples at the LOQ of the method (Appendix 16). Taking into account the EDI_{max} for the notified substance of 0.18 mg/kg BW/day at the target rate (800 TMU/kg feed), and the lack of significant differences in safety-related outcomes in broiler between the control feed up to the 125-fold excess of the target rate (EDImax of 26 mg TOS/kg BW/day), we consider the risk from the potential presence of TeA to be minimal. This risk evaluation is further supported by the high

NOAEL for the notified substance fed to Wistar rats during the subchronic oral toxicity study (> 1100 mg TOS/kg BW/day).

In summary, the demonstrated absences of (a) significant levels of mycotoxins or (b) protein homologies to known fungal toxins support our evaluation of *T. thermophilus* strain DSM 33149 to be a safe production organism for the notified substance, the mannanase 19287 enzyme preparation commercialized as Natupulse® TS and TS L.

The mannanase 19287 enzyme preparation by *T. thermophilus* DSM 33149, the subject of this AGRN, has been tested in standard toxicology tests (Kern, A. *et al.*, 2020); the results demonstrate that the enzyme material does not contain toxic or genotoxic substances. The publication and results are discussed in further detail in Part 6, Section F.3.a-d, below.

The safety of the production strain DSM 33149 (*T. thermophilus* expressing the synthetic mannanase 19287 gene) was evaluated based on the flow chart for animal feed enzymes outlined in (Pariza, M.W. and Cook, M., 2010). The flow chart is modified from Pariza and Johnson's decision tree for evaluating microbial enzyme safety (Pariza, M.W. and Johnson, E.A., 2001) and asks several questions relating to the introduced DNA in the genetically modified production microorganism. **Table 10** outlines the answers to the questions of the decision tree.

Table 10: *T. thermophilus* DSM 33149 Decision Tree

1. Is the production strain genetically modified?	**Yes**, go to 2	Both mutagenic and recombinant techniques have been used in the development of DSM 33149.
2. Is the production strain modified using rDNA techniques?	**Yes**, go to 3	Standard recombinant techniques have been used to generate the production strain.
3. Issues relating to the introduced DNA are addressed in 3a–3e.	See below	
3a. Do the expressed enzyme product(s) which are encoded by the introduced DNA have a history of safe use in food or feed?	**No**, go to 3b	The expressed mannanase 19287 enzyme preparation does not have a history of safe use in food or feed. In addition, the final products Natupulse TS/TS L have been commercialized and used safely in other international jurisdictions for over 2 years.
3b. Is the NOAEL for the test article in appropriate short-term oral studies sufficiently high to ensure safety?	**Yes**, go to 3c	The NOAEL for the test article identified in the subchronic rodent study represents an approx. 6000-fold excess of the maximal EDI consumed by the broiler at the target rate (Kern, A. *et al.*, 2020). A published tolerance study in the target animal (Fickler, A. *et al.*, 2024) shows no significant differences in safety-related outcomes (incl. body weight) up to a 125-fold excess of the target rate (see Part 6.F. below). The rat NOAEL is 40 – 50 times higher than the rate of 100,000 TMU/kg feed in the published tolerance study in the target animal.
3c. Is the test article free of transferable antibiotic resistance gene DNA?	**Yes**, go to 3e	All transferable antibiotic resistance genes present during the construction of the expression vector have been removed prior to transformation of the host organism and the WGS does not show the presence of ARM genes.
3e. Is all other introduced DNA well characterized and free of attributes that would render it unsafe for constructing microorganisms to be used to produce food-grade products?	**Yes**, go to 4	The rDNA sequence has been characterized by sequence analysis and contains the synthetic gene coding for mannanase 19287 as well as the presence of fungal selection marker genes incorporated into the host genome.
4. Is the introduced DNA randomly integrated into the chromosome?	**Yes**, go to 5	Southern Blot analysis demonstrates multiple integration sites within the *T. thermophilus* genome.
5. Is the production strain sufficiently well characterized so that one may reasonably conclude that unintended	**Yes**, go to 6.	The recombinant production strain and its host strain have been thoroughly characterized:

pleiotropic effects which may result in the synthesis of toxins or other unsafe metabolites will not arise due to the genetic modification method that was employed?		Gene knockouts of endogenous genes during host strain development as well as the insertion sites of the manF expression cassettes coding for mannanase 19287 have been analyzed for the presence of open reading frames for homologous proteins of known fungal toxins and virulence factors. No significant homologies have been identified. Antibiotic resistance markers present in plasmids during the assembly of the expression cassette are not found in the production strain genome.
6. Is the production strain derived from a safe lineage, as previously demonstrated by repeated assessment via this evaluation procedure?	**No**, go to 7.	The production strain DSM 33149 is derived from a lineage that has been evaluated once before for an endogenous cellulase expressed in *T. thermophilus* (GRN 292).
7. Is the organism nonpathogenic?	**Yes**, go to 8.	See part 6 section C
8. Is the test article free of antibiotics?	**Yes**, go to 9.	No antibiotics are present during the manufacturing process; in addition, no antimicrobial activity has been identified in accordance with JECFA requirements.
9. Is the test article free of oral toxins known to be produced by other members of the same species?	**Yes**, go to 11.	Mannanase CC, the liquid concentrate of the notified substance has tested negative for mycotoxins of regulatory concern in five Test Article batches and five commercial production batches. No treatment related adverse effects were observed in 90-day subchronic oral toxicity study or target animal tolerance study (Fickler, A. *et al.*, 2024; Kern, A. *et al.*, 2020).
11. Is the NOAEL for the test article in appropriate oral studies sufficiently high to ensure safety?	**Yes**, the test article is **ACCEPTED.**	The NOAEL determined in the subchronic oral toxicity study is in excess of the 100-fold requirement per 21 CFR 170.22 (Kern, A. *et al.*, 2020). This is further confirmed in a tolerance study (Part 6, section F.4.(a)) up to 125-fold excess of the target rate.

The answers to the above questions indicate that there are no safety concerns regarding the production strain and introduced DNA in question; thus, all of the criteria used in the published decision tree for evaluating the safety of a new enzyme (the mannanase 19287) have been met.

In summary, the lack of safety concerns after applying the well-established Pariza and Cook decision tree to the mannanase 19287 enzyme preparation supports the safety of the notified substance.

D. Safety of the Donor Organism

The synthetic(b) (4) sequence used for the expression of mannanase 19287 is a novel recombinant variant of the native *T. reesei* (b) (4) gene (GenBank ID L25310) (Kensch, O., 2015; Kensch, O. *et al.*, 2010). (b) (4) .

(b) (4)

The UniProtKB/Swiss-Prot Database was queried for keyword "virulence" and taxon "aspergillus nidulans". 70 hits were listed, 37 "reviewed" (Swiss-Prot listing) and 33 "unreviewed" (UniProtKB) proteins were found. The list was searched for the "(b) (4)_EMENI" keyword; no match was found. Furthermore, the Swiss-Prot database for "fungal toxin" with 114 entries was searched for the "(b) (4)_EMENI" keyword; as before, no toxin hits were found.

In summary, we have evaluated the safety of the *A. nidulans* (b) (6), (b) (4) gene and have determined that the presence of the (b) (4) gene in the genome of the production strain *T. thermophilus* DSM 33149 does not impact the safety evaluation of the mannanase 19287 enzyme preparation.

E. Safety of the Manufacturing Process

As described in Part 2, Section B, the dry Natupulse® TS and liquid Natupulse® TS L enzyme products are manufactured using generally known and accepted methods for the production of microbial enzymes (Aunstrup, K. *et al.*, 1979; Pariza, M.W. and Foster, E.M., 1983). In addition, Natupulse® TS and Natupulse® TS L enzyme products are manufactured in accordance with both current Good Manufacturing Practices (cGMP) for animal food and the 1992

Organization for Economic Co-operation and Development's criteria for Biosafety Level 1 (OECD, 1992).

Raw materials and processing aids evaluation

Raw materials and processing aids used in the manufacture of the mannanase 19287 enzyme preparation, the notified substance of this AGRN, are of suitable grade for the production of human and animal food. Using the published data for test articles used in the toxicological studies of Mannanase CC and (b) (4) , as well as the EDI_{max} calculations from two target animal studies (Fickler, A. *et al.*, 2024; Fickler, A. *et al.*, 2023; Kern, A. *et al.*, 2020), we can estimate the intake of raw materials or processing aids (Appendix 13).

Antifoams (AFs) are used during the submerged fed-batch fermentation process to suppress the formation of air-filled bubbles, since they limit the necessary gas and nutrient exchange. The substances are used at levels of 0.05 – 1 % during fermentation and are removed during the filtration steps in down stream processing (DSP). For a worst-case estimate, we presume that none of the AF is removed and Mannanase CC contains 1 % of the processing aid. Using the data from the safety publication (Kern, A. *et al.*, 2020), the Mannanase CC test article had an activity of approximately 90,000 TMU/g Mann CC. At 1% of AF (10 mg AF/g MannCC) this calculates to 0.00011 mg antifoam/TMU:

10 mg AF/g MannCC ÷ 90,000 TMU/g MannCC = 0.00011 mg AF/TMU

Broiler chicken consumed an EDI_{max} of 65 TMU/kg BW/day (target rate tolerance study) and therefore 0.0072 mg AF/kg BW/day:

65 TMU/kg BW/day x 0.0001 mg AF/TMU = 0.0072 mg AF/kg BW/day
= 7.2 µg AF/kg BW/day

At the tolerance rate of 100,000 TMU, the maximum intake of AF would be:

8125 TMU/kg BW/day x 0.00011 mg AF/TMU = 0.9 mg AF/kg BW/day

The published tolerance study Fickler *et al* 2024 demonstrated that there were no significant differences in safety-related outcomes at the tolerance rate; therefore, the safety of process ingredients, including AF, is supported by the publication (Fickler, A. *et al.*, 2024).

Safety of Natupulse® TS and Natupulse® TS L

The commercial final products Natupulse® TS and Natupulse® TS L enzyme products meet the general and additional requirements for enzyme preparation of the *Food Chemicals Codex* and JECFA (FAO/WHO, 2006; US Pharmacopeia, 2020).

In summary, the manufacturing process for the Natupulse® TS and Natupulse® TS L products is considered safe and supports our evaluation of the notified substance as GRAS for its intended use to reduce the viscosity of intestinal digesta in poultry diets.

F. Safety Studies

The safety of the mannanase 19287 enzyme preparation has been evaluated in established toxicological studies. The results of the toxicology studies have been published (Kern, A. *et al.*, 2020). The following sections provide short summaries of the published safety studies and a discussion of the safety aspects of the broiler utility study.

1. Test materials

Two Mannanase CC test article batches (00000477L0 and 00000877L0) and one (b) (4) test article batch (012708) were analyzed for chemical and microbial characteristics. **Table 11** lists the results which confirmed that the test materials comply with JECFA and FCC requirements (FAO/WHO, 2006; Kern, A. *et al.*, 2020; US Pharmacopeia, 2020).

Table 11: Purity of Test Article Batches for Toxicology

Test article batch ID		[redacted]		
Enzyme activity [TMU/g]		[redacted]		
Total Organic Solids (TOS), calc.[g/100g]		23.1	23.3	67.5
Chemical Composition	**Limits**			
Lead	≤5 mg/kg	pass	pass	pass
Antimicrobial activity	absent	pass	pass	pass[1]
Mycotoxins	absent	pass	pass	pass
rDNA	≤ 10 ng/g	pass	pass	pass[1]
Microbial Composition	**Limits**			
Total microbial count	≤5 x 10^4 CFU/g	pass	pass	pass
Coliform bacteria	<30 CFU/g	pass	pass	pass
Salmonella [CFU]	absent in 25 g	pass	pass	pass
Escherichia coli [CFU]	absent in 25 g	pass	pass	pass
Production Organism [CFU]	< 3/ g	pass	pass	pass[1]

[1]based on corresponding Mannanase CC 877L0 result

2. Shelf life

Mannanase CC >18 months at 20 °C

[redacted] >24 months at 20 °C

3. Toxicity studies

a. Acute inhalation toxicity

In order to evaluate the acute inhalation toxicity of the active substance mannanase 19287 per OECD Guideline 403, the Wistar rat test animals were exposed to the test article (b) (4) for four hours at the rate of 5.09 mg (b) (4) . The animals were housed for 14 days and checked for clinical observations, body weight and mortality rate. No mortality was observed after testing and clinical observations reported intermittent respiration in the animals up to day 5; no clinical signs and findings were observed from day 6 to day 14. Mean body weights decreased on the first day post-inhalation; male rats increased their weights starting on day 2, female rats after day 3 post-testing. Gross pathology examination showed no signs of pathological abnormalities during necropsy after the animals were euthanized on day 14.

Based on the results of the study, the LC50 is > 5.1 mg/L air in male and female rats after the four-hour exposure to dust aerosol of the mannanase 19287 preparation (Kern, A. *et al.*, 2020).

b. 90-Day subchronic oral toxicity

Test article 477L0 (89.55 TMU/mg) was well tolerated by Wistar rats when administered in rat feed for 3 months and **Table 12** details the associated feeding data. No deaths or overt signs of toxicity were observed during the course of the study. No clinical findings, no test substance-related changes in body weight or clinical pathology parameters were observed in male and female animals of any test group (Kern, A. *et al.*, 2020).

Table 12: Feed intake in 90-day subchronic oral toxicity study

Group #	Test article in rat feed [ppm]	Number of animals		Mean daily intake enzyme [mgTOS/kg bw/day]	
		Male	Female	Male	Female
1 - Control	0	10	10	0	0
2 - Test article, low dose	6500	10	10	105	116
3 - Test article, mid dose	21700	10	10	352	380
4 - Test article, high dose	65000	10	10	1117	1297

Based on these results the NOAEL may be >1117 mg TOS/kg bw/day (4.33×10^5 TMU/kg bw/day) for male rats, and >1297 mg TOS/kg bw/day (5.03×10^5 TMU/kg bw/day) for female rats.

c. Genotoxicity studies

The potential for Mannanase CC to induce any genotoxicity was thoroughly evaluated in two different pertinent assays, the bacterial reverse mutation assay (AMES test) and the chromosomal aberrations assay in cultured human peripheral blood lymphocyte (PBL assay). The results of both studies are summarized below and thoroughly discussed in (Kern, A. *et al.*, 2020).

The purpose of the AMES test was to investigate the potential of Test Article 877L0 to induce reverse mutations in independent *Salmonella typhimurium* and *E. coli* strains in the presence and absence of an exogenous metabolic activation system. No increase in the number of revertant were observed at any concentration of mannanase 19287. The reference mutagens induced an increase in reversion rate, comparable to historical data obtained at the test site.

The purpose of the PBL Assay was to evaluate the potential of 877L0 to induce micronuclei in human lymphocytes at 250 – 2000 µg/mL in the presence and absence of an exogenous S9 metabolic activation system. No cytotoxicity or induction of micronuclei was detected up to the highest concentration of the test article after either pulse or continuous exposure. The presence of mutagens (MMC, Decolcine or CPA) significantly increased cytostasis (as the measurement of cytotoxicity) and the appearance of micronuclei.

These results demonstrate that β-mannanase 19287 is considered to be non-cytotoxic/non-mutagenic when tested up to 2000 µg/mL.

d. Worker safety studies

Additional studies were conducted to verify the safety of mannanase 19287 for users. Primary eye irritation in the (b) (4) in vitro system (OECD 492 guideline) and primary skin irritation in the (b) (6), (b) (4) in vitro system (OECD 439 guideline) were conducted using mannanase 19287 spray-dried (b) (4) concentrate. The test material was found to be non-irritating in primary eye irritation (viability > cut-off value OECD 492) as well as in primary skin irritation (viability > cut-off value OECD 439) (Kern, A. *et al.*, 2020).

In addition, Natupulse® TS and Natupulse® TS L test articles were also tested for eye irritation in the (b) (4) in vitro system (OECD 492 guideline) and primary skin irritation in the (b) (4) in vitro system (OECD 439 guideline). The test materials were found to be non-irritating in primary eye irritation (viability > cut-off value OECD 492) as well as in primary skin irritation (viability > cut-off value OECD 439).

In summary, the published results of the toxicity studies support our evaluation of the notified substance as GRAS for its intended use to reduce the viscosity of intestinal digesta in poultry diets.

4. Target animal studies

a. Introduction

We have determined that the scientific literature clearly demonstrates that the intended use of endo-β-mannanases (E.C. 3.2.1.78) to reduce viscosity in digesta has no bearing on target animal safety and that therefore no utility data are required for the determination of safety of the mannanase 19287 enzyme preparation produced by *T. thermophilus* strain DSM 33149.

Control diets, either standard diets in the absence of enzyme supplementation, or diets where soybean meal has been substituted with other protein sources containing elevated levels of soluble NSPs, can be regarded as examples for the lack of enzyme functionality (no enzyme supplemented activity). In a recent broiler study (Fickler, A. *et al.*, 2023), the negative control (NC) diet contained 7.5% guar meal, a protein source with significant levels of soluble galactomannans. A standard corn/soybean meal diet served as the positive control (PC). Three

additional treatments were based on the NC diet and contained increasing amounts of the mannanase 19287 enzyme preparation at 400 – 1200 TMU/kg feed. The NC diet without Natupulse® TS supplementation can be viewed as a stand-in for the lack of enzyme activity. No significant differences in mortality of the target animals were observed.

The safety of the mannanase 19287 enzyme preparation produced by *T. thermophilus* strain DSM 33149 can also be demonstrated by comparing a feeding study of the target animal using a diet at the target dose with a control diet without enzyme supplementation and with a tolerance diet containing a significant excess of the notified substance.

b. Tolerance in broiler chicken

Poultry show the highest daily feed intake among common food-producing target animal species when normalized by dry matter of feed: 53 – 79 g feed/kg BW/day). In particular, broiler chicken consume approximately 79 g feed/kg BW/day (EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) *et al.*, 2017).

The tolerance of broiler chicken to the mannanase 19287 preparation was evaluated using the Natupulse® TS test article at >100-fold excess of the target rate of 800 TMU/kg feed and the outcome of the study has been published recently (Fickler, A. *et al.*, 2024). Final mortality, feed consumption, and daily weight gain can be considered as outcome measures supporting the safety of the animals (Manning, L. *et al.*, 2007). We view these outcome measures as pivotal data for our determination that the mannanase 19287 enzyme preparations (marketed as Natupulse® TS and Natupulse® TS L), when used as intended to reduce the viscosity of intestinal digesta in poultry diets, are generally recognized as safe (GRAS), based on scientific procedures.

Broiler chickens were fed for 35 days with a standard corn/soybean diet in three phases. A total of five treatment groups received diets with varying levels of mannanase 19287 enzyme preparation from 0 – 100,000 TMU/kg added to the standard diet. During the tolerance study, the average male Ross 308 broiler chicken was expected to consume (b) (4)). For the tolerance diet of (b) (4) (Appendix 22).

Animals were fed a standard corn/soybean meal diet over 35 days; no other protein sources with elevated levels of non-starch polysaccharides (NSPs) were added (**Table 13**). The Control

diet did not contain the mannanase 19287 enzyme preparation (Treatment 1, T1). Test diets T2 – T4 were supplemented with Natupulse® TS at (b) (4). The tolerance diet (T5) included the test article at (b) (4) over the target rate of 800 TMU/kg feed (**Table 14**). Total feed intake and total weight gain were determined and used to calculate the average body weight, average daily gain, and average daily feed intake.

Gross analyses confirmed the calculated contents for all diets between 95 % - 103%. The expected enzyme activities enzyme recoveries ranged from approx. 90 % to 110 % with the exception of the grower diet of T3 at the target rate (800 TMU/kg feed). Mannanase 19287 activity was measured in that ration at almost 200 % of the target rate.

The average body weight, average daily gain, and average daily feed intake data (**Table 15**) as well as mortality rates (**Table 16**) (b) (4), including the mannanase 19287 supplementation at the (b) (6) dose. This finding was further confirmed by the fact that (b) (4)

No adverse effects on target animal safety parameters were observed at any level of Natupulse® TS inclusion in the tolerance study. Furthermore, the average bodyweights of broilers in all treatment groups (2.46 – 2.52 kg) matched or exceeded the expected value of 2.44 kg per Ross 308 2022 Performance Objectives (Aviagen, 2022).

Table 13: Fickler *et al,* 2024 data Table 1

Table 1. Ingredient and nutrient composition of basal feeds

(Feed ingredients and their common names).

Ingredients (%)	**Starter 0-14 d**	**Grower 14-28 d**	**Finisher 28-35 d**
Ground corn	49.87	51.30	54.84
Soybean Meal	35.15	32.33	26.33
Ground Extruded Whole Soybeans	6.69	7.63	8.90
Vegetable Oil	3.00	4.00	2.00
Animal Fat	-	-	3.00
L-Lysine	0.194	0.098	0.102
L-Threonine	0.096	0.038	0.030
DL-Methionine	0.315	0.256	0.235
Salt	0.383	0.356	0.353
Calcium carbonate	0.667	0.604	0.549
Dicalcium phosphate	1.962	1.727	1.555
Choline Chloride	0.046	0.034	0.039
Vitamin-mineral premix[1]	0.300	0.300	0.300
Noxyfeed[2]	0.020	0.020	0.020
Maxiban G160[3]	0.063	0.063	-
Titanium dioxide	-	-	0.500
Wheat bran[4]	1.250	1.250	1.250
[1]The premix provides per kg feed: Vitamin A acetate 10000 UI; Cholecalciferol 4800 UI; Tocopherol 45 mg; Thiamine mononitrate 3 mg; Riboflavin 9 mg; Pyridoxine hydrochloride 4.5 mg; Vitamin B12 supplement 40 µg; Menadione sodium bisulfite complex 3 mg; Calcium panthotenate 16.5 mg; Nicotinic acid 51 mg; Folic acid 1.8 mg; Biotin 0.15 mg			
The premix provides per kg feed: Ferrous sulfate monohydrate 54 mg; Calcium iodate 1.2 mg; Copper sulfate pentahydrate12 mg; Manganese oxide 90 mg; Zinc oxide 66 mg; Sodium selenite 0.18 mg.			
Calculated nutrient levels (g/kg)			
ME (kcal/kg)	2940	3038	3136
Crude protein	225	215	195
Ash	61.4	57.2	57.4
Crude fibre	35.2	34.3	31.9
Crude fat	60.3	72.0	84.4
Digestible lysine	12.54	11.27	10.09
Digestible threonine	8.43	7.55	6.76
Digestible methionine	6.17	5.51	5.05
Digestible Met+Cys	9.31	8.53	7.84
Total calcium	9.60	8.70	7.90
Total phosphorus	7.39	6.89	6.38
Non-phytate phosphorus	4.80	4.35	3.95

[2] Contains BHT+ propyl gallate (56%) and citric acid (14%).

[3] Providing 50 g narasin + 50 g nicarbazin/ton of feed.

[4] Wheat bran was replaced by mannanase premix at 0.005% (T-2), 0.010% (T-3), 0.015% (T-4) and 1.25% (T-5).

Table 14: Fickler *et al*, 2024 data Table 2

Table 2. Mannanase recoveries in finished feeds for starting, growing and finishing phases

		Starter		Grower		Finisher	
	Expected TMU/kg	Measured TMU/kg	Recovery %	Measured TMU/kg	Recovery %	Measured TMU/kg	Recovery %
T-1		<100		<100		<100	
T-2	400	360	90	410	103	380	95
T-3	800	730	91	1540	193	740	93
T-4	1200	1110	93	1330	111	1150	96
T-5	100000	94340	94	96260	96	97060	97

T1= Negative Control (NC, no Natupulse TS); T2= NC + 400 TMU/kg, T3= NC + 800 TMU/kg, T4= NC + 1200 TMU/kg, T5= NC + 100,000 TMU/kg. One TMU is defined as the amount of enzyme that liberates 1 µmol reducing sugars determined as Mannose per minute under the conditions of the test method (pH 3.5 and 50 °C).

Table 15: Fickler *et al*, 2024 data Table 3

Table 3. Average body weight, average daily gain, and average daily feed intake of broiler chickens.

		Treatments						P-values			
		T-1 0 TMU/kg feed	T-2 400 TMU/kg feed	T-3 800 TMU/kg feed	T-4 1200 TMU/kg feed	T-5 100,000 TMU/kg feed	Standard error	Treatment effect	Co vs. mannanase	*Linear mannanase effect*[1]	*Quadratic mannanase effect*[1]
ABW (g)	0 d	42.4	42.4	42.0	42.5	42.0	0.25	0.491	-	-	-
	14 d	502	501	496	489	480	6.70	0.161	0.175	*0.172*	*0.712*
	28 d	1775	1756	1779	1766	1742	19.70	0.673	0.519	*0.967*	*0.878*
	35 d	2521	2506	2513	2520	2461	25.30	0.434	0.474	*0.959*	*0.675*
ADG (g/d)	0-14 d	32.8	32.7	32.4	31.9	31.30	0.49	0.177	0.186	*0.177*	*0.689*
	14-28 d	90.9	89.7	91.7	91.2	90.1	1.03	0.649	0.813	*0.553*	*0.703*
	28-35 d	106.5	107.2	104.9	107.7	102.7	1.65	0.217	0.639	*0.853*	*0.513*
	0-35 d	70.8	70.4	70.6	70.8	69.1	0.72	0.441	0.478	*0.957*	*0.681*
ADFI (g/d)	0-14 d	37.7	37.7	37.1	36.0	35.8	0.61	0.105	0.150	*0.055*	*0.383*
	14-28 d	121.4	120.5	121.9	121.1	119.5	1.27	0.709	0.637	*0.950*	*0.976*
	28-35 d	178.4	175.4	177.4	179.2	171.8	1.96	0.081	0.269	*0.610*	*0.220*
	0-35 d	99.2	98.3	99.0	98.6	96.4	0.96	0.263	0.289	*0.774*	*0.797*

Values are least-square means of nine replicates of 41 chickens per treatment at the beginning of the study.

[1] *Using Mannanase at 0, 400, 800 and 1200 TMU/kg.*

One TMU is defined as the amount of enzyme that liberates 1 µmol reducing sugars determined as Mannose per minute under the conditions of the test method (pH 3.5 and 50 °C)

ABW: average body weight, ADG: average daily gain, ADFI: Average daily feed intake

Table 16: Fickler *et al*, 2024 data Table 4

Table 4. Effect of dietary treatment on percentage of dead and culled birds during the study (0-35d of age)

	% Dead birds				% Culled birds			
	0-14 d	**14-28 d**	**28-35 d**	**0-35 d**	**0-14 d**	**14-28 d**	**28-35 d**	**0-35 d**
T-1 0 TMU/kg feed	0.8	0.3	0.3	1.4	0.3	0.5	0.3	1.1
T-2 400 TMU/kg feed	0.5	0.3	0.3	1.1	0.3	0.0	0.0	0.3
T-3 800 TMU/kg feed	0.0	0.5	0.3	0.8	0.3	0.5	0.0	0.8
T-4 1200 TMU/kg feed	0.3	0.5	0.3	1.1	0.3	0.6	0.3	1.1
T-5 100,000 TMU/kg feed	0.8	1.1	0.3	2.2	0.0	1.1	0.0	1.1
Standard error	0.32	0.45	0.28	0.55	0.26	0.33	0.17	0.47
P-values								
Treatment effect	0.306	0.796	1.000	0.593	0.920	0.261	0.580	0.668
C vs. mannanase	0.256	0.504	0.997	0.638	0.817	0.992	0.304	0.550
Mannanase effect[1]								
Linear	*0.132*	*0.516*	*0.994*	*0.591*	*0.990*	*0.699*	*1.000*	*0.910*
Quadratic	*0.401*	*1.000*	*1.000*	*0.411*	*0.993*	*0.406*	*0.129*	*0.275*

Values are least-square means of nine replicates of 41 chickens per treatment at the beginning of the study.
[1]Using mannanase at 0, 400, 800 and 1200 TMU/kg. One TMU is defined as the amount of enzyme that liberates 1 µmol reducing sugars determined as Mannose per minute under the conditions of the test method (pH 3.5 and 50 °C).

Please note that Wistar rats ingested approximately 1100- 1300 mg of the mannanase 19287 enzyme preparation (per TOS) /kg BW / day during the 90-day oral subchronic toxicity study and showed no significant adverse effects. Broiler chicken in the tolerance study consumed up to 26 mg /kg BW / day; these amounts represent approximately a 40-50-fold excess of the tolerance ration during the rat study (Kern, A. *et al.*, 2020). While there are significant differences in the digestive systems of mammals and fowl, this comparison nonetheless supports our finding that there were no safety concerns related to feeding the mannanase 19287 enzyme produced with *T. thermophilus* DSM 33149 to broilers.

In summary, the results of the published broiler tolerance study (Fickler, A. *et al.*, 2024) support our conclusion that the mannanase 19287 enzyme preparation, the notified substance of this dossier, is GRAS for its intended use to reduce the viscosity of intestinal digesta in poultry diets.

G. Results and Conclusion

BASF Corporation has reviewed all available data and information and is not aware of any data and information that are, or may appear to be, inconsistent with our conclusion of GRAS status.

Safety evaluation regarding target animal consumption of the notified substance

The data included and discussed in this Animal GRAS Notification support the conclusion of BASF Corporation that the mannanase 19287 enzyme preparation in Natupulse® TS and Natupulse® TS L products is GRAS for use in poultry feed to decrease the viscosity of intestinal digesta by increasing the digestibility of β-mannans in poultry diets. This conclusion is based on the following:

•The notified substance mannanase 19287 is an enzyme preparation and enzymes are generally considered to be non-toxic (Olempska-Beer, Z.S. *et al.*, 2006).

•The safety of the mannanase 19287 enzyme preparation by *T. thermophilus* DSM 33149 is further supported by publications demonstrating the effects of endo-β-1,4-mannanase preparations manufactured by bacterial and fungal production organisms (Jackson, M.E., 2010; Kiarie, E.G. *et al.*, 2021; Kipper, M. *et al.*, 2020; Shastak, Y. *et al.*, 2015).

•The notified substance mannanase 19287 has been identified as an endo-β-1,4-mannanase enzyme through sequence analysis and functionality testing with galactomannans; endo-β-1,4-mannanase enzymes have a history of safe use as additives for both human and animal food. Two GRAS Notifications to FDA-CFSAN evaluated the intended use of mannanase enzymes in fruit juices and coffee extracts (GRASN 566 and 739) and FDA issued "No questions" responses in both cases. Beta-mannanases produced by fermentation of bacterial and fungal production organisms are listed in Table 30.1 of the AAFCO OP (Association of American Feed Control Officials (AAFCO), 2023b). In addition, endo-β-1,4-mannanases have been authorized in the EU for food and feed use.

•The safety of the mannanase 19287 enzyme preparation and its production organism *T. thermophilus* DSM 33149 is supported by the outcome of its evaluation according to the well-established decision tree for feed enzymes (Pariza, M.W. and Cook, M., 2010). Using this evaluation approach, the notified substance (the mannanase 19287 enzyme preparation) is not toxic.

•The safety evaluation of the mannanase 19287 enzyme preparation has been published (Kern, A. *et al.*, 2020). The paper reported the toxicological analysis results of the enzyme preparation in a subchronic rat 90-day oral toxicity study, inhalation toxicity, *in vitro* testing for genotoxicity, skin and eye irritation.

•The safety of Natupulse® TS and TS L products is supported by the results of a published 35-day broiler tolerance study (Fickler, A. *et al.*, 2024). Animals consuming a standard corn/soybean diet containing Natupulse® TS demonstrated the tolerance of broiler chicken to a (b) (4) of the mannanase 19287 enzyme preparation over the target rate of 800 TMU/kg feed.

•The safety of the mannanase 19287 enzyme preparation is supported by the comparison of the NOAEL determined in the published 90-day rat study (Kern, A. *et al.*, 2020) with the estimated daily intake (EDI) of mannanase 19287 at the tolerance rate of (b) (4) TMU/kg feed in the published tolerance study in broiler chicken (Fickler, A. *et al.*, 2024). Rat consumption at the highest intake is 40-50 times higher than the EDI for broiler chicken at the tolerance rate. In conclusion, this published tolerance study allows us to leverage the rat study data to conclude that there are no target animal safety concerns in the target animal.

•The safety of the notified substance is supported by the increased digestibility of β-mannans in a standard corn/soybean diet containing 7.5% of guar meal and the presence of the mannanase 19287 enzyme preparation through the reduced ileal digesta viscosity. Enzyme activities at all rates result in significant reductions in digesta viscosity compared to the feed containing guar meal (Fickler, A. *et al.*, 2023).

•The safety of mannanase 19287 is supported by the observed lack of significant differences in mortality rates between diets evaluated in a published broiler study of a standard corn/soybean diet containing 7.5% of guar meal and the presence of Natupulse® TS (Fickler, A. *et al.*, 2023).

•The BASF mannanase 19287 enzyme preparation has been commercialized as Natupulse® TS and TS L for 4 years and no adverse effects have been reported. Natupulse® is currently approved in many countries worldwide, including Australia, Bangladesh, India, Korea, Philippines, Thailand, Argentina, Brazil, Chile, South Africa.

• The mannanase 19287 enzyme preparation produced by *T. thermophilus* DSM 33149 has been evaluated by the European Food Safety Agency (EFSA) based on a request by the European

Commission to deliver a scientific opinion on the safety and efficacy of endo-1,4-β-D-mannanase produced by *T. thermophilus* DSM 33149 (Natupulse® TS/TS L). The responsible committee for Animal Feed Additives reviewed the data and published its scientific opinion on Natupulse TS and TS L in 2024 (EFSA Panel on Additives Products or Substances used in Animal Feed (FEEDAP) *et al.*, 2024). According to the scientific opinion, the committee concluded that the production strain does not pose a safety risk and that the enzyme preparation has the potential to be safe and effective for the intended use in broiler feed.

Based on all the available information in this GRAS Notification, BASF Corporation concludes that the mannanase 19287 enzyme preparation produced by *T. thermophilus* DSM 33149 is safe for its intended use to reduce the viscosity of intestinal digesta in poultry diets.

Safety assessment regarding human consumption of target animal products

Enzyme preparations are used in small quantities during feed production, and it is expected that the target animals will digest any residual salts, carbohydrates and proteins to use their constituents (sugars, amino acids and minerals) as nutrients during metabolism.

The mannanase 19287 enzyme, like all β-mannanase enzymes, catalyzes the hydrolysis of beta-mannan components of hemicellulose(Association of American Feed Control Officials (AAFCO), 2023b). Therefore, the mannanase 19287 enzyme will liberate mannan oligomers from the hemicellulose polymer constituents (Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB), 1972). The resulting β-(1,4)-mannan oligosaccharides can be hydrolyzed further by endogenous glycosidases, including β-mannosidases (EC 3.2.1.25) that act on the terminal β-glycosidic bond, and α-galactosidases (EC 3.2.1.22) removing the side chains (Dhawan, S. and Kaur, J., 2007). The oligo- and monosaccharides generated by these enzymatic activities will be metabolized as nutrients and will not remain in the edible animal tissues. The processing aids used in the manufacture of the mannanase 19287 enzyme preparation are suitable for their intended use and are not expected to affect target animal safety.

Mannanase 19287 is a protein and will be digested into amino acids in the animal's digestive tract. The Enzyme Marketing Coordination document of the AAFCO OP confirms in its Safety section that enzymes as protein catalysts are expected to be digested by the target animals(Association of American Feed Control Officials (AAFCO), 2023a). Enzymes evaluated

by the safety assessment process developed by Pariza and Foster (Pariza, M.W. and Foster, E.M., 1983) and updated for microbial enzyme preparations in animal feed (Pariza, M.W. and Cook, M., 2010) should raise no human safety concerns.

In summary, based on all the available information in this GRAS Notification, BASF Corporation concludes, through scientific procedures, that the mannanase 19287 enzyme preparation produced by *T. thermophilus* DSM 33149 is GRAS for its intended use in the target animals and is safe for humans consuming edible products derived from these animals.

PART 7: LIST OF SUPPORTING DATA AND INFORMATION IN GRAS NOTICE

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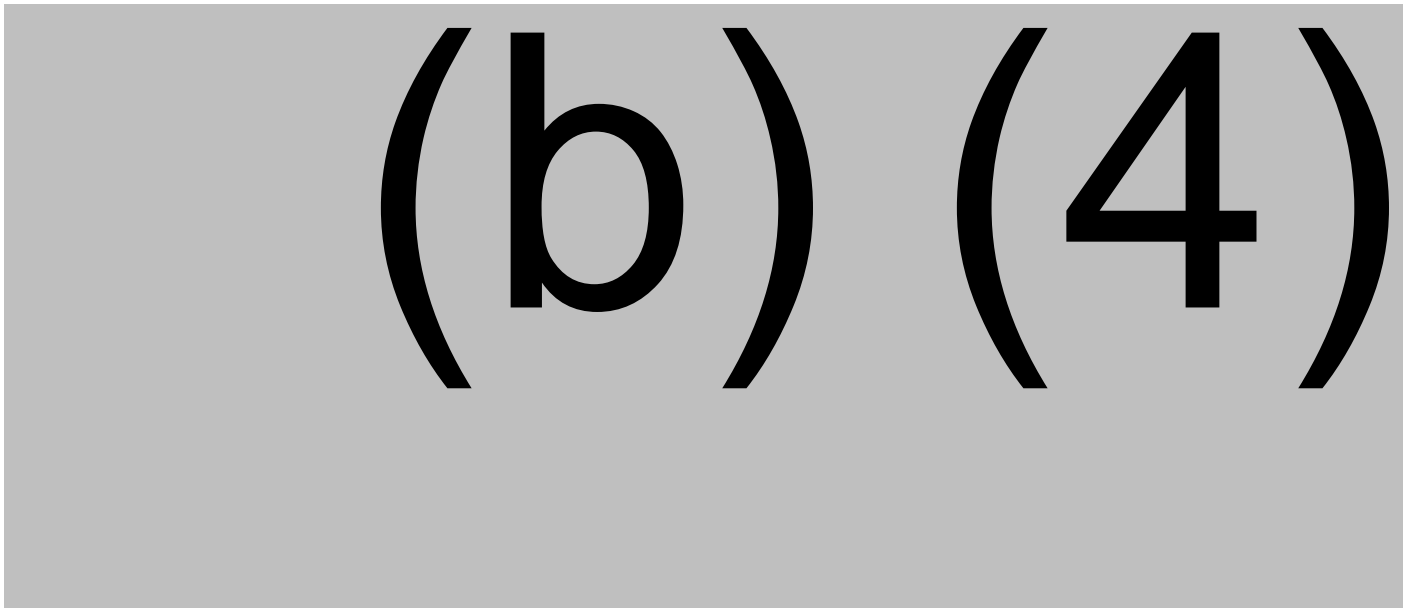
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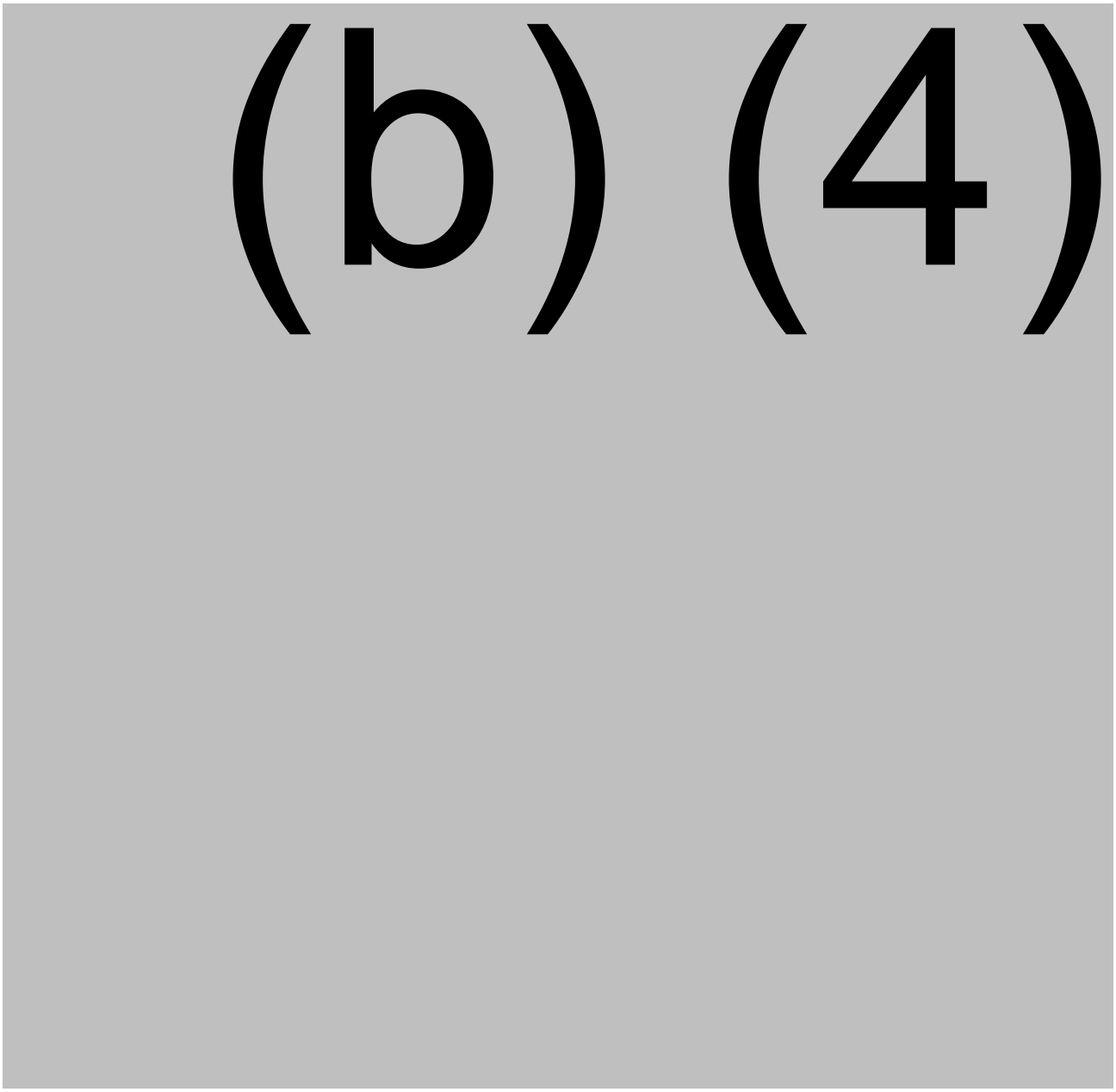
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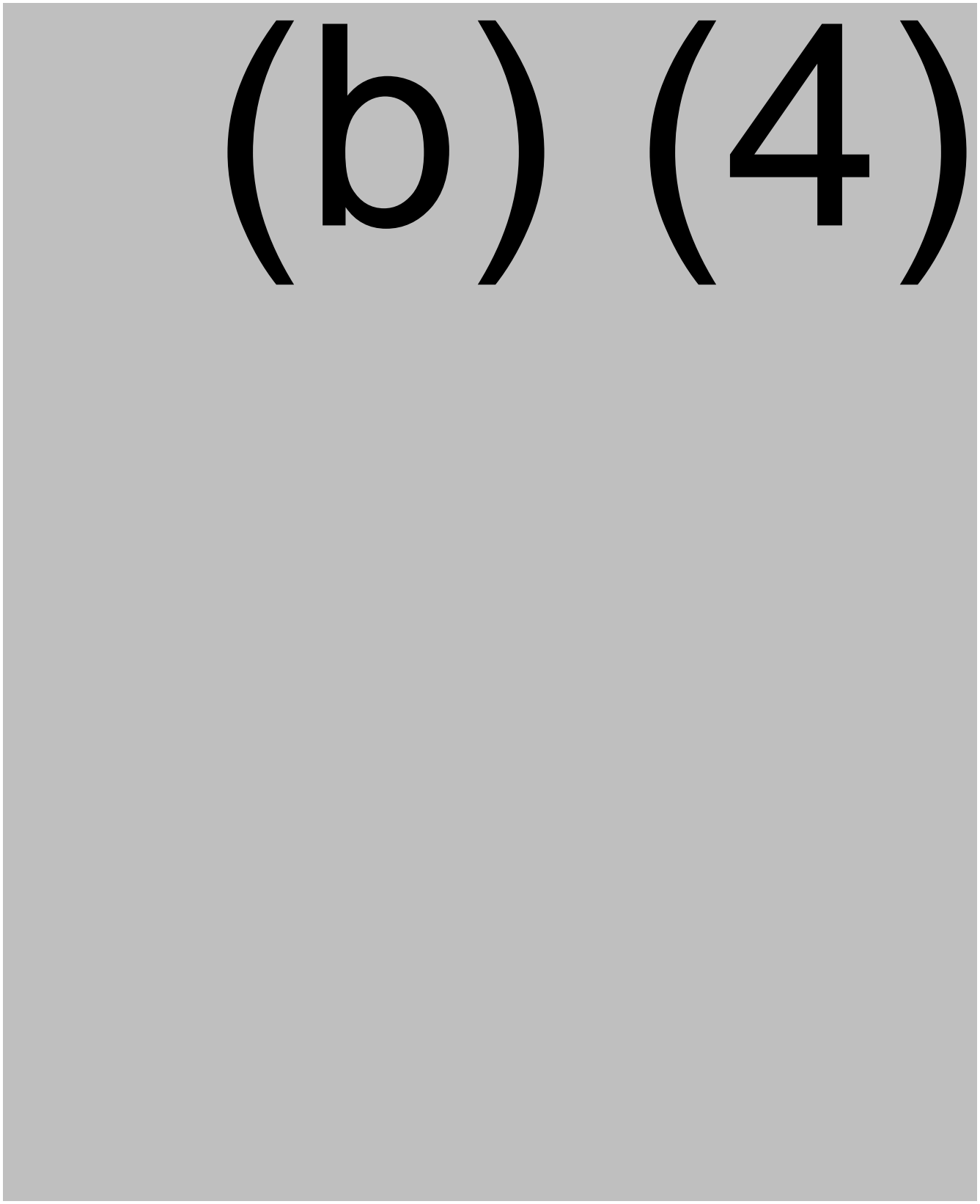
Appendix 01: Mannanase 19287 Amino Acid Sequence


(b) (4)

Appendix 02: Amino Acid Alignment Mannanase 19287 vs. *T reesei* (b) (4)


(b) (4)

Appendix 03: Coding Region of Synthetic (b) (4) Gene


(b) (4)

Appendix 4: Biochemical Characterization Mannanase 19287

Biochemical Characterization Mannanase 19287

Andreas Kern
Sr. Regulatory Affairs Specialist

April 16, 2020

Updated March 12, 2024

Biochemical Characterization of Mannanase 19287 Preparations

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Biochemical Characterization of Mannanase 19287 Preparations

Introduction

This report provides the results of studies used to develop basic biochemical characterization data for the mannanase 19287 enzyme preparation derived from Mannanase CC test article batches and production samples. The basic characterization data includes: 1) the specific activity of the enzyme, 2) the demonstration of endo-b-mannanase activity on glucomannan substrates, 3) the effect of pH on mannanase 19287 activity, 4) the effect of temperature on mannanase 19287 enzyme activity, 5) thermotolerance of the enzyme preparation, and 6) evaluation of additional enzyme activities in the mannanase 19287 preparation.

Summary

Characterization studies relating to mannanase 19287 activity were performed under standard laboratory conditions. The mannanase activity assays were performed according to the standard operating procedure "Method of Analysis FEN/0004/01 - Photometric assay of mannanase activity in feed additives." The studies included the determination of total organic solubles (TOS) as well as UV and IR spectroscopy.

Specific activity of the mannanase 19287 was determined by testing the activity of a lyophilized enzyme concentrate (Batch (b) (4) C1-R83-2017-03) after dialysis against an (b) (4). The volatile buffer compounds evaporated together with the water, leaving behind a purified enzyme powder. The specific mannanase activity of mannanase 19287 was determined to be 366.13 TMU/mg of the purified mannanase 2018-01 using enzyme activity SOP FEN 0004/01. One mannanase unit (TMU) is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one µmol mannose from mannan in one minute under the standard conditions of the assay (50 °C, pH 3.5).

The effect of pH on mannanase 19287 activity was determined by modifying the pH of the assay buffer. The pH of the buffer was modified to values above and below the standard conditions of the assay (pH 3.5) and used to prepare the substrate, dilution buffer, and mannan standard curve. The results at each pH were compared to the result at the standard assay pH of 3.5. It was determined that mannanase 19287 activity remained at 100 ±15% between pH 2.65 and 5.30; more alkaline conditions rapidly reduced enzyme activity to background levels.

The temperature dependent mannanase 19287 activity in the standard buffer (pH 3.5) was determined by modifying the assay temperature during the activity determination per FEN 0004/01. The assay was run at various temperatures (b) (4) . Maximum activity was measured at (b) (4), when tested under temperature assay conditions of 40°C to 75°C.

Thermotolerance

(b) (4)

Enzyme Side Activities

Mannanase 19287 preparations were evaluated for the presence of related glucohydrolase- or protease activities. Specifically, the enzyme concentrates were tested for amylase, cellulase, xylanase, and acid protease. Both amylase and cellulase activities were below the LOQ of each method; (b) (4) (Visser, H. *et al.*, 2011).

Test Material

1. **Mannanase 2018-01**

The material for determination of specific activity was derived from test batch (b) (4) produced in (b) (4) m

(b) (4)

2. **Mannanase SD powder (batch # 012267-L, derived from test batch 00000077L0)**

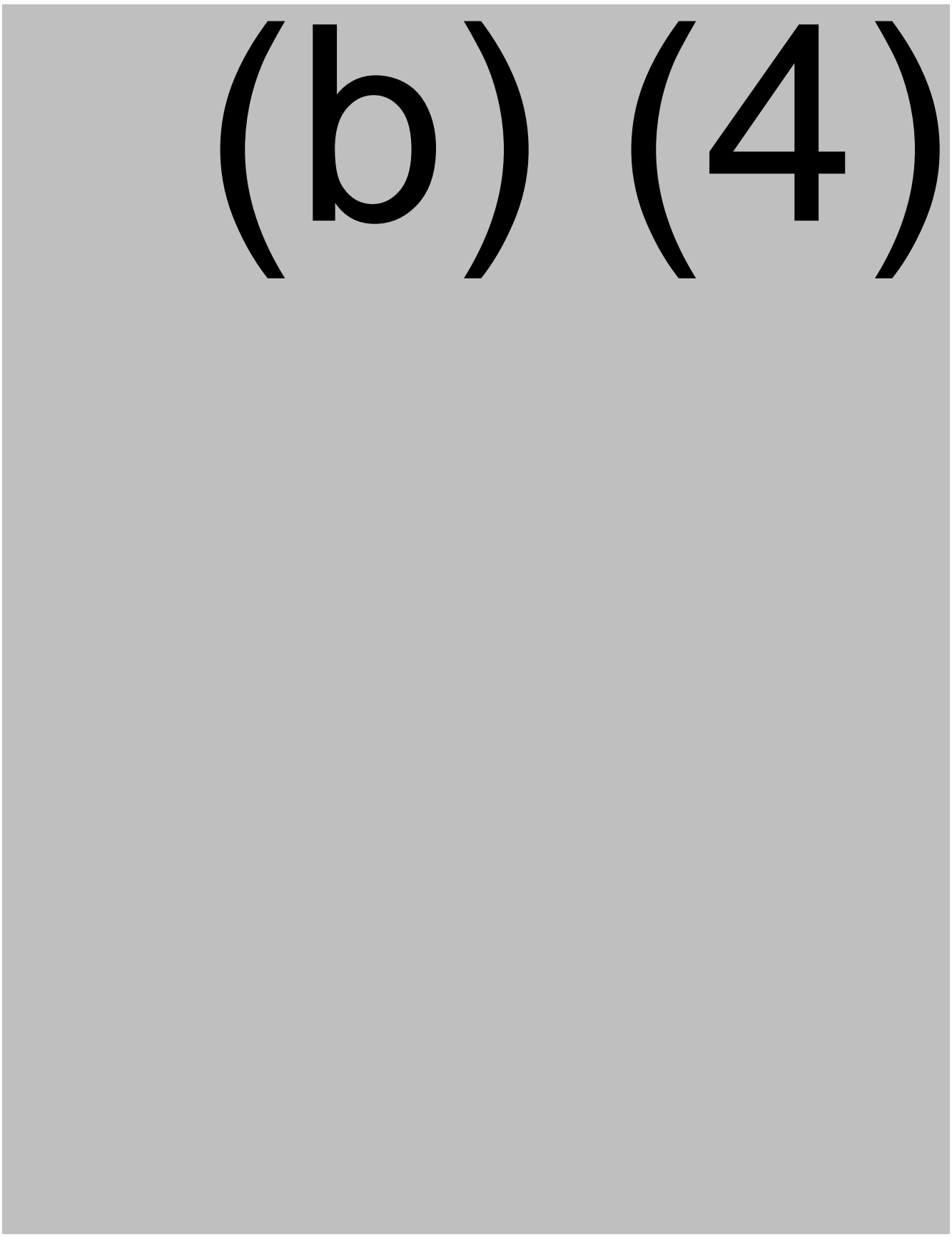
The material for determination of the effect of pH on activity was derived from the same test batch (b) (4) produced in (b) (4)

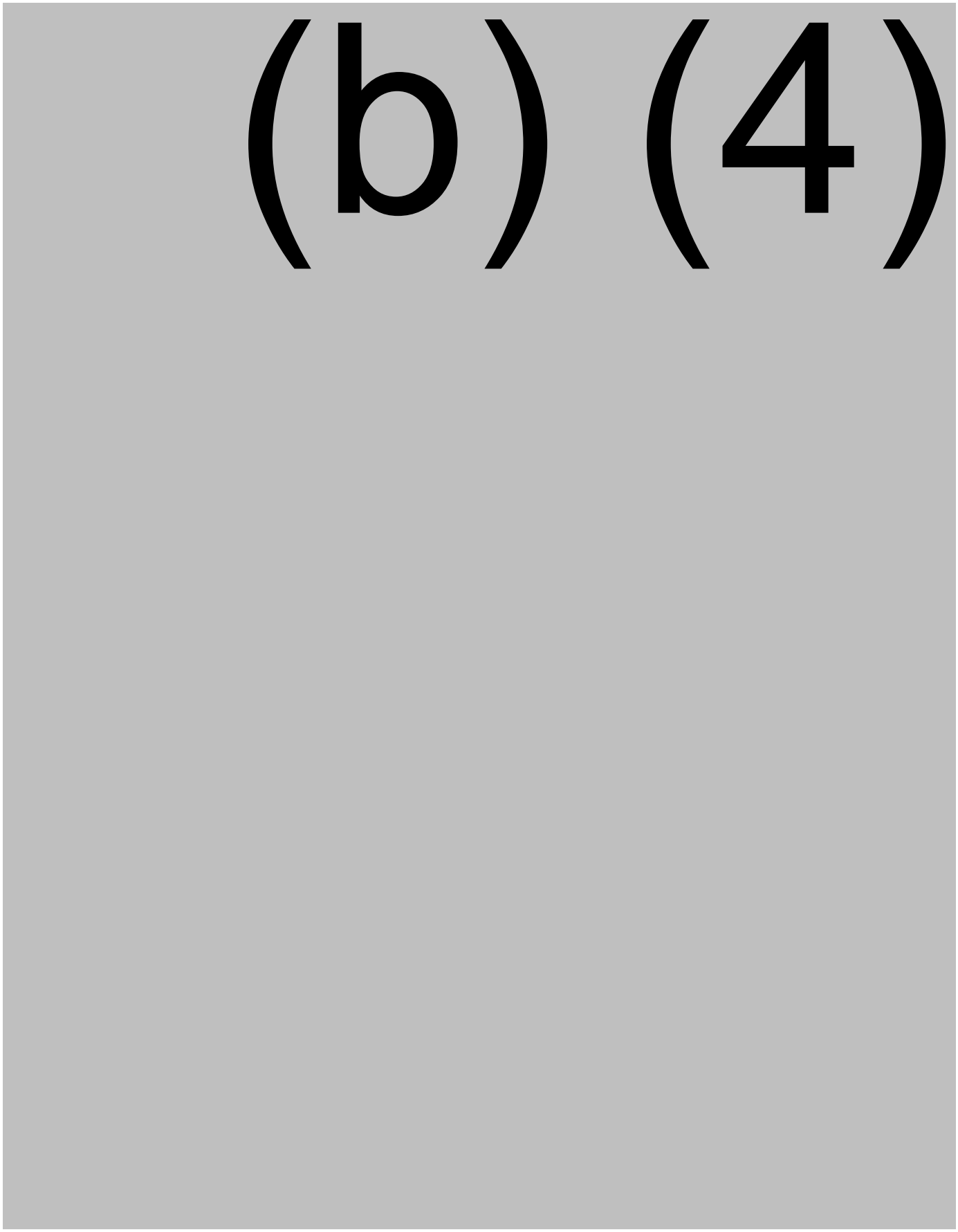
3. **Mannanase CC Concentrate Test Article (Lot # (b) (4) Registry Batch 2)**

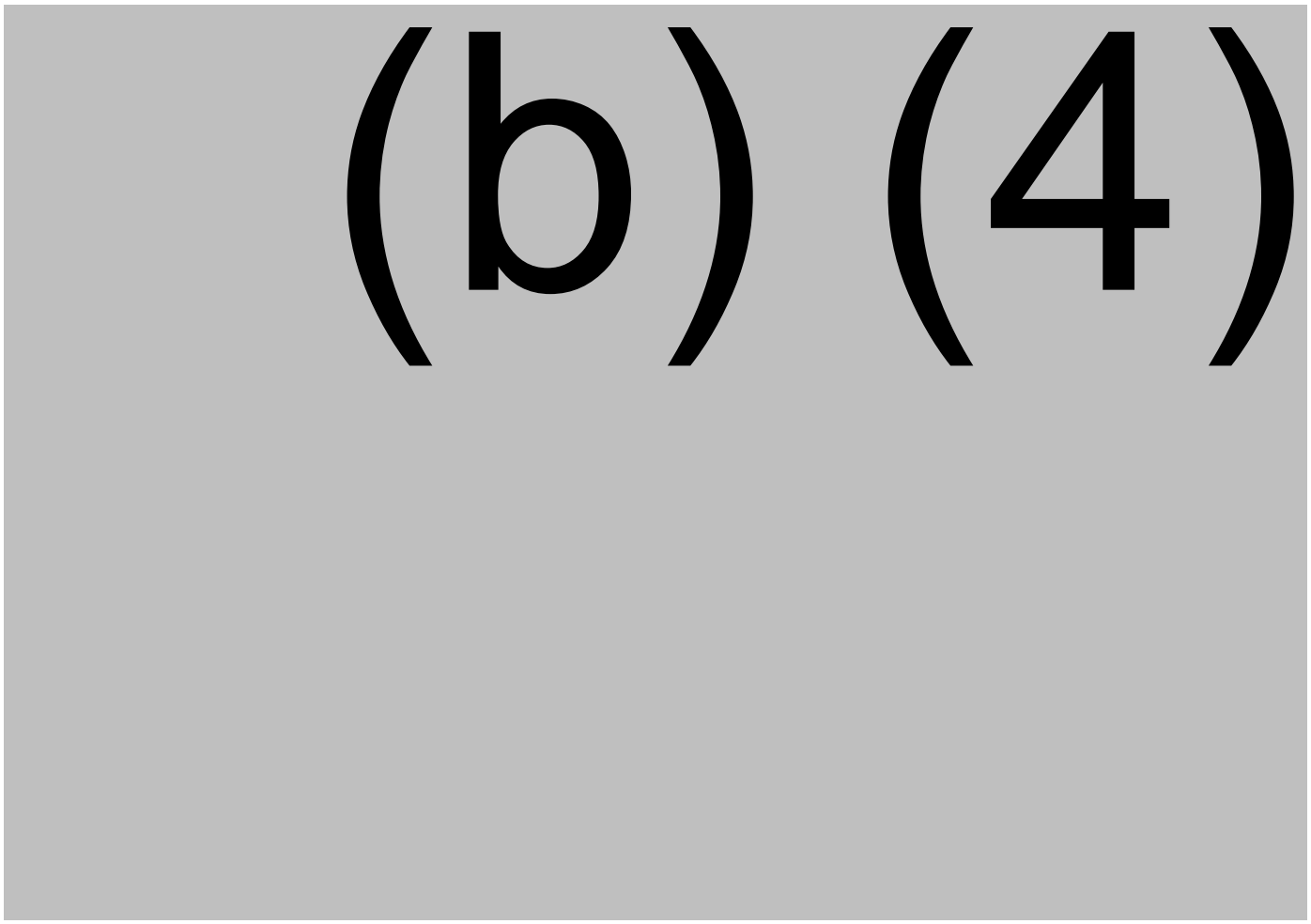
The material for determination of the effect of temperature on activity was derived from registry batch (b) (4) produced in (b) (4)

4. (b) (4) **for identification of oligosaccharide masses**

(b) (4)


(b) (4)


(b) (4)


(b) (4)

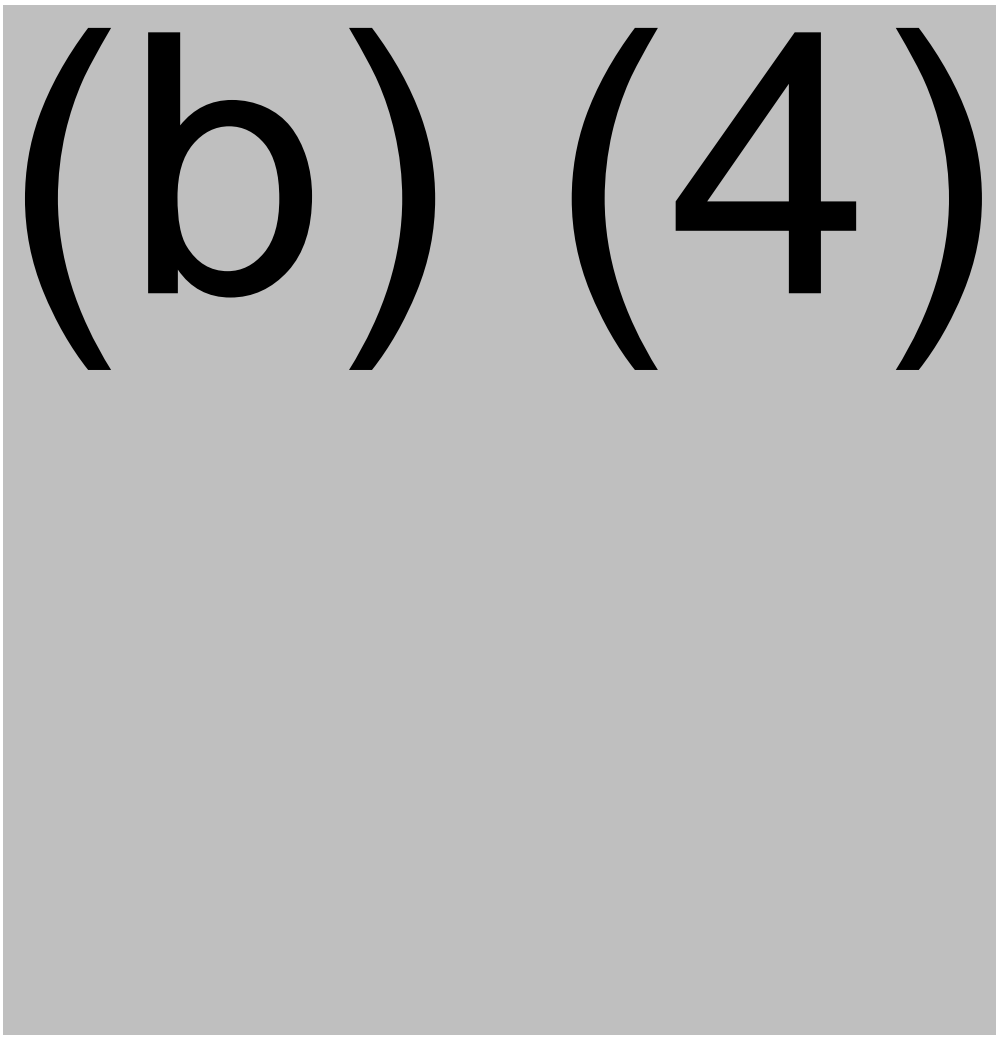
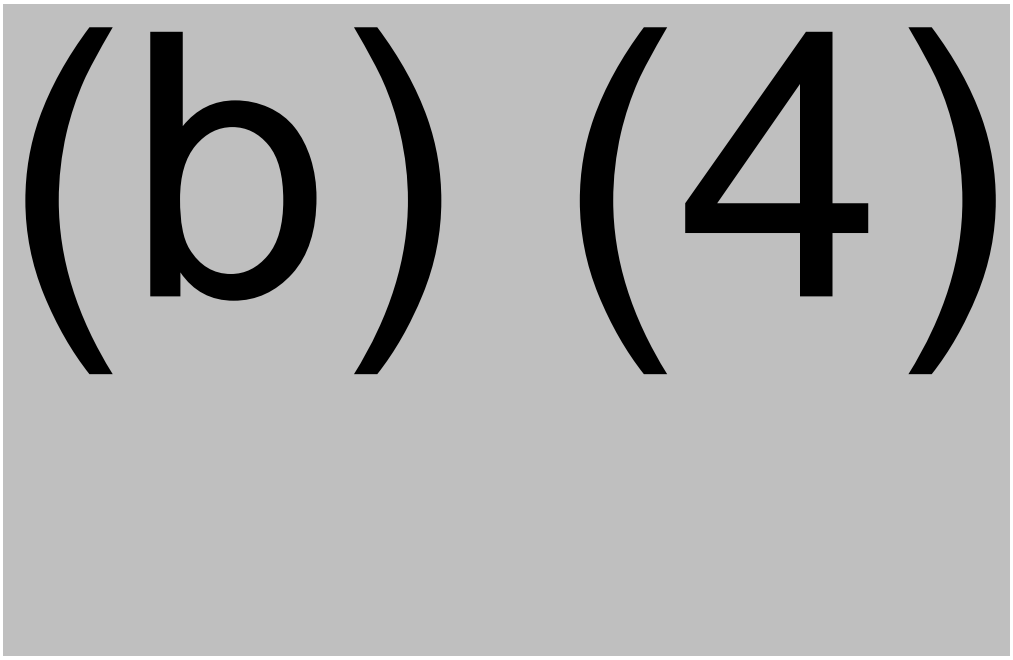
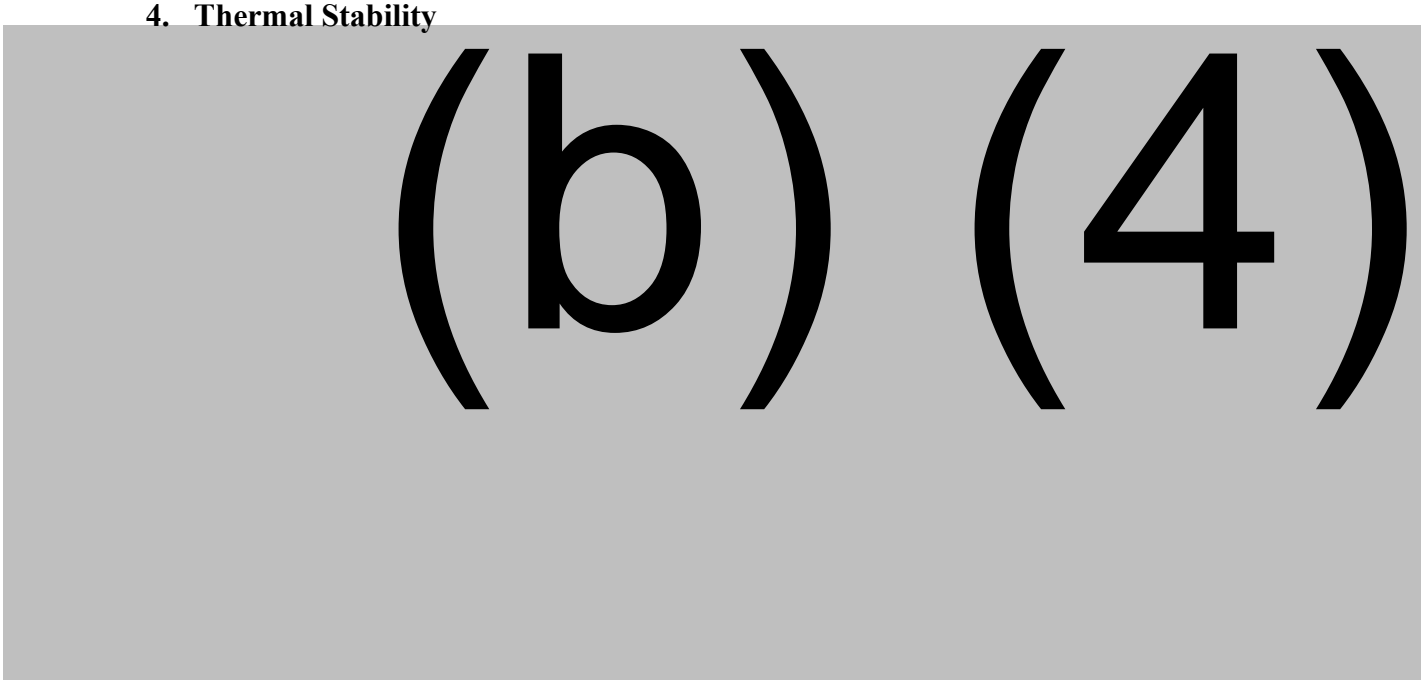

(b) (4)

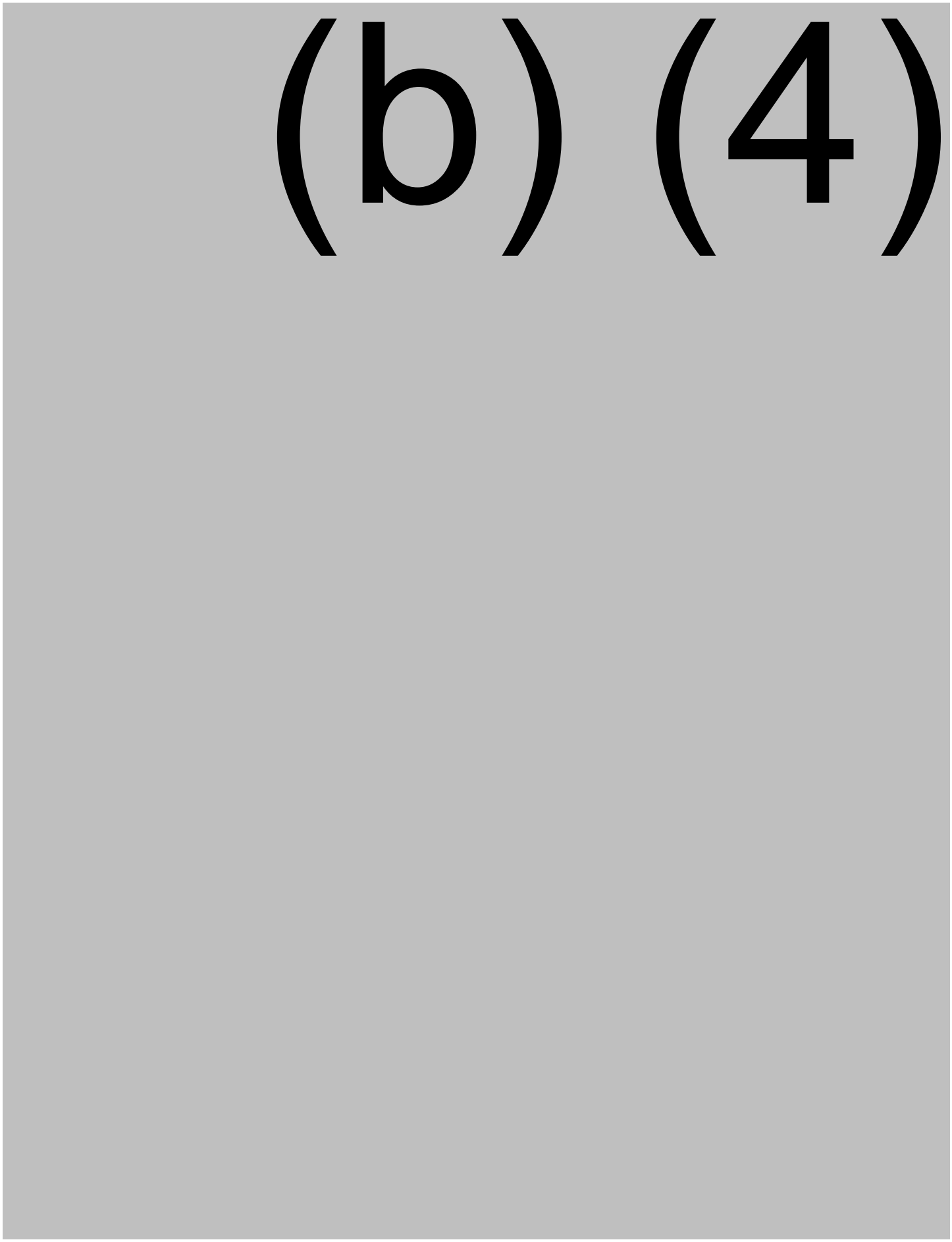
Figure 2. Effect of pH on Mannanase 19287 Activity

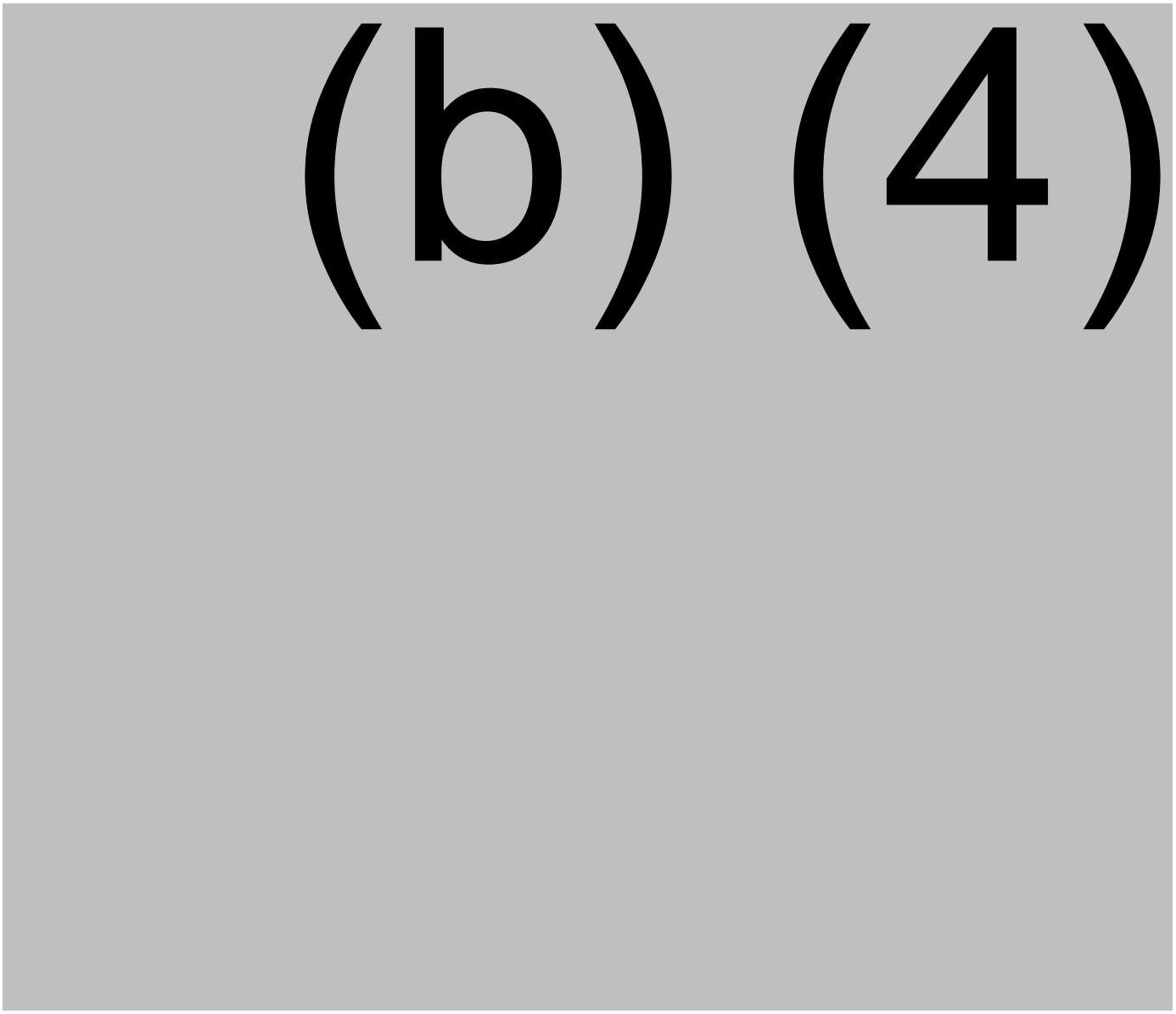


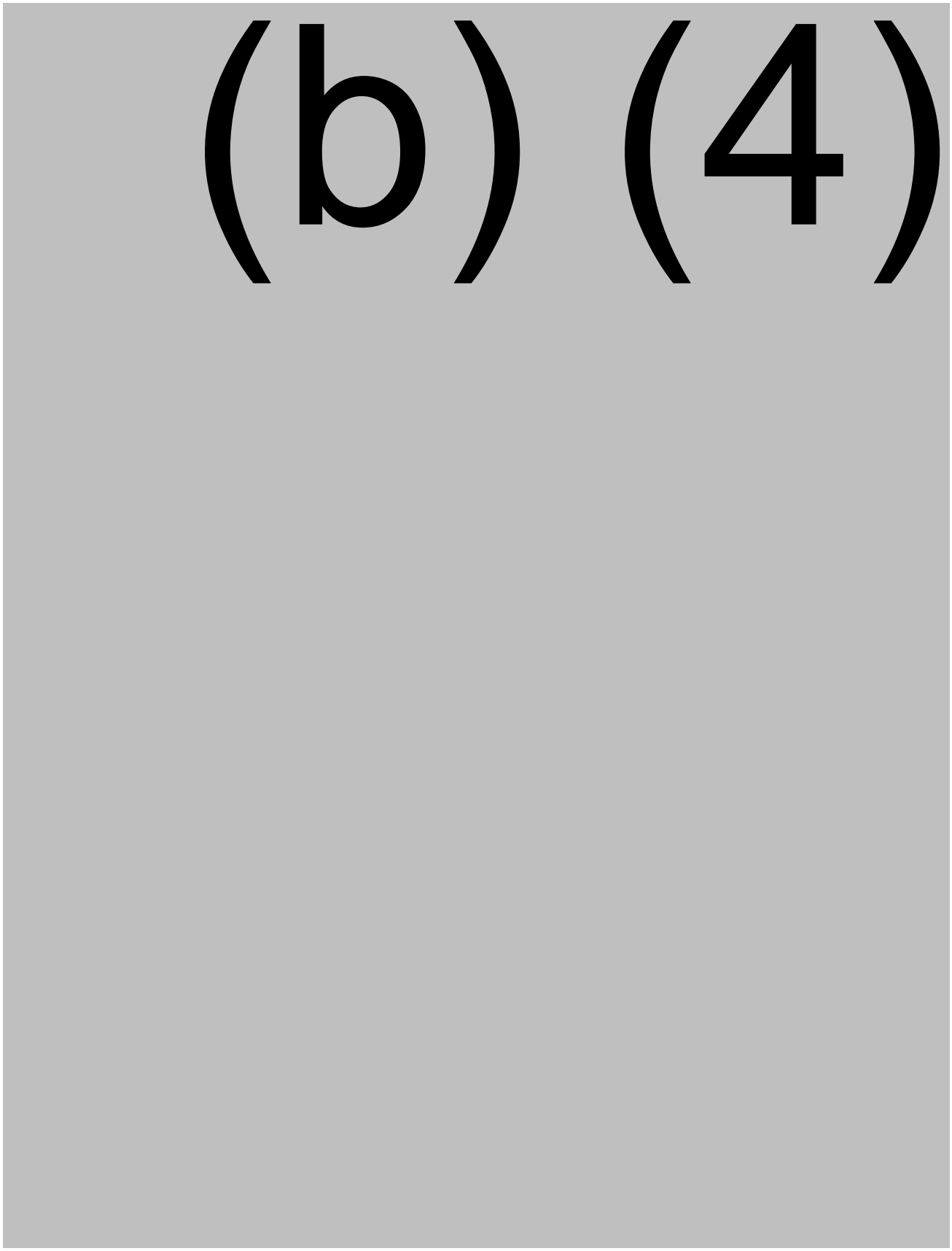
The optimal pH of mannanase 19287 activity was determined to be at pH 3.0. Activity remained stable (100 % ± 15%) up to pH 5.3. As the pH dropped into neutral and alkaline conditions, enzyme activity reached background levels (< 20%) around pH 7.5.

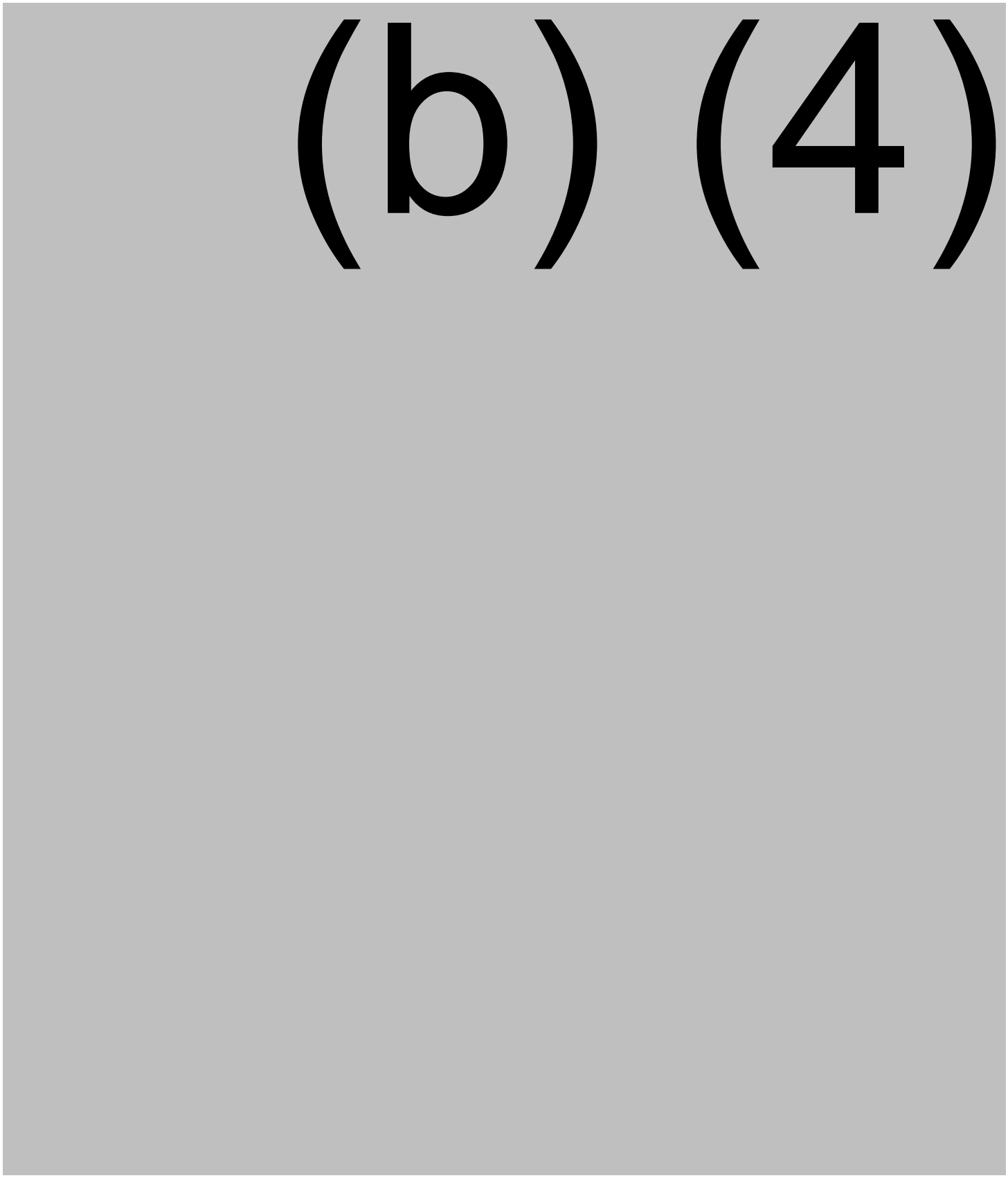
4. Thermal Stability

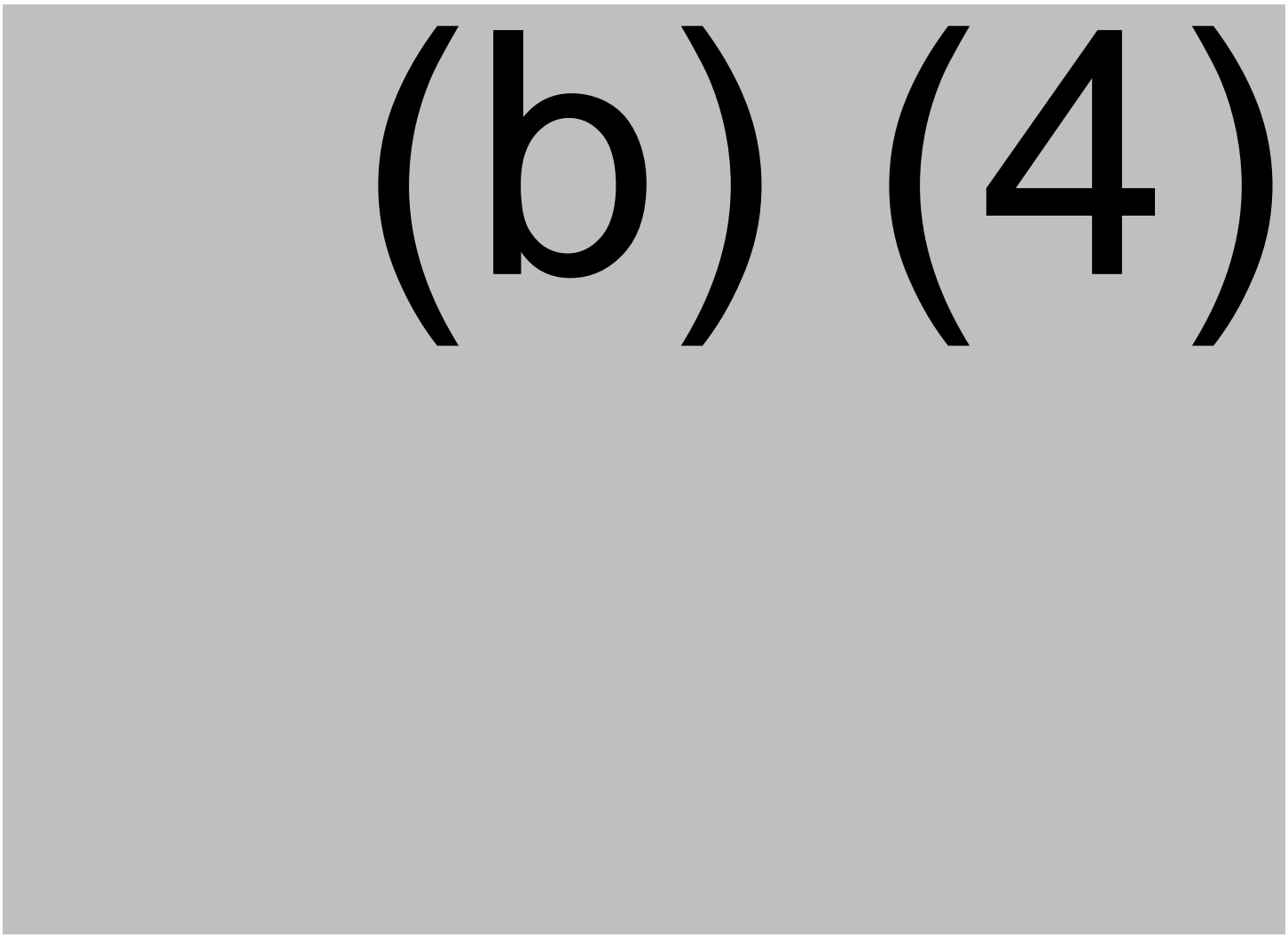



(b) (4)


(b) (4)


(b) (4)


(b) (4)


(b) (4)

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Annex 1: Analytical Report Mannanase 2018

Final Report

Characterization of "Mannanase, endo-1,4-Beta-"
Study No. 18L00272 (confidential)



Test item	"Mannanase, endo-1,4-Beta-"
Chemical identity	Mannanase, endo-1,4-Beta-
Batch identification	Mannanase 2018-01
Date of production (test item)	08/2018
Origin of test item	(b) (6)
PSN	None
CAS no.	37288-54-3
Sponsor	(b) (6)
Date of receipt of order	Sep 20, 2018
Date of receipt of test item	Sep 21, 2018
Testing facility	Competence Center Analytics, BASF SE, D-67056 Ludwigshafen
Study director	Dr. Bader, RAA/AR – E 210
Storage cond. test item	Ambient temperature
Test period	Oct 22 - Nov 23, 2018
Storage of records	GLP archives, Competence Center Analytics
Storage of sample of test item	Archives, Competence Center Analytics

(b) (4)

1 Appearance and homogeneity

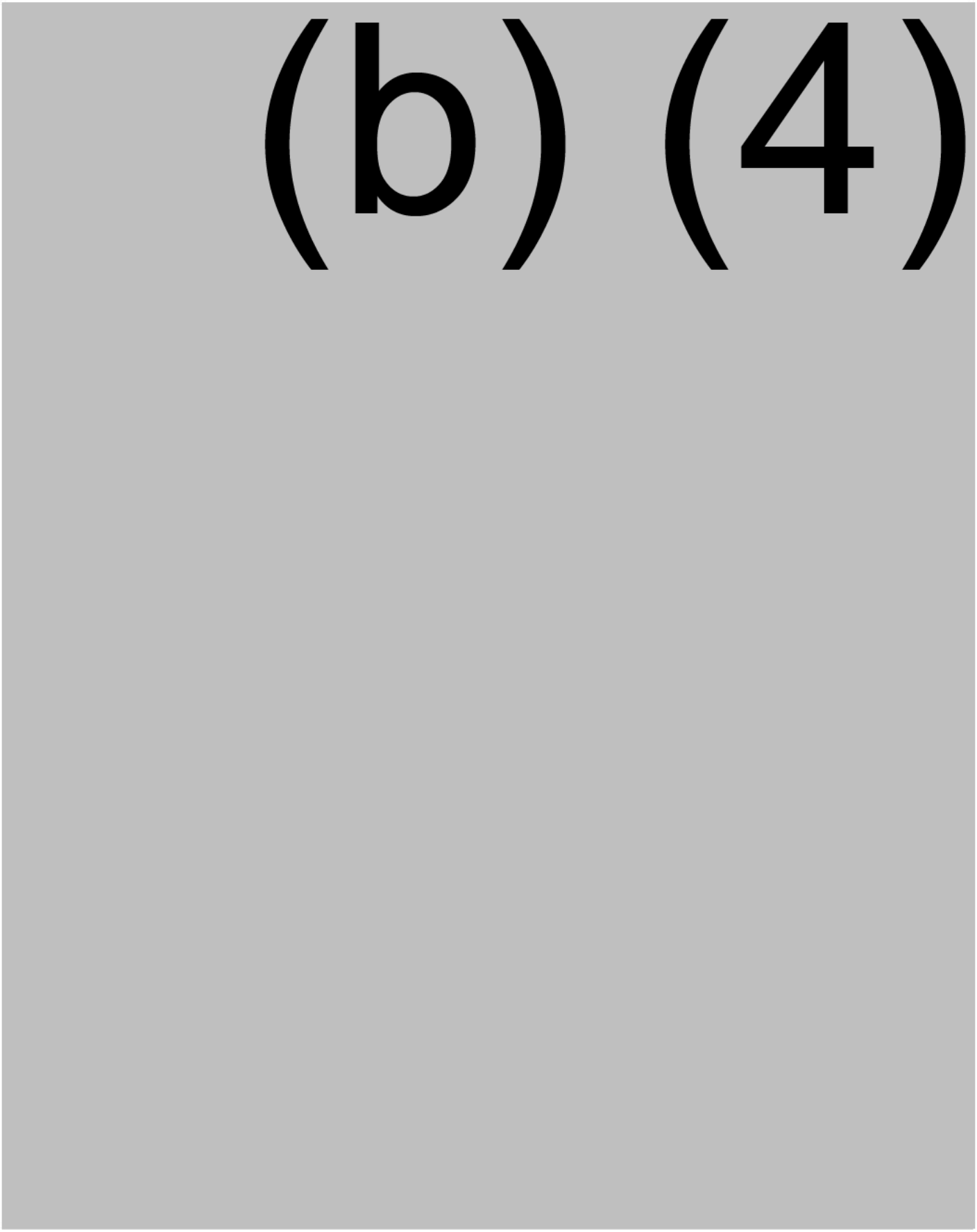
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Study director
(Dr. Bader)

Date

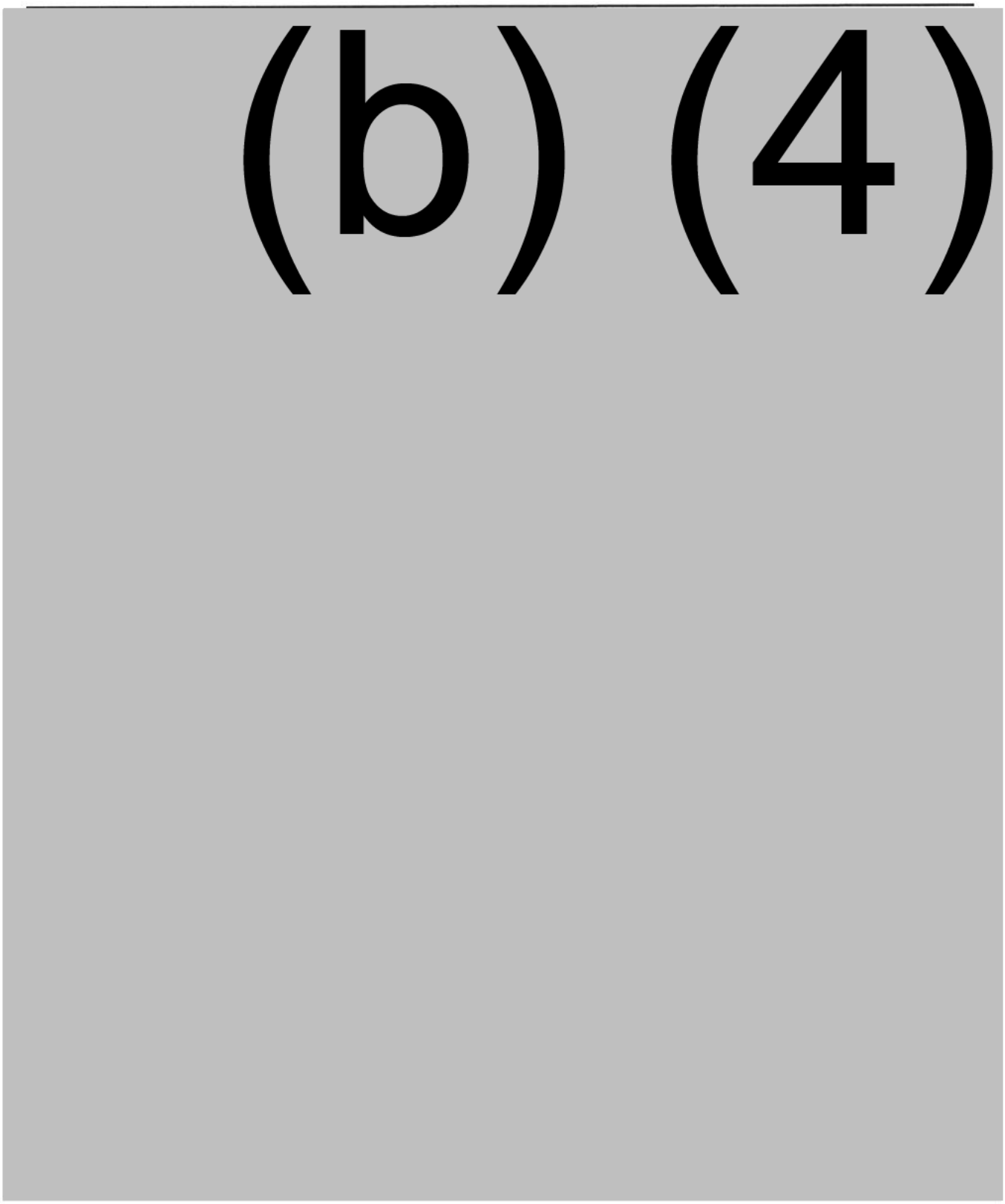
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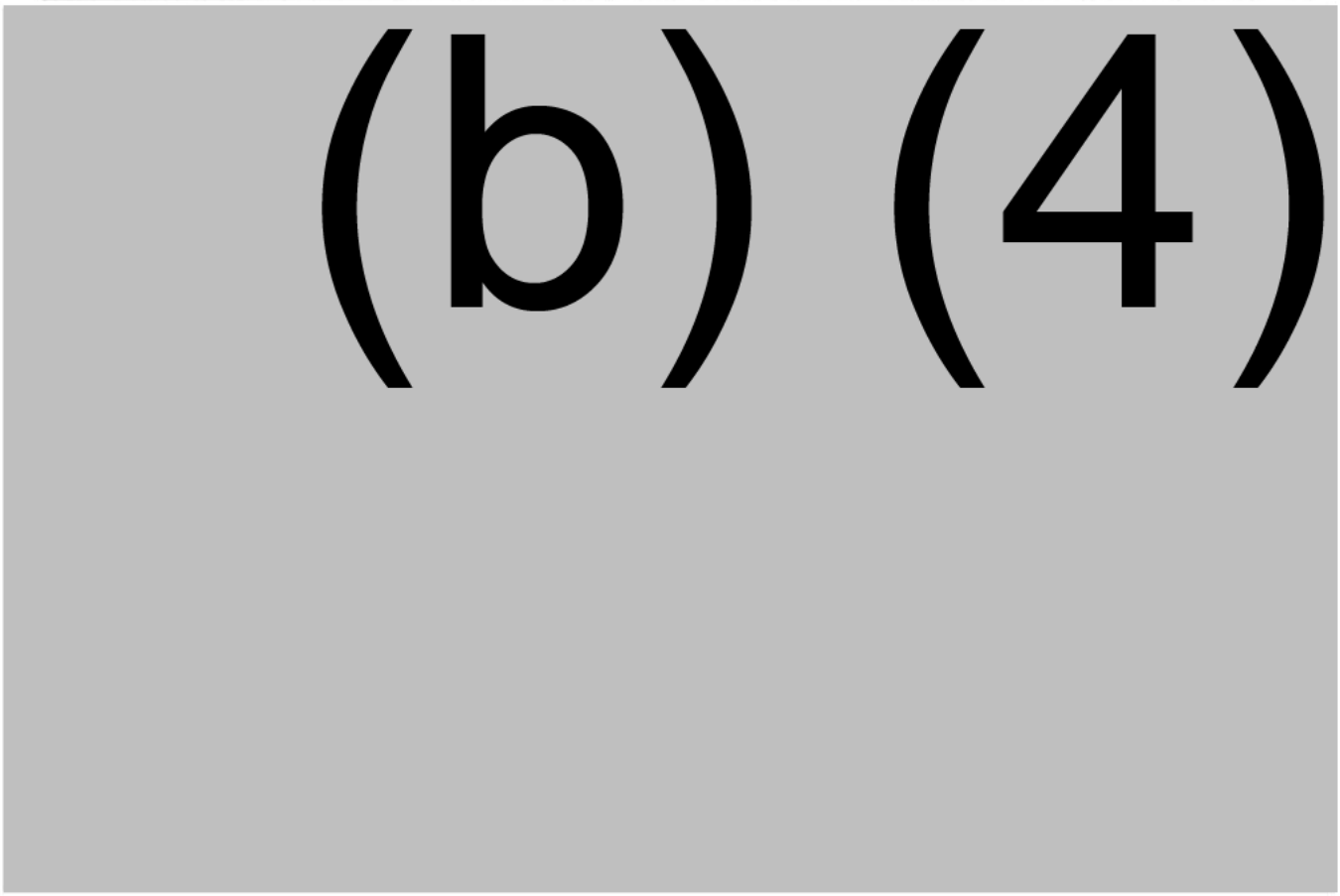
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(b) (4), (b) (6)

Study director
(Dr. Bader)

Date

Statement of the Quality Assurance Unit

(b) (4)

Annex 2: Endo-β-Mannanase Activity of Mannanase 19287

BASF
We create chemistry

Endo-β-Mannanase Activity of Mannanase 19287 Produced by *Thermothelomyces thermophilus* DSM 33149

Analysis Performed By

(b) (6)

December 2022

Final Report

Andreas Kern, PhD
Sr. Regulatory Affairs Manager
Animal Nutrition

February 7, 2024

BASF
We create chemistry

Endo-β-Mannanase Activity of Mannanase 19287 Produced by *Thermothelomyces thermophilus* DSM 33149

SIGNATURE PAGE

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Technical Expert:

(b) (6)

Hao Li Date

Sr. Scientist I
Enzyme Technologies

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Endo-β-1,4-D-Mannanase Activity of Mannanase 19287

Summary

The endo-β-1,4-D-mannanase protein (EC 3.2.1.78) of the mannanase 19287 enzyme preparation is encoded by a synthetic (b) (4) The protein sequence shows >96% homology to a (b) (4) from *Trichoderma reesei* (*T. reesei*, GenBank Q99036), and both β-mannanases belong to Family 5 Subfamily 7 of glycosyl hydrolases (GH). Endo-β-1,4-D-mannanases in GH-family 5-7 share a highly conserved beta/alpha fold structure with identical amino acids in strictly conserved positions. These amino acids are also preserved in mannanase 19287 derived from gene (b) (6). The identity of mannanase 19287 protein expressed in the production strain *T. thermophilus* DSM 33149 has been confirmed by MS protein analysis and gene sequence analysis.

Endo-β-1,4-D-mannanase activity has been confirmed through LC/MS analysis of mannose-oligosaccharides in the reaction supernatants of purified (b) (4) show the appearance of galactomannan oligosaccharides of varying lengths during the incubation with the enzyme preparation. These results confirm the endo-β-1,4-D-mannanase activity of mannanase 19287, randomly cleaving the β-1,4-D-mannose backbone of the substrates.

Introduction

Several β-mannanase enzymes and their source organisms are listed in Table 30.1 of the 2023 Official Publication from the Association of American Feed Control Officials (AAFCO OP) for their respective intended uses in poultry (Association of American Feed Control Officals (AAFCO), 2023):

- Source Organism *Aspergillus niger*, var.
- *Trichoderma longibrachiatum* (a.k.a. *T. reesei* or *T. viride*).
- *Bacillus lentus* / *Paenibacillus lentus*.

Mannanase 19287 shows high levels of amino acid identities and similarities with the *A. niger* and *T. reesei* β-mannanases in GH-family 5-7 (Henrissat, B., 2024). Mannanases in GH-

family 5-7 share a highly conserved beta/alpha fold structure with identical amino acids in strictly conserved positions (Hilge, M. *et al.*, 1998). These amino acids are also preserved in mannanase 19287 derived from gene (b) (4)

The current study was performed to confirm the endo-β-1,4-D-mannanase (EC 3.2.1.78) activity in the mannanase 19287 enzyme preparation produced with *T. thermophilus* strain DSM 33149.

Materials and Methods

1. **Galactomannan sources**

(b) (4)

2. **Hexose determination in** (b) (6)

(b) (4)

3. **Hydrolytic activity of spray-dried mannanase 19287 enzyme intermediate concentrate** (b) (4) **galactomannans**

(b) (4)

(b) (4)

4. **MALDI-TOF MS identification of oligosaccharide masses**

(b) (4)

Results

1. **Mannose/Galactose content of** (b) (4)

(b) (4)

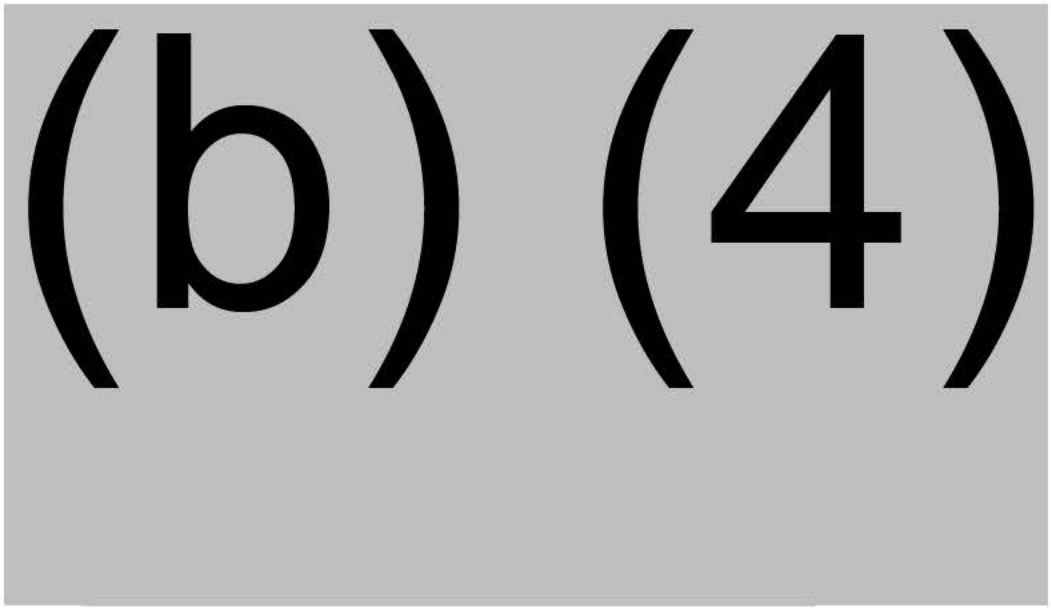
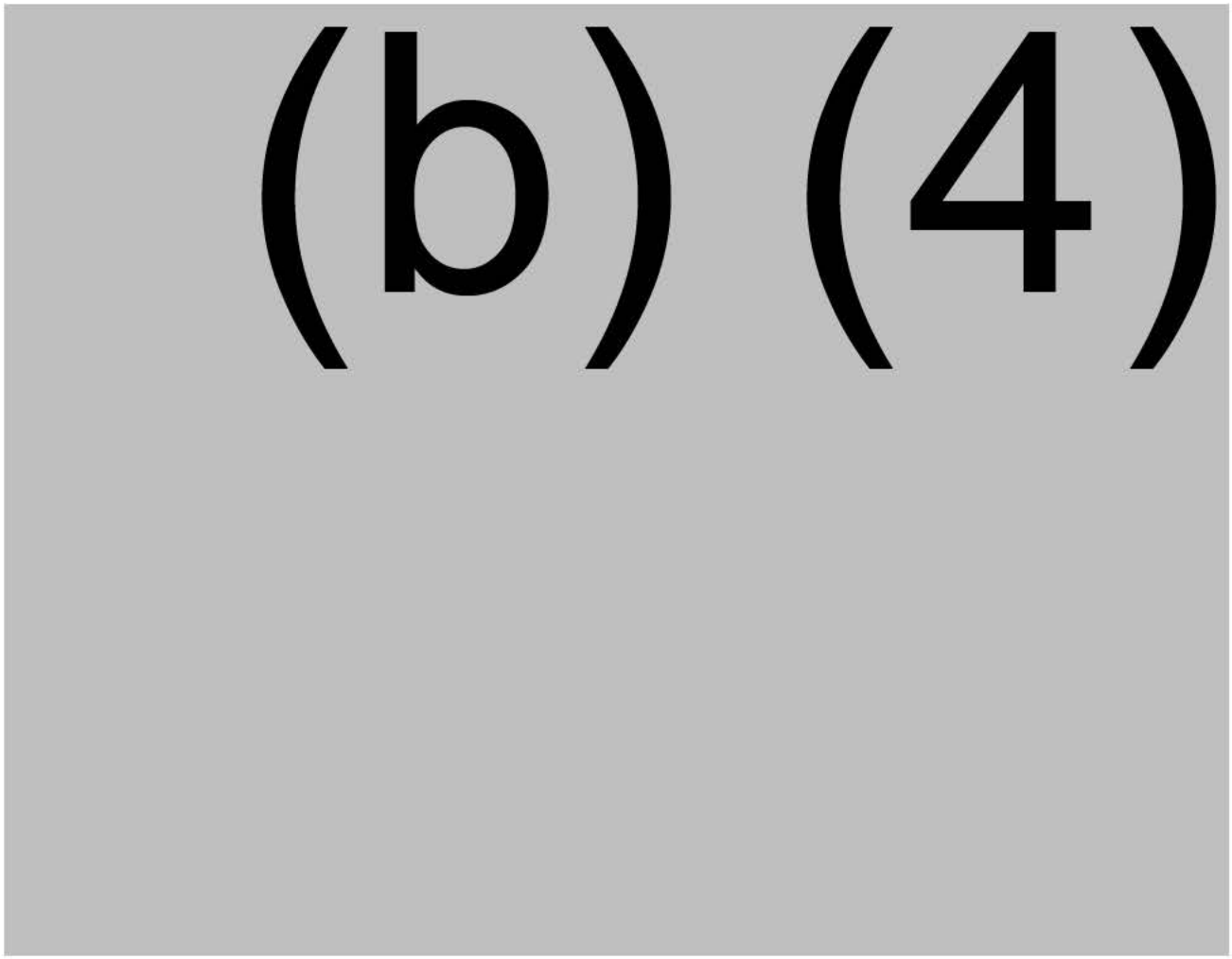
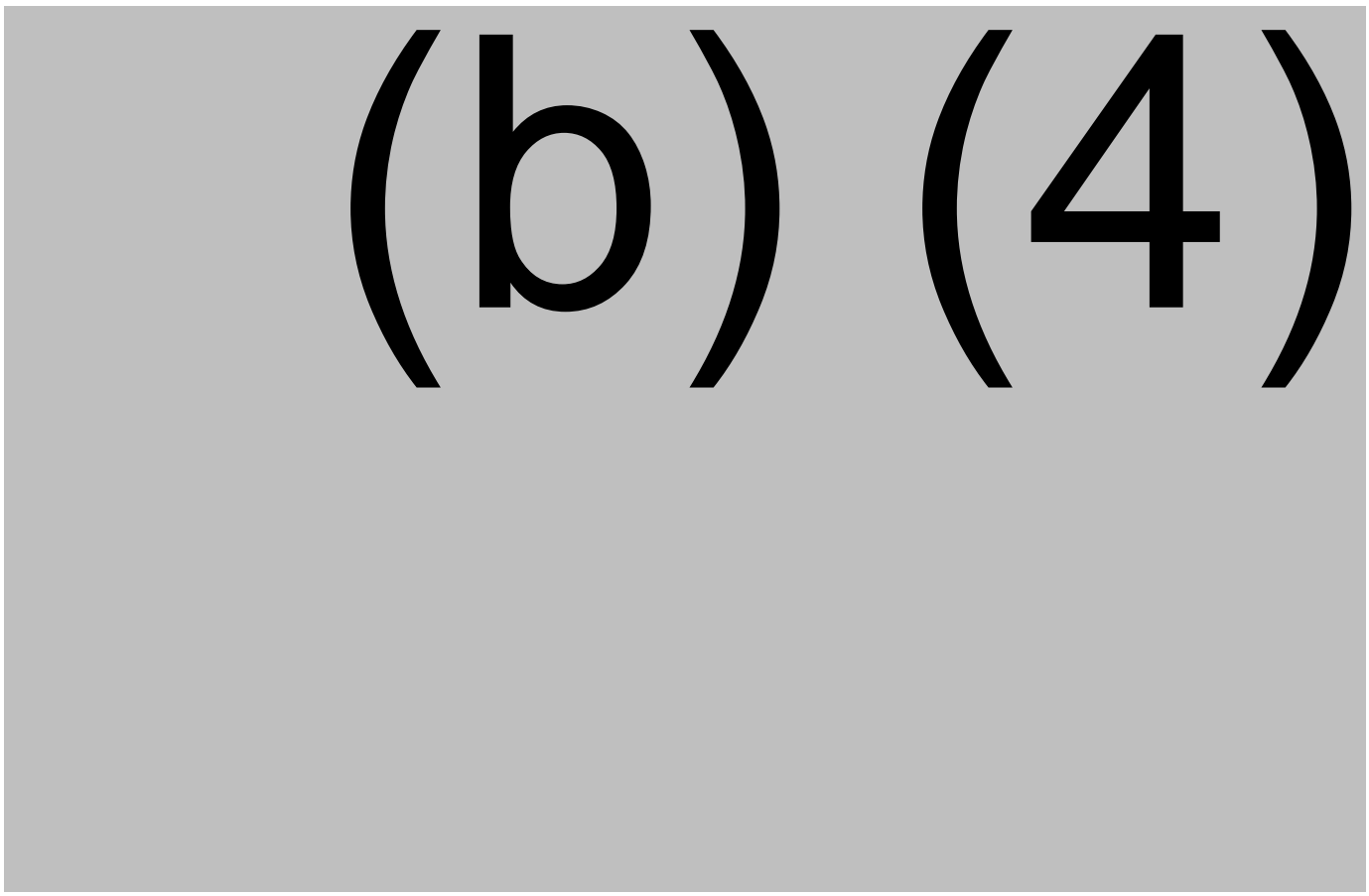


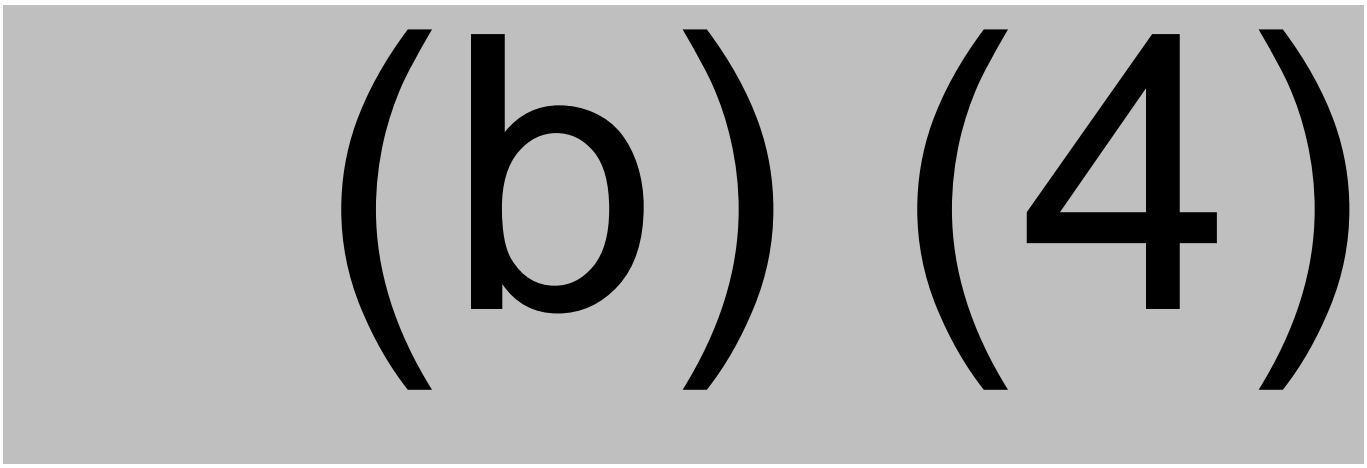
Figure 1: HPLC Chromatogram of hydrolyzed (b) (6), (b) (4) **galactomannan.** Galactomannans

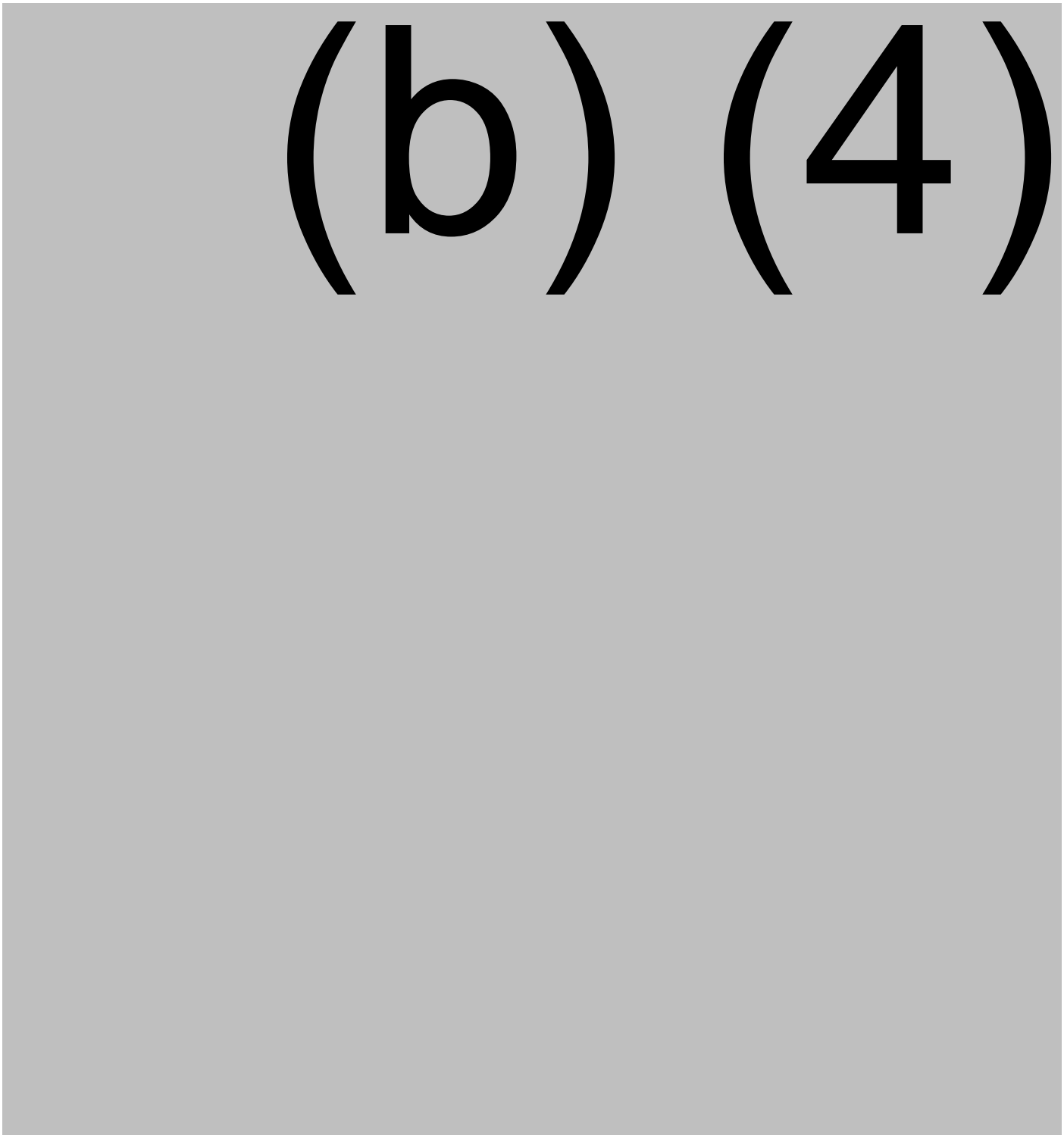


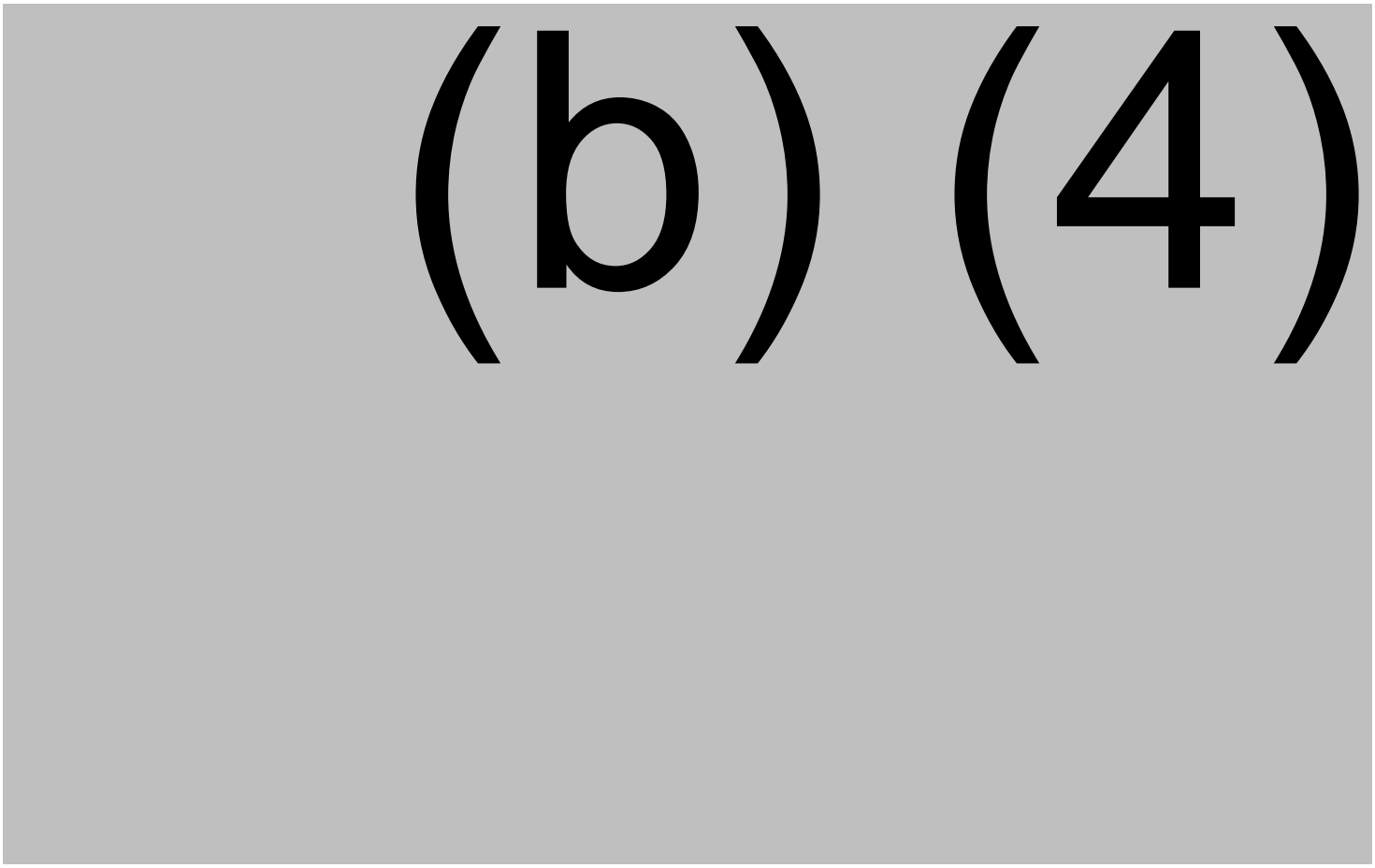




3. Oligosaccharide identification




(b) (4)



References:

Association of American Feed Control Officals (AAFCO). *Table 30.1 Enzymes/Source Organism Acceptable for Use in Animal Feeds*. *2023 Official Publication*. (2023).

Henrissat, B. *Glycosyl hydrolase families: classification and list of entries* Last Accessed 1/26/2024, Available from: https://ftp.uniprot.org/pub/databases/uniprot/knowledgebase/complete/docs/glycosid.txt (2024).

Hilge, M., Gloor, S.M., Rypniewski, W., Sauer, O. et al. *High-resolution native and complex structures of thermostable beta-mannanase from Thermomonospora fusca - substrate specificity in glycosyl hydrolase family 5*. Structure 6 (11), pp. 1433-1444 (1998).

Whistler, R.L. and Saarnio, J. *Galactomannan from Soy Bean Hulls*. Journal of the American Chemical Society 79 (22), pp. 6055-6057 (1957).

Annex 3: Biochemical Characterization Report Mannanase CC Enzyme Concentrate Test Article

We create chemistry

Biochemical Characterization Report
Mannanase CC Enzyme Concentrate Test Article

Report Number: 23R022

(b) (6)

QC Scientist II

August 16, 2023

BASF Enzymes LLC
3550 John Hopkins Court
San Diego, CA 92121

Confidential

Biochemical Characterization Report
Mannanase CC Enzyme Concentrate Test Article

APPROVERS

Department	Name, Role
Author	(b) (6)
Quality Control	(b) (6)
Quality Assurance	

Biochemical Characterization Report

Mannanase CC Enzyme Concentrate Test Article

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Biochemical Characterization Report

Mannanase CC Enzyme Concentrate Test Article

Introduction

This document provides the results of studies used to develop basic biochemical characterization data for the Mannanase CC enzyme concentrate test articles, lot # (b) (4) and lot# (b) (4) The basic characterization data includes: 1) the presence of other enzyme activities (see side activities) and 2) the enzyme's thermal tolerance.

Summary

Characterization studies relating to the presence of other enzyme activities and thermal tolerance were conducted under standard laboratory conditions.

Mannanase CC, lot # (b) (4) and lot# (b) (4) was tested for the presence of alpha-amylase, cellulase, protease, and xylanase activity. Enzyme activity was not detected or was below the limit of quantitation of the assay when testing for alpha-amylase, and cellulase. Protease activity is observed to be 44 U/g for lot# (b) (4) and 35 U/g for lot# (b) (4) Mannanase CC lot # (b) (4) and lot# (b) (4) contain 6.5 U/mL and 6.6 U/mL xylanase activity respectively.

The thermal tolerance was determined by exposing the Mannanase CC enzyme concentrate to a temperature of (b) (4) for a prolonged period prior to testing for activity. It was observed that the mannanase activity at (b) (4) decreases 19% in the first 4 minutes and by 87% after 64 minutes of exposure. Mannanase activity at (b) (4) decreases 30% in the first 4 minutes and by 86% after 64 minutes of exposure.

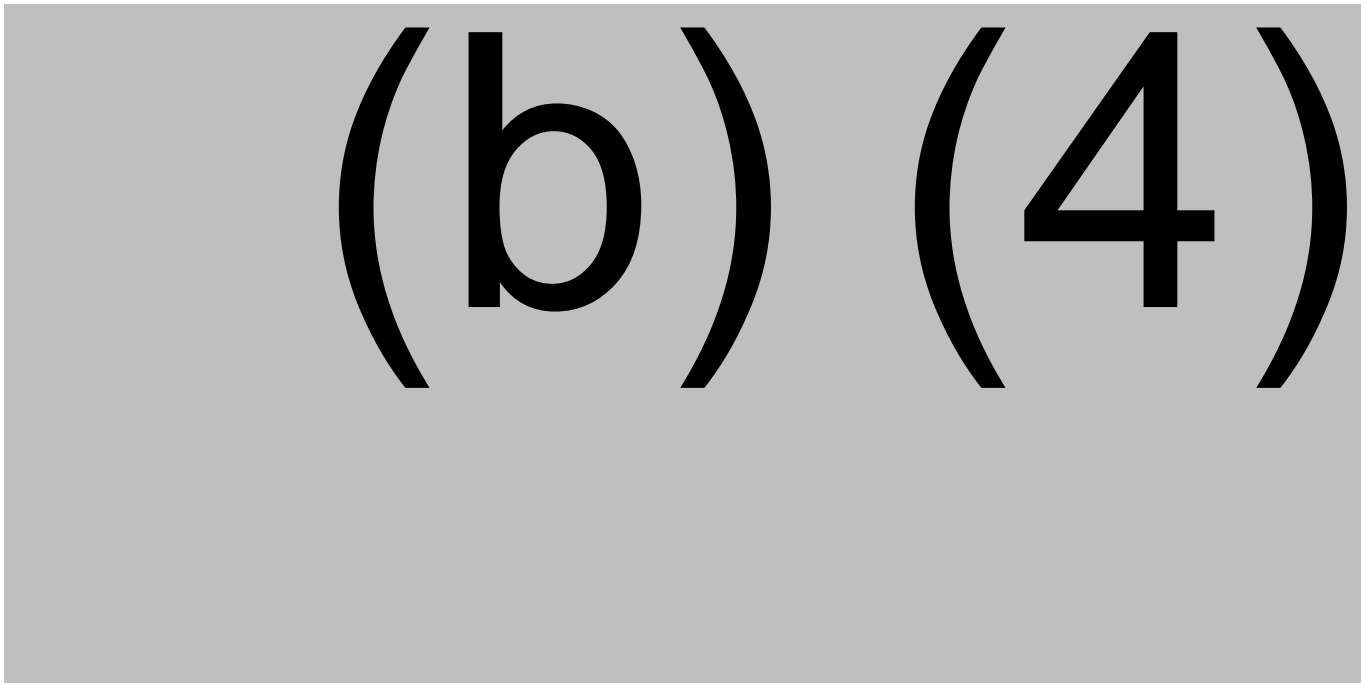
The raw data that supports these studies is maintained in BASF Enzymes LLC QA/QC files.

Test Material

- Mannanase CC enzyme concentrate, lot# (b) (4) and lot# (b) (4)

(b) (4)

.



Results and Conclusions

1. Side Activities

The Mannanase CC enzyme concentrate lot# (b) (4) and lot# (b) (4) were each tested for various enzyme activities. The presence of alpha-amylase, cellulase, protease, and xylanase side activities were determined using test article Mannanase CC. The results obtained from these assays are summarized in Table 1.

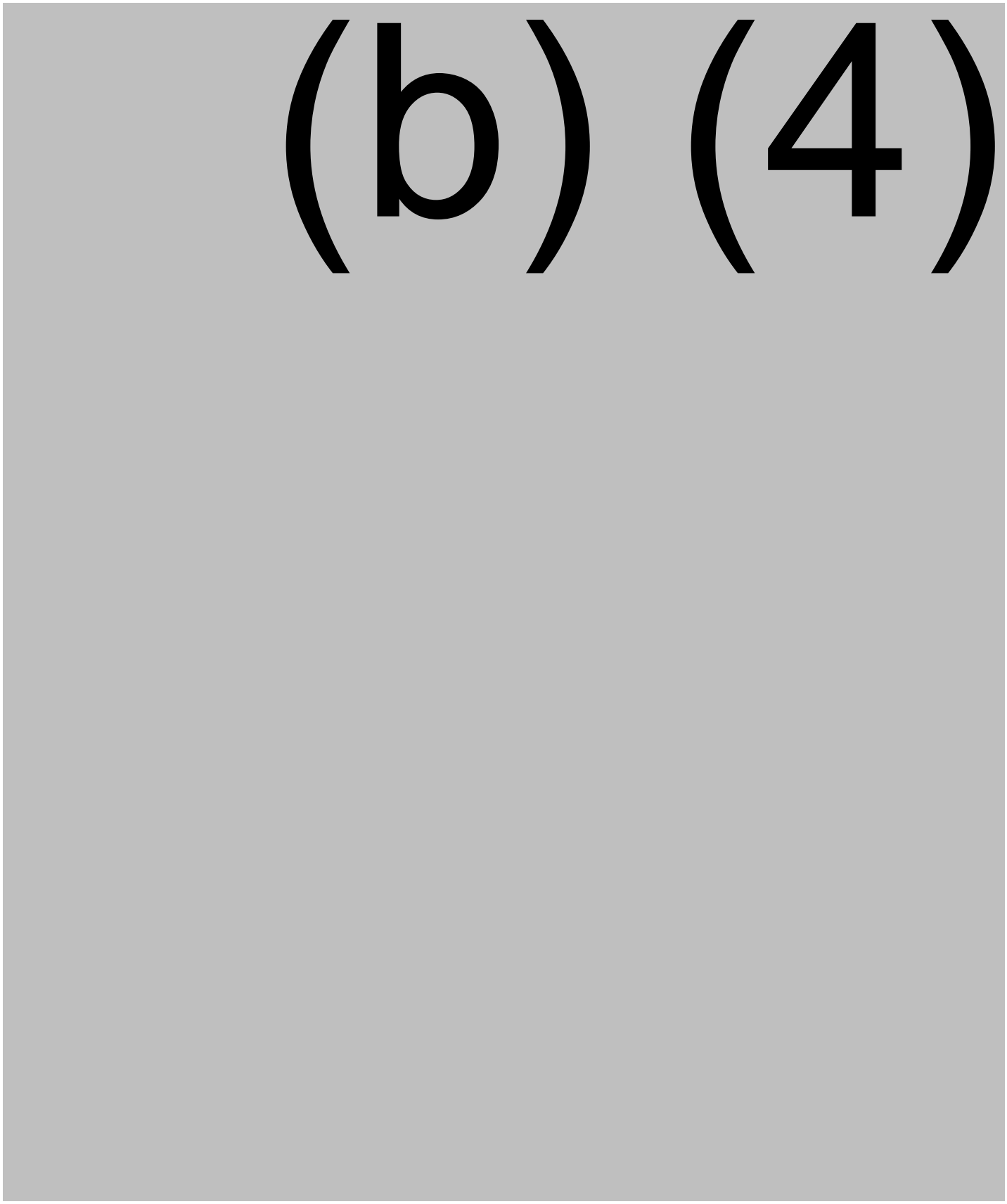
Table 1. Evaluation of Possible Side Activities in Mannanase CC

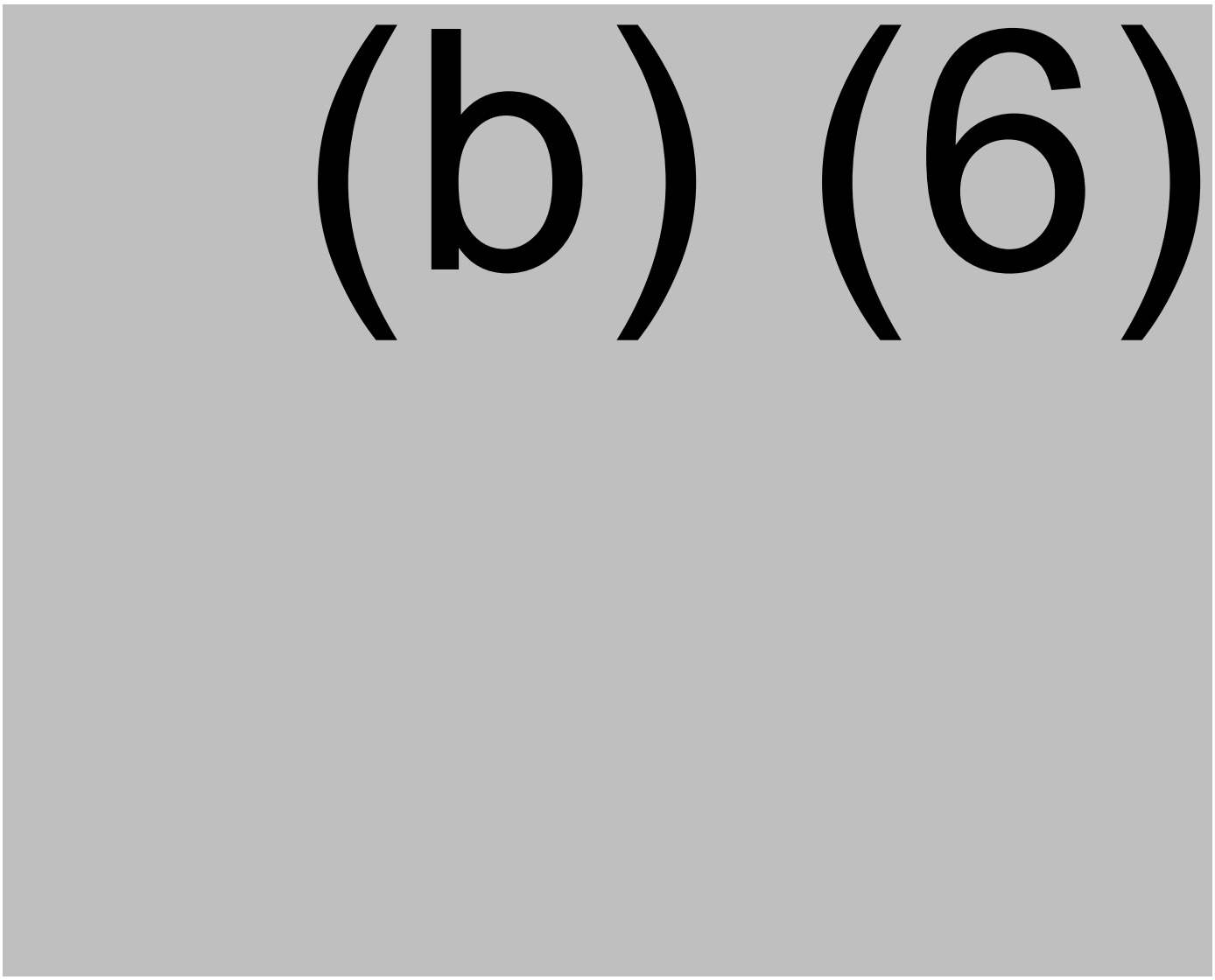
(b) (4)

2. **Thermal Tolerance**

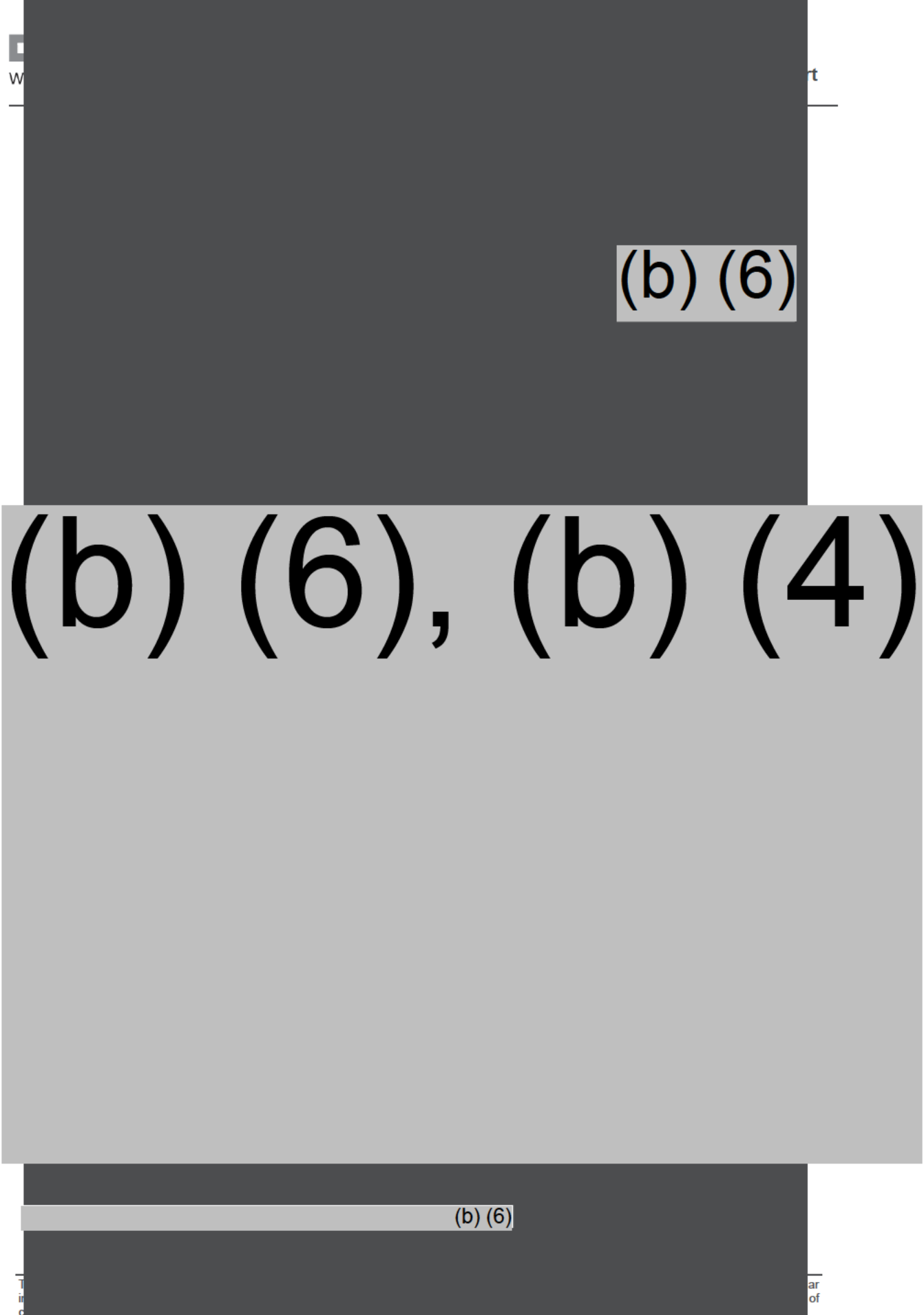
(b) (4)

(b) (4)


(b) (4)


(b) (6)

Annex 4: Mannanase CC Assay Results


(b) (6)
(b) (6), (b) (4)
(b) (6)

Annex 5: Xylanase FEN-002-01 Method of Analysis

Method of Analysis FEN/0002/01

BASF
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Title: **Photometric assay of xylanase activity in feed additives**

Effective date: August 18th, 2017

Prepared by: (b) (6)

Reviewed and approved:

Signature, date

1 Keywords

1.1 To be determined

Xylanase activity

1.2 Matrix

Feed additives such as

- Xylanase TS SD
- Xylanase CC
- Natugrain® Wheat TS
- Natugrain® Wheat TS L
- Natugrain® TS
- Natugrain® TS 5XG
- Natugrain® TS L
- Natugrain® TS DL
- Natuphos® E 5000 Combi G
- Natuphos® E 5000 Combi L
- Natuphos® 5000 Combi G
- Natuphos® 5000 Combi L

2 Principle

This assay procedure is specific for *endo*-1,4-ß-D-xylanase activity. (b) (4)

One Thermostable Endoxylanase Unit (TXU) is defined as the amount of enzyme which liberates 5.00 micromole reducing sugars, measured as xylose equivalents per minute as defined by the sugar release method.

3 Working range

(b) (4)

4 Safety note

The method described involves the handling of hazardous substances. Attention is therefore drawn to the various provisions governing the handling of potentially dangerous material.

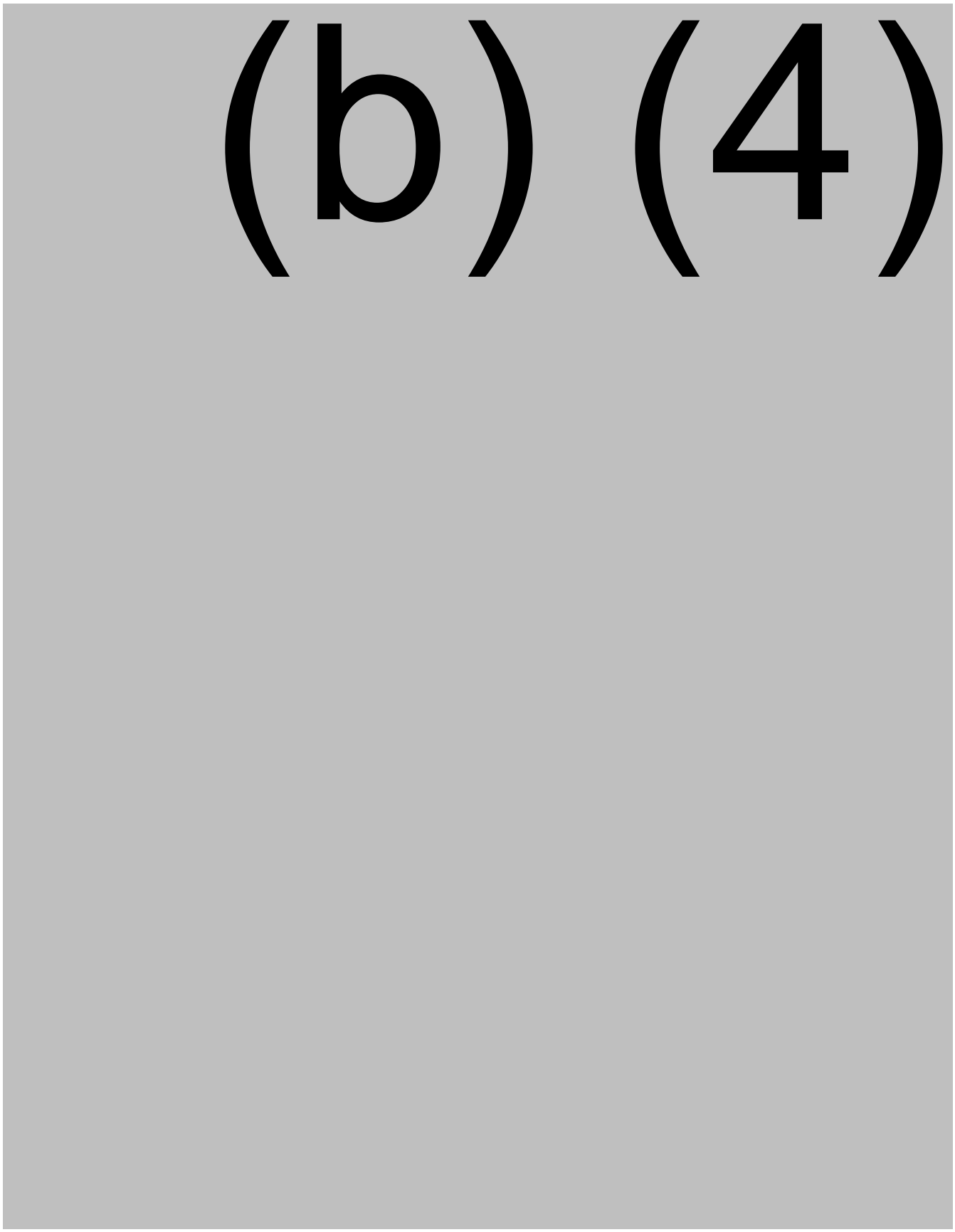
Reagents

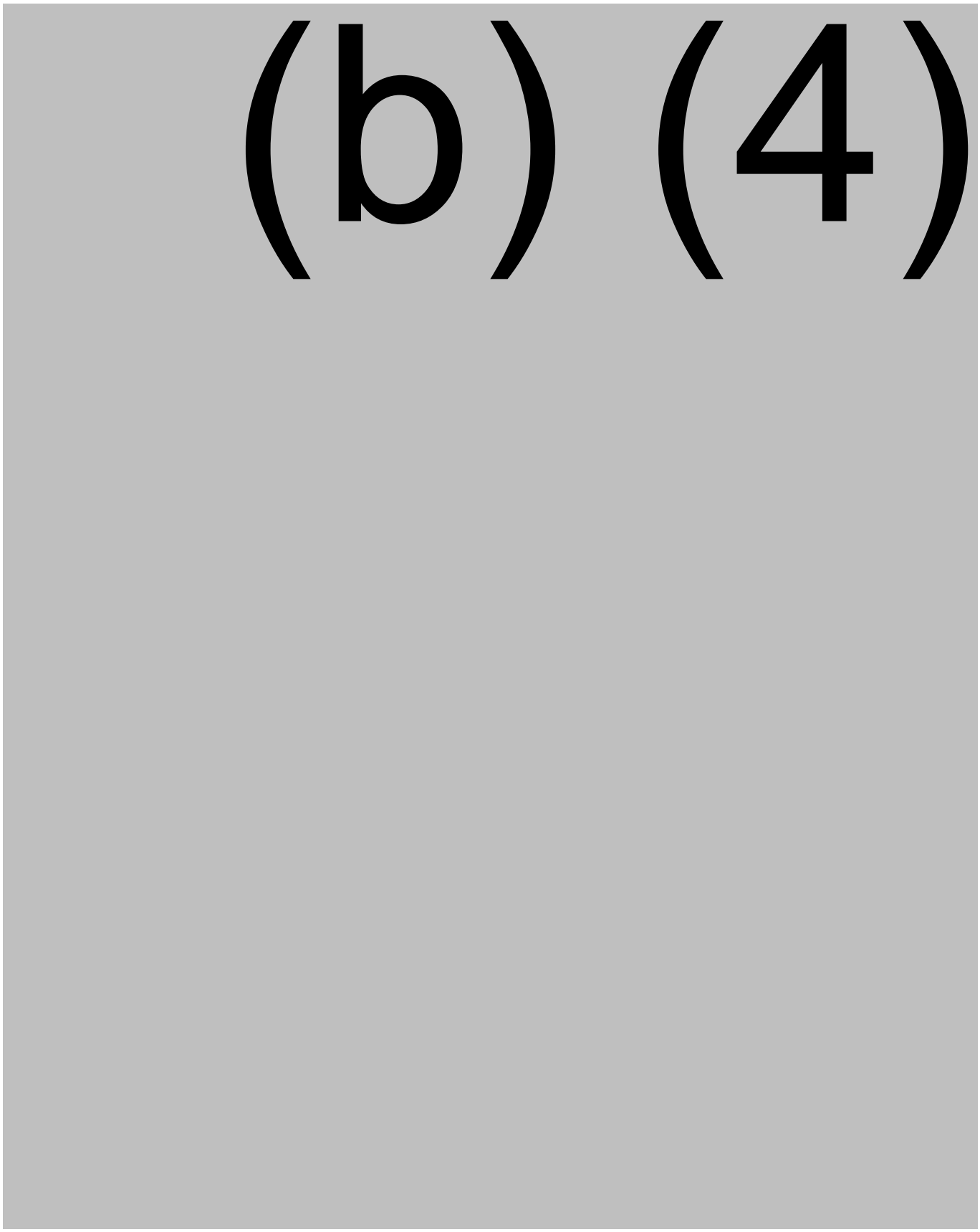
(b) (4)

(b) (4)

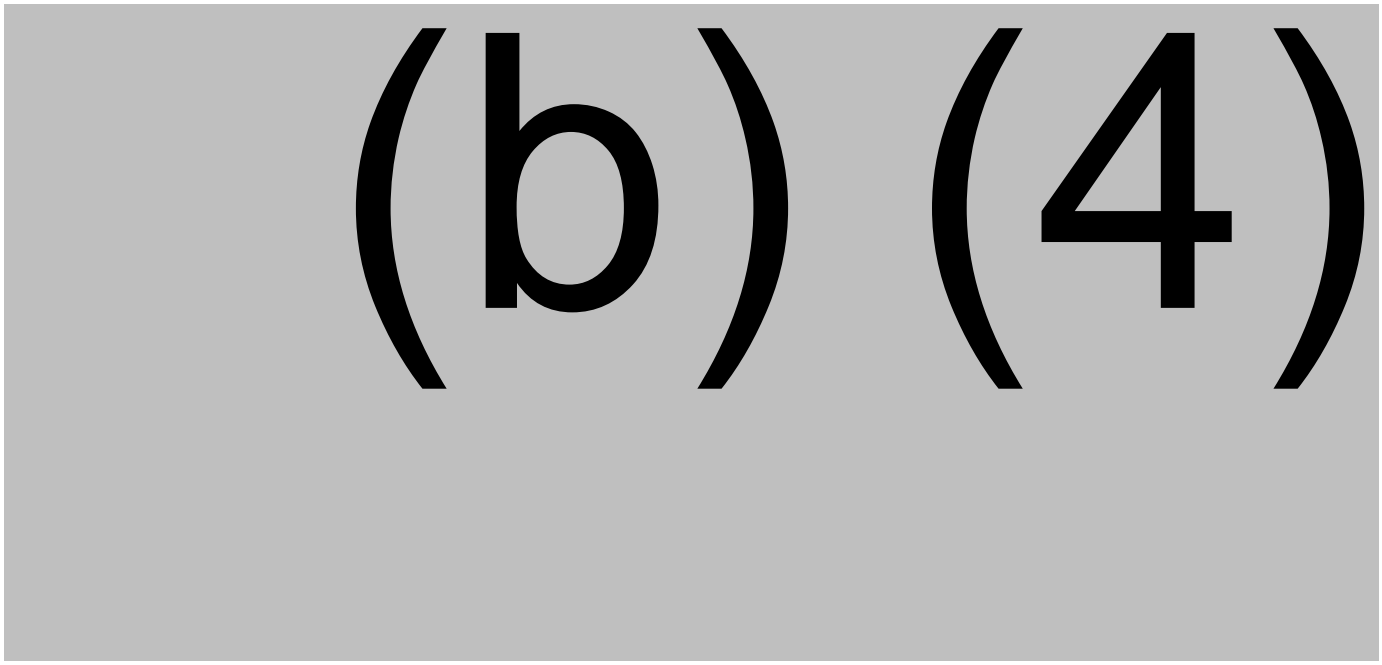
(b) (4)

(b) (4)


(b) (4)


(b) (4)

(b) (4)


(b) (4)

Annex 6: β-Glucanase FEN-007-01 Method of Analysis

Title: **Photometric assay of glucanase activity in feed additives**

Effective date: August 18th, 2017

Prepared by: (b) (6)

(b) (6)

Reviewed and approved: Dr. (b) (6)

(b) (6)

1 Keywords

1.1. To be determined:

Glucanase activity

1.2. Matrix

Feed additives such as

- Glucanase TS SD
- Glucanase CC
- Natuphyt Combi CC
- Phytase Combi CC
- Natugrain® TS
- Natugrain® TS 5XG
- Natugrain® TS L
- Natugrain® TS DL
- Natuphos® E 5000 Combi G
- Natuphos® E 5000 Combi L
- Natuphos® 5000 Combi G
- Natuphos® 5000 Combi L

2 Principle

This assay procedure is specific for the endo-1,4-ß-D-glucanase activity (endo-Cellulase) present in glucanase preparations.

(b) (4)

A thermostable β-glucanase unit (TGU) is defined as the amount of enzyme that releases 1 µmol of reducing sugar, determined as glucose equivalents, per minute under the given assay conditions

3 Working range

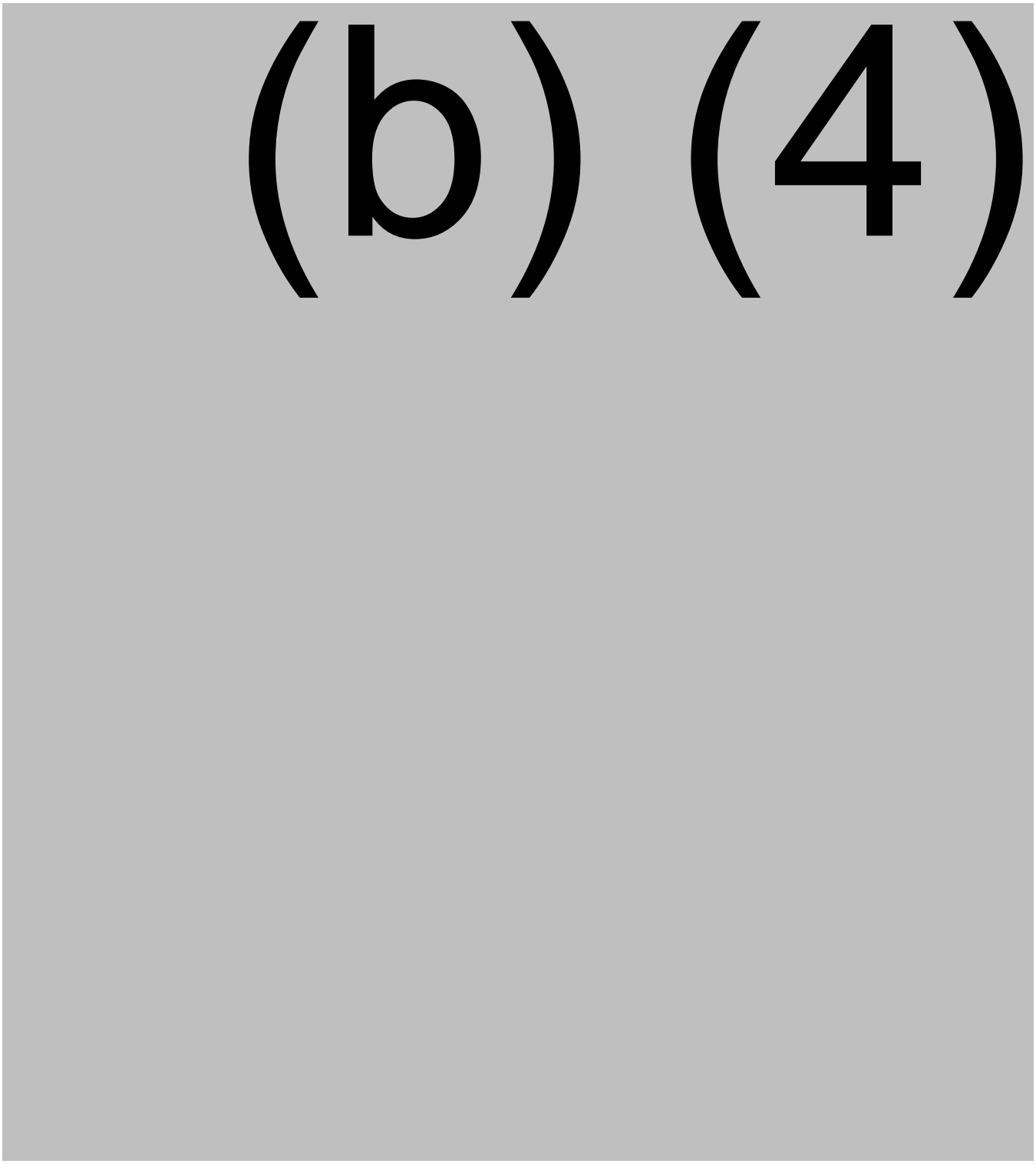
(b) (4)

4 Safety note

When using the test method, the substance-specific or substance group-specific safety documents available in the laboratory for the matrices and reagents described in the method must be observed. Safety instructions included in the analysis order or supplied by the manufacturer of the reagents must also be observed. For the work described in the execution, the safety operating instructions correspondingly available in the laboratory are to be considered. The guidelines for safe working in the laboratory apply.

5 Reagents

(b) (4)


(b) (4)

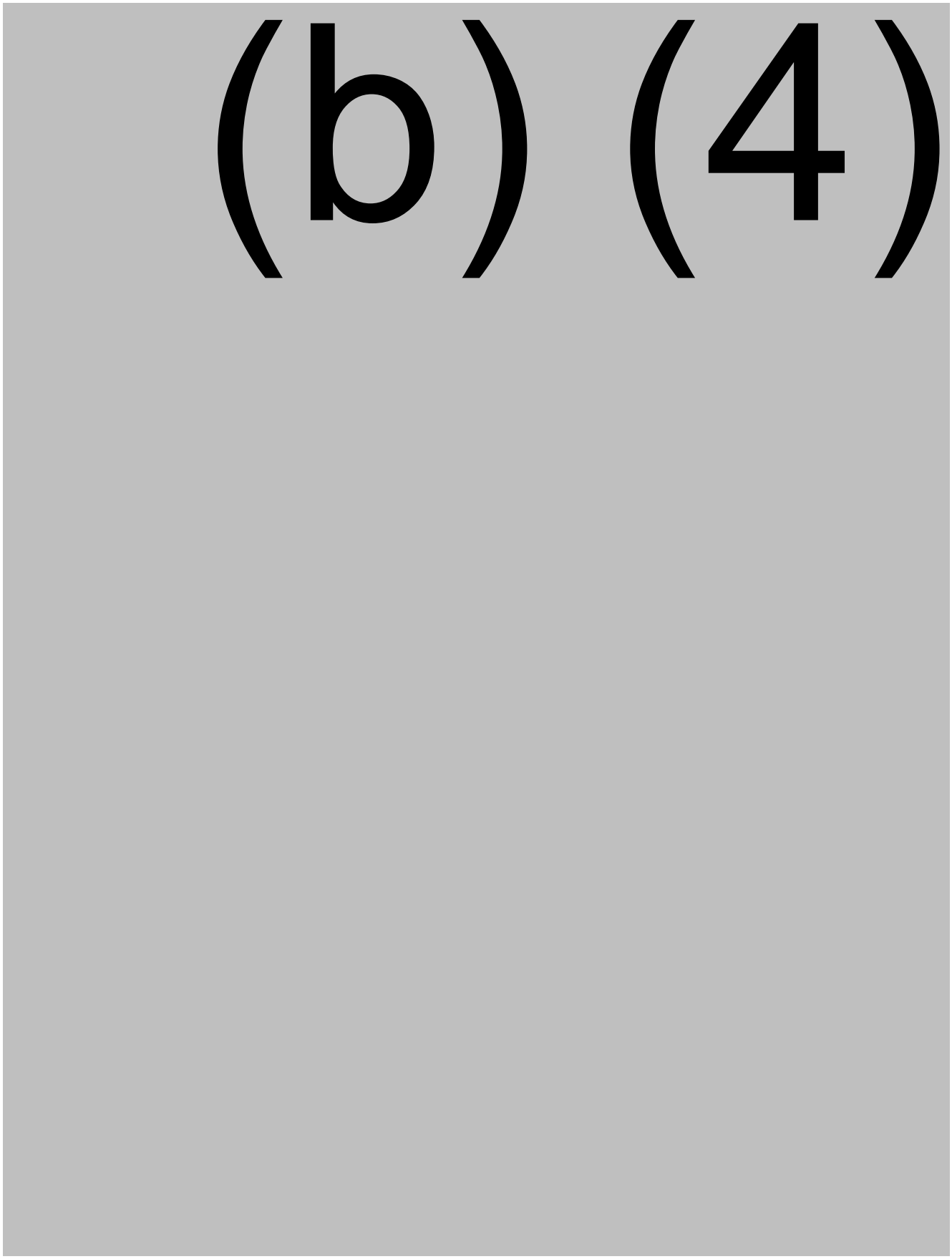
6 Apparatus

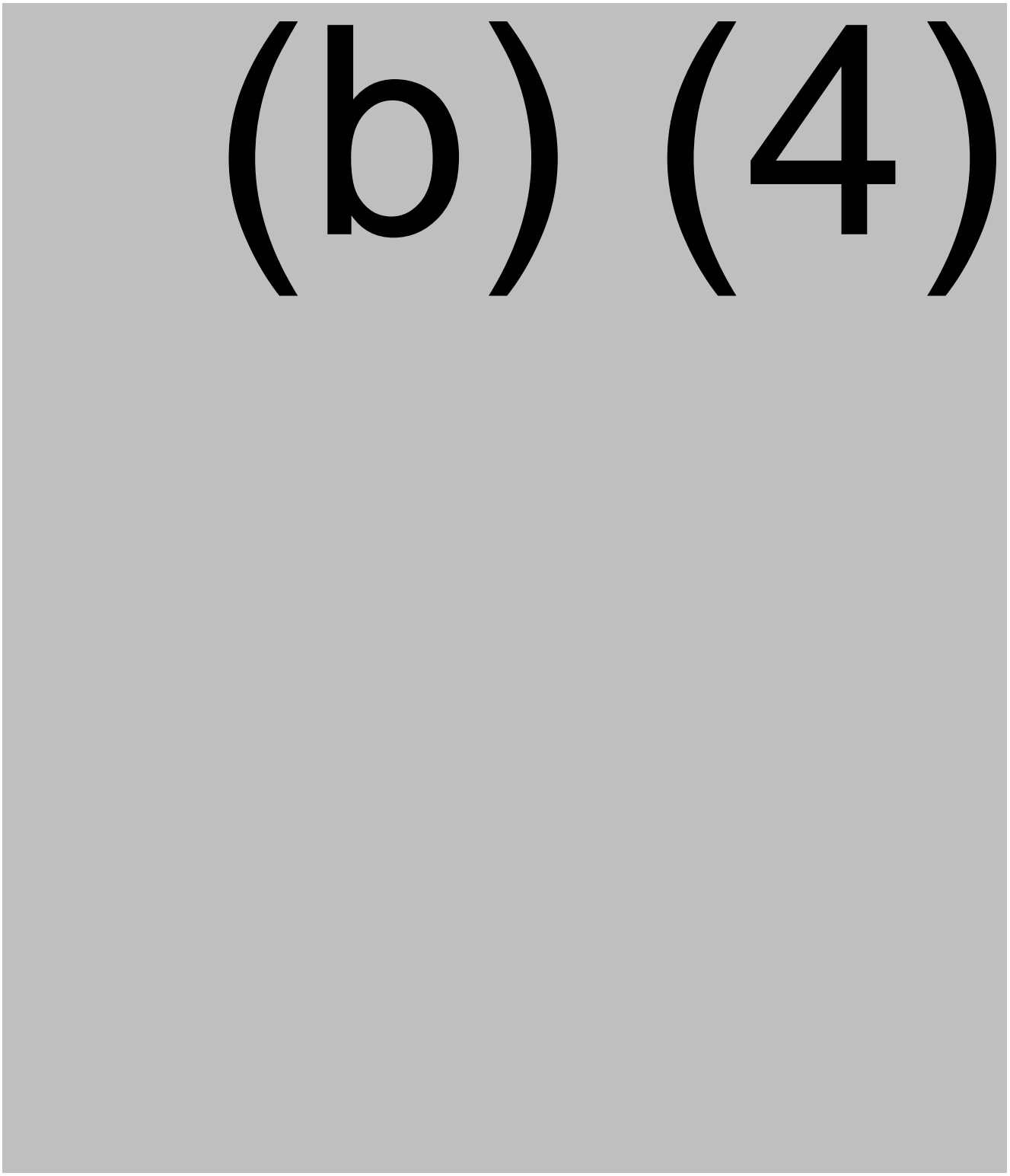
(b) (4)

7 Preparation of standard, sample and blank solution

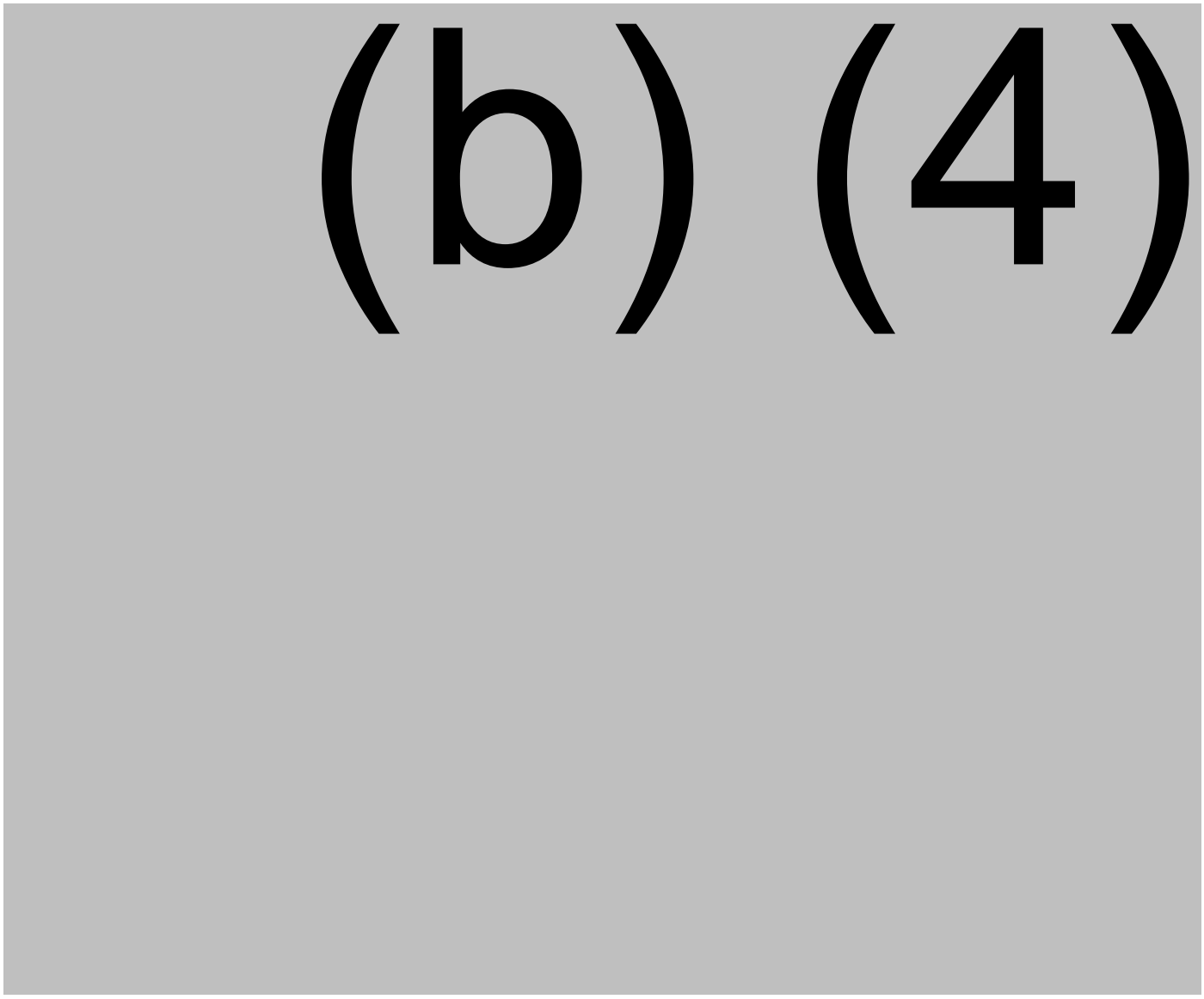
(b) (4)

(b) (4)


(b) (4)


(b) (4)

8 Procedure



9 Calculation of glucanase activity

(b) (4)

10 Measurement uncertainty

(b) (4)

11 Comments

(b) (4)

12 Modification history

12.1 Changes in Version 0

Initial version

Appendix 05: Production Strain Genetic Development Report

BASF
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Production Strain DSM 33149

Genetic Development Report

Production Strain DSM 33149

Genetic Development Report

Author:

KernA14

Digitally signed by KernA14
Date: 2024.02.14 06:34:38 -08'00'

Andreas Kern, Ph.D. **Date**
Sr. Regulatory Affairs Manager
Animal Nutrition

Technical Reviewer:

(b) (6)

Enzymes Process and Strain

Production Strain DSM 33149

Genetic Development Report

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Introduction

The filamentous fungus *T. thermophilus* is able to secrete large quantities of enzymes and may serve as an alternative to similar well-known production hosts, including *Aspergillus niger* or *Trichoderma reesei* (Singh, B., 2016). A natural strain of *T. thermophilus* named C1 was isolated from soil in Russia, deposited at the All-Russian Collection of Microorganisms (VKM, accession number VKM F-3500-D) and has been developed into an expression platform for industrial-scale quantities of endogenous and recombinant enzymes and other proteins (Visser, H. *et al.*, 2011). BASF has licensed this platform to manufacture enzymes for food, feed and technical applications. See separate reports for mutagenic and recombinant approaches during the development of the host strain.

Dyadic selected host strain (b) (4) based on its ability to secrete a highly enriched enzyme preparation with a very low background of secreted endogenous proteins, including *T. thermophilus* enzymes that may degrade the target protein in the fermentation supernatant. The purpose of transforming *T. thermophilus* host strain (b) (4) with the BASF-proprietary synthetic (b) (4) gene is to generate a genetically modified production strain carrying multiple copies for the large-scale manufacture of the mannanase 19287 feed enzyme. Thermotolerance is designed into the mannanase enzyme to maintain activity after exposure to high temperature during the pelleting process of feed. In addition, (b) (4) is also optimized for enzyme activity at an acidic pH optimum to allow for enzyme activity in the digestive system of the target species.

Characteristics of the inserted sequences

Design of the Synthetic Gene manF

Several bacterial and fungal species express enzymes that degrade the hemicellulosic components of plant cell walls, including mannanases(Bhat, M.K., 2000; Chauhan, P.S. *et al.*, 2012; Dhawan, S. and Kaur, J., 2007; Moreira, L.R.S. and Filho, E.X.F., 2008; van Zyl, W.H. *et al.*, 2010).

The biotech company (b) (4) developed genetically modified enzymes by introducing point mutations into a *T. reesei* wild-type mannanase in a protein-engineering approach. Modified sequences were heterologously expressed in *Saccharomyces cerevisiae* to evaluate the impact of the amino acid variations on the enzyme activity under different temperature and pH conditions; several variants also demonstrated resistance to pepsin digestion (Kensch, O., 2015; Kensch, O. *et al.*, 2010). BASF acquired synthetic enzyme sequences from (b) (4) that were optimized for their use as enzyme supplements in animal feed.

Mannanase 19287 is a recombinant feed enzyme that demonstrates improved temperature tolerance and high enzymatic activity under acidic pH conditions.

The described characteristics of the mannanase 19287 enzyme produced with *T. thermophilus* DSM 33149 allow for continued activity after exposure to high temperature during the pelleting process of animal feed. In addition, mannanase 19287 is also optimized for enzyme activity in the presence of pepsin at acidic pH conditions; this allows for residual high enzyme activity in

the digestive tract of the target species.

The impact of the amino acid modifications on the mannanase 19287 enzyme are described in detail in the Mannanase 19287 Protein Characterization Report.

Additional modifications were introduced into the (b) (4) DNA sequence to improve expression in the fungal organism *T. thermophilus*. Neither additional modification changed amino acids or characteristics of the mature mannanase 19287 enzyme protein.

Genetic modification

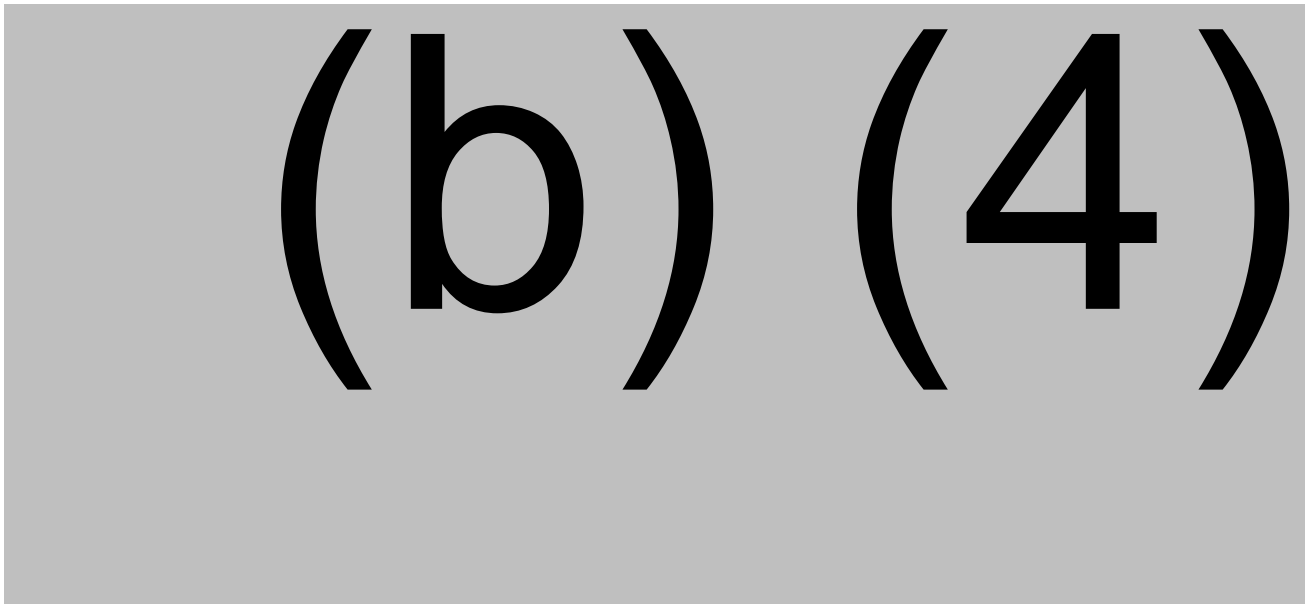


Figure 1: Mannanase 19287 protein sequence and synthetic gene (b) (4)

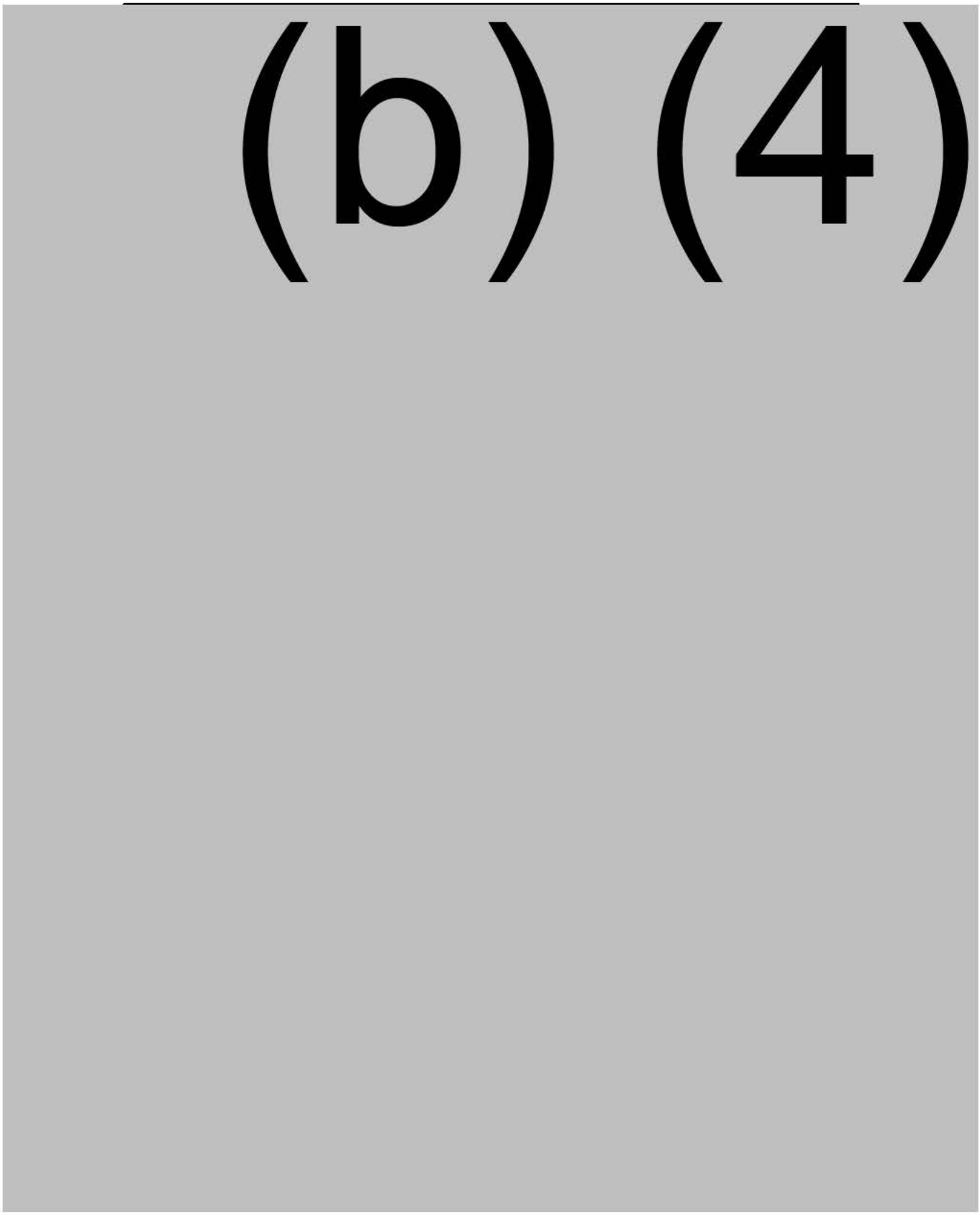


Figure 2: Plasmid map of the synthetic (b) (4) gene and the endogenous *T. thermophilus* cbh1 signal sequence.

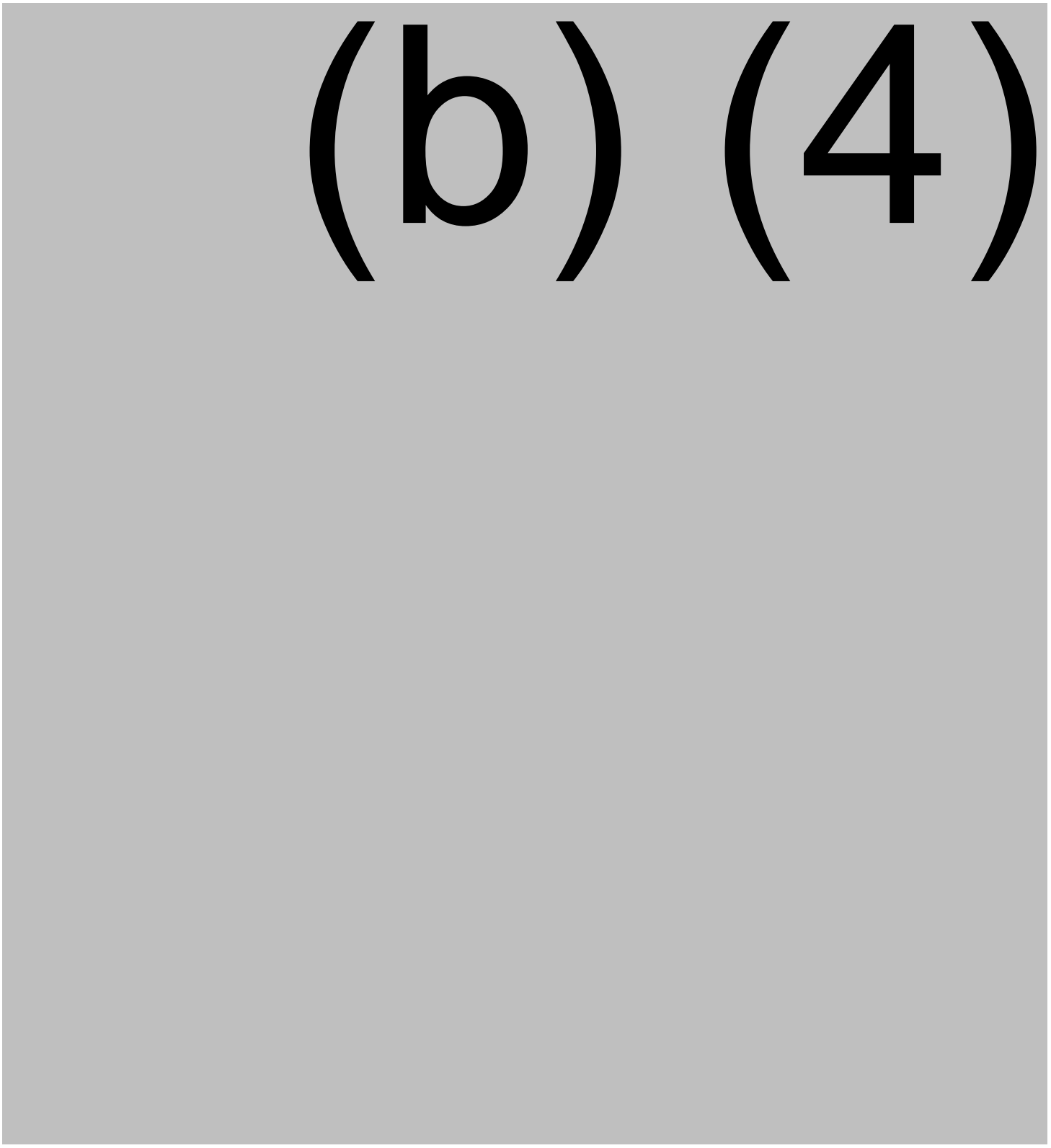
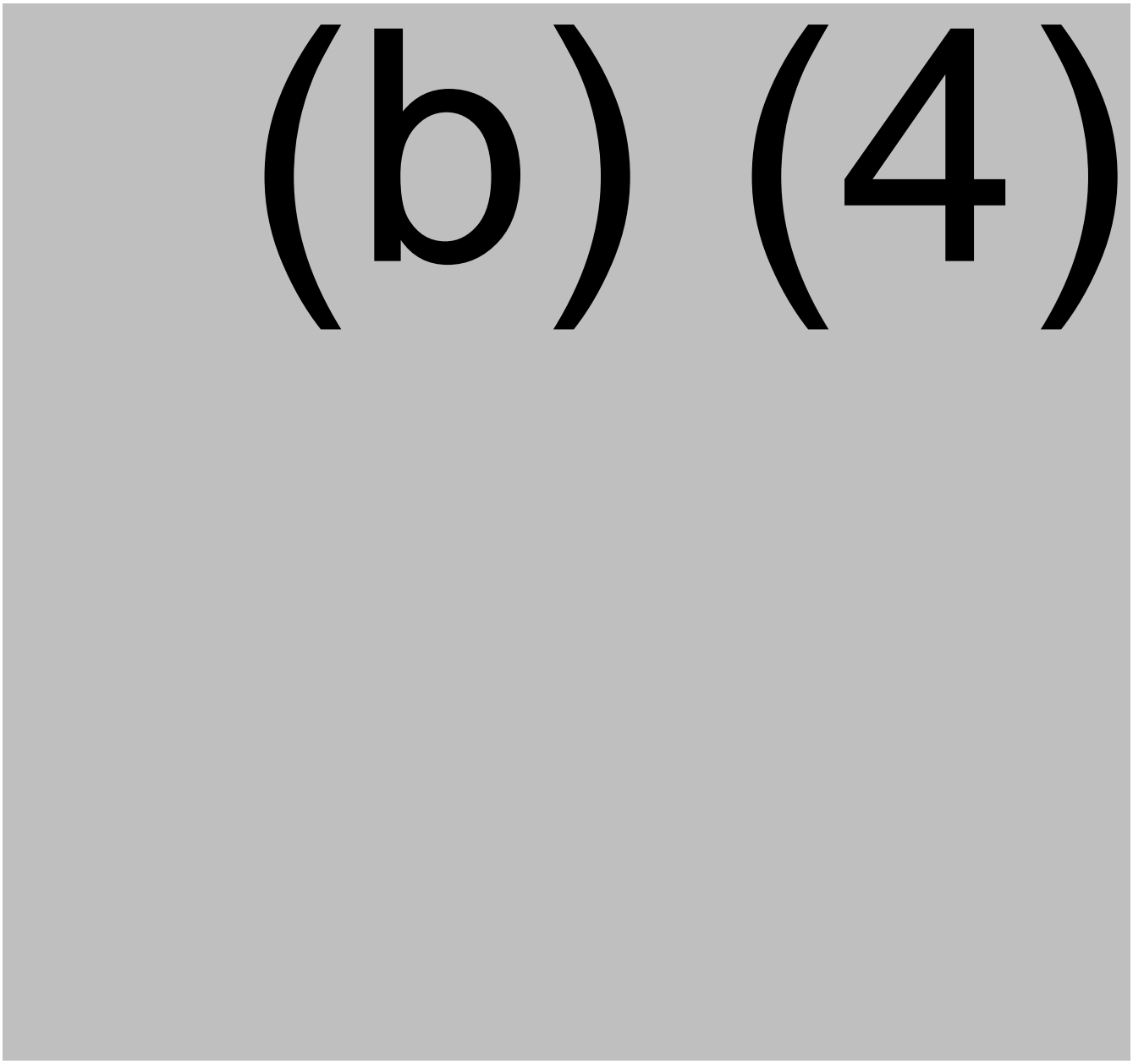
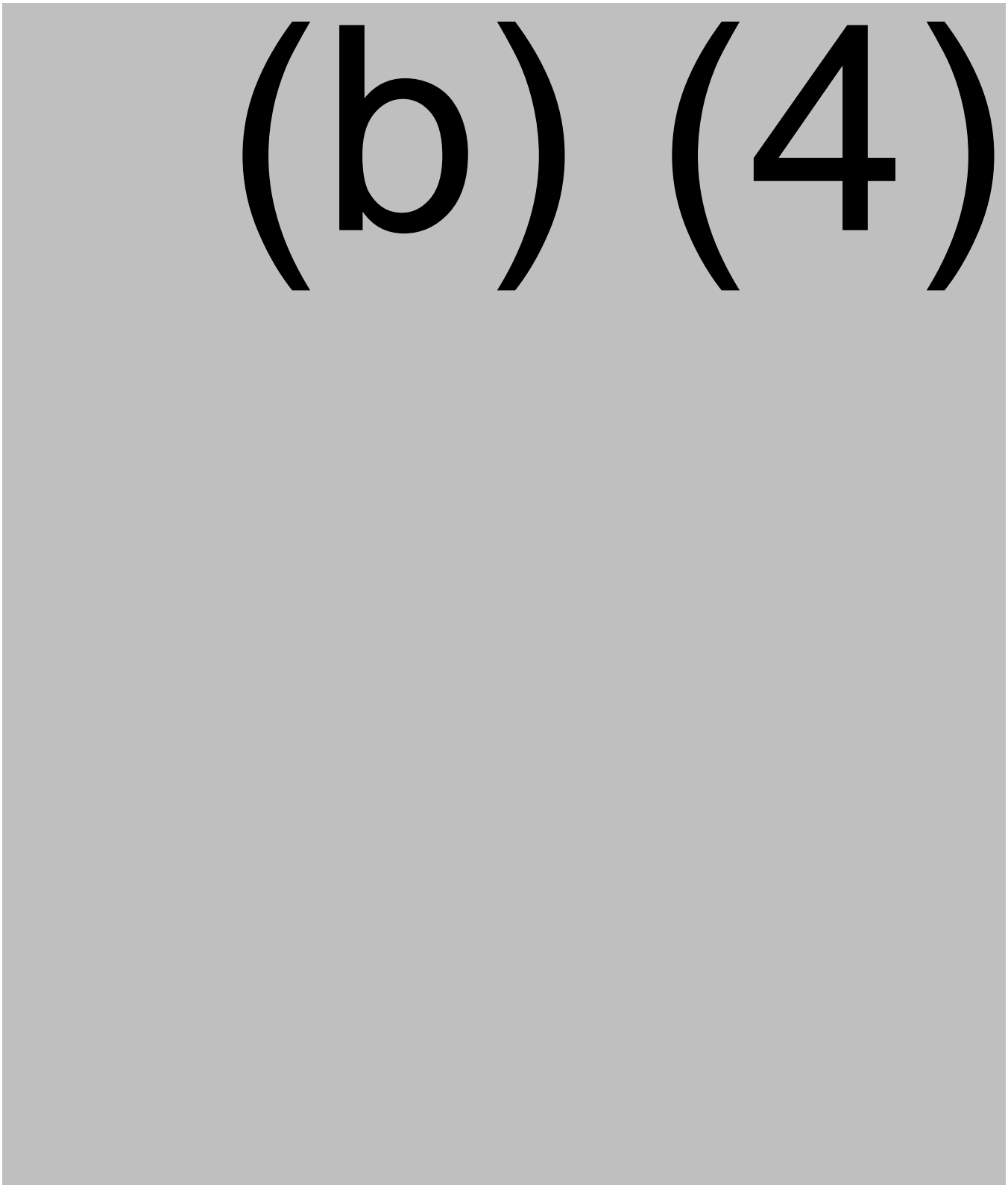
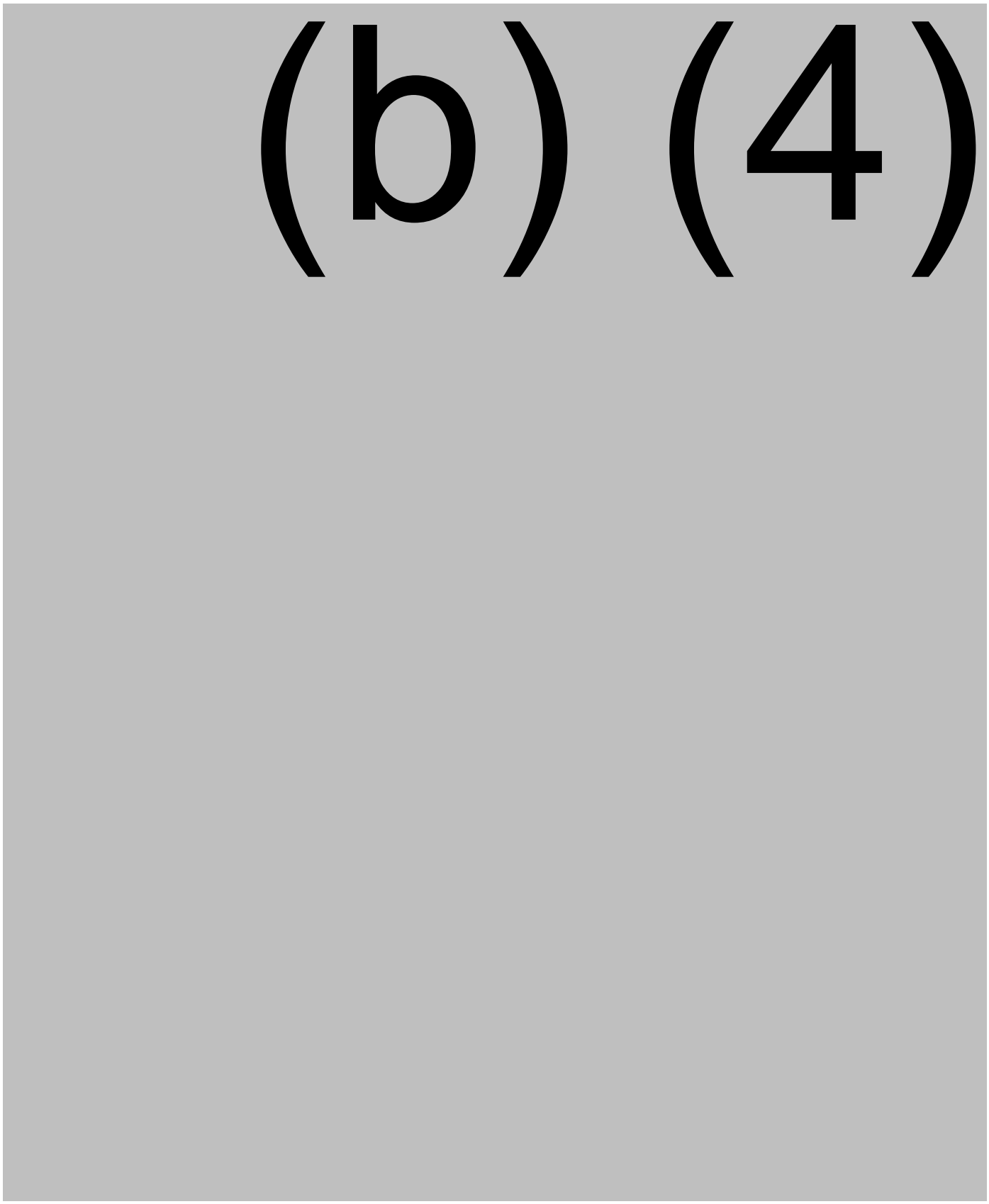
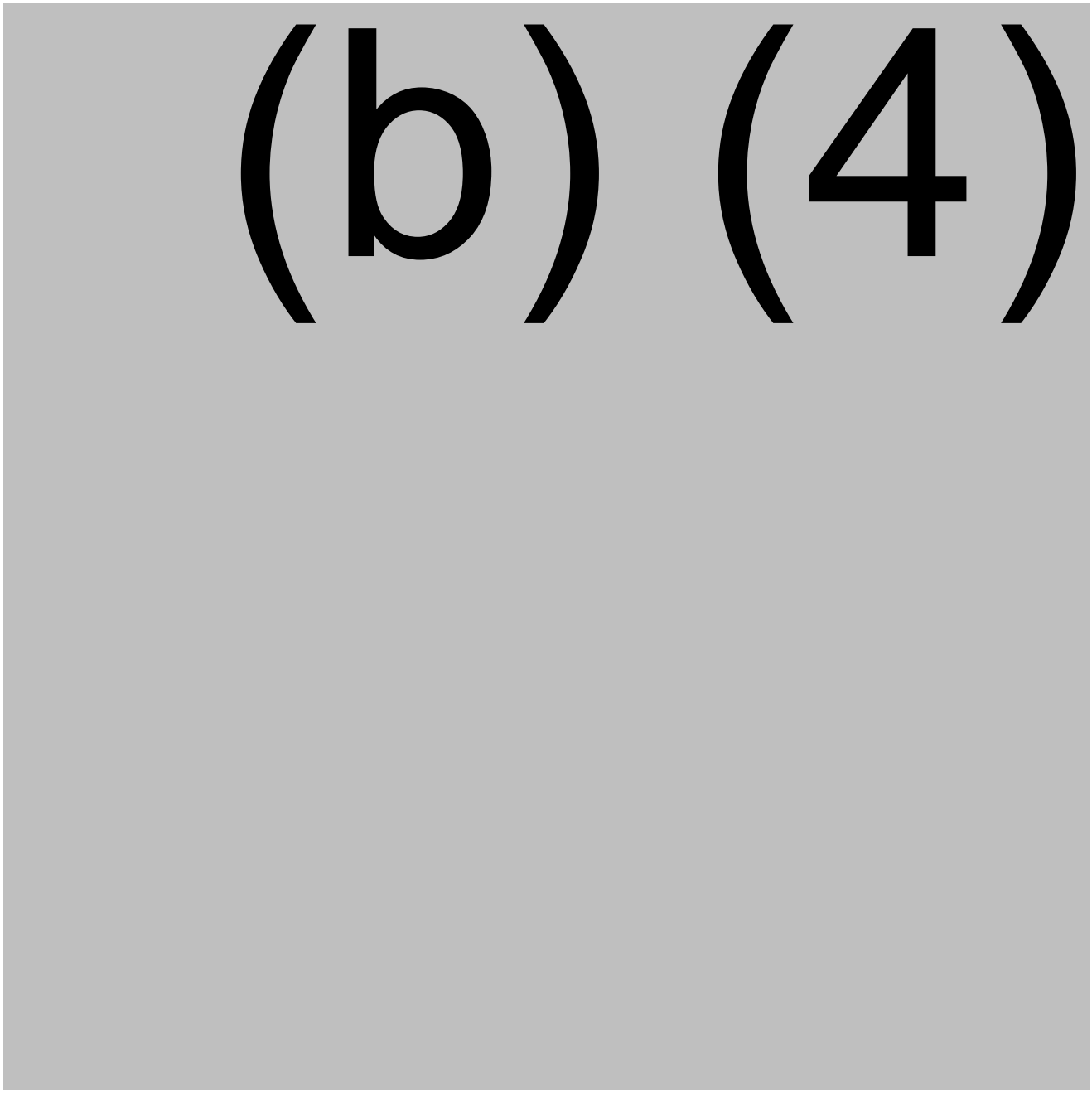


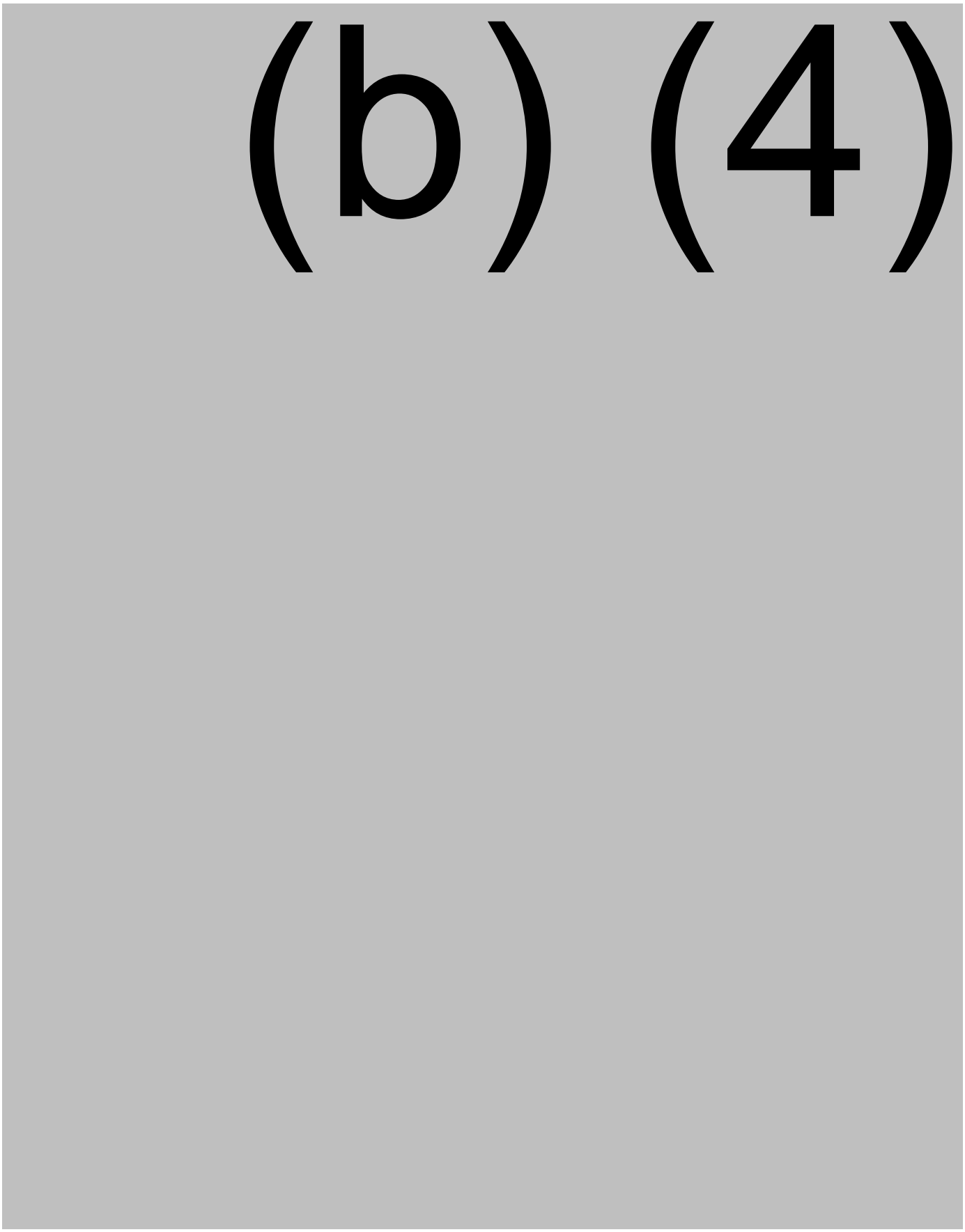
Figure 3: Cloning vectors used during the (b) (4) expression cassette construction.

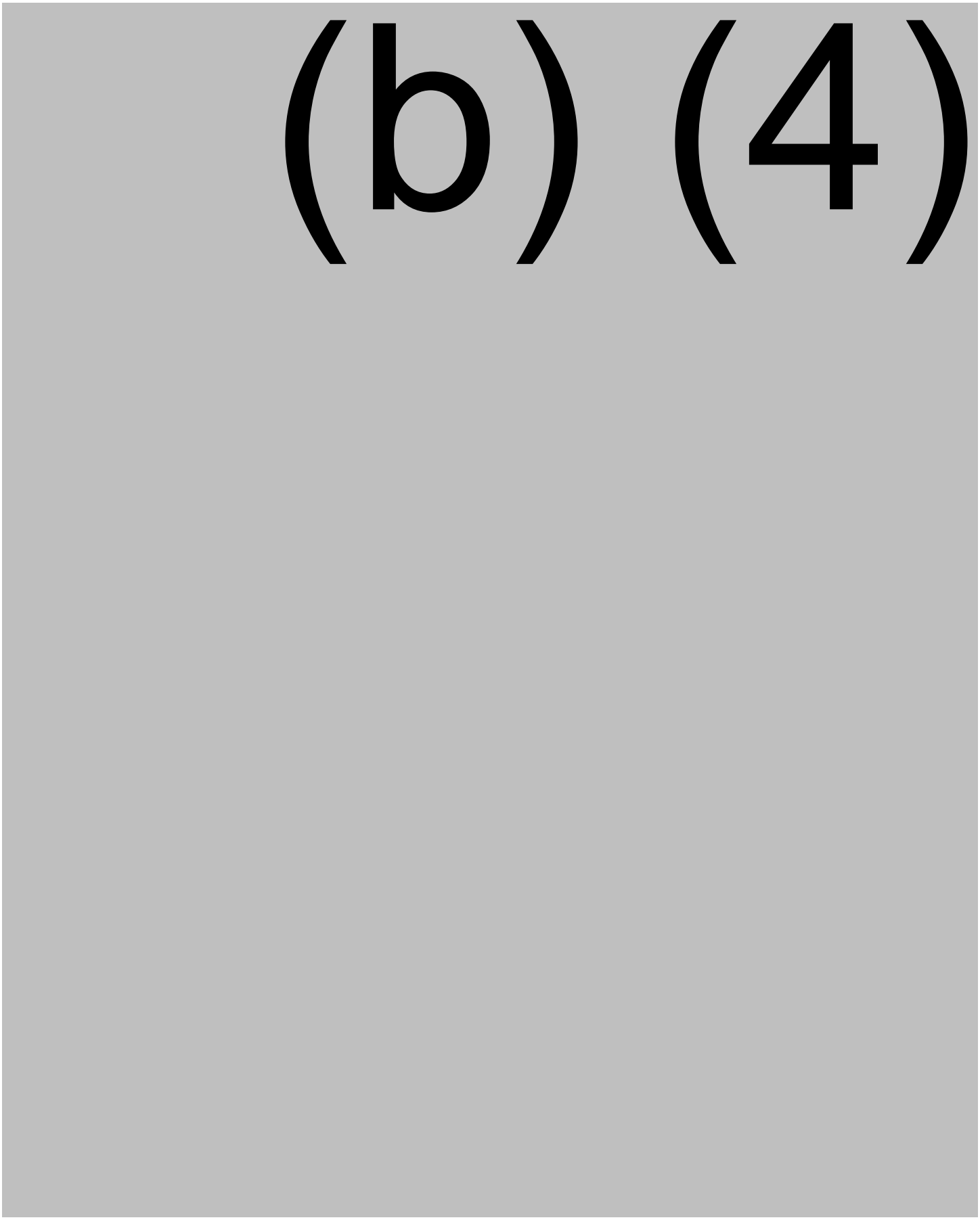


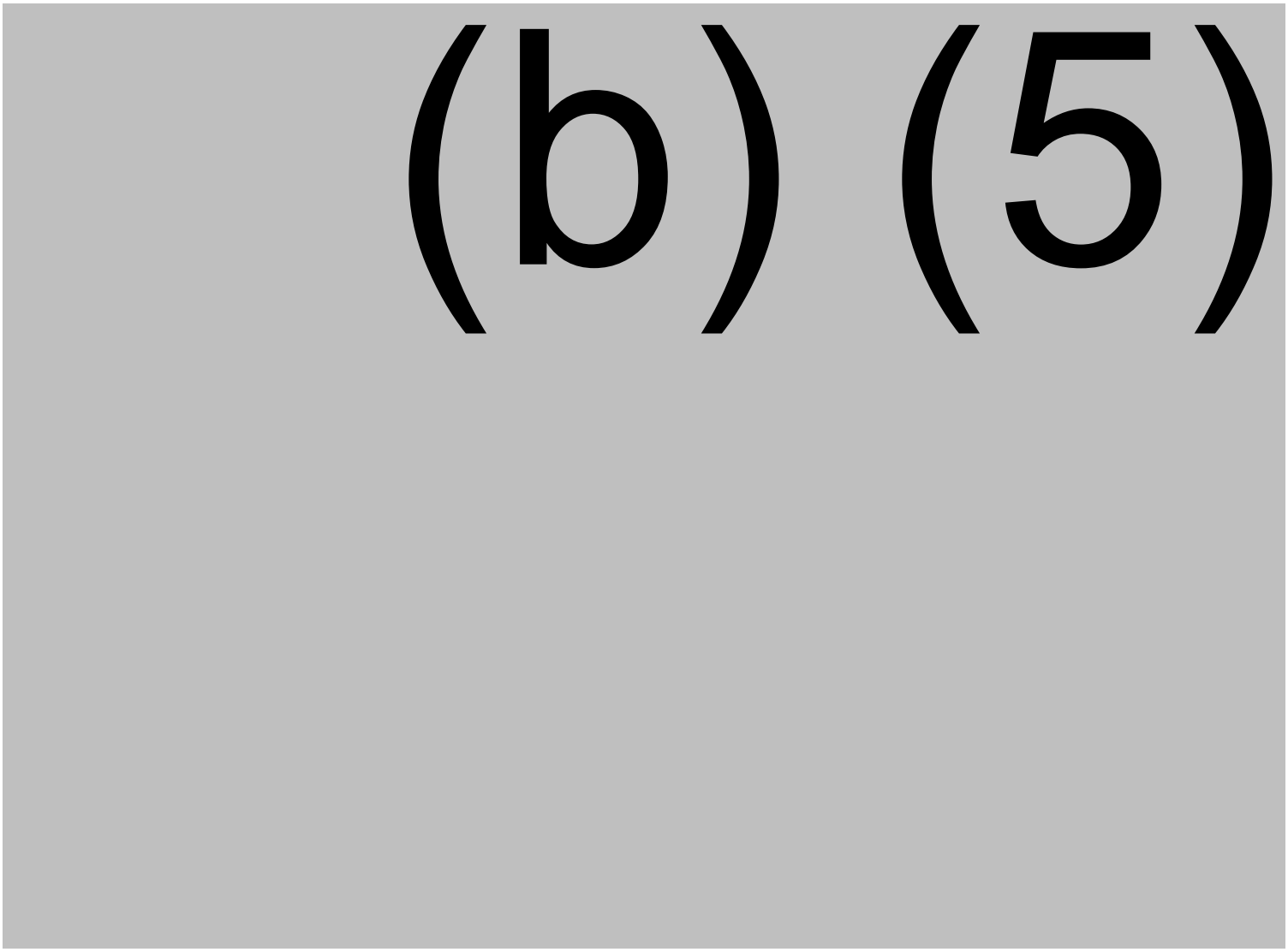

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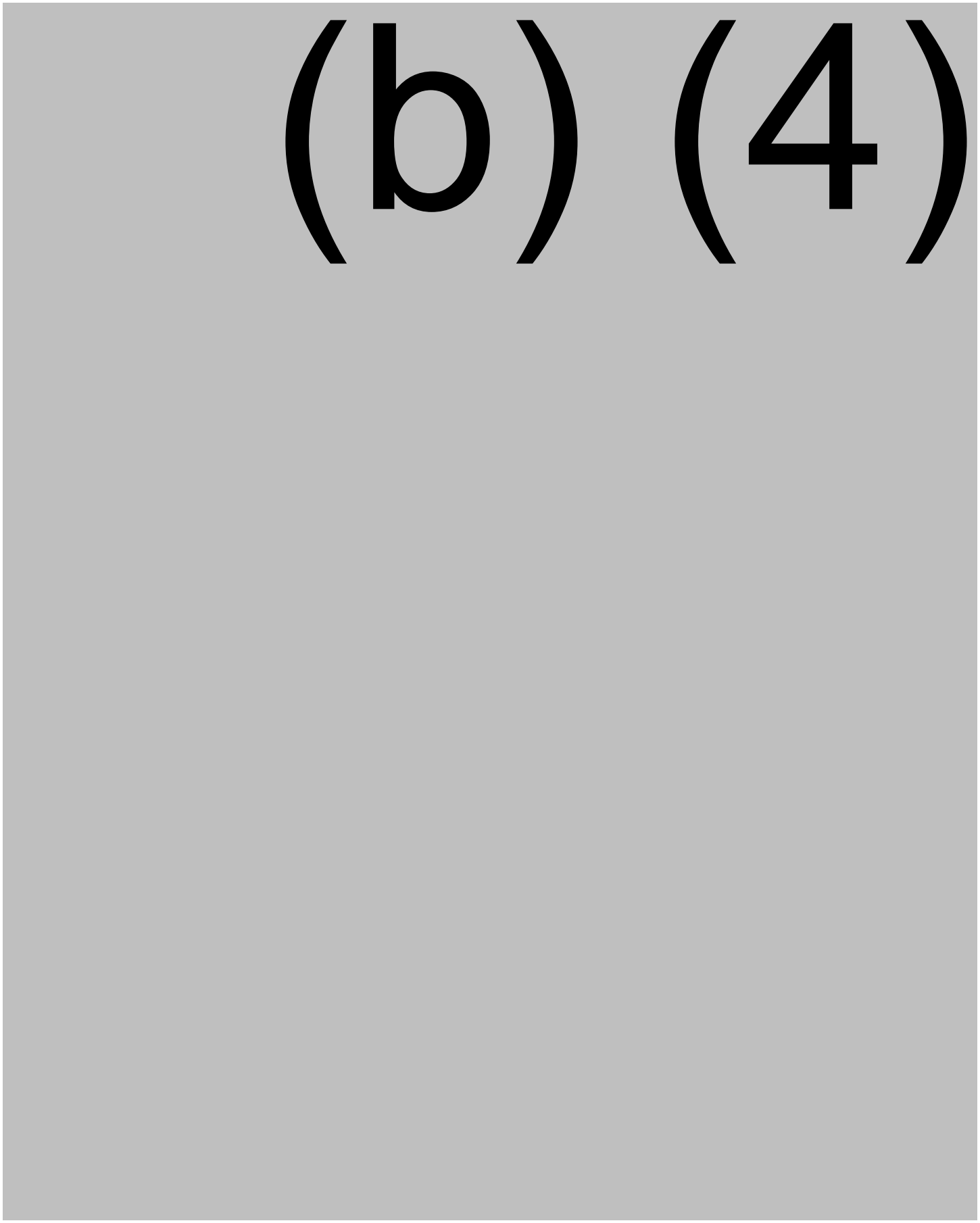

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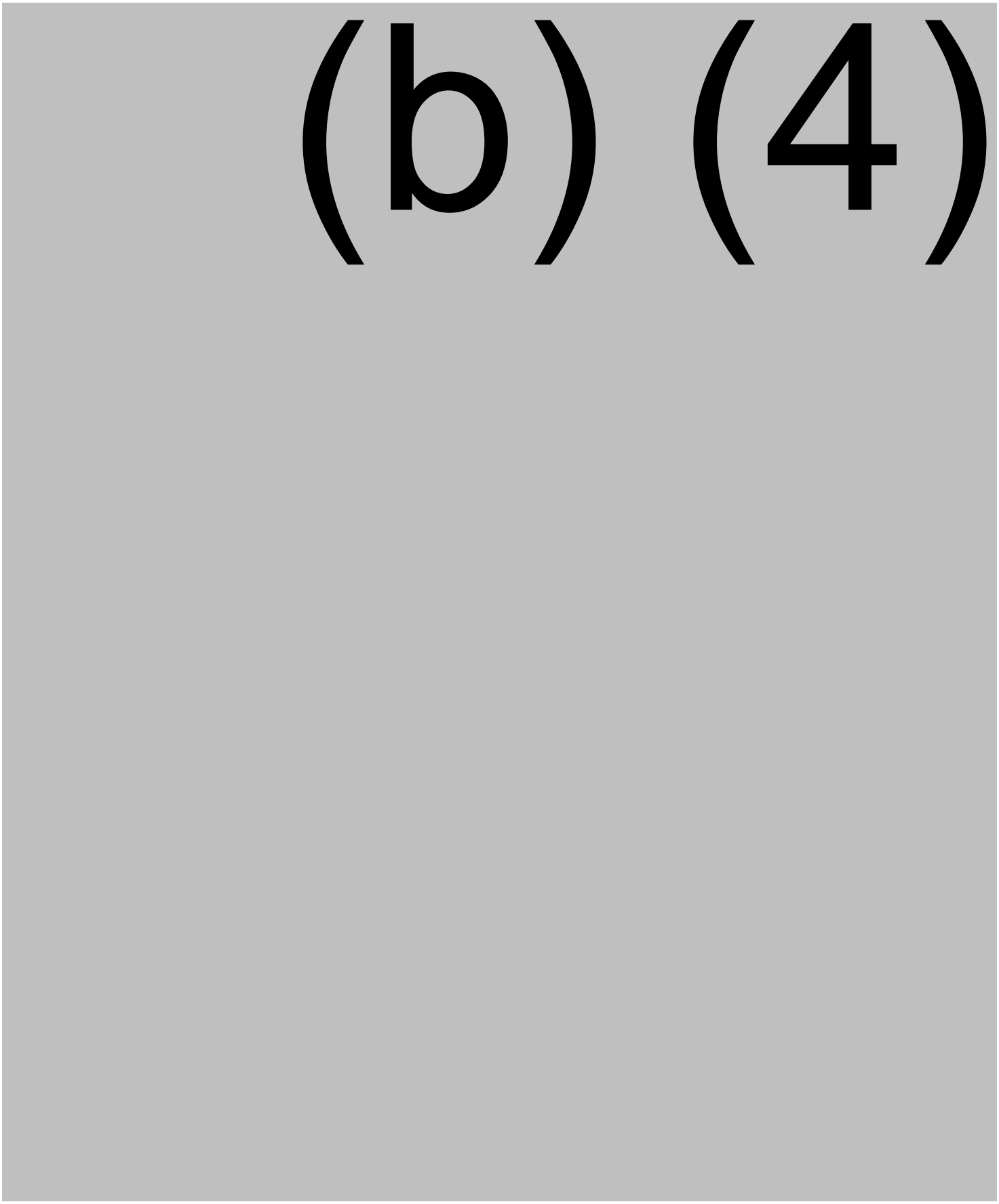

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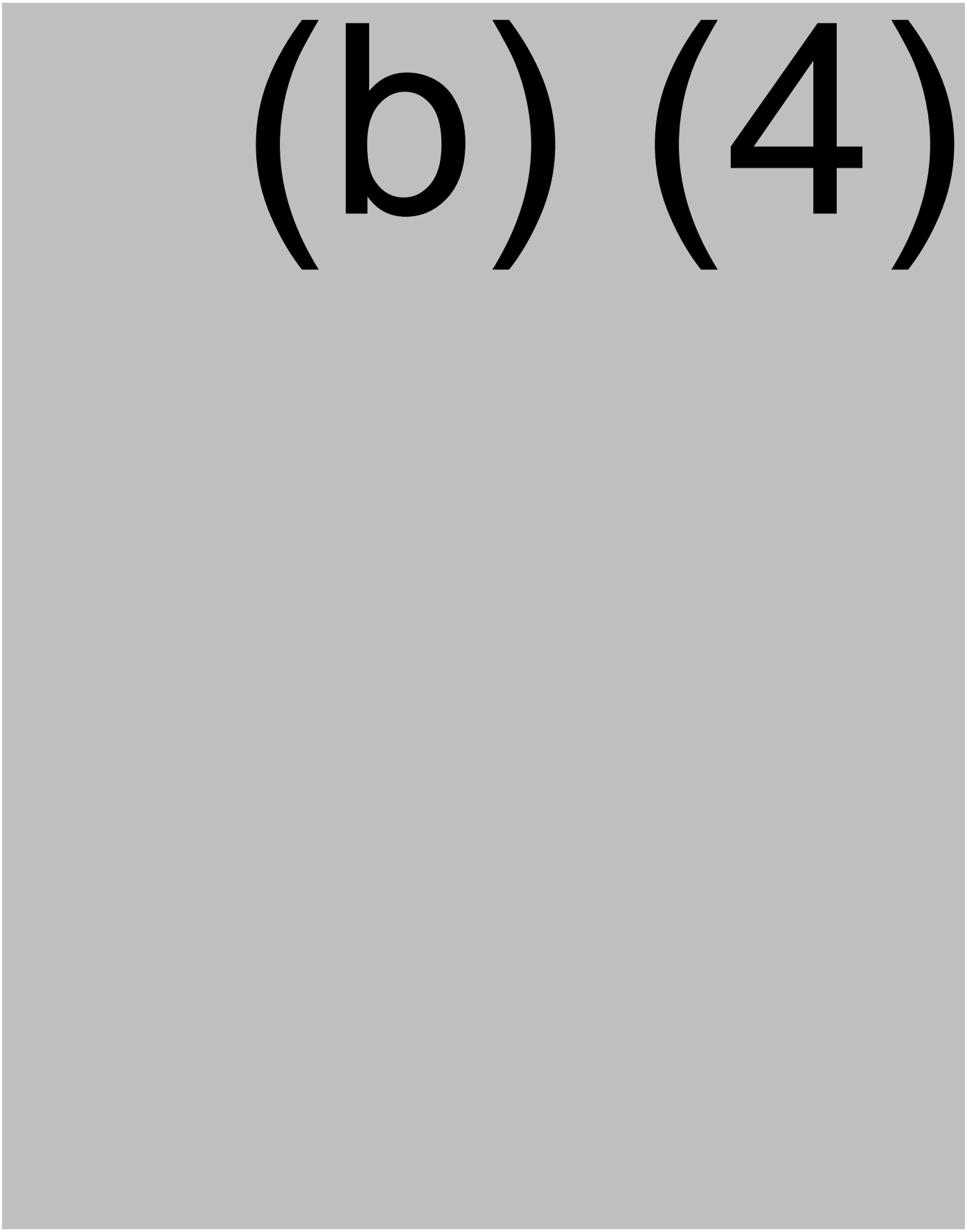

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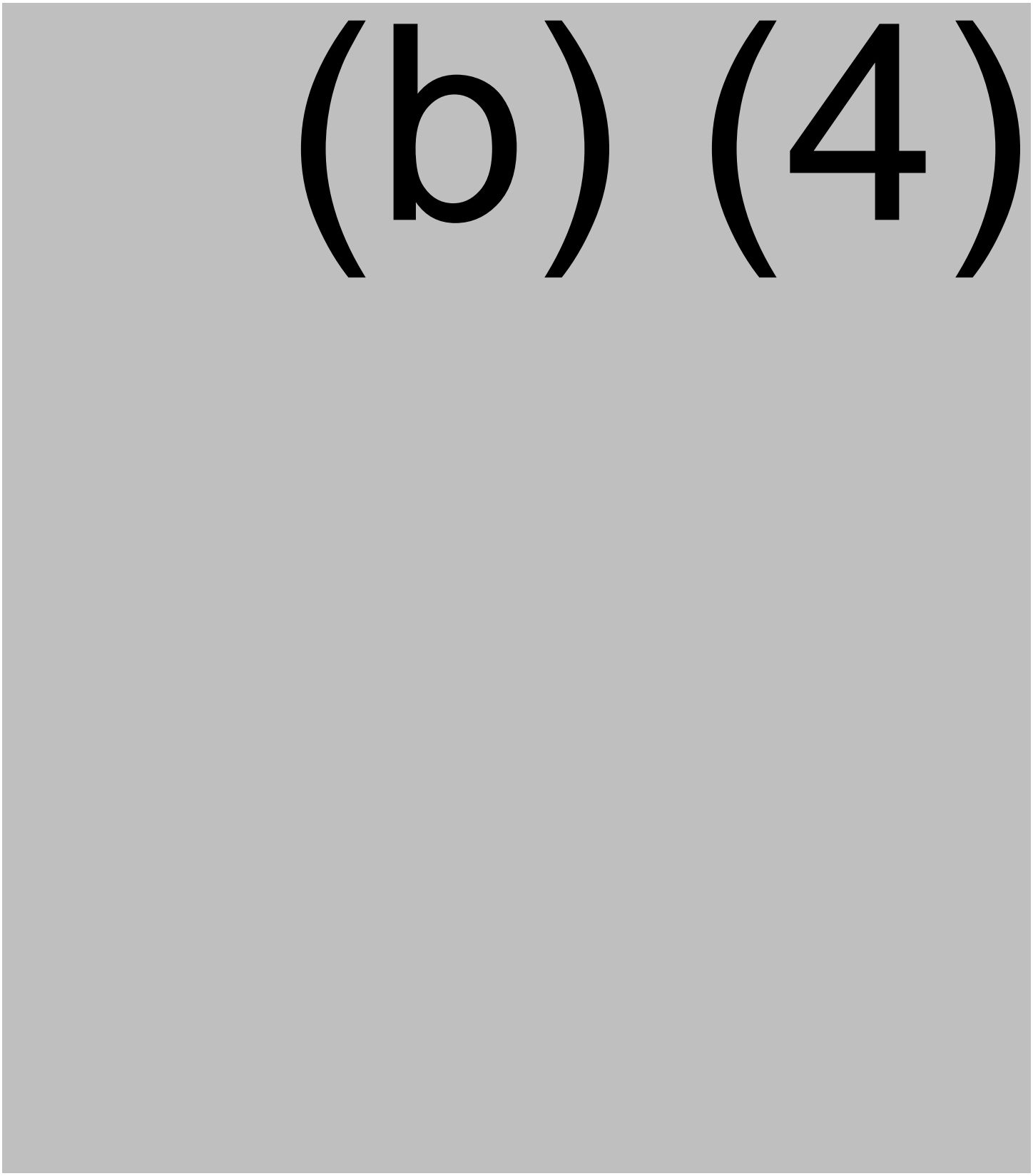

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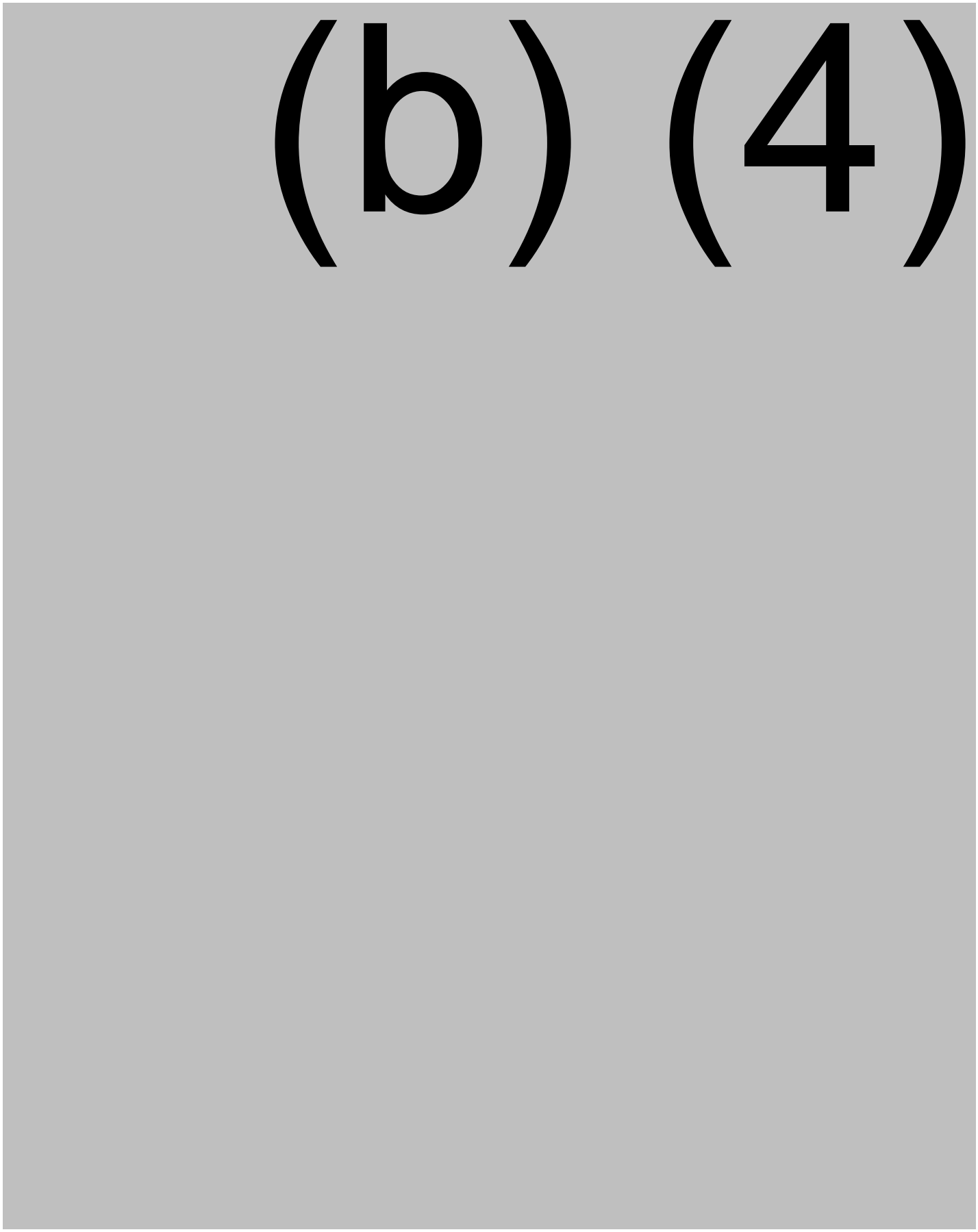

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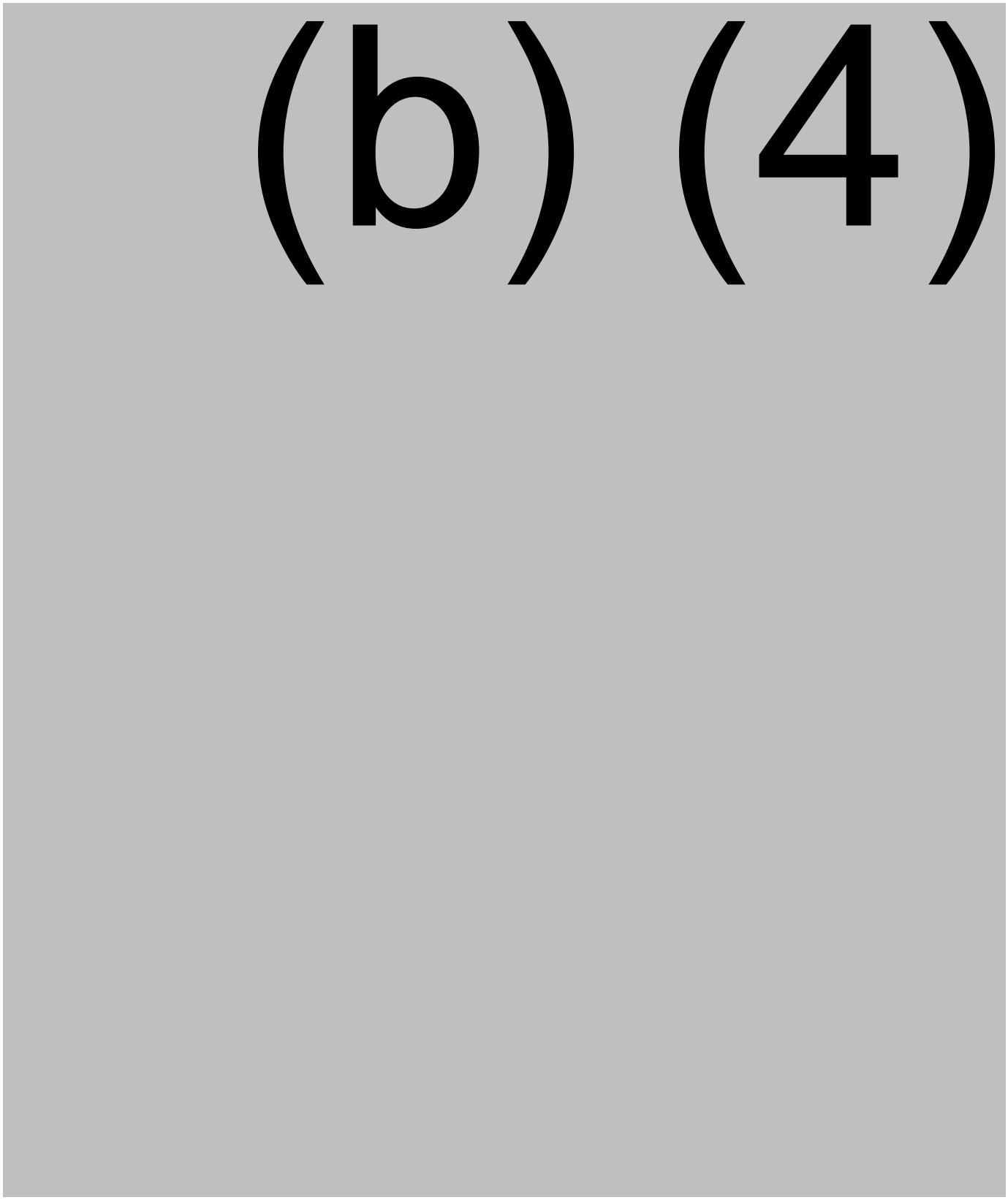

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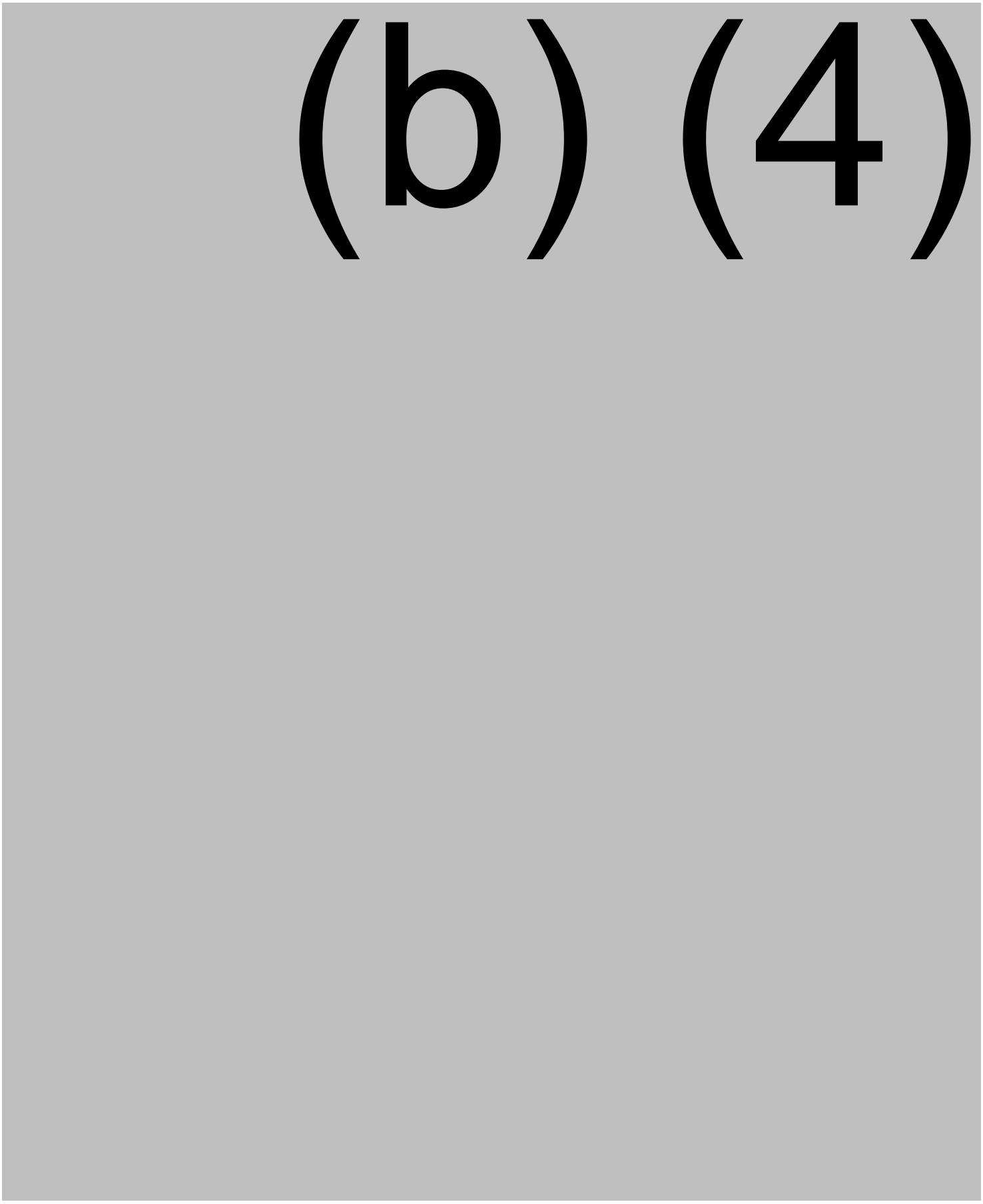

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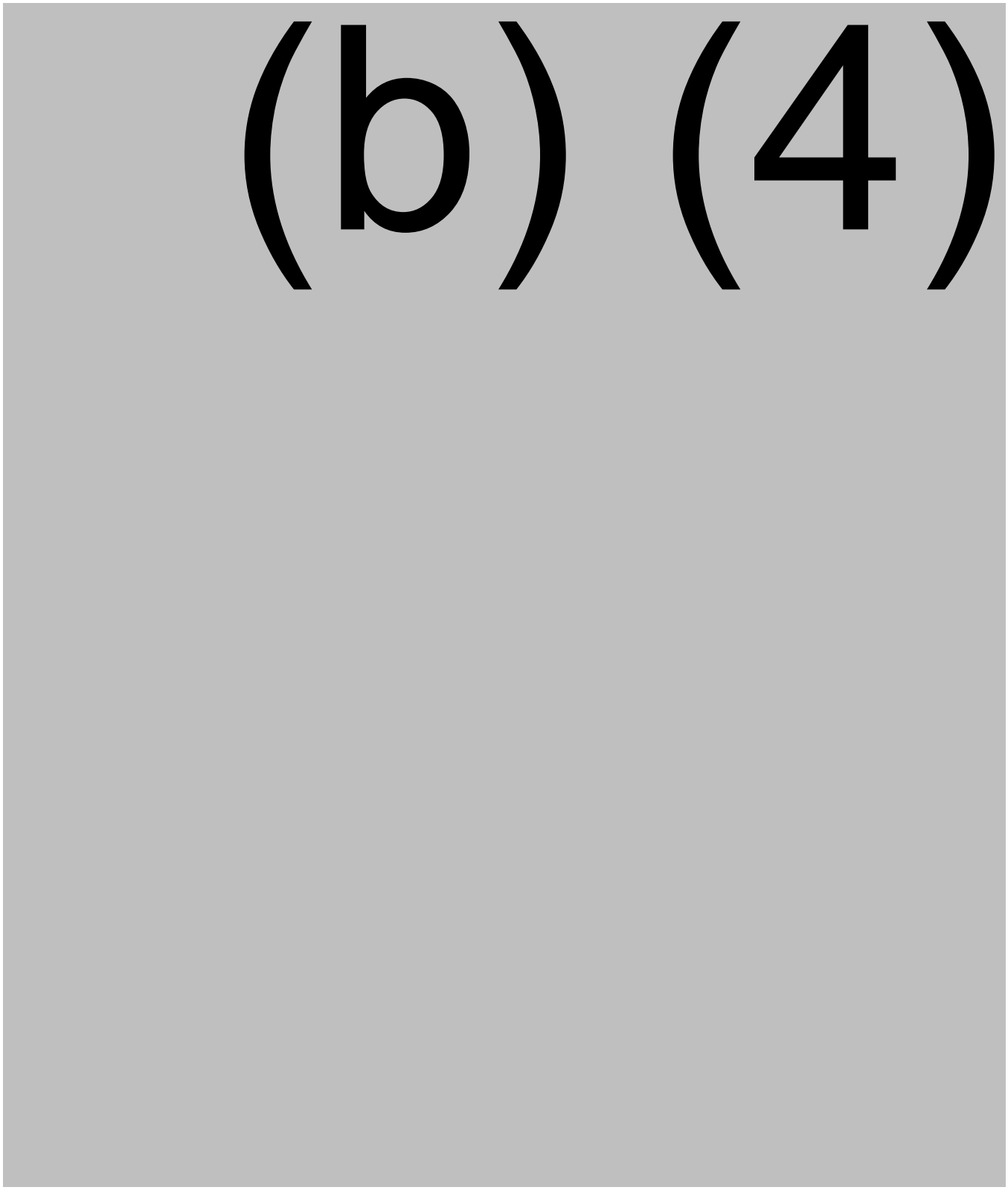

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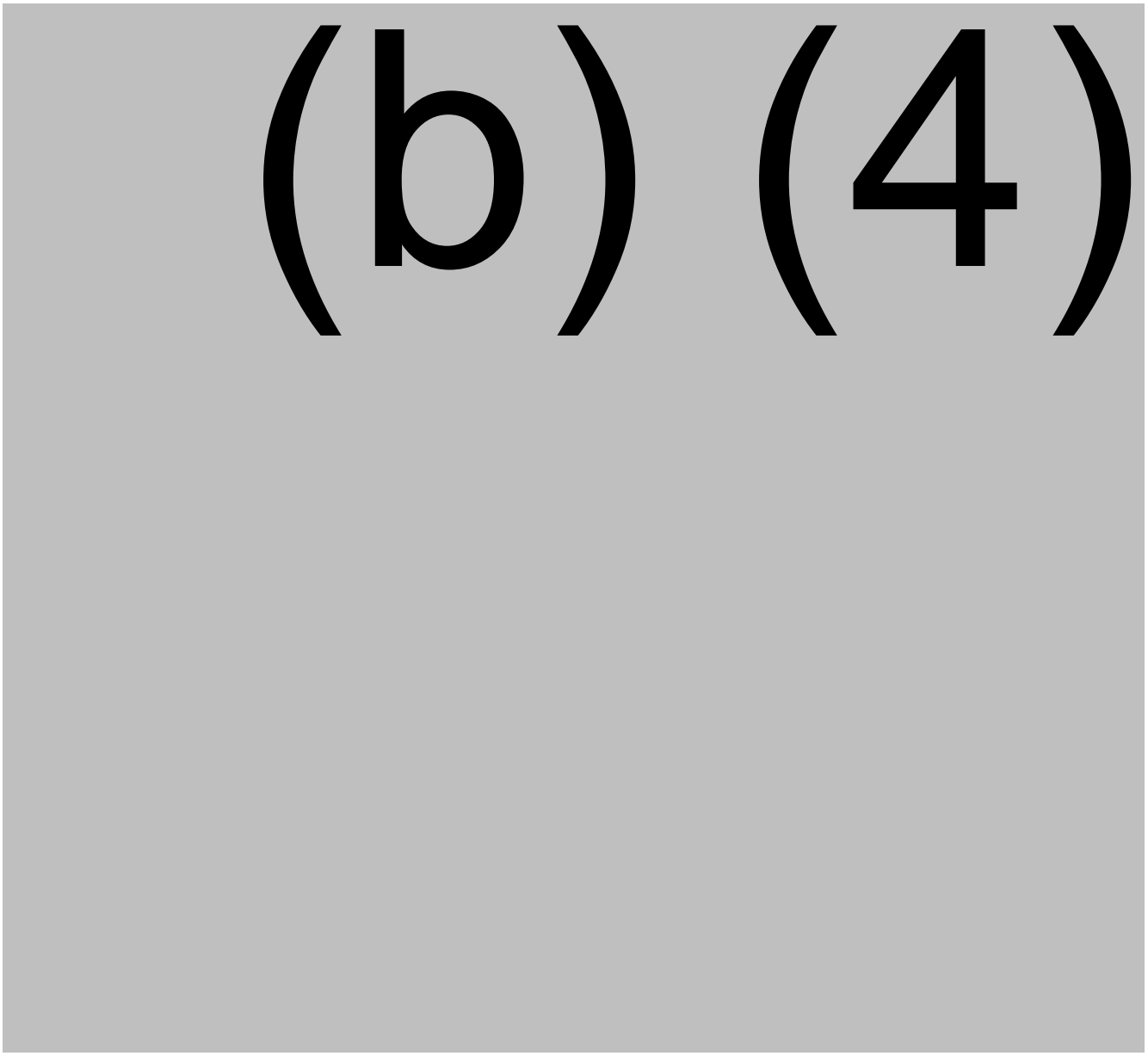

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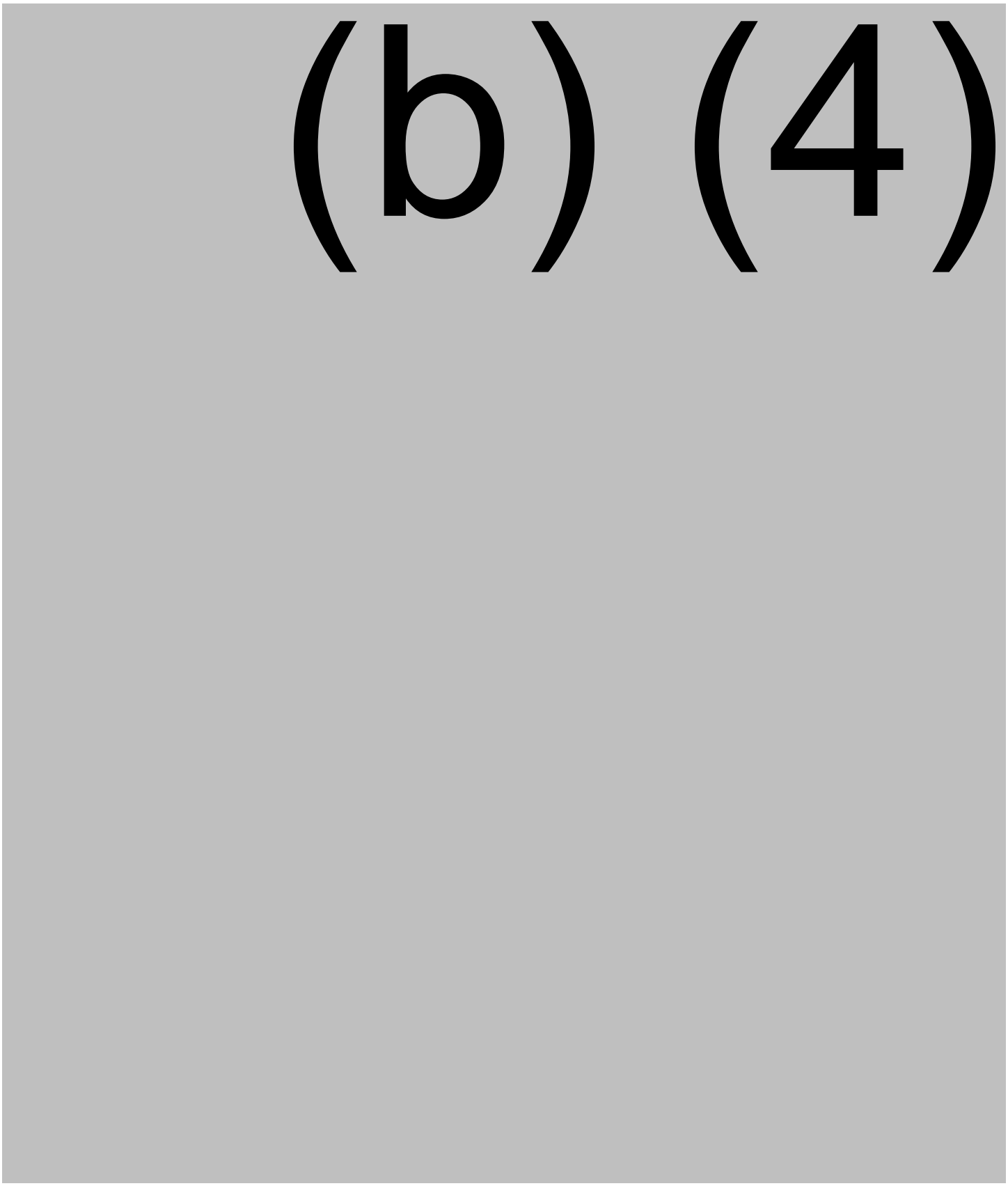

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Genetic stability of production strain *T. thermophilus* DSM 33149




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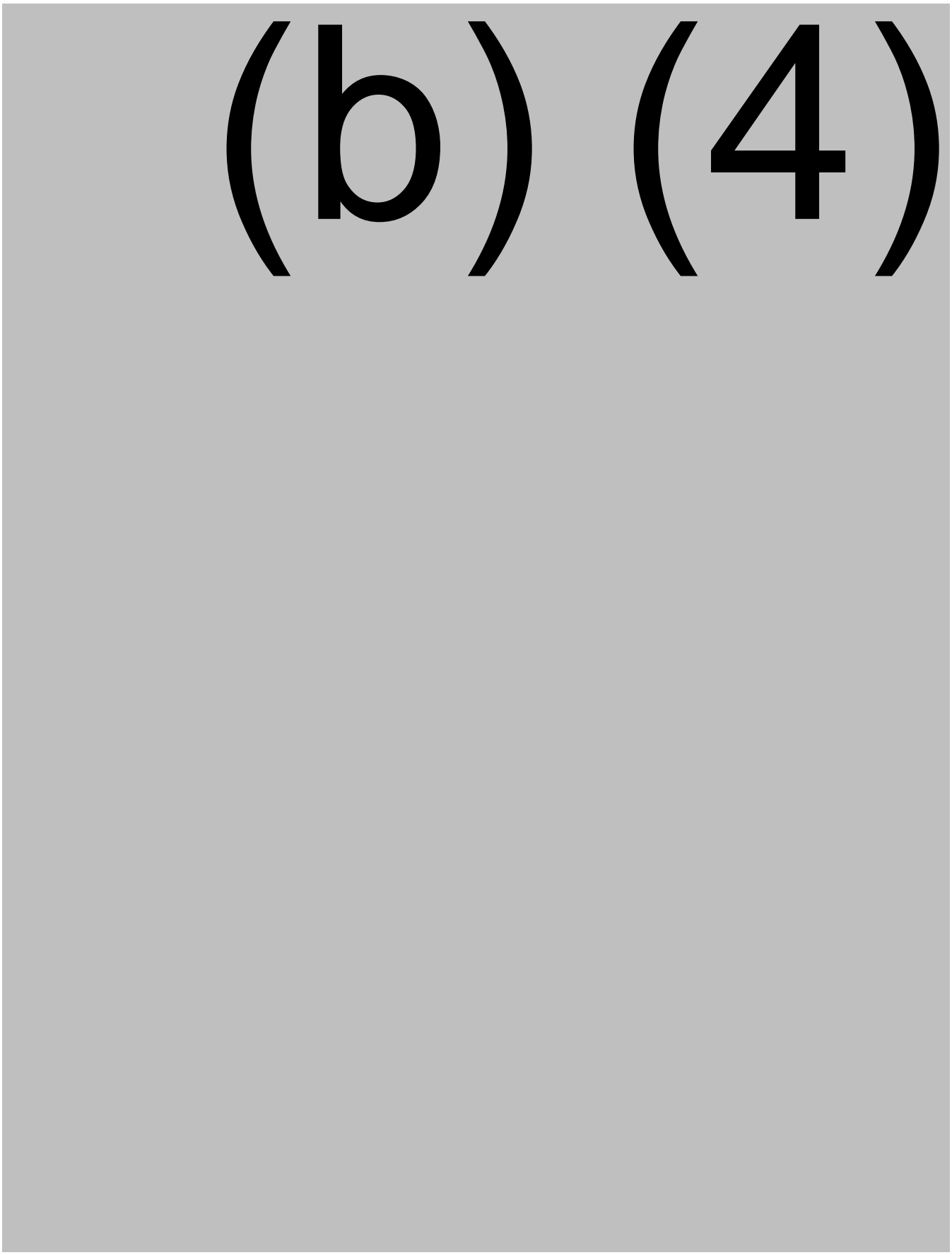
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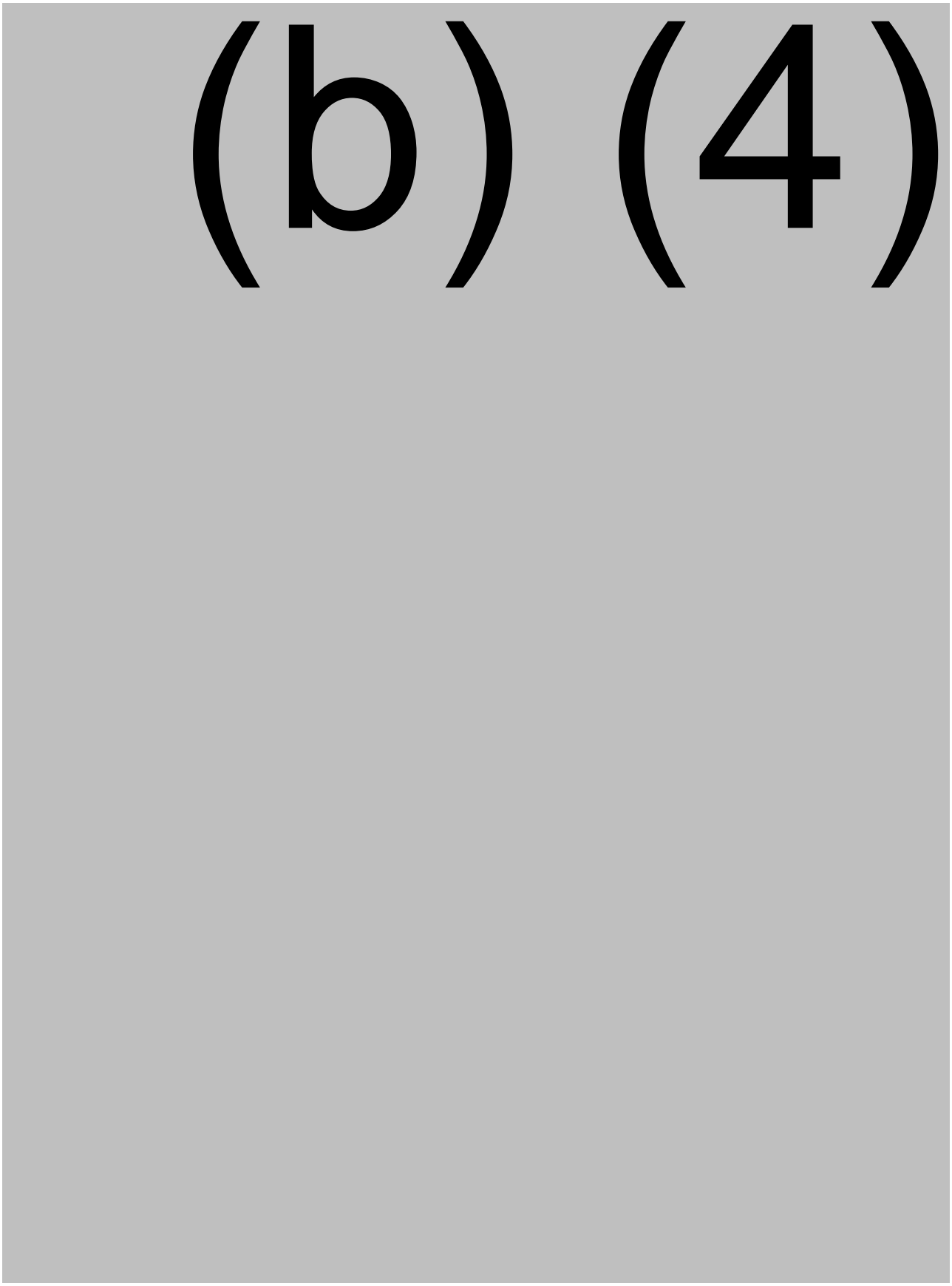
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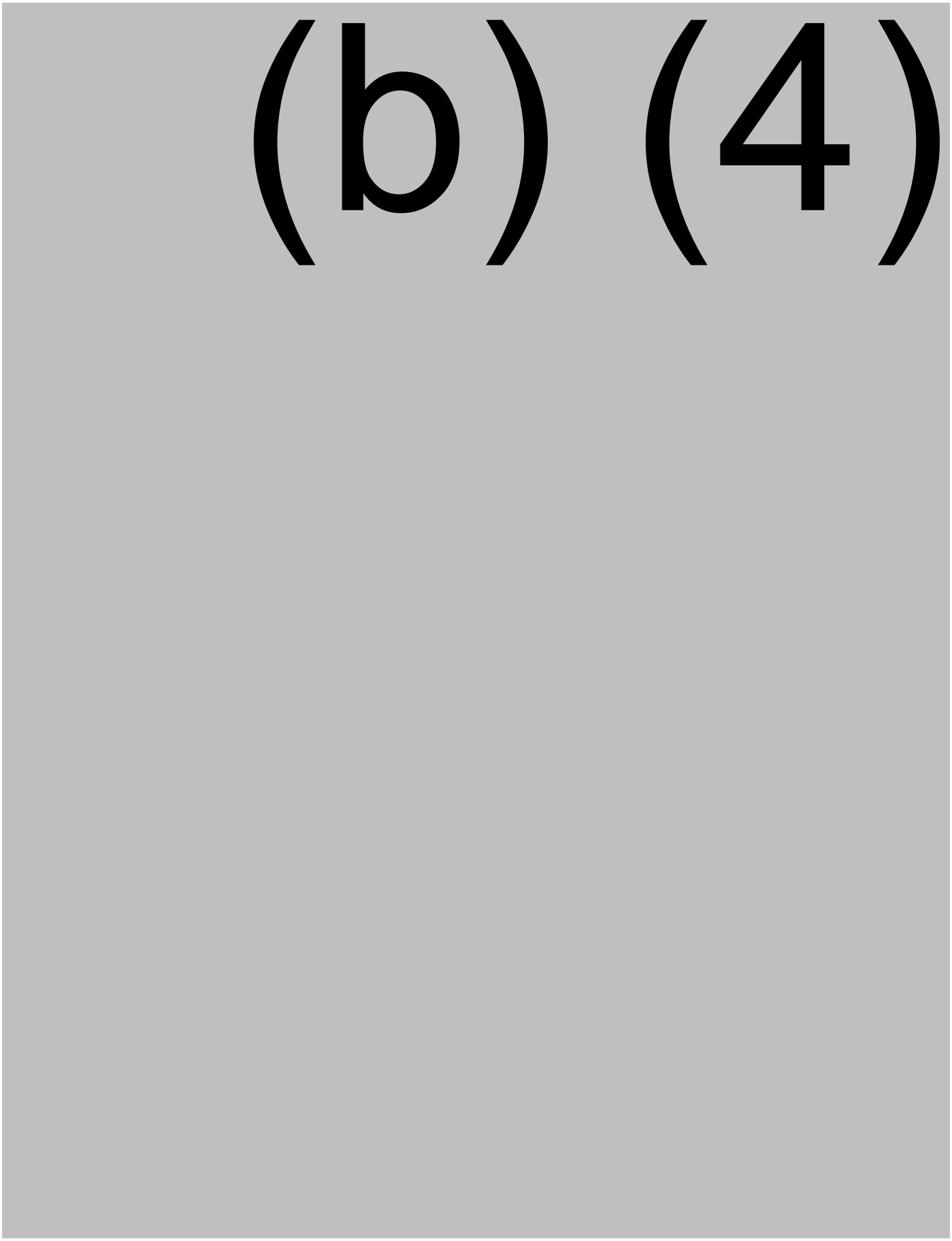
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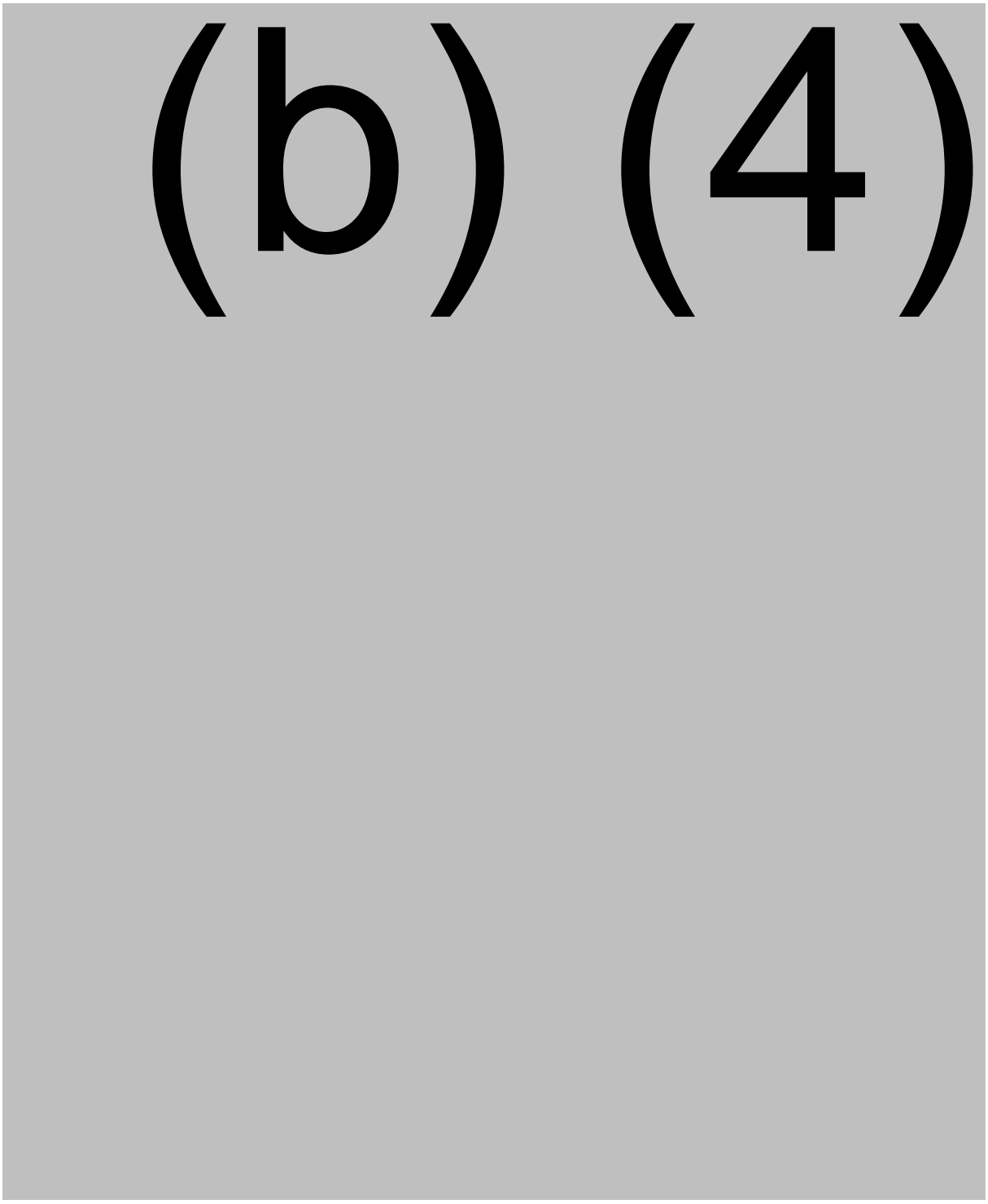
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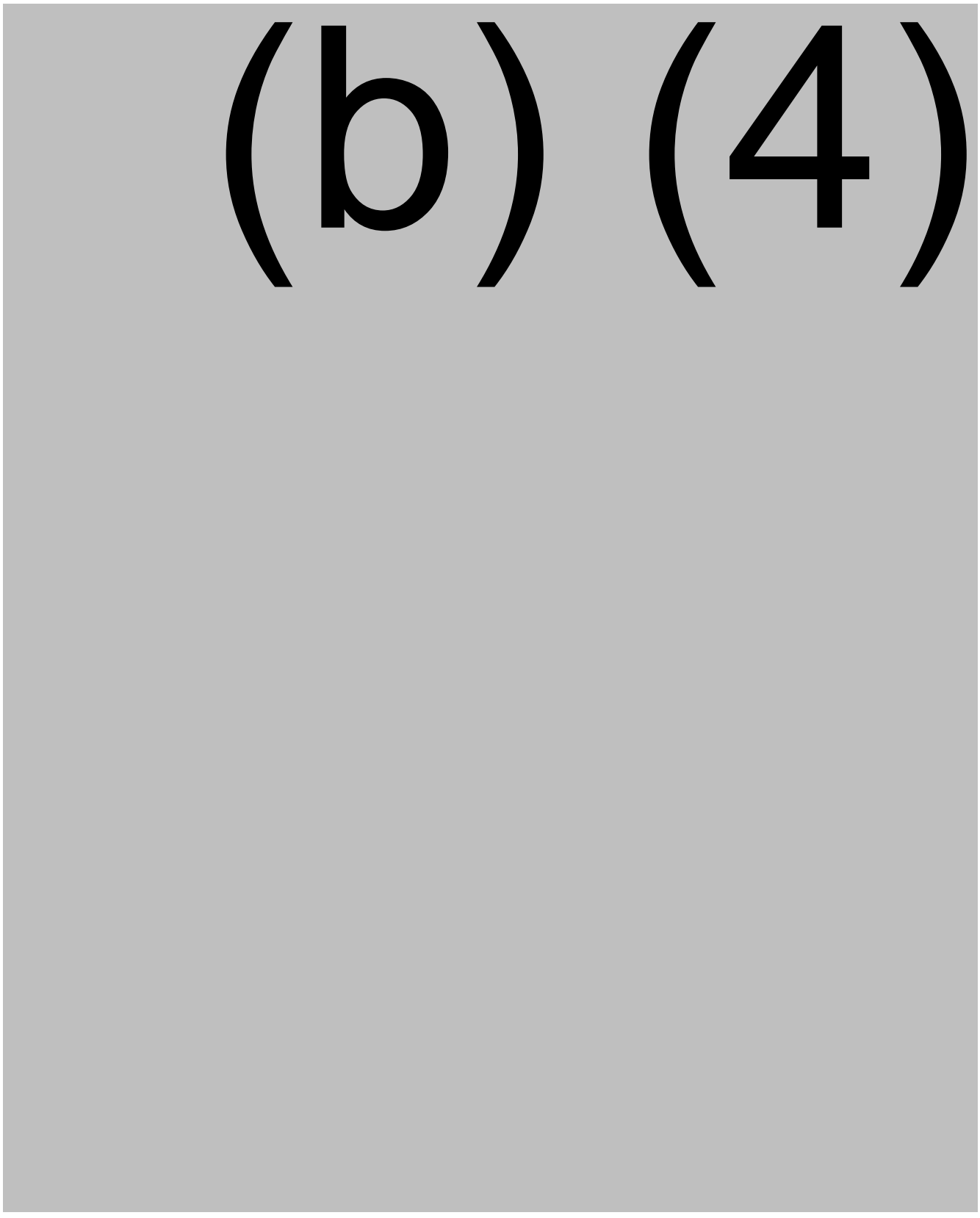

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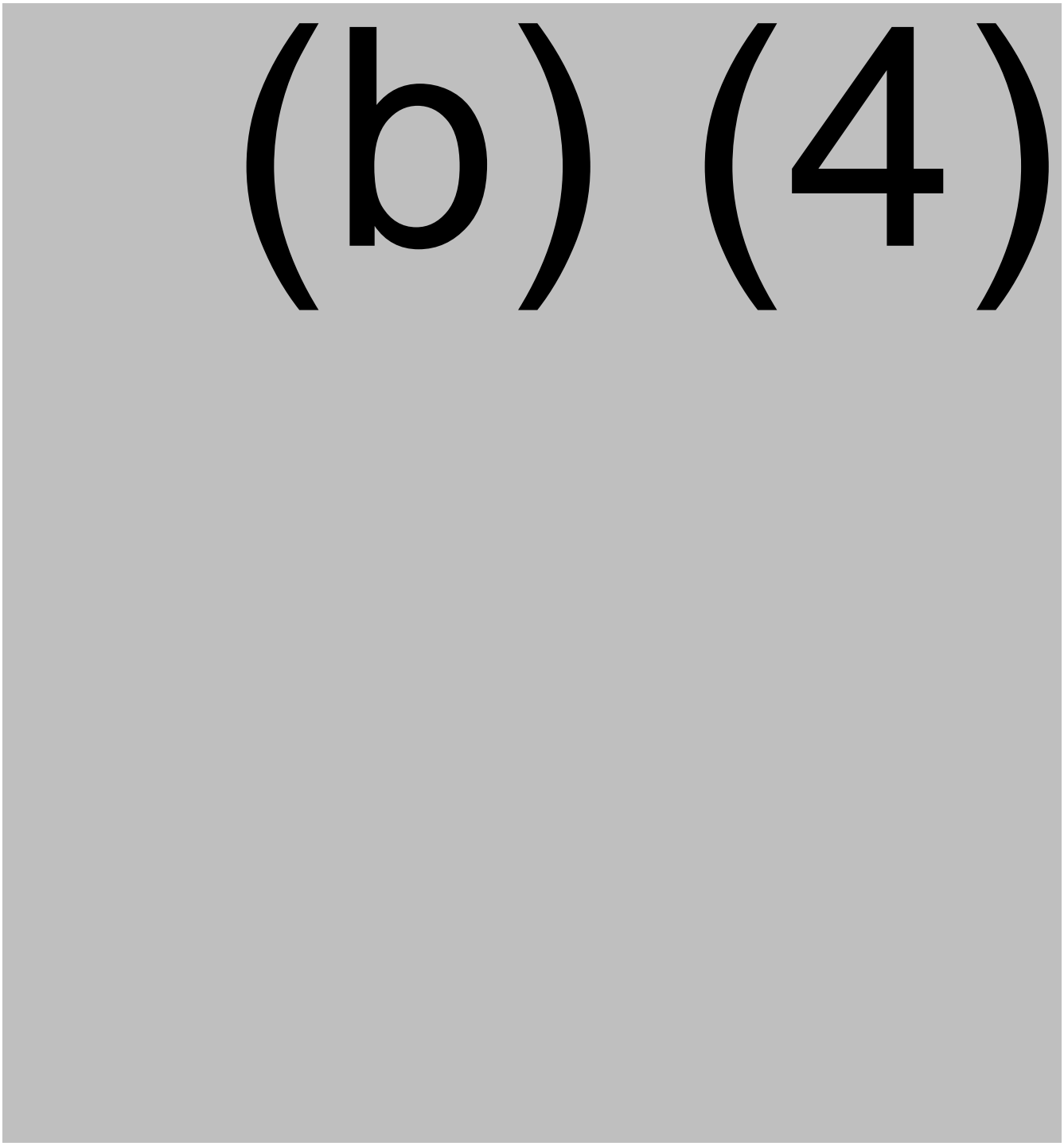


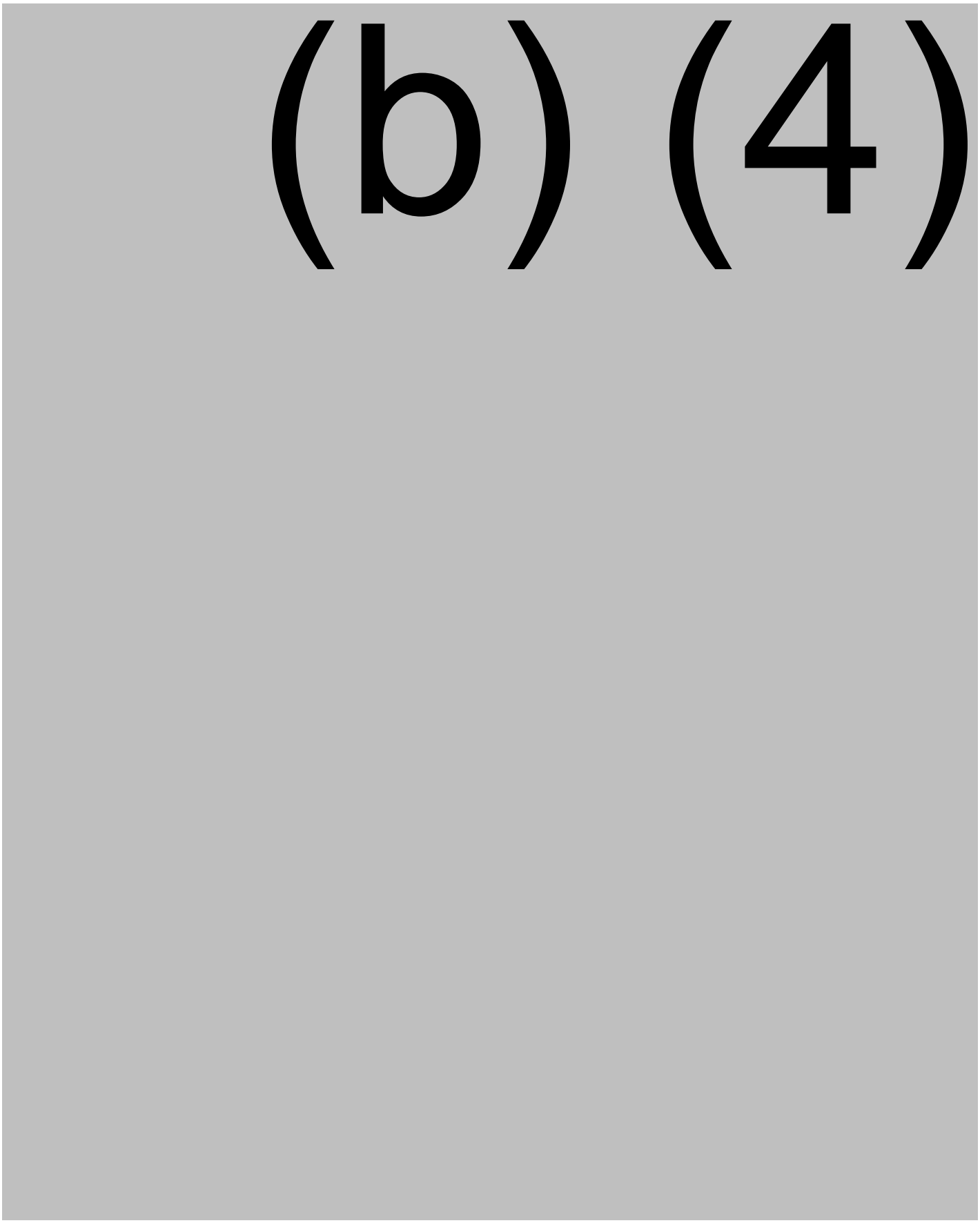
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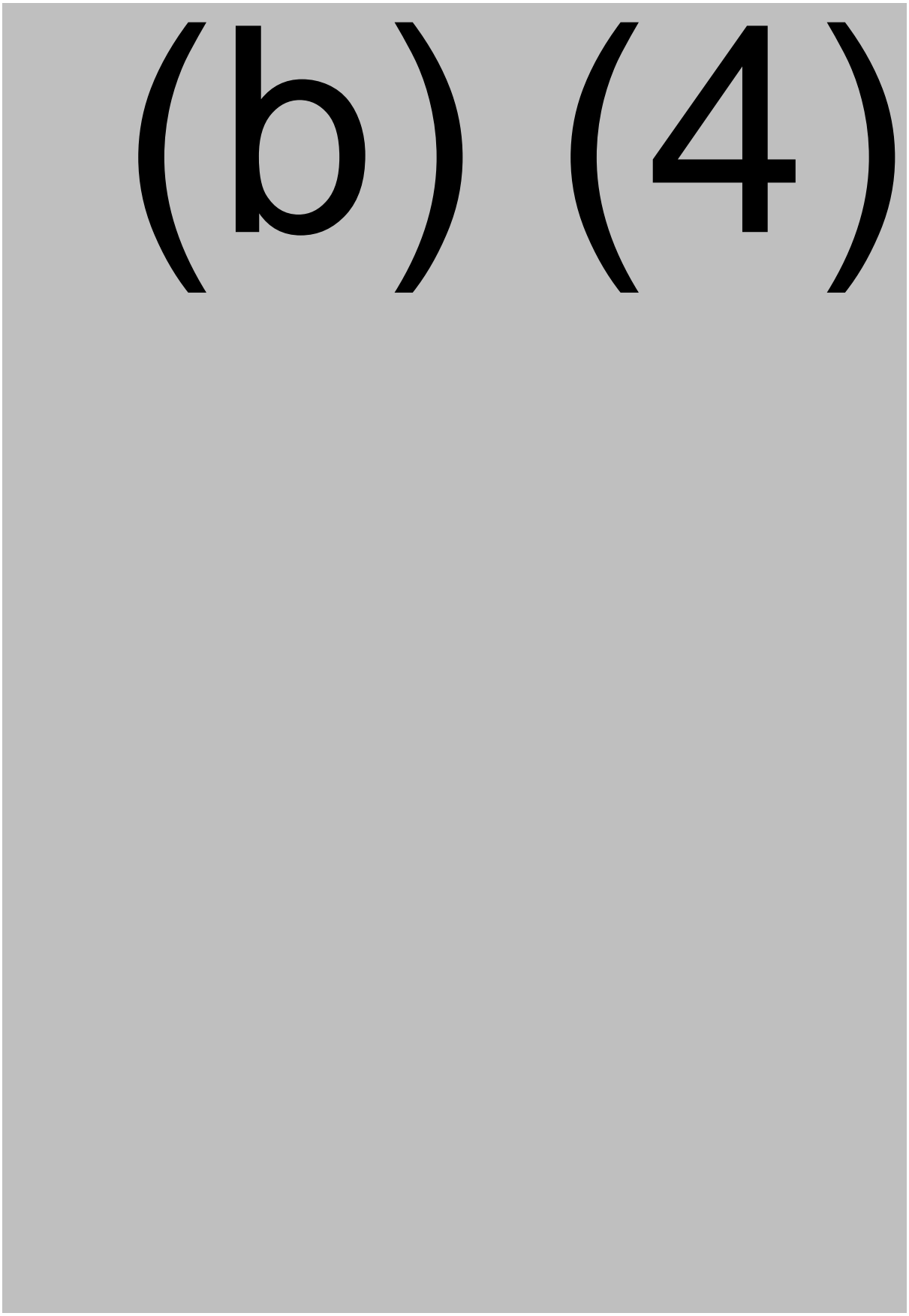

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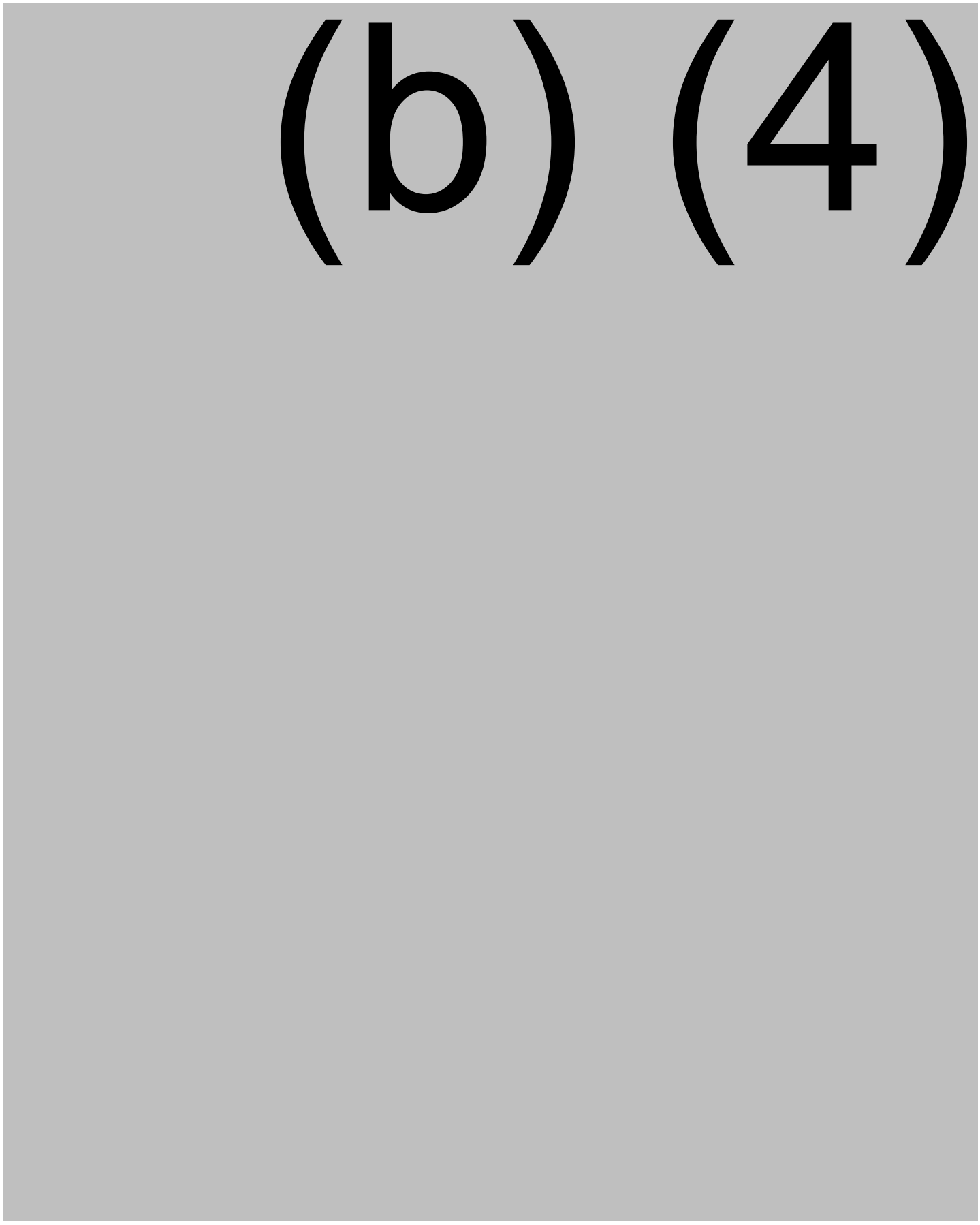

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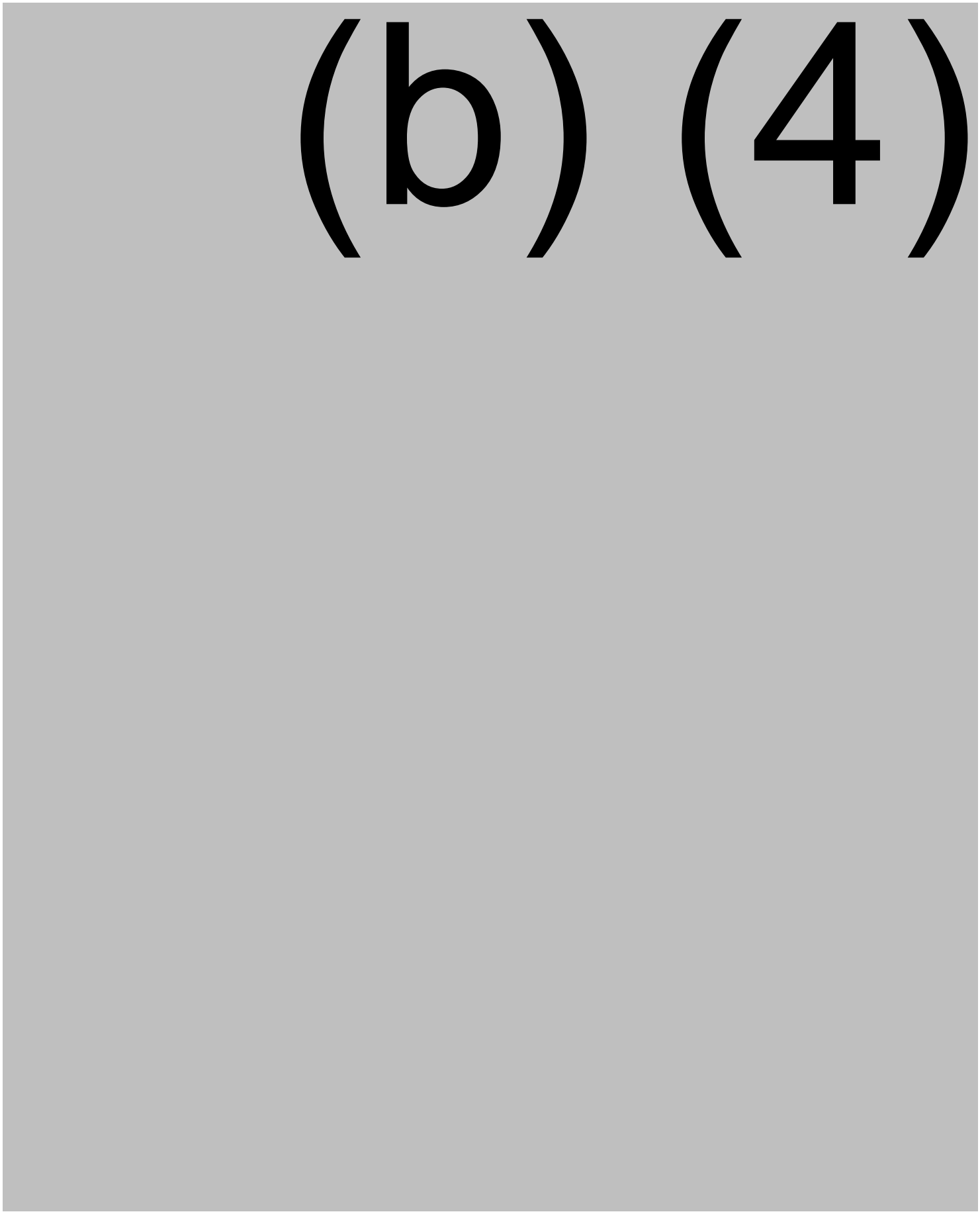

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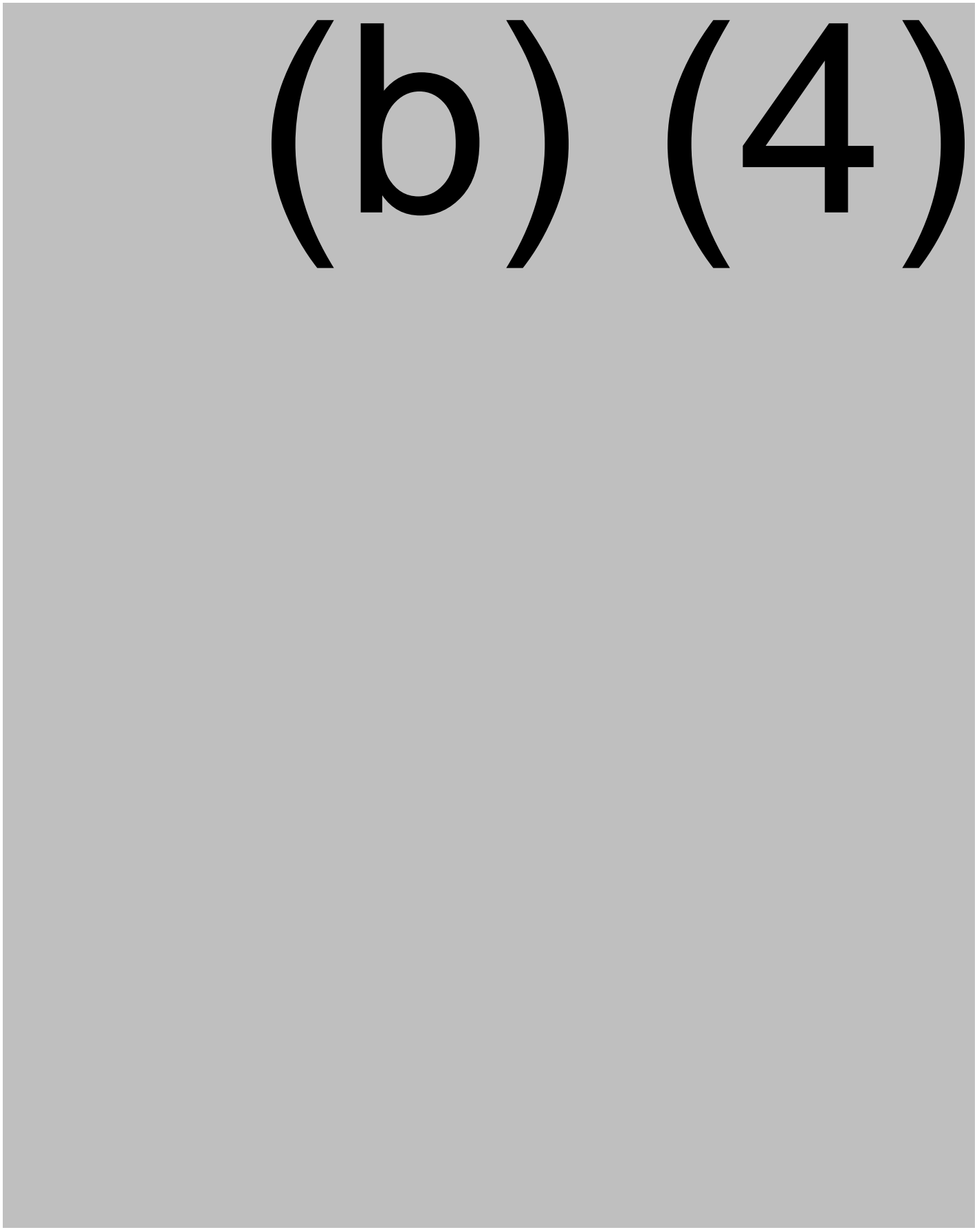

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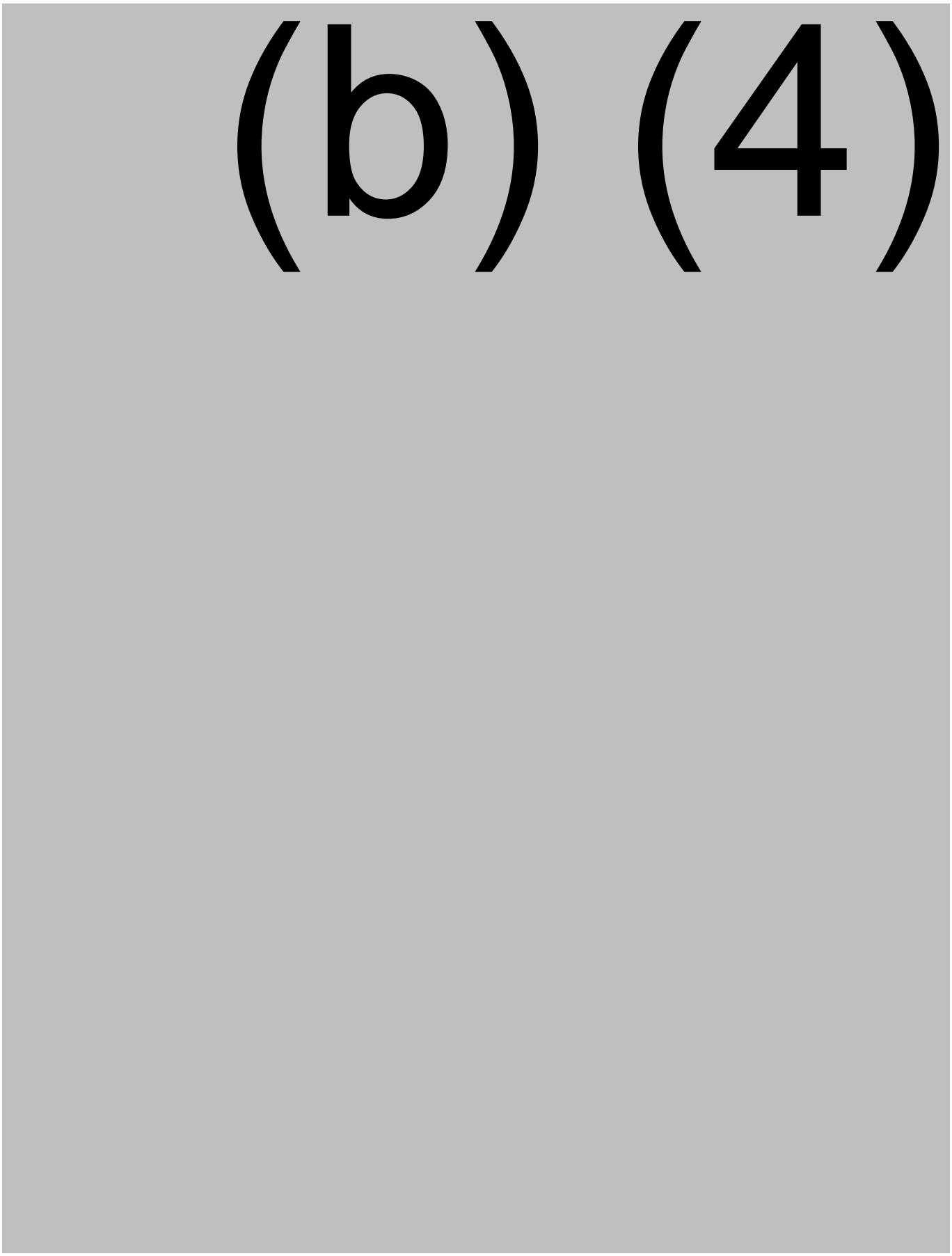

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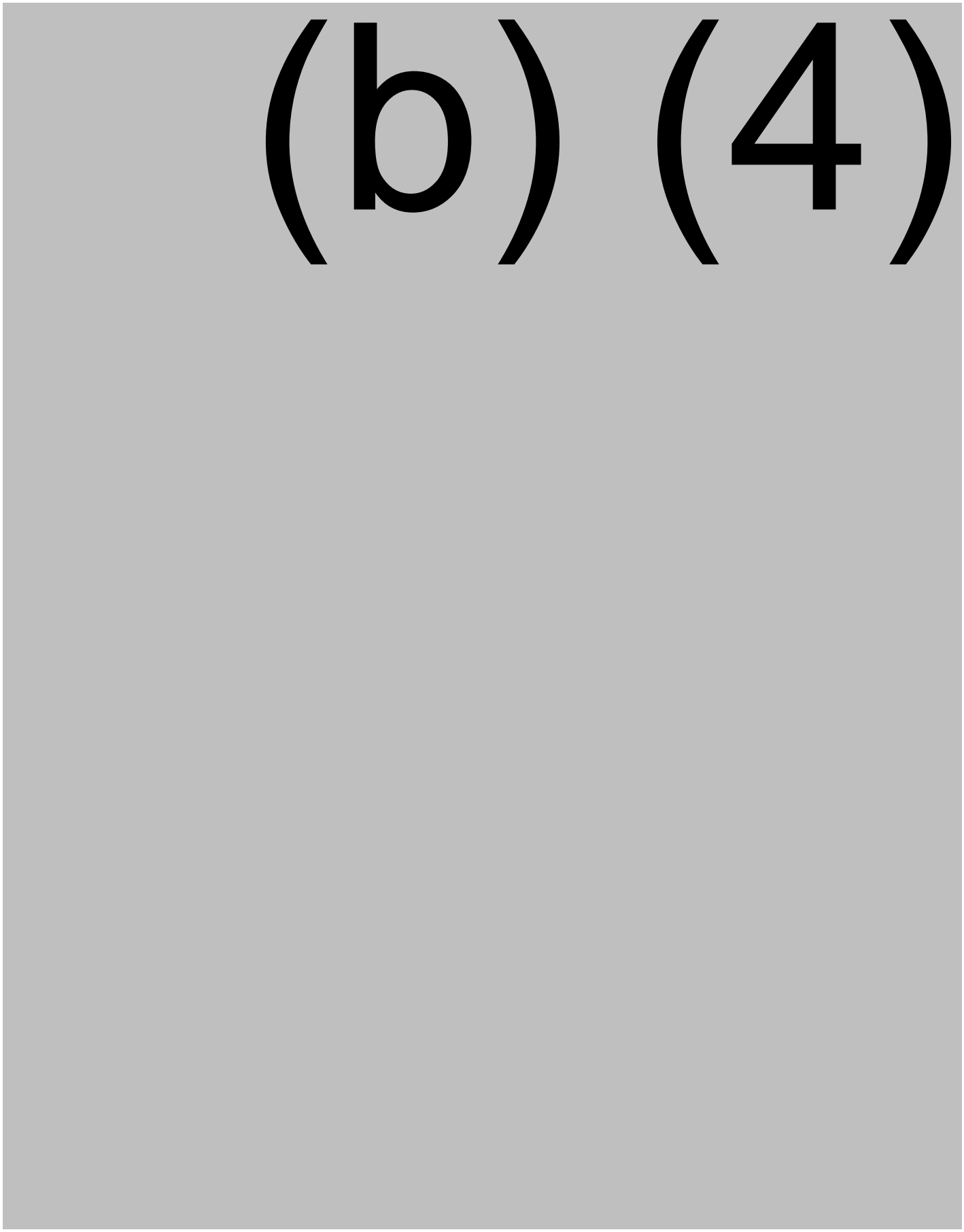

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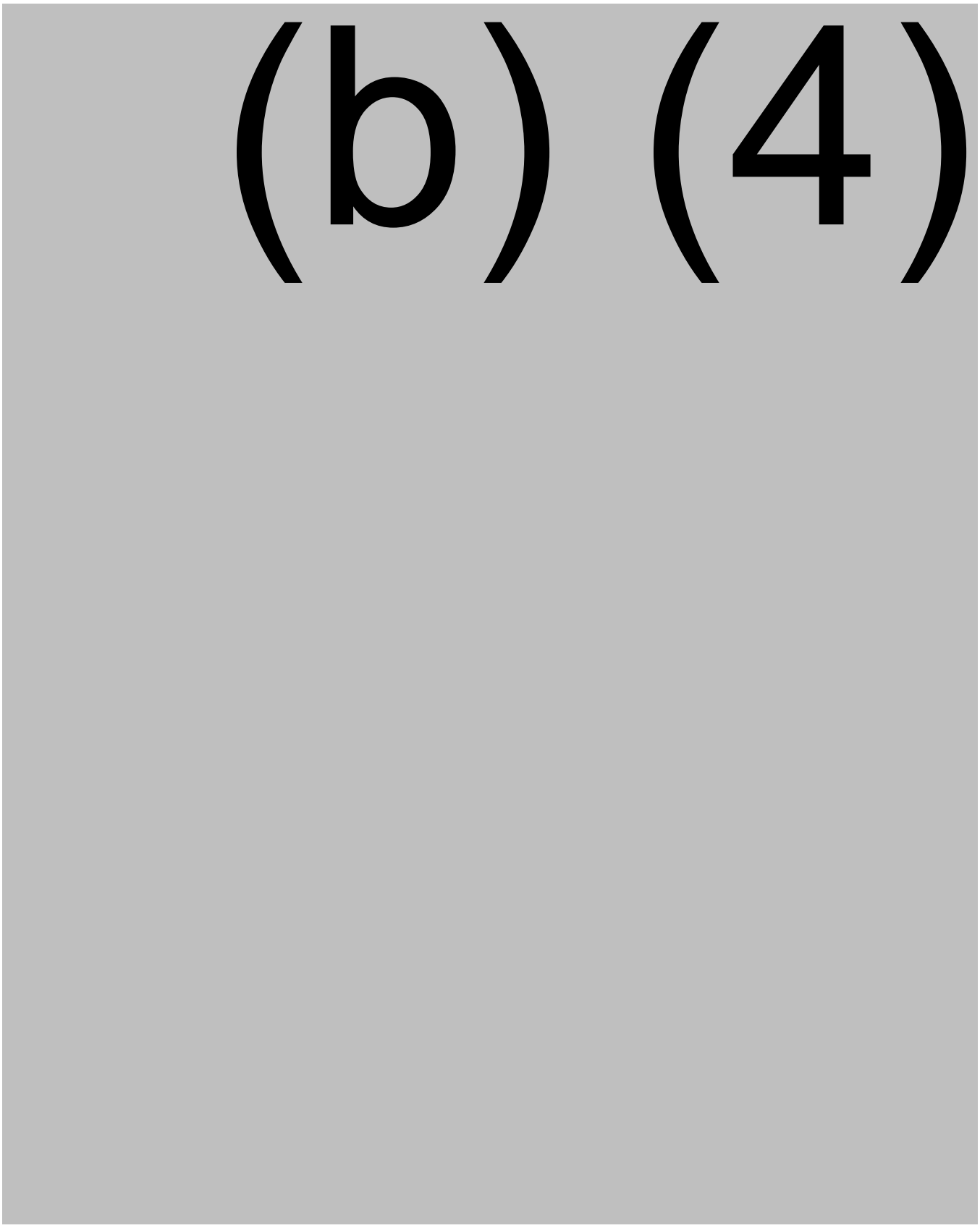

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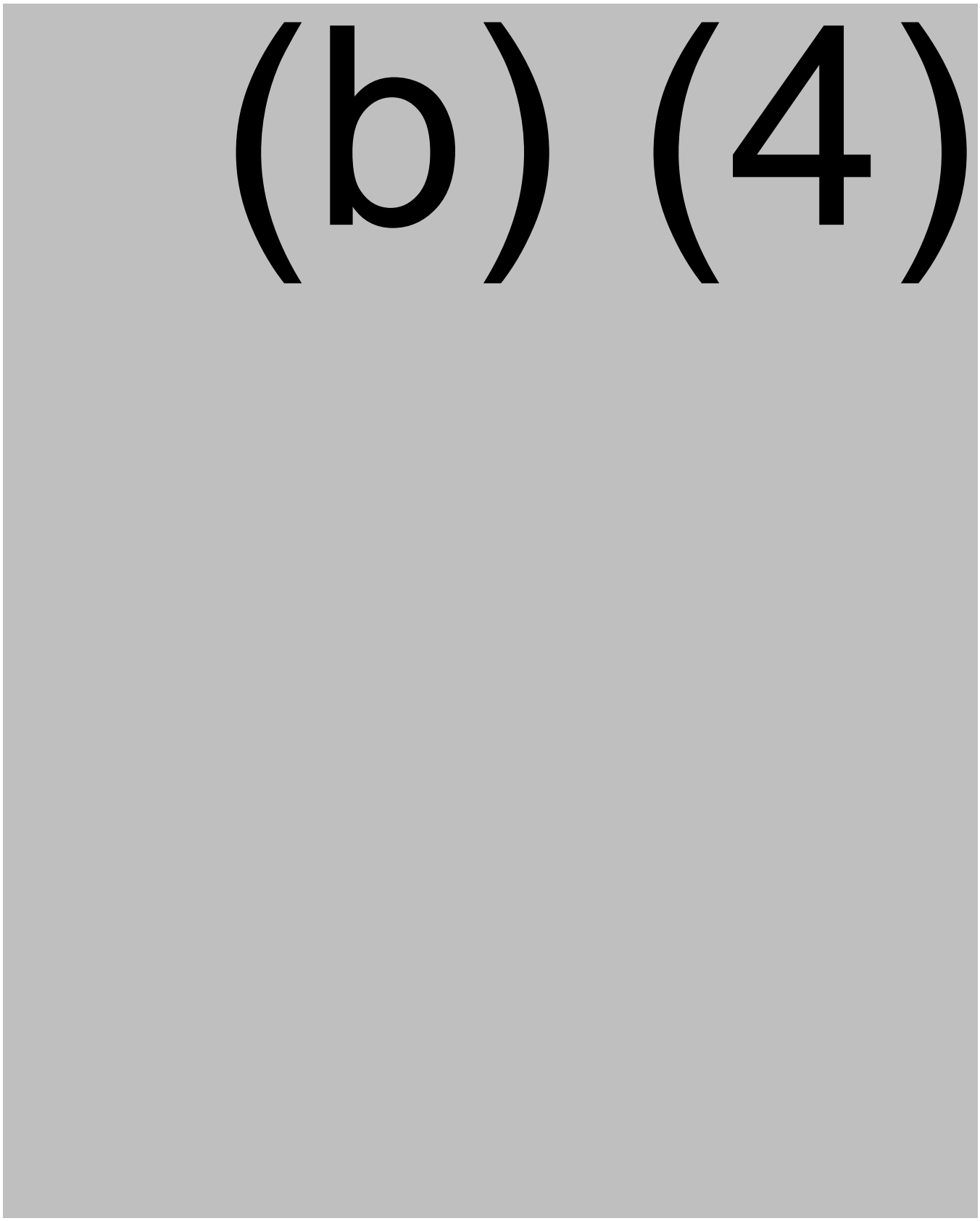

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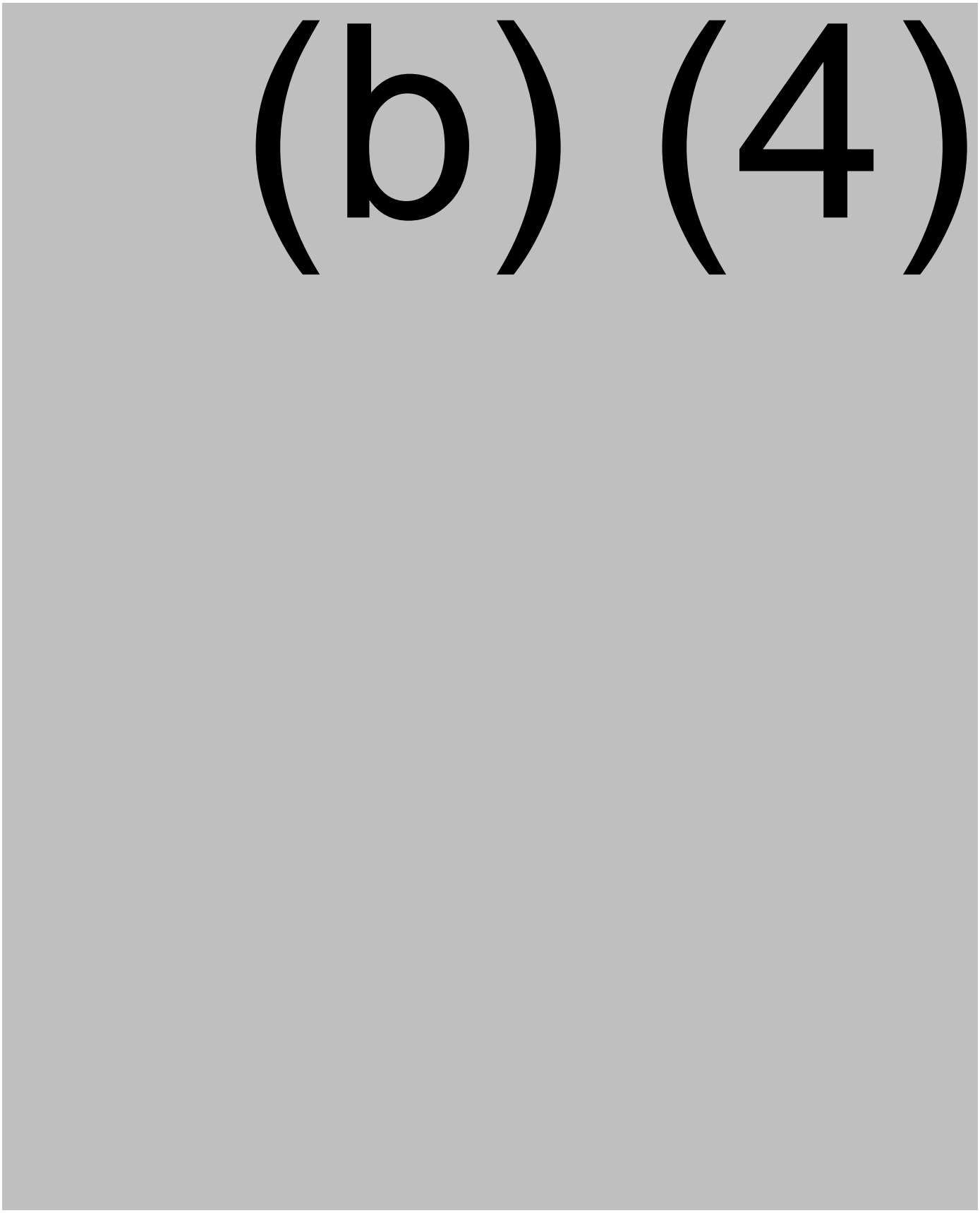

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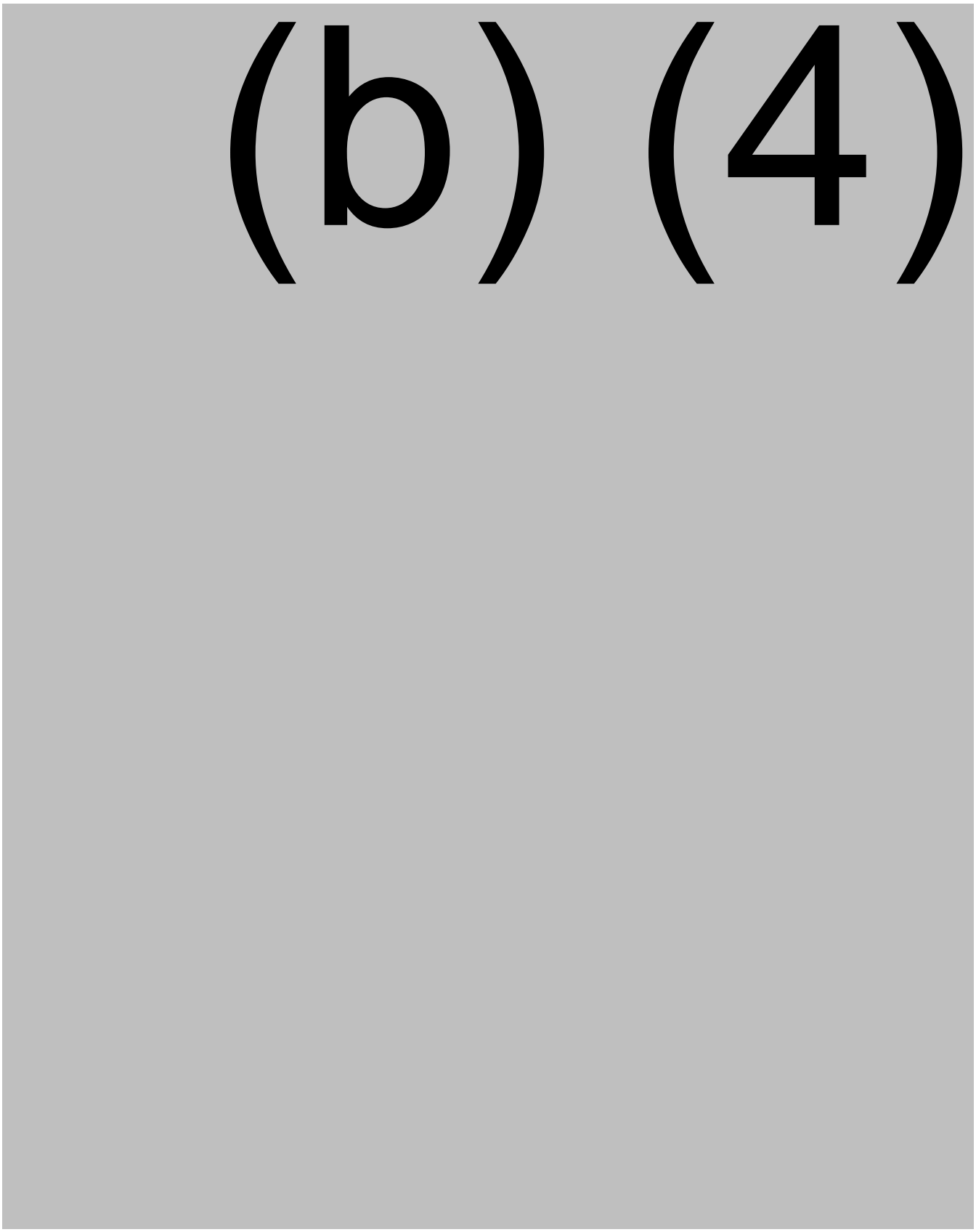

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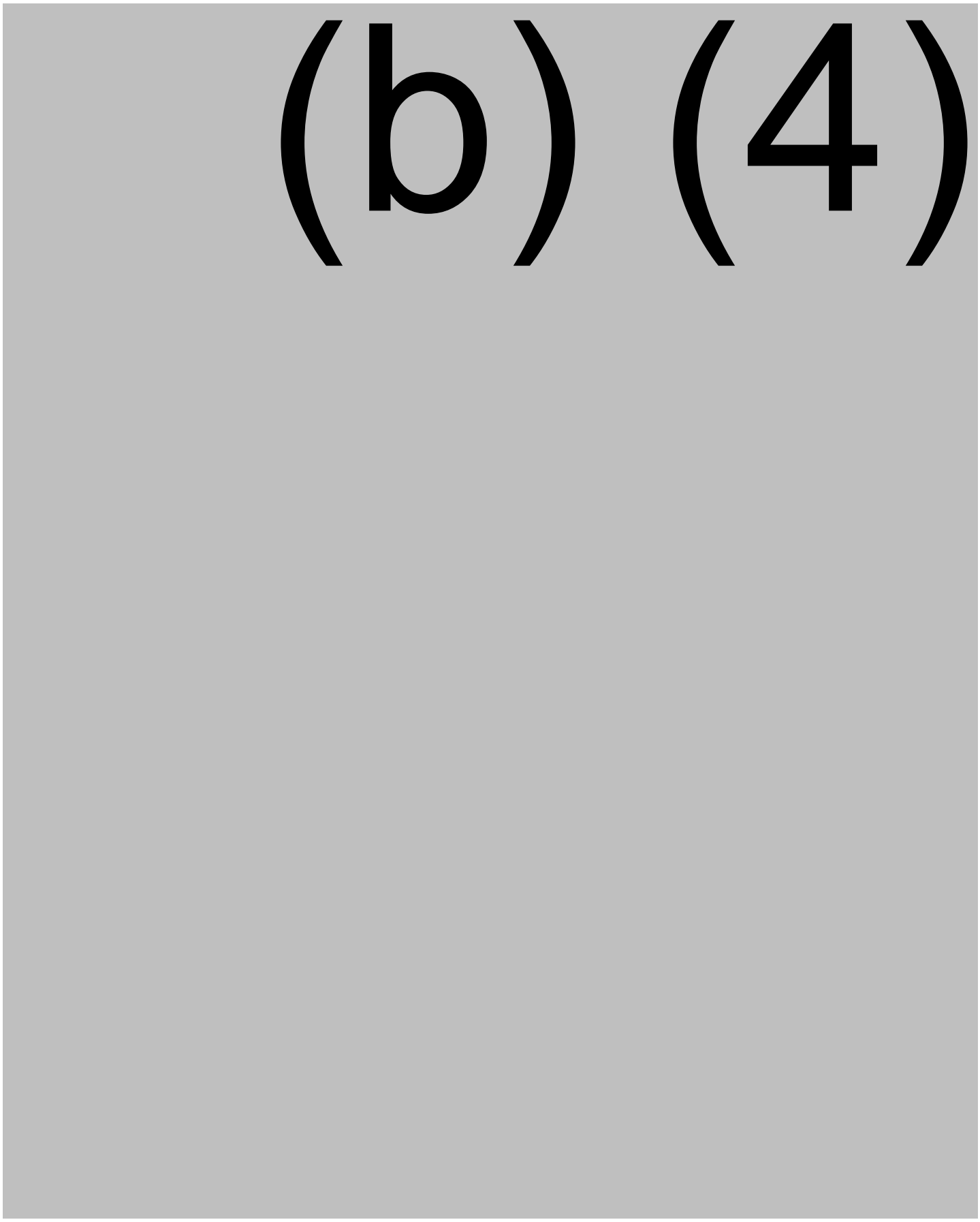

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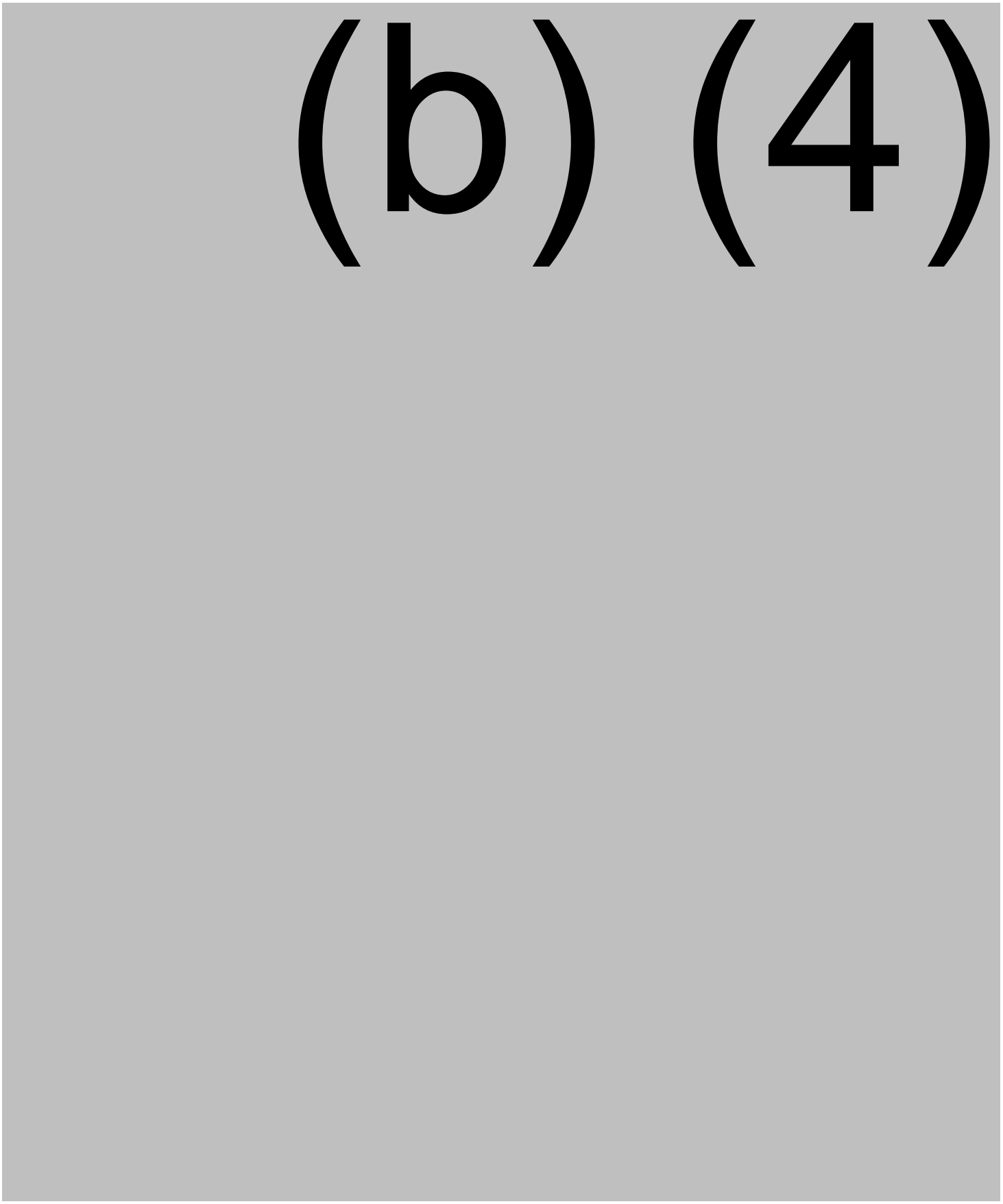

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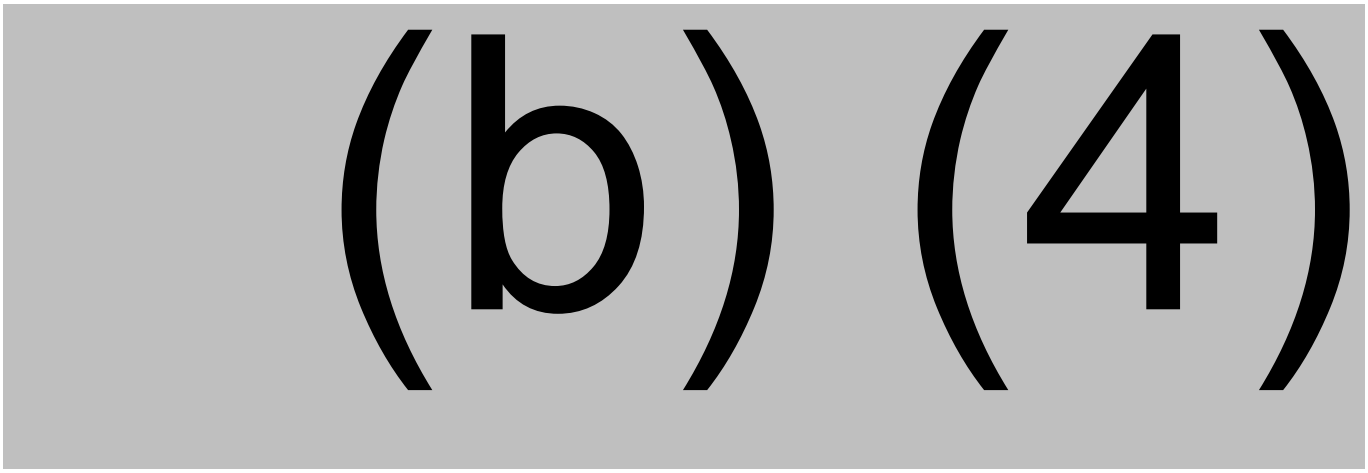

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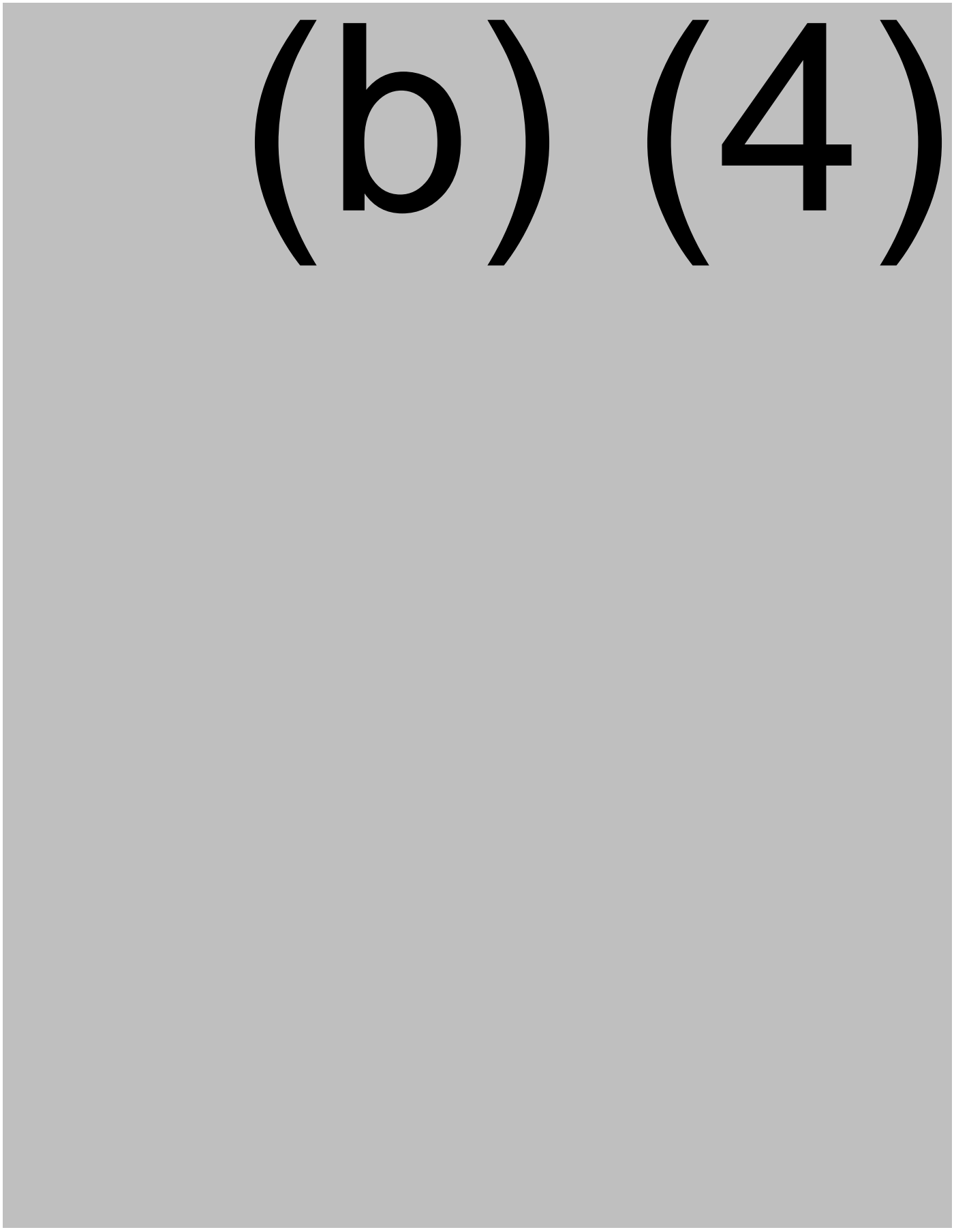

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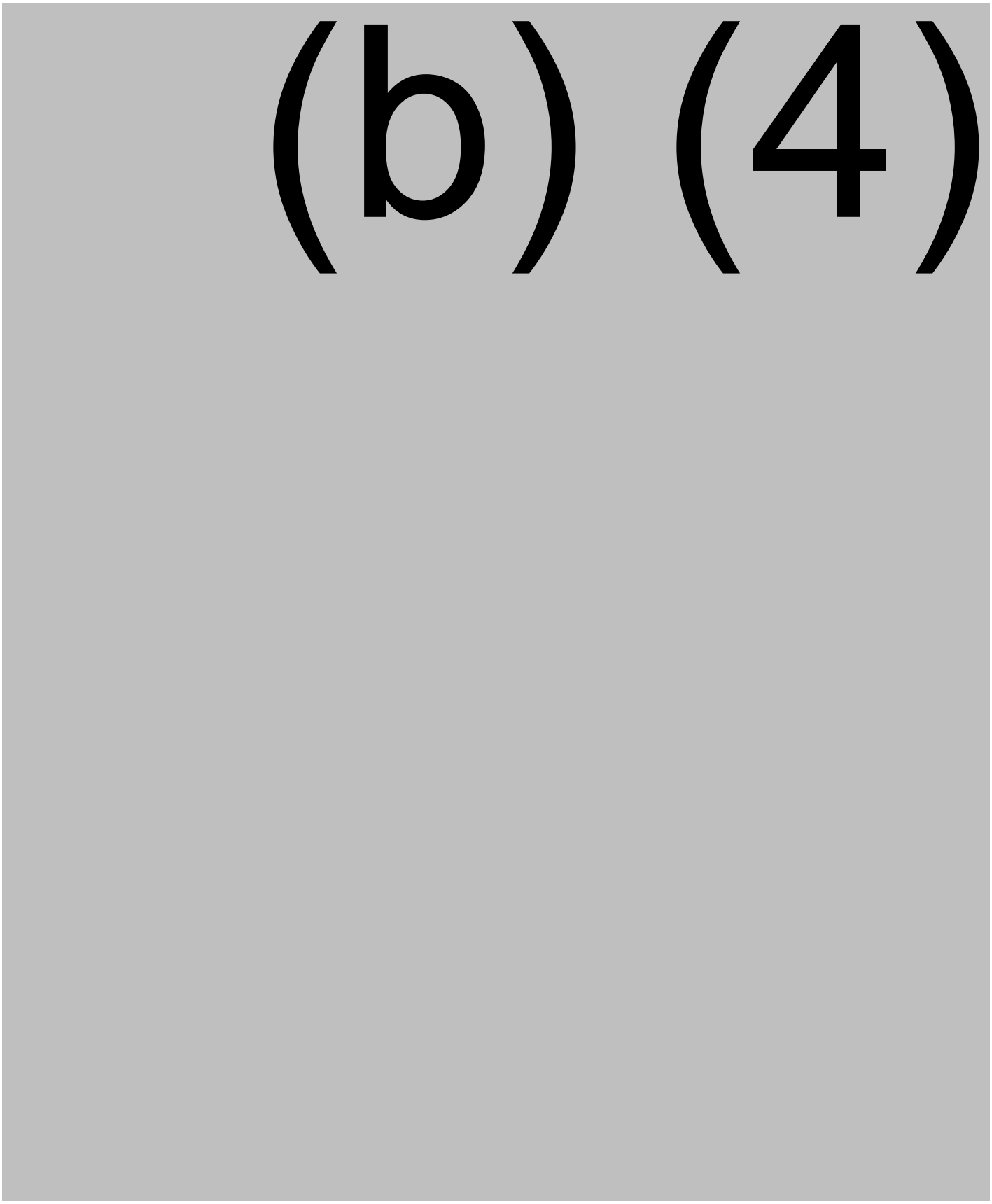

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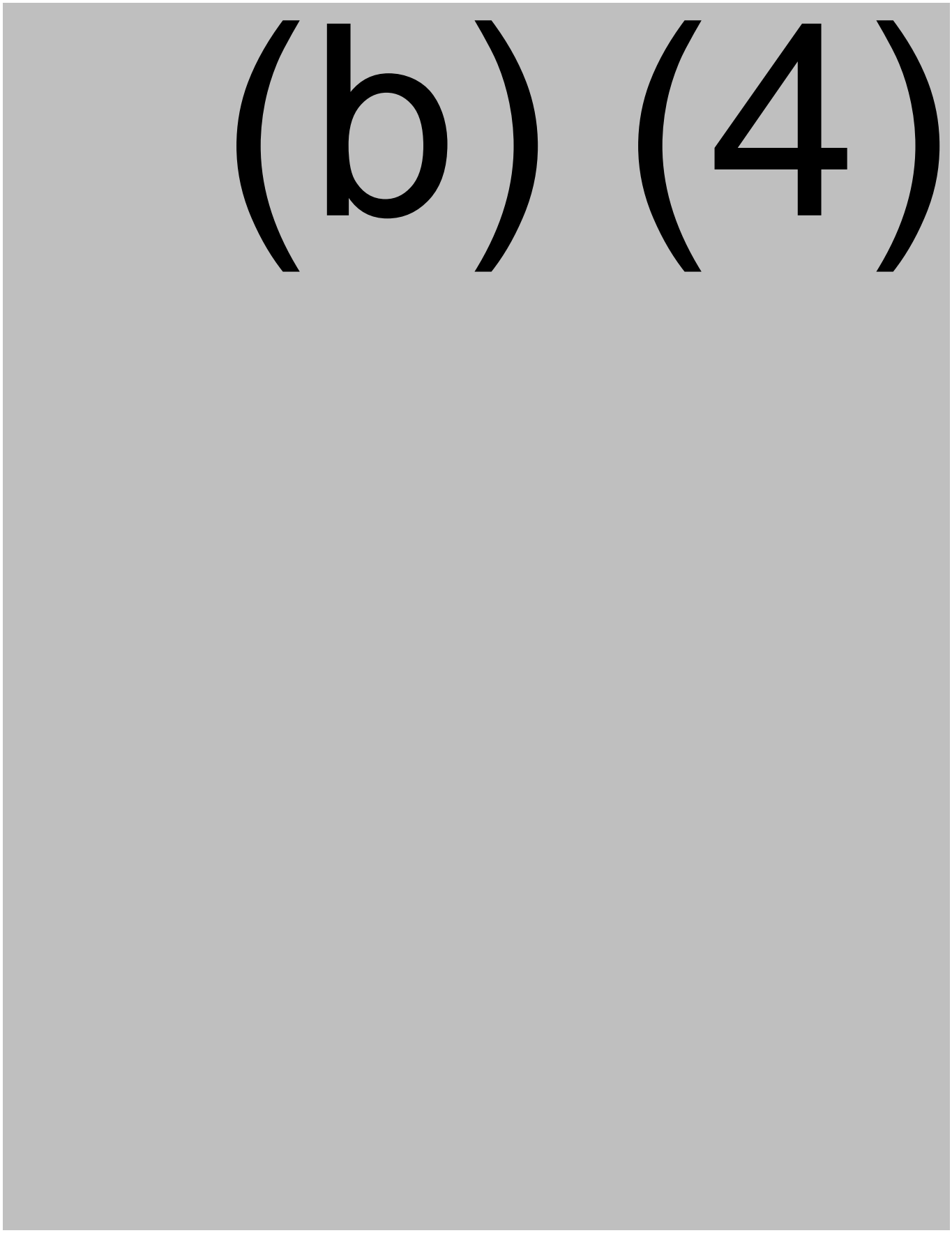

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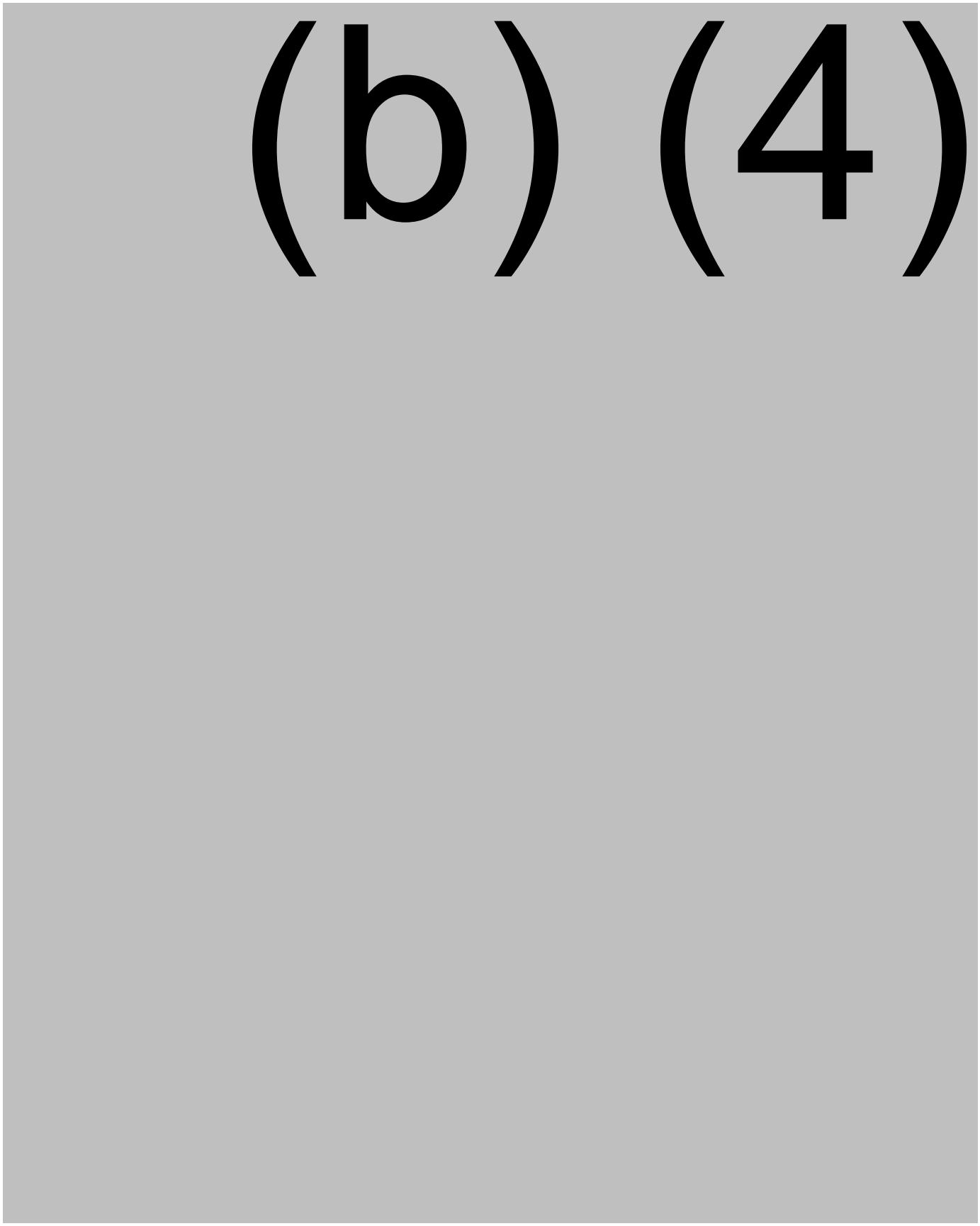

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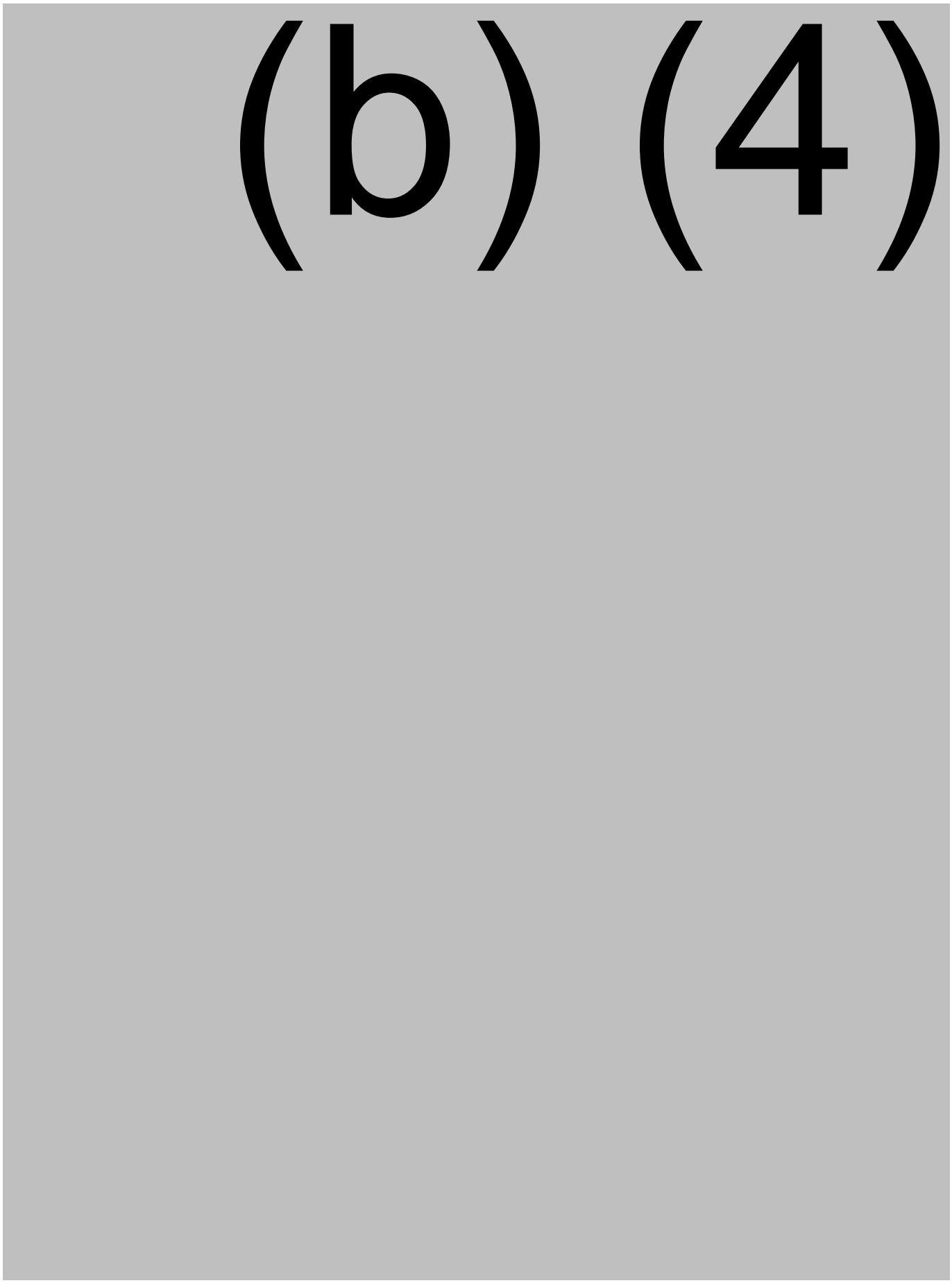

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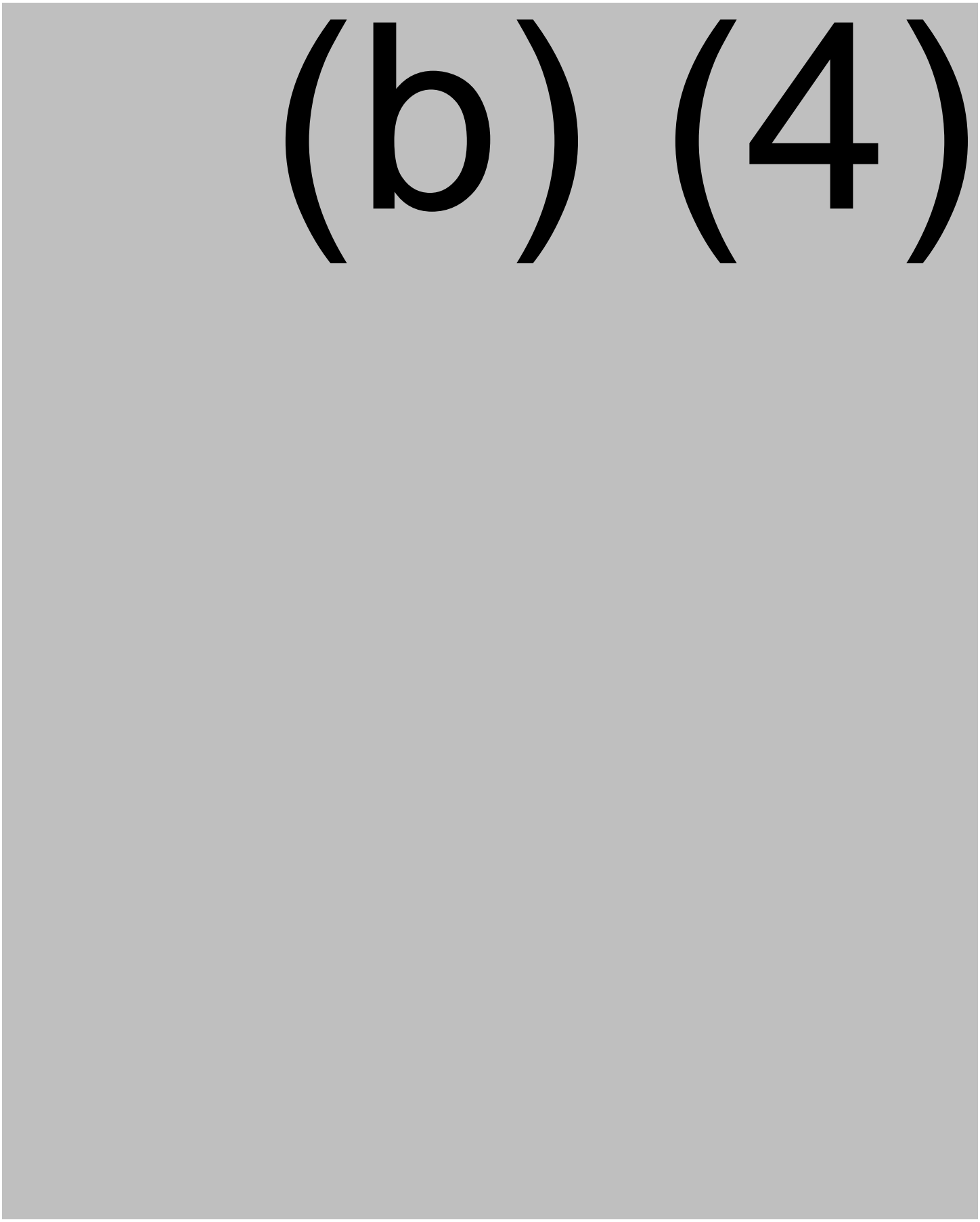

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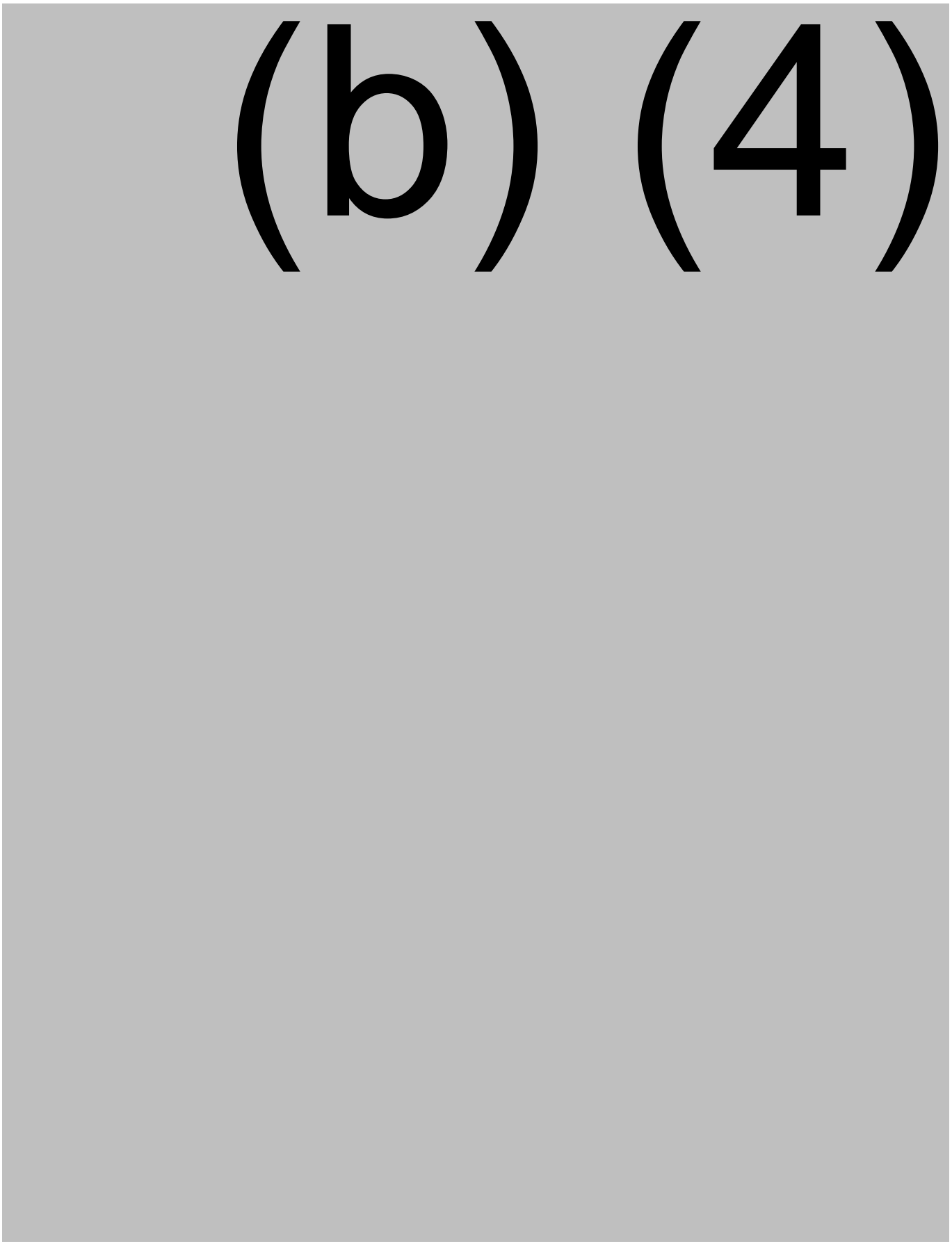

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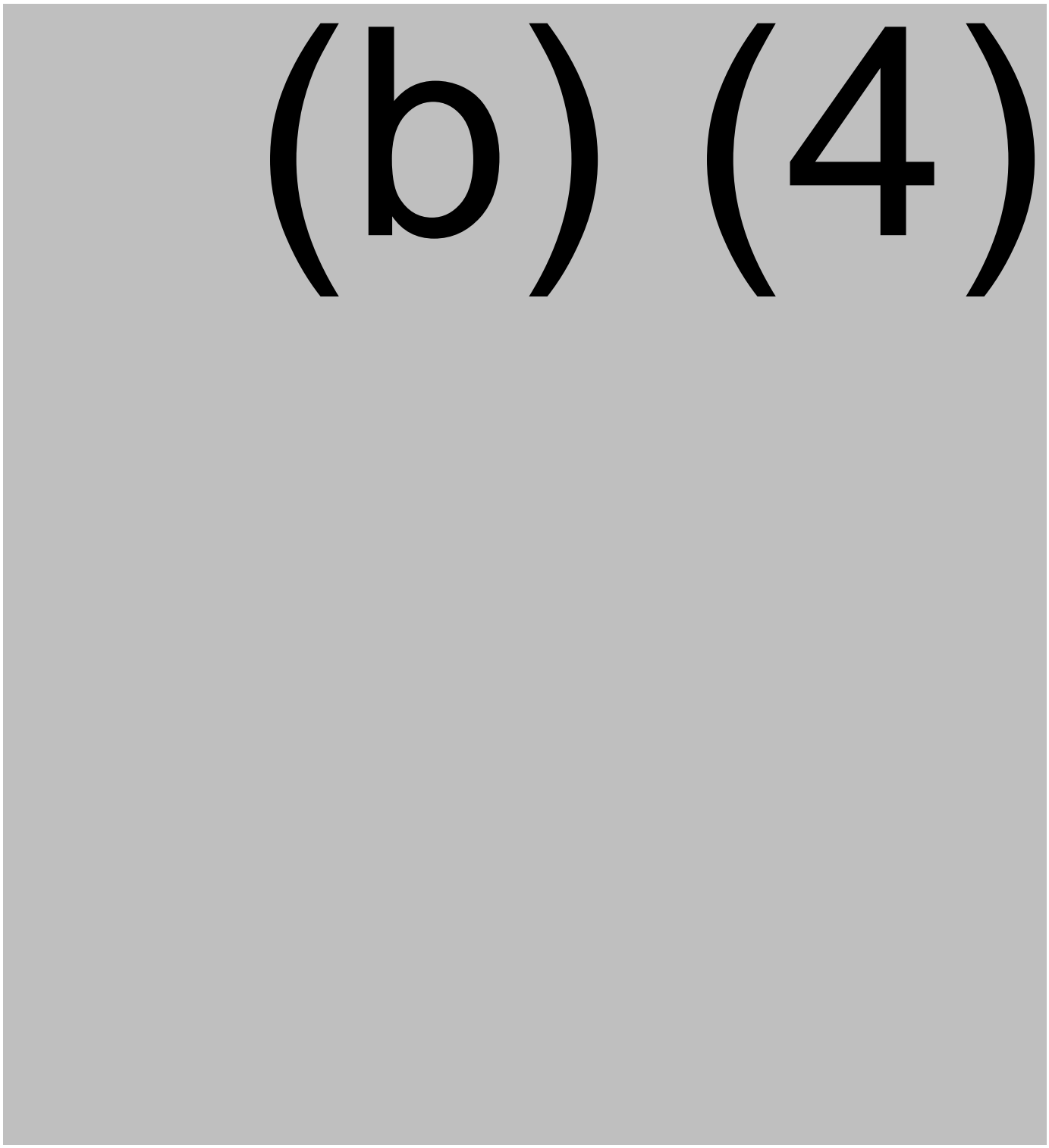

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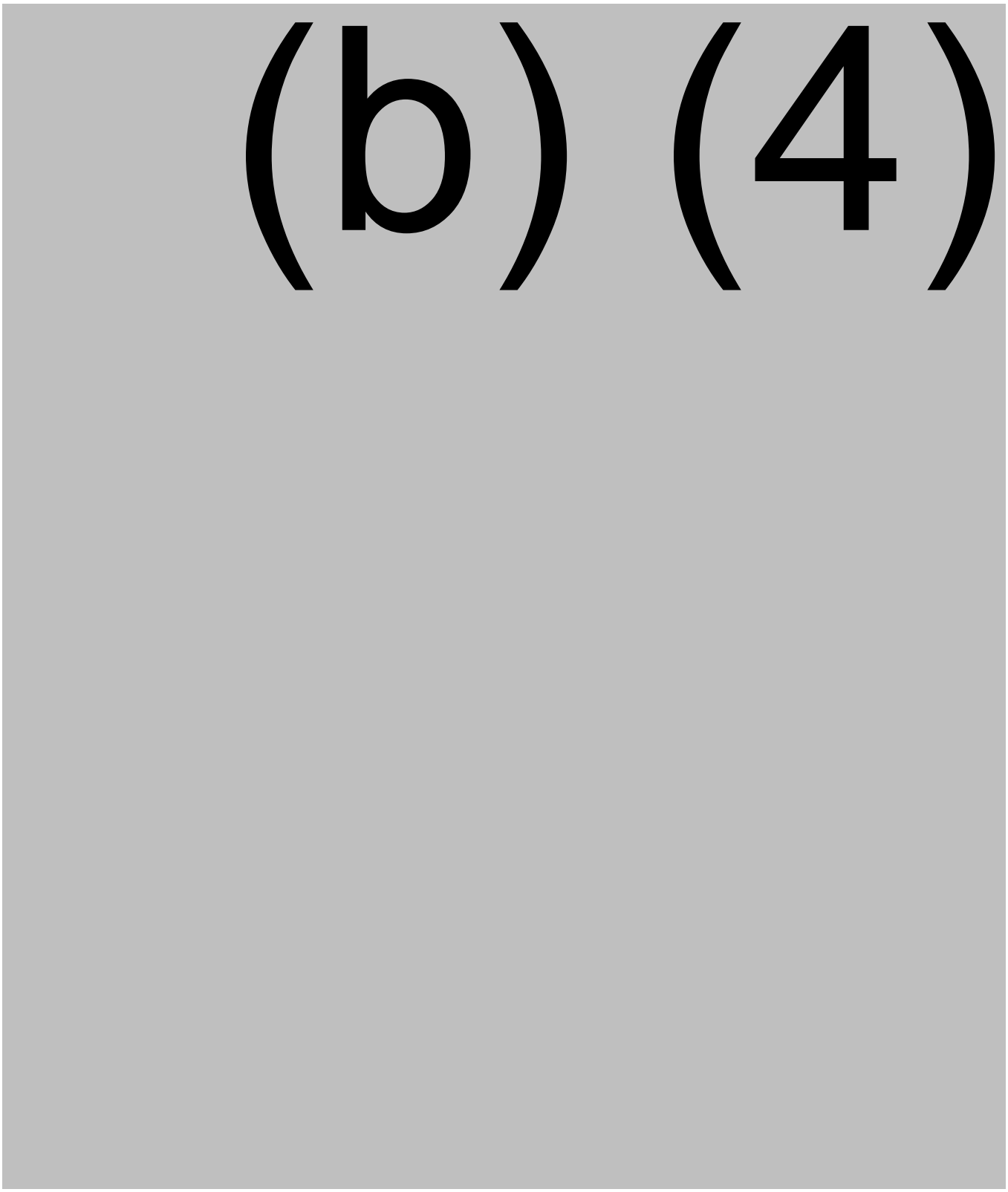

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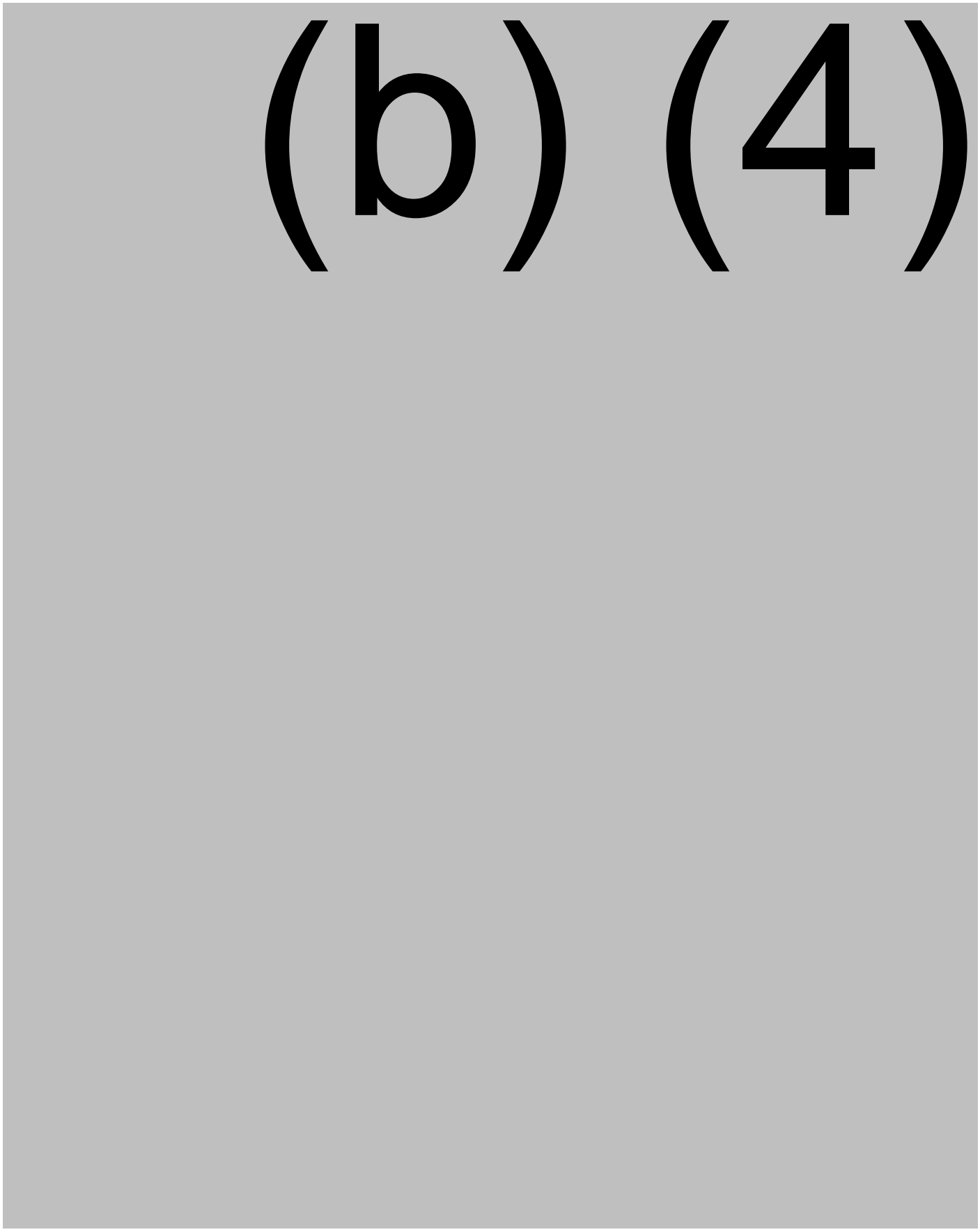

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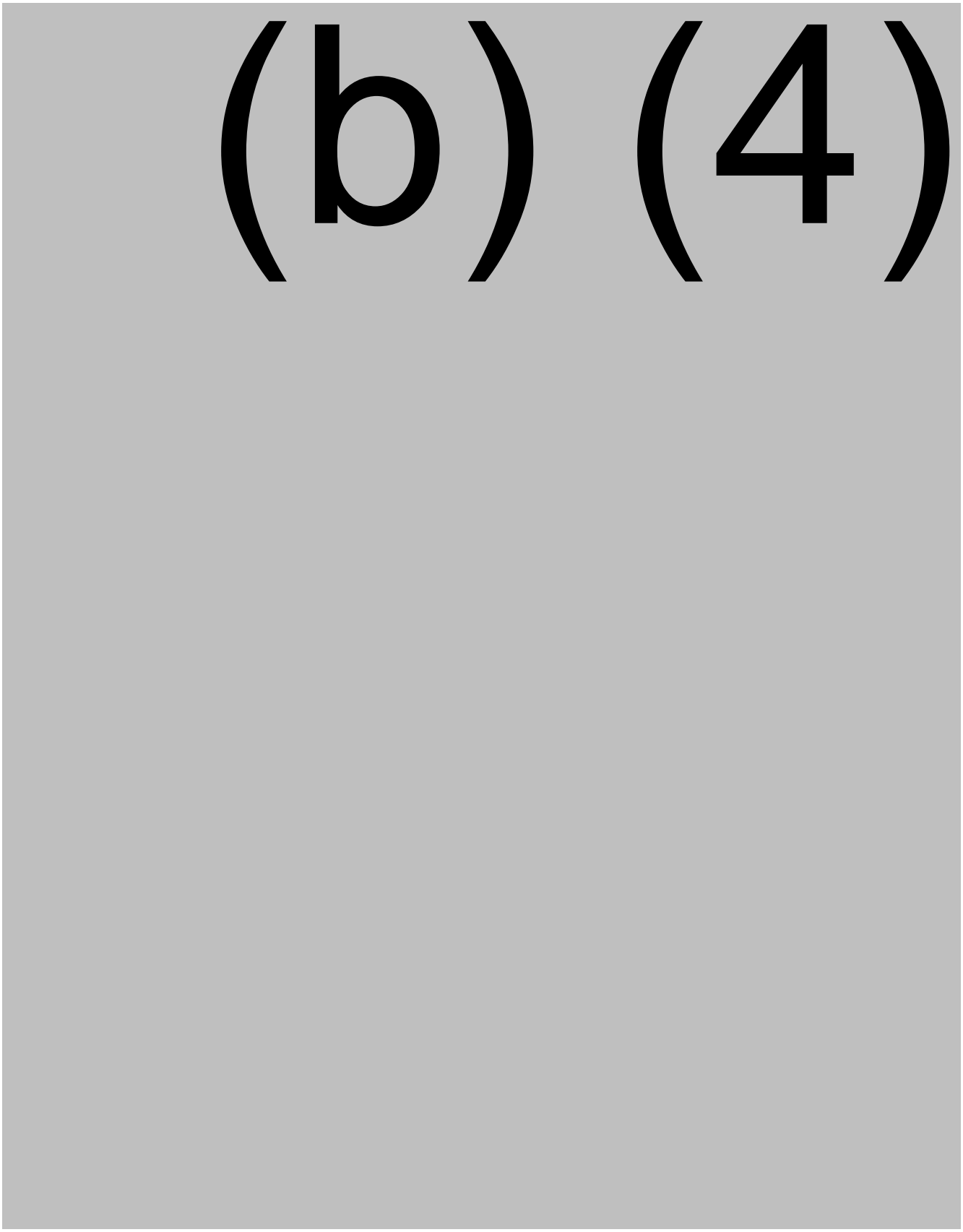

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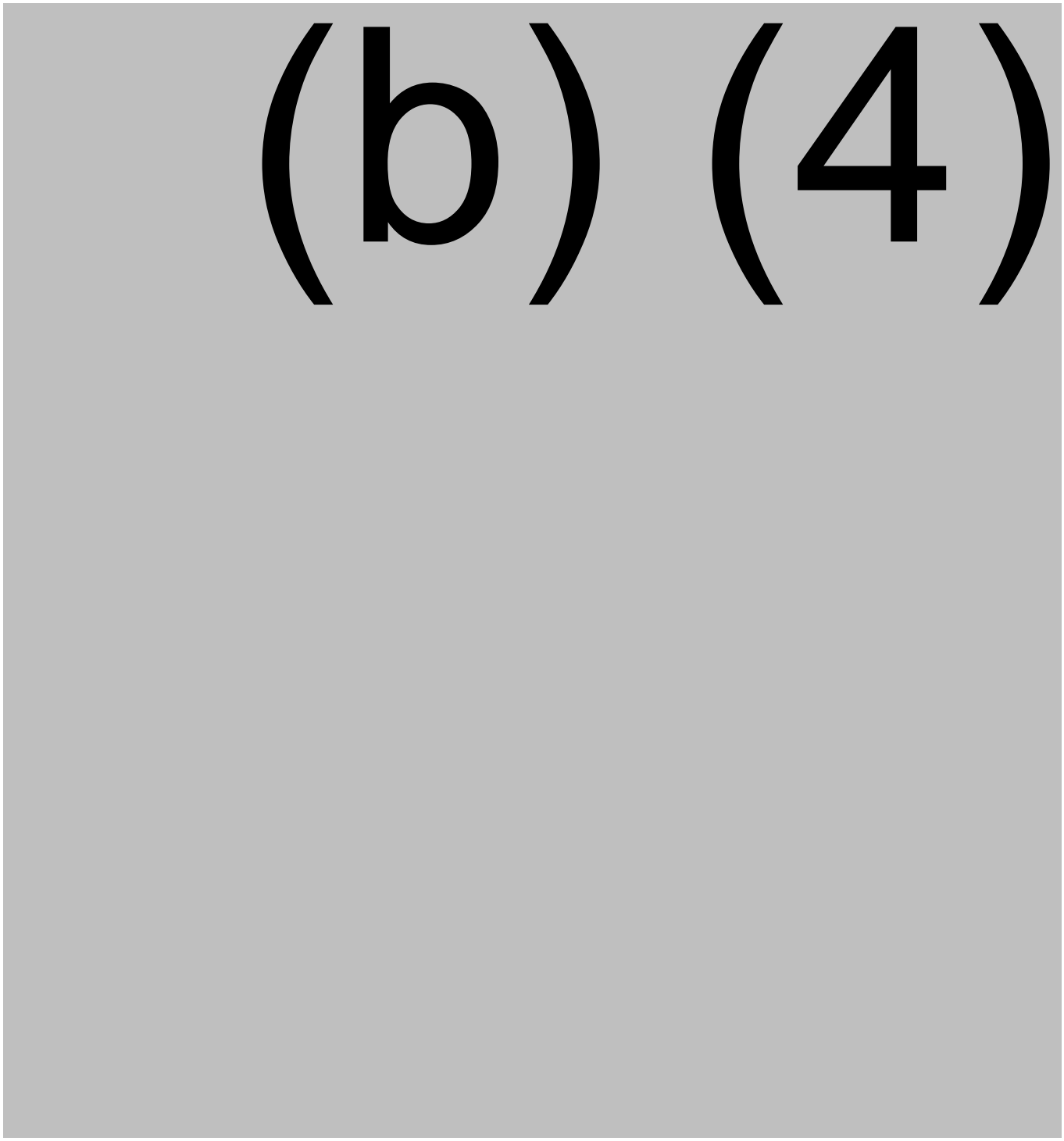

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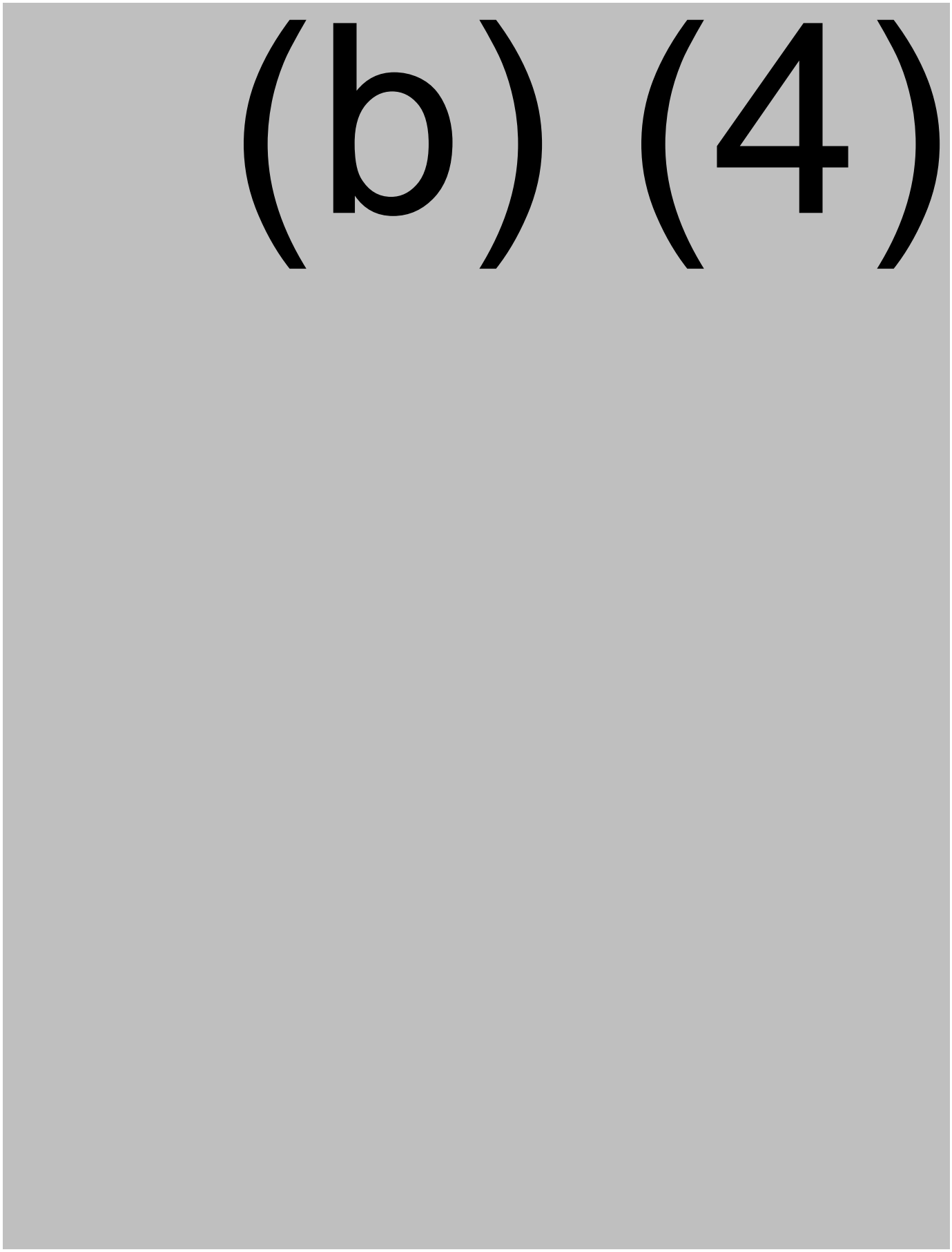

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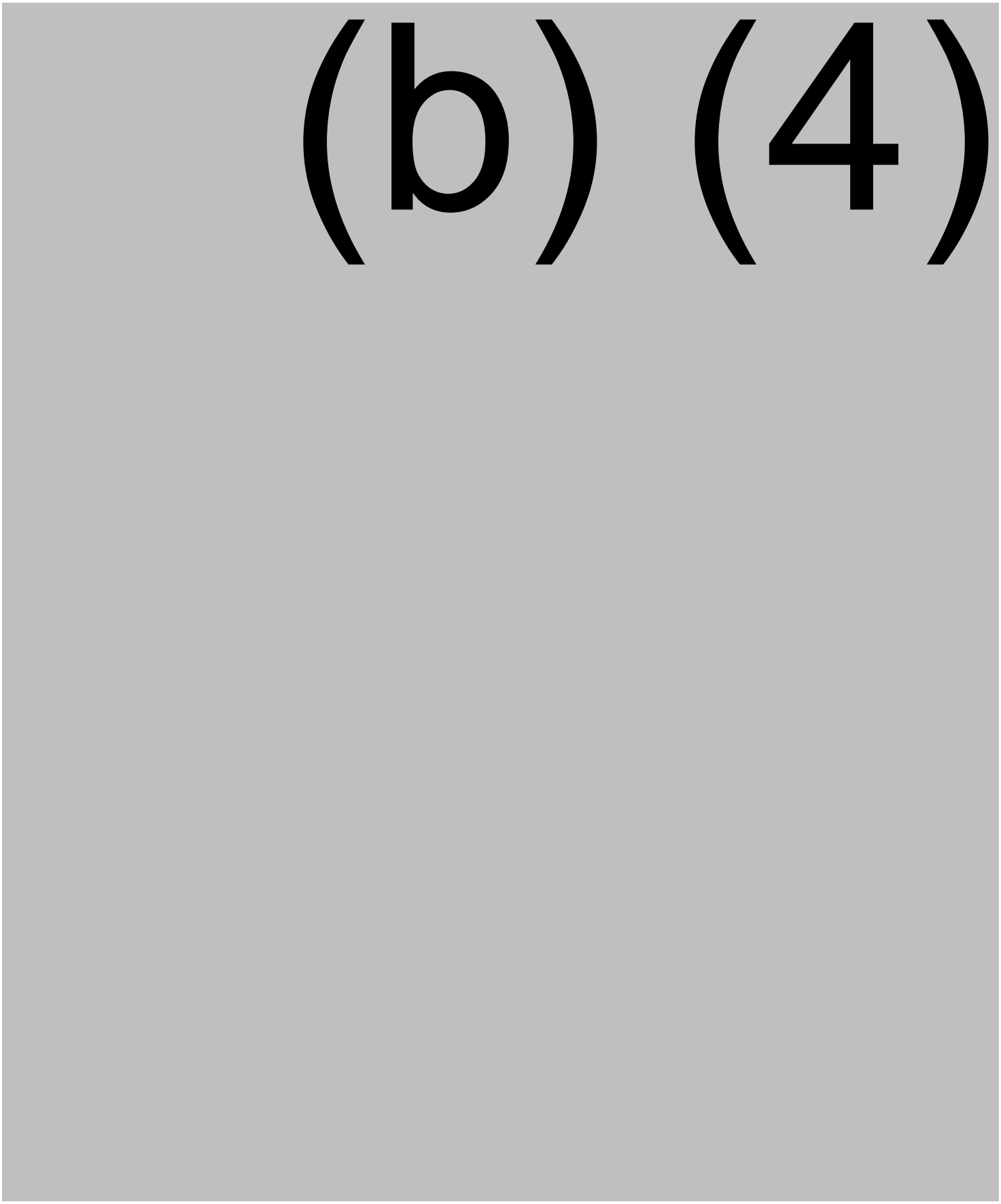
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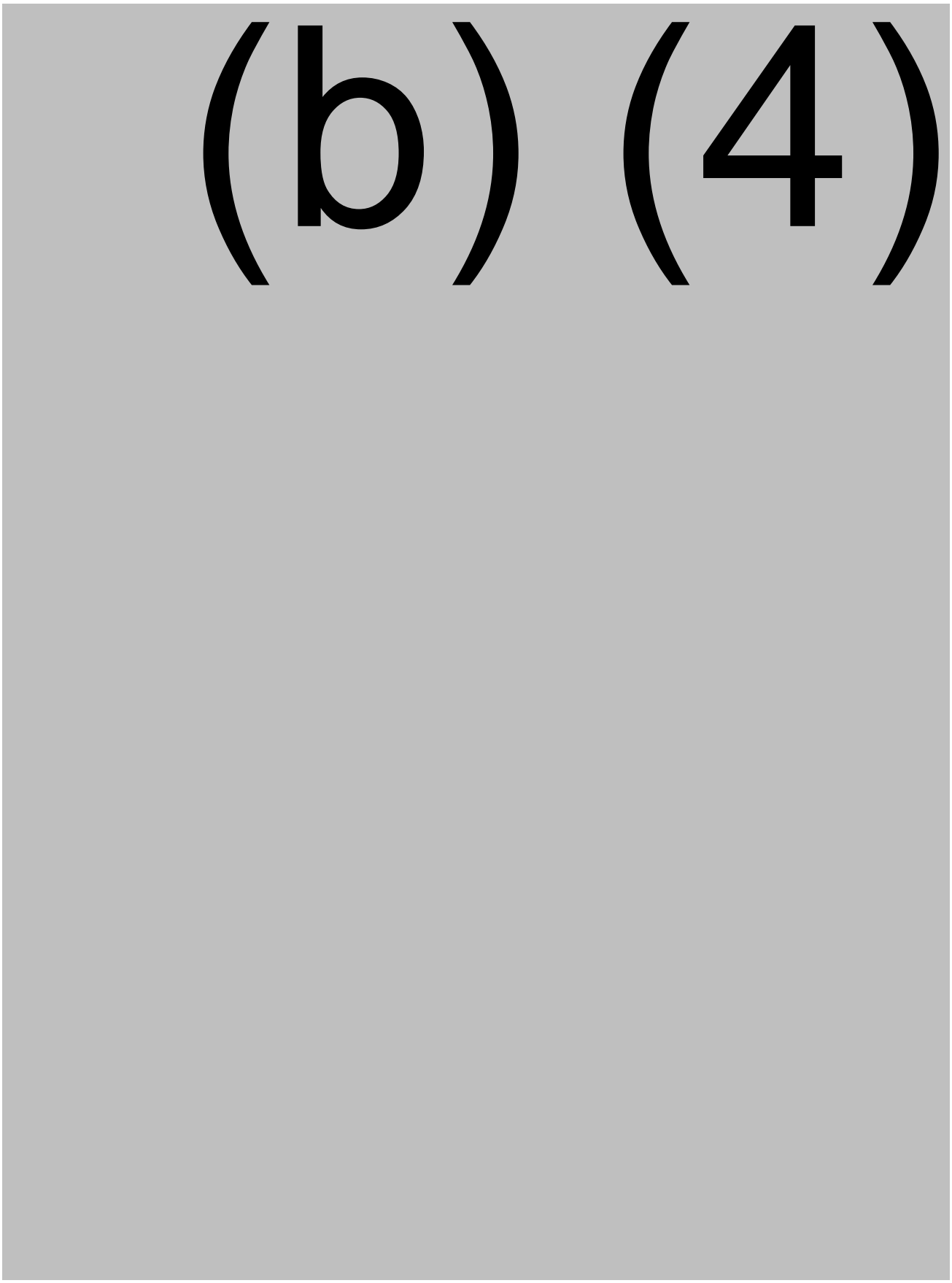

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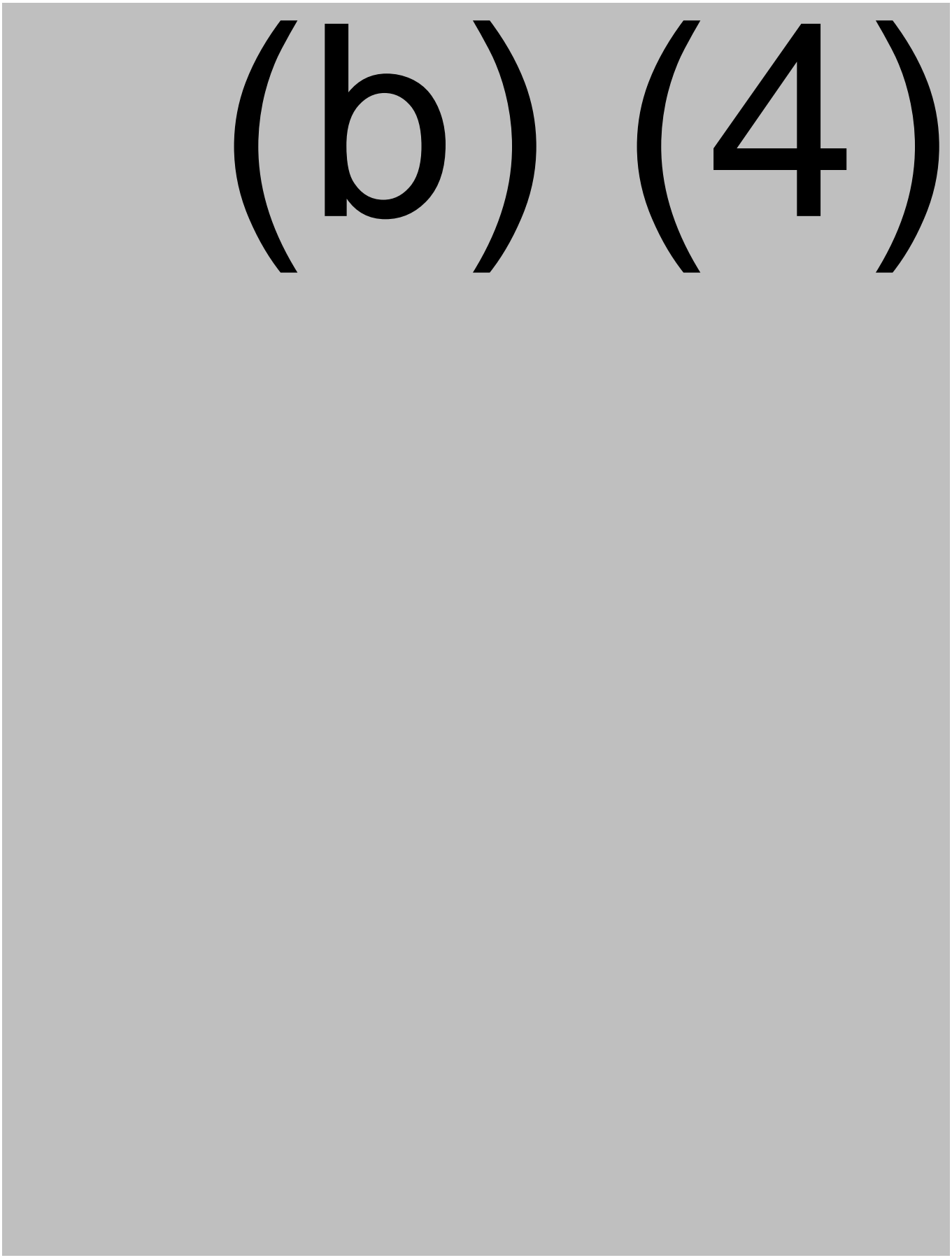

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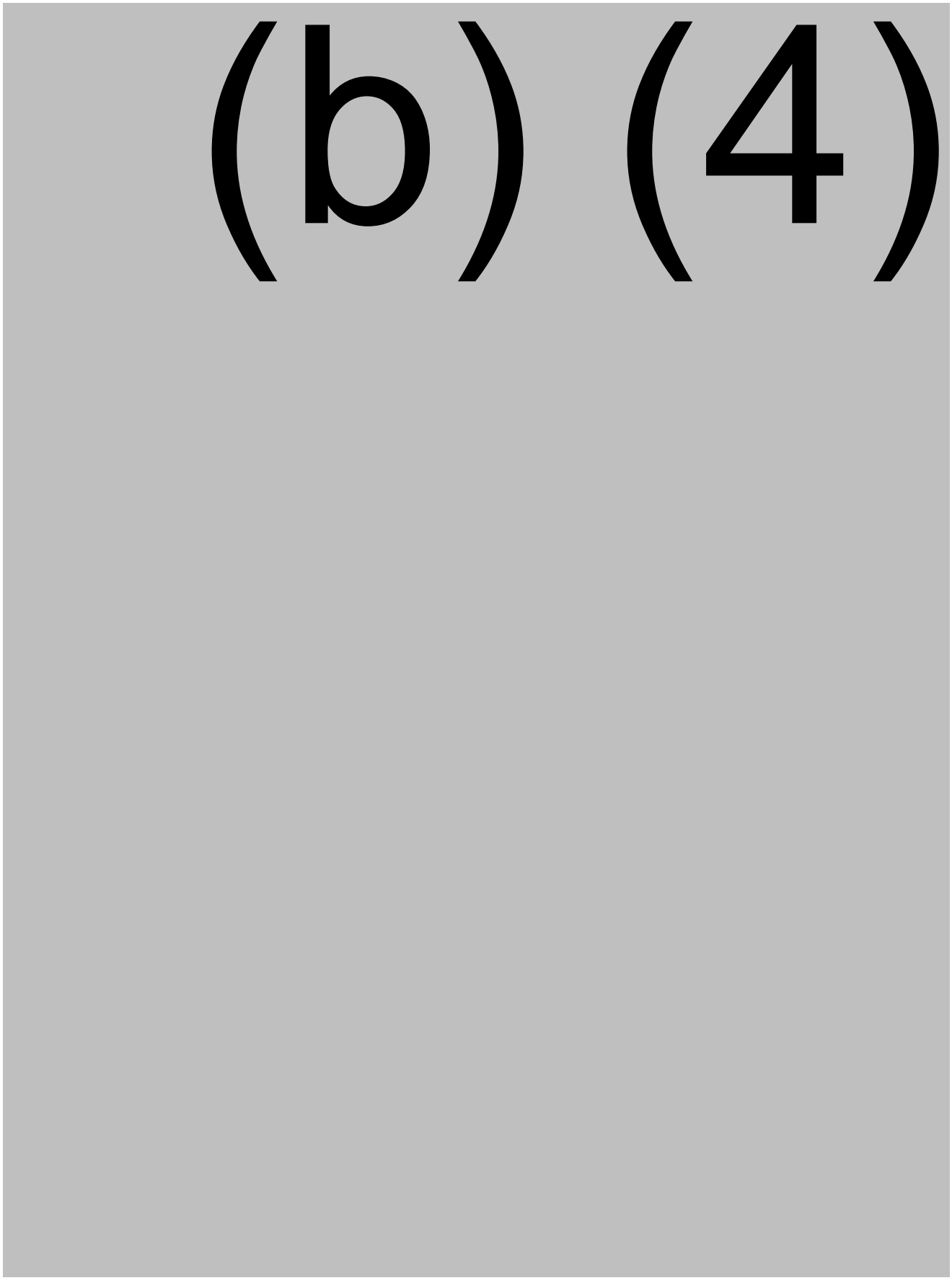

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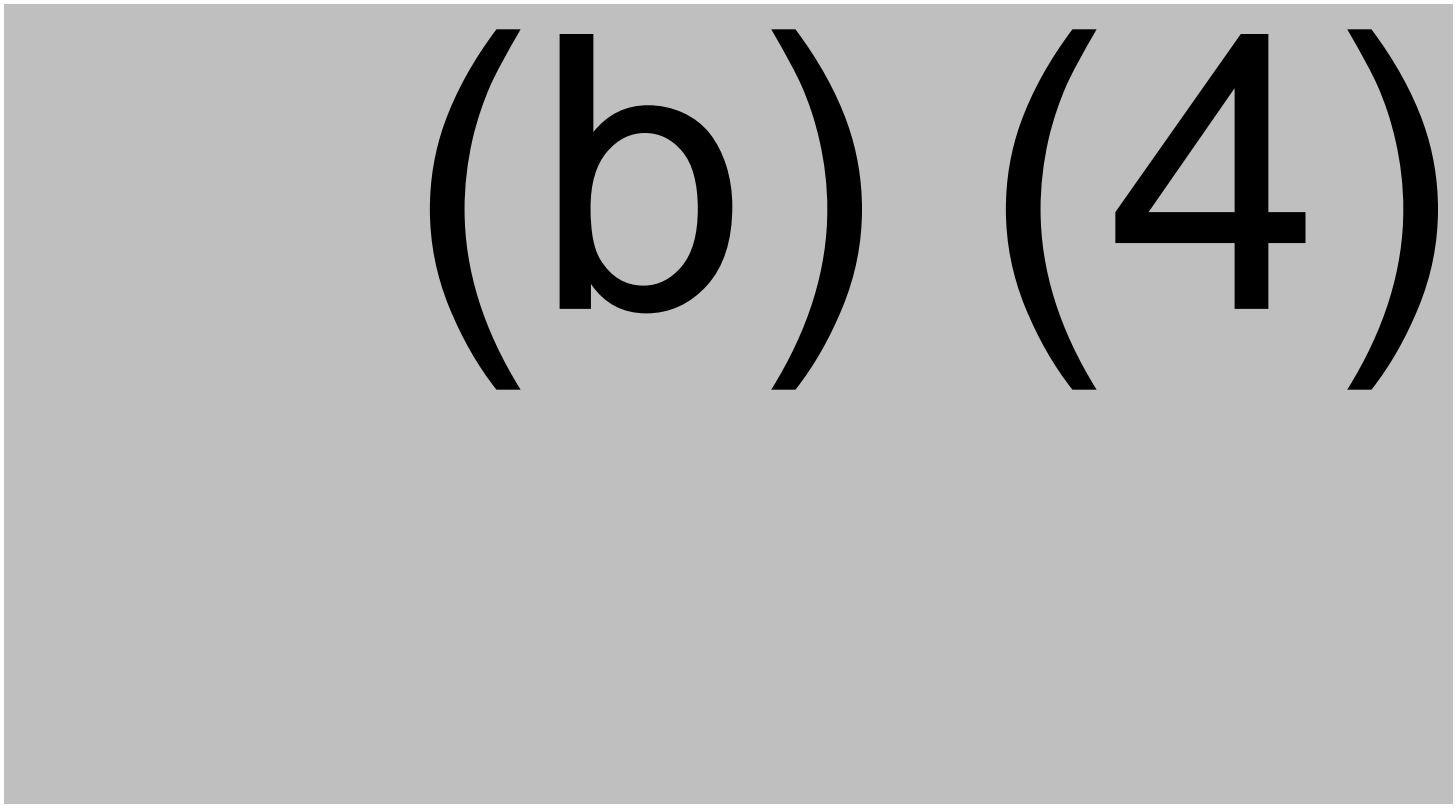

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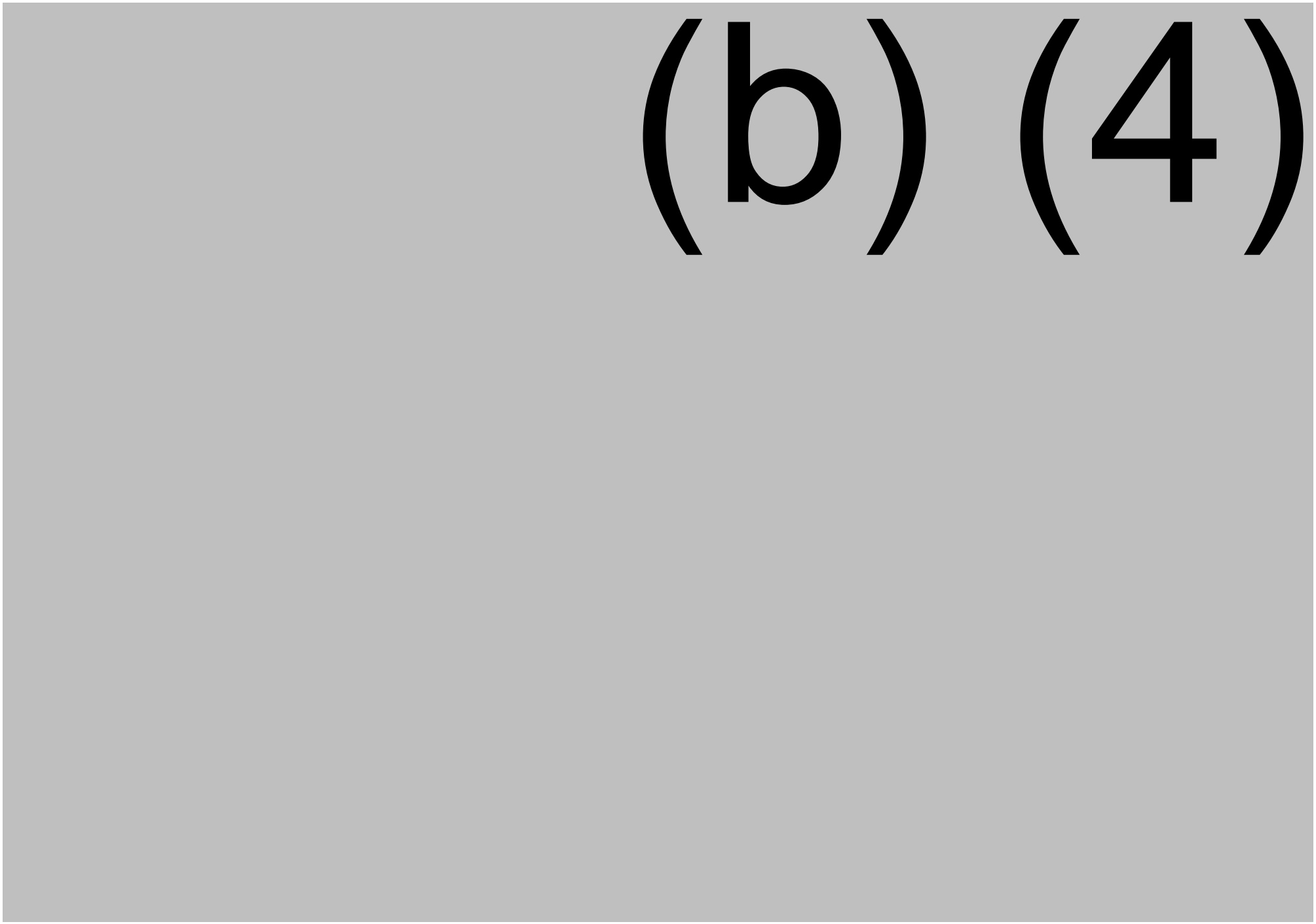

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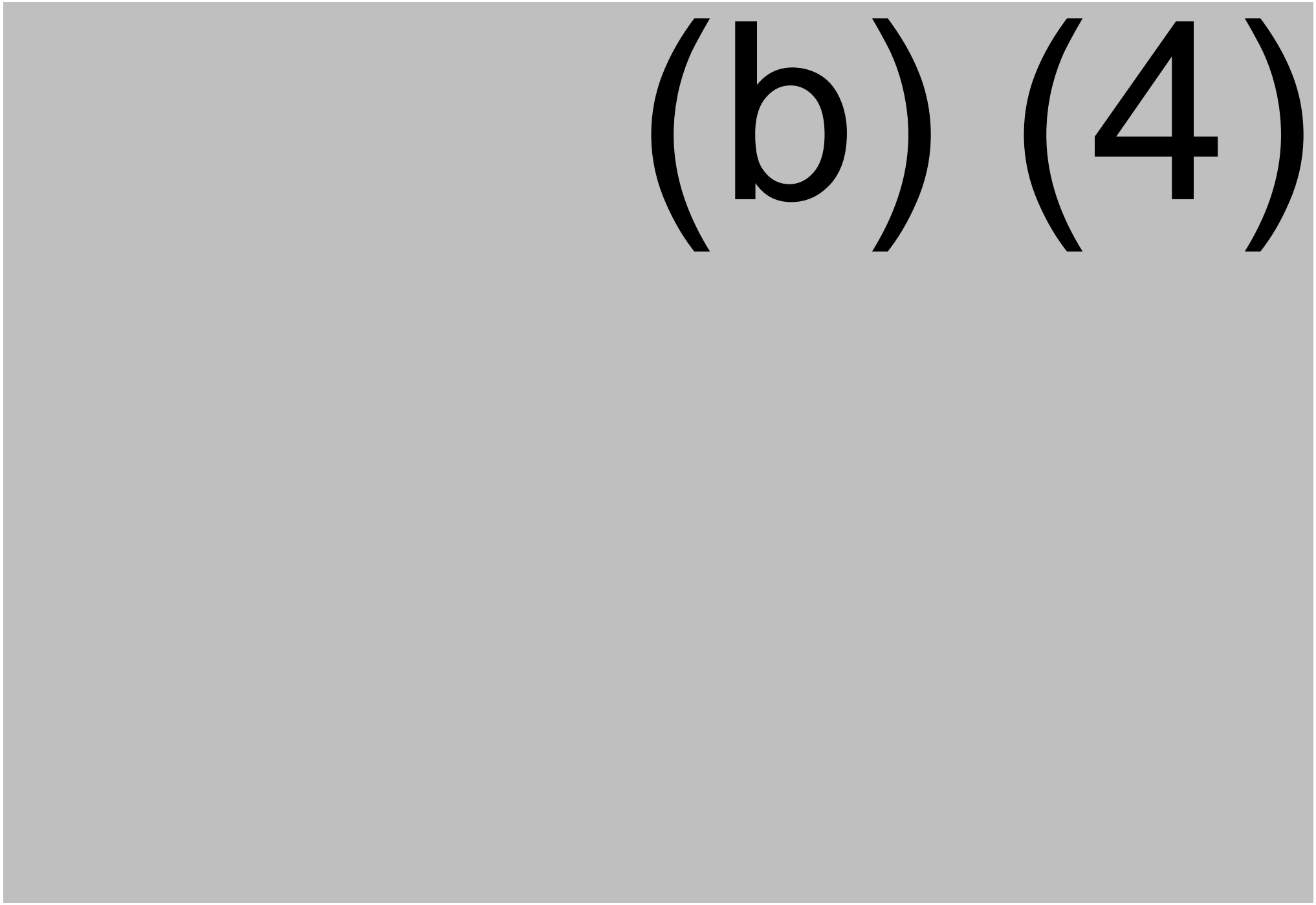

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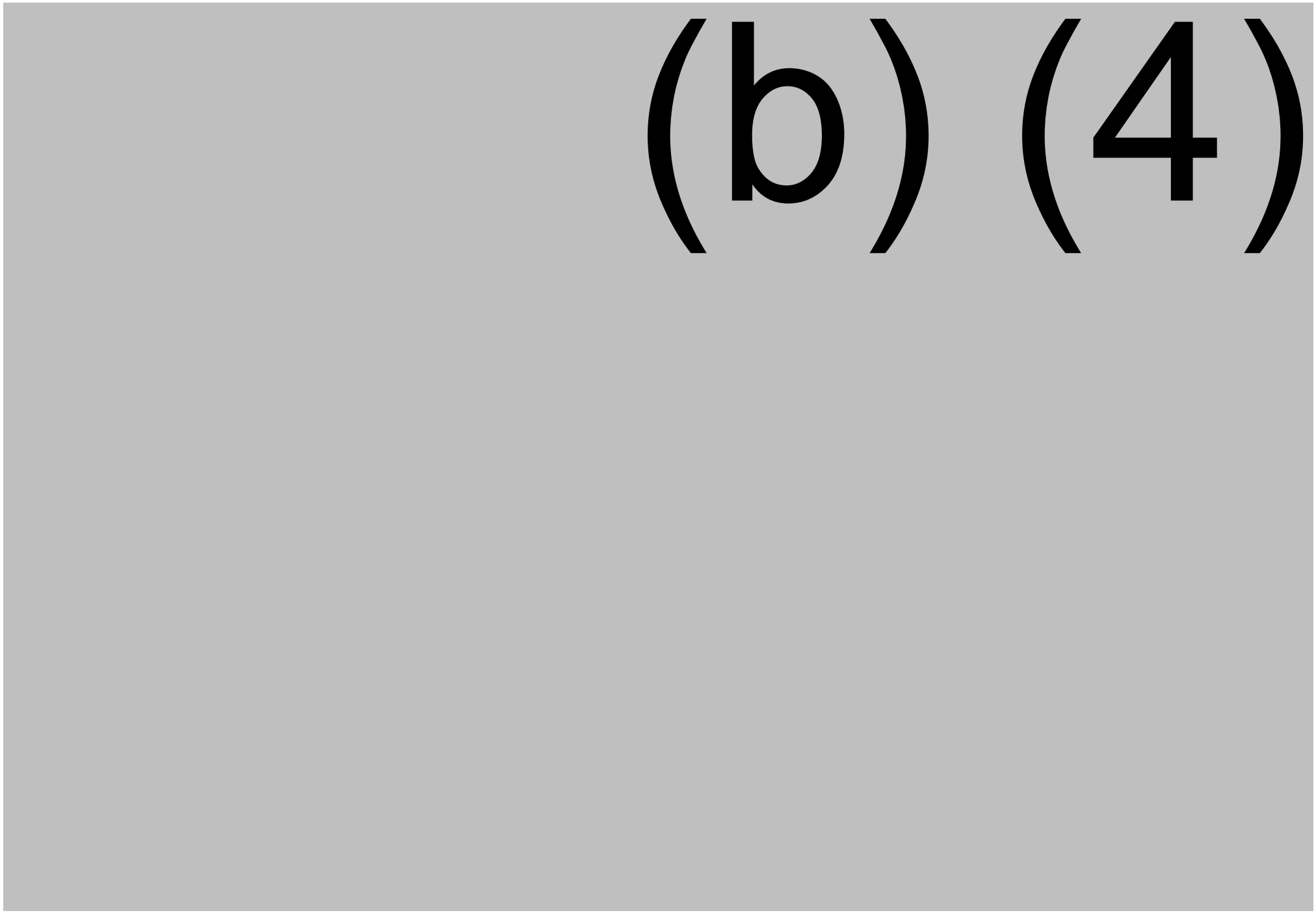

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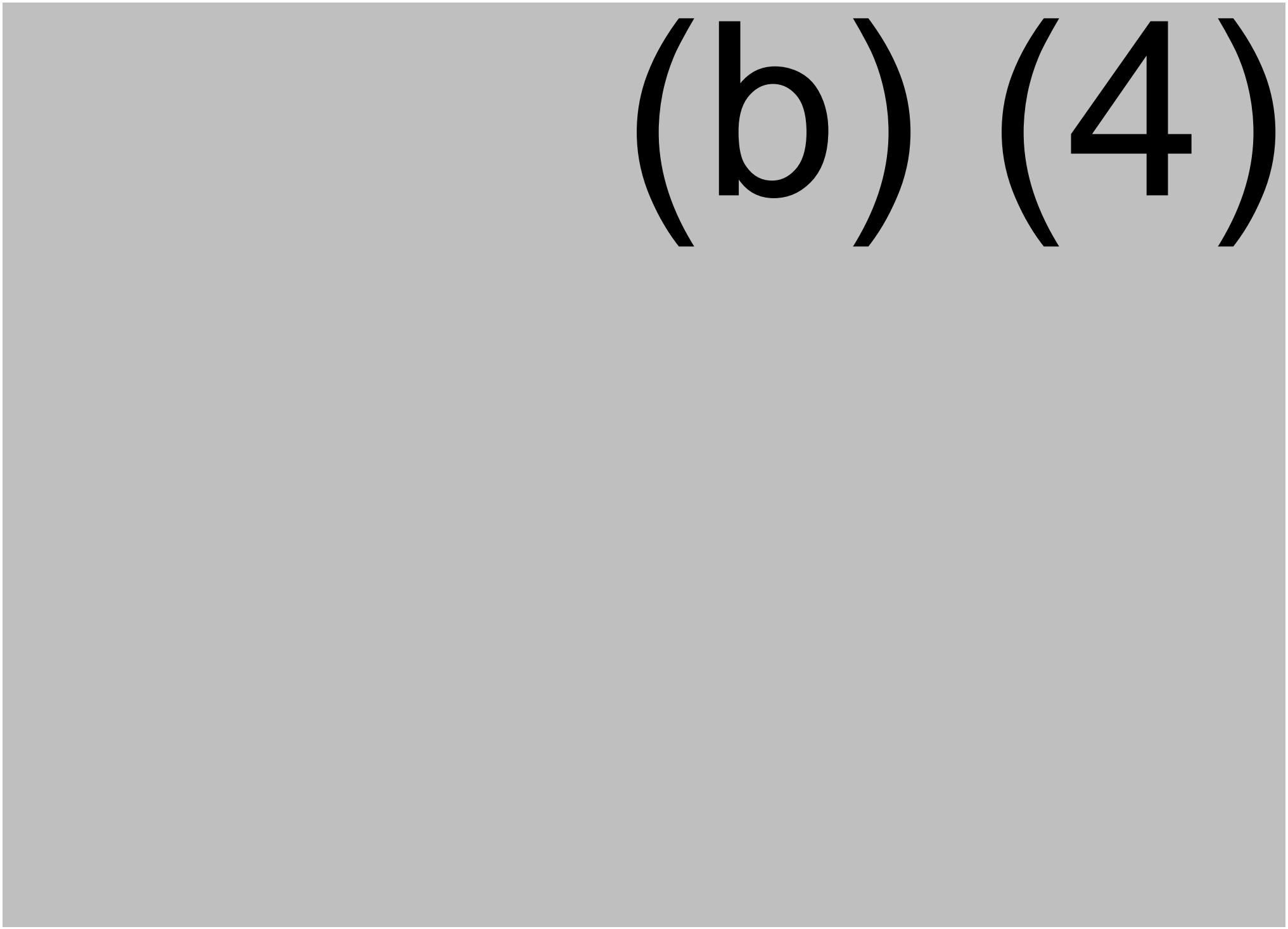

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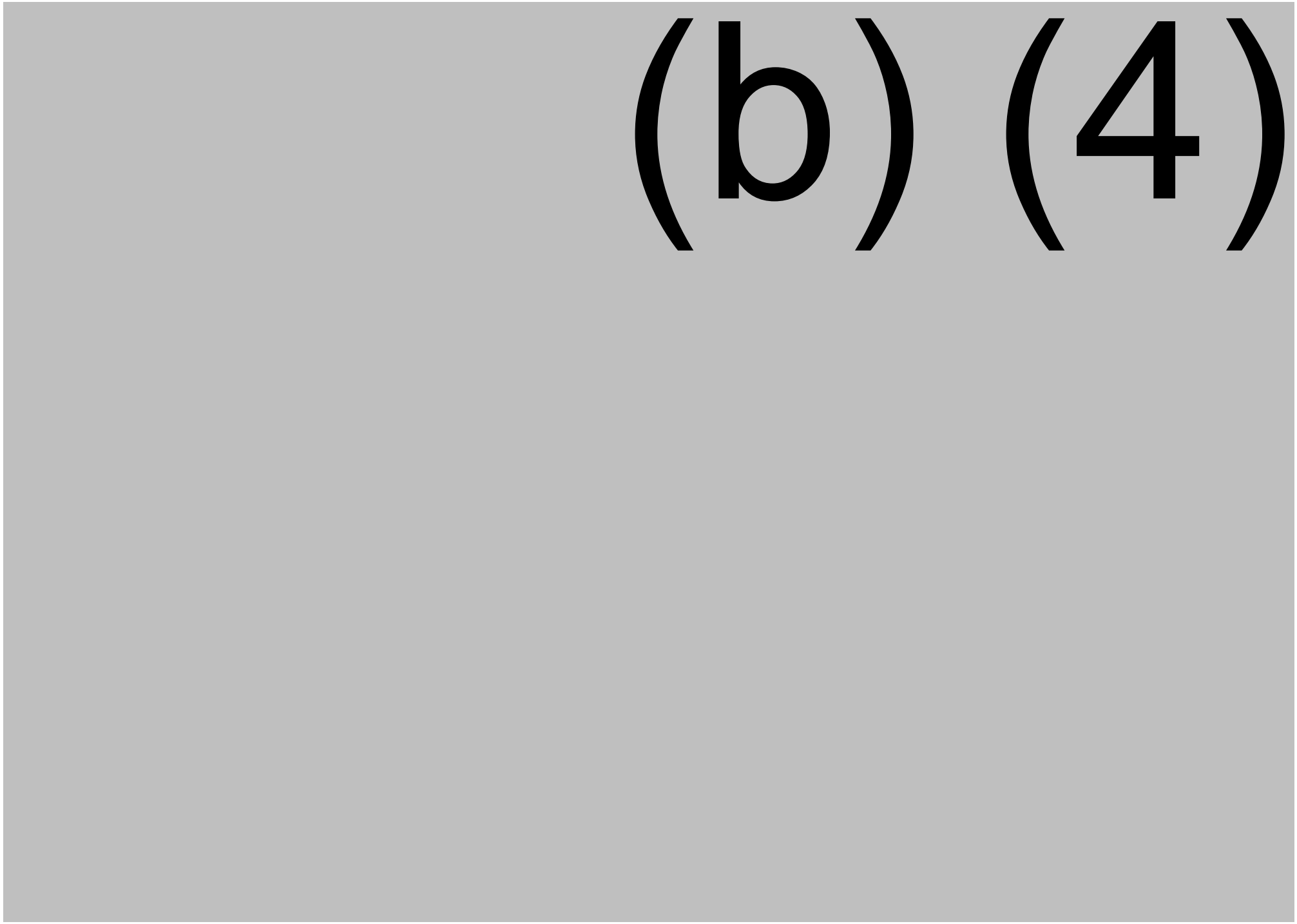

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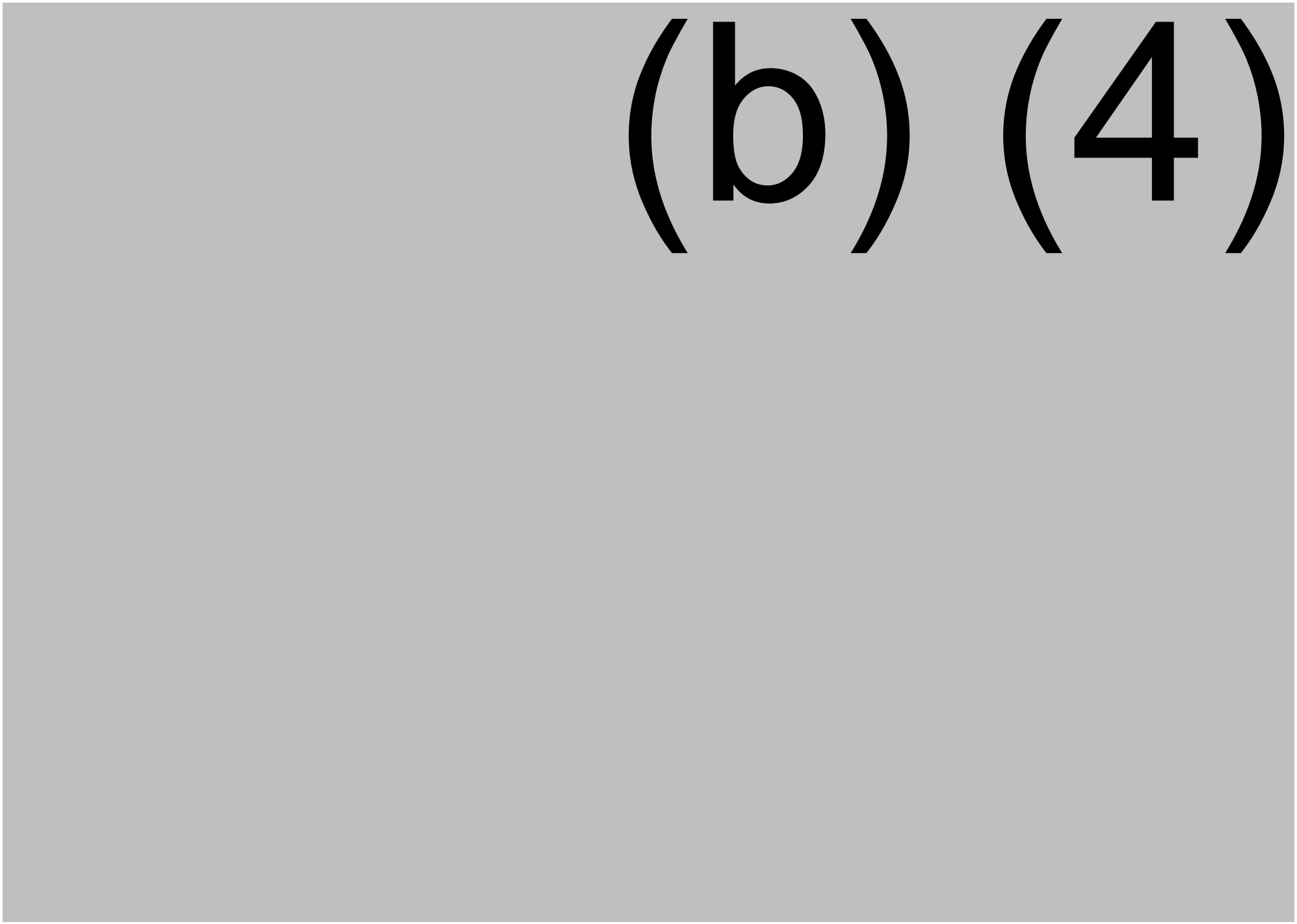

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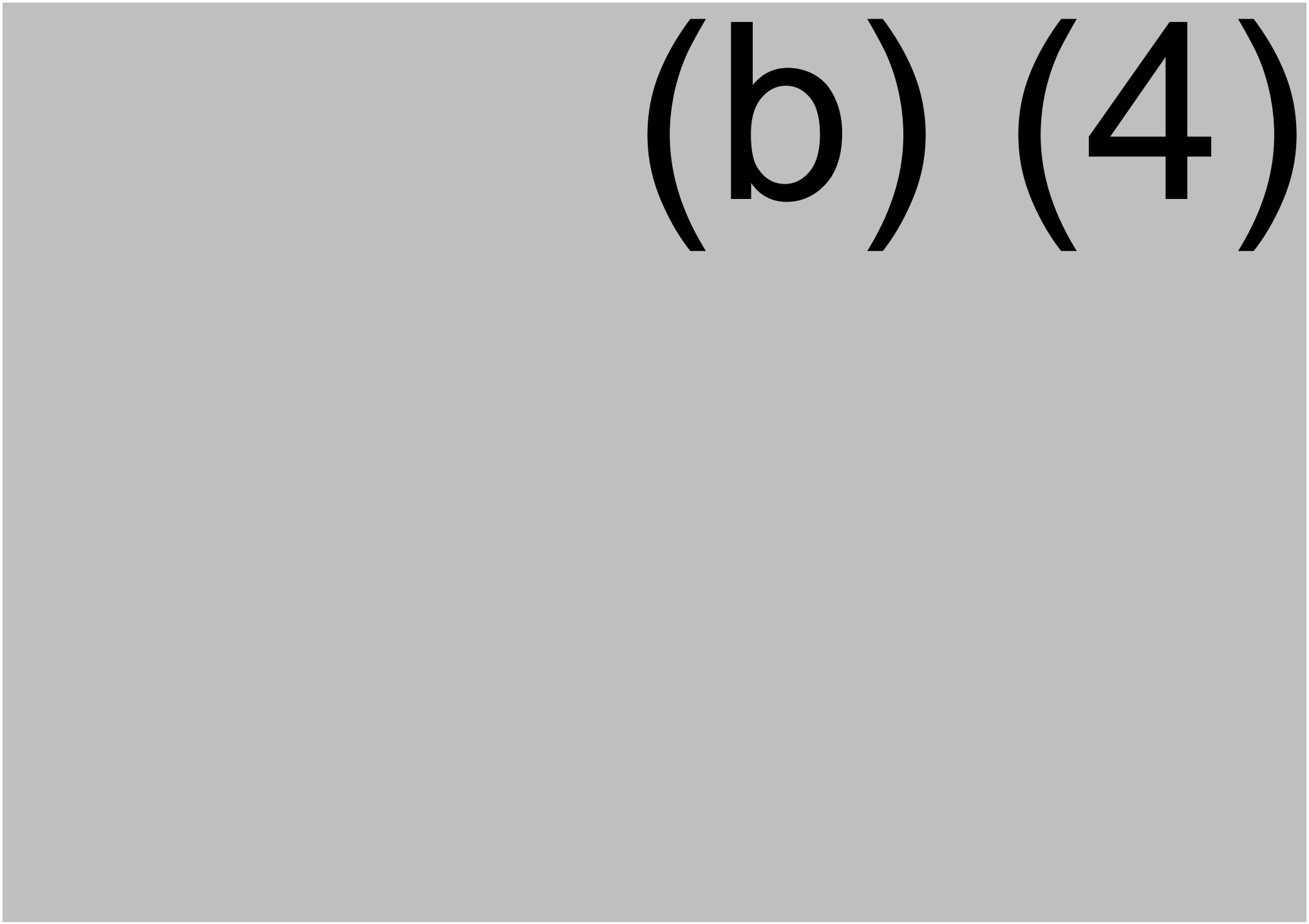

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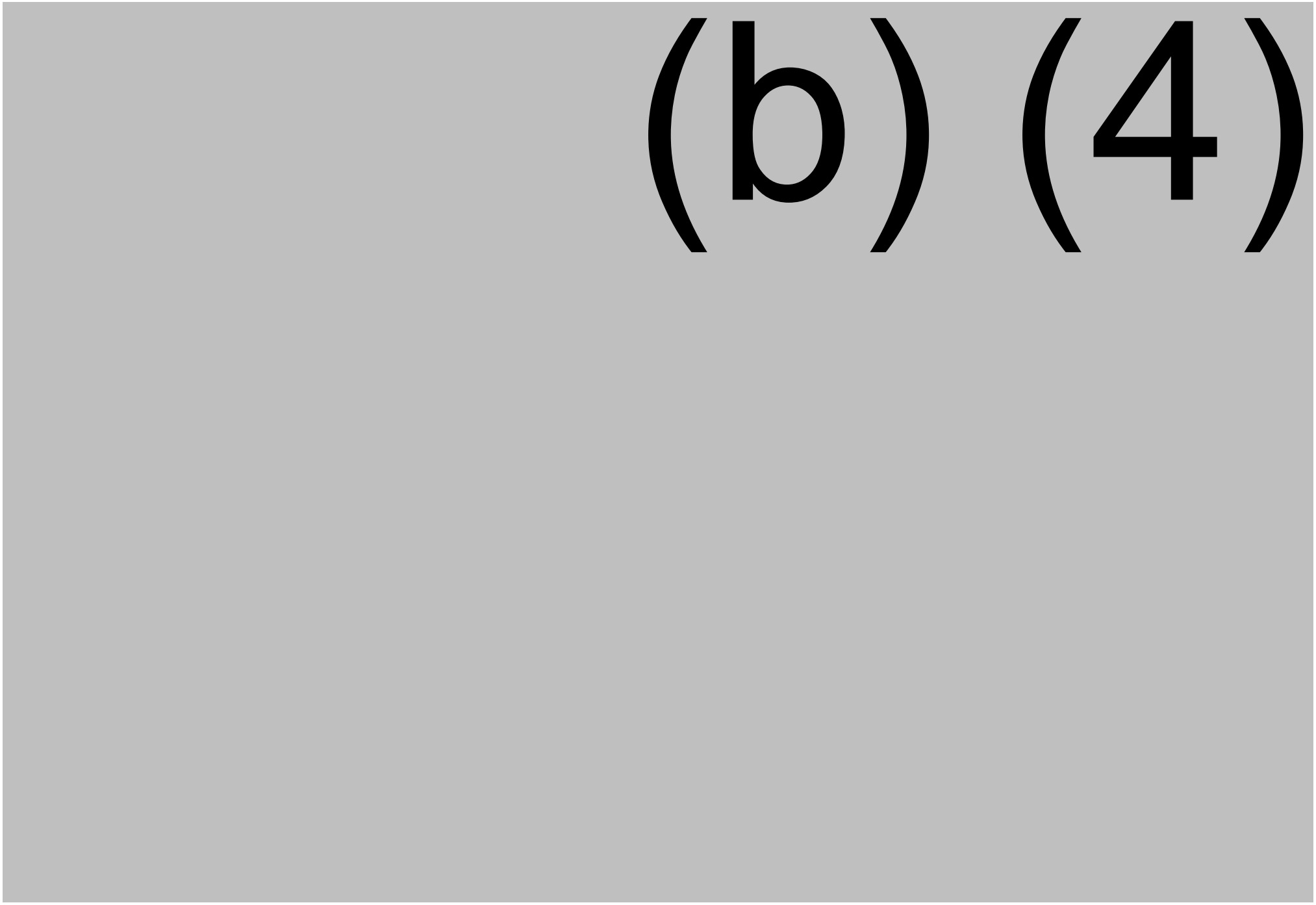

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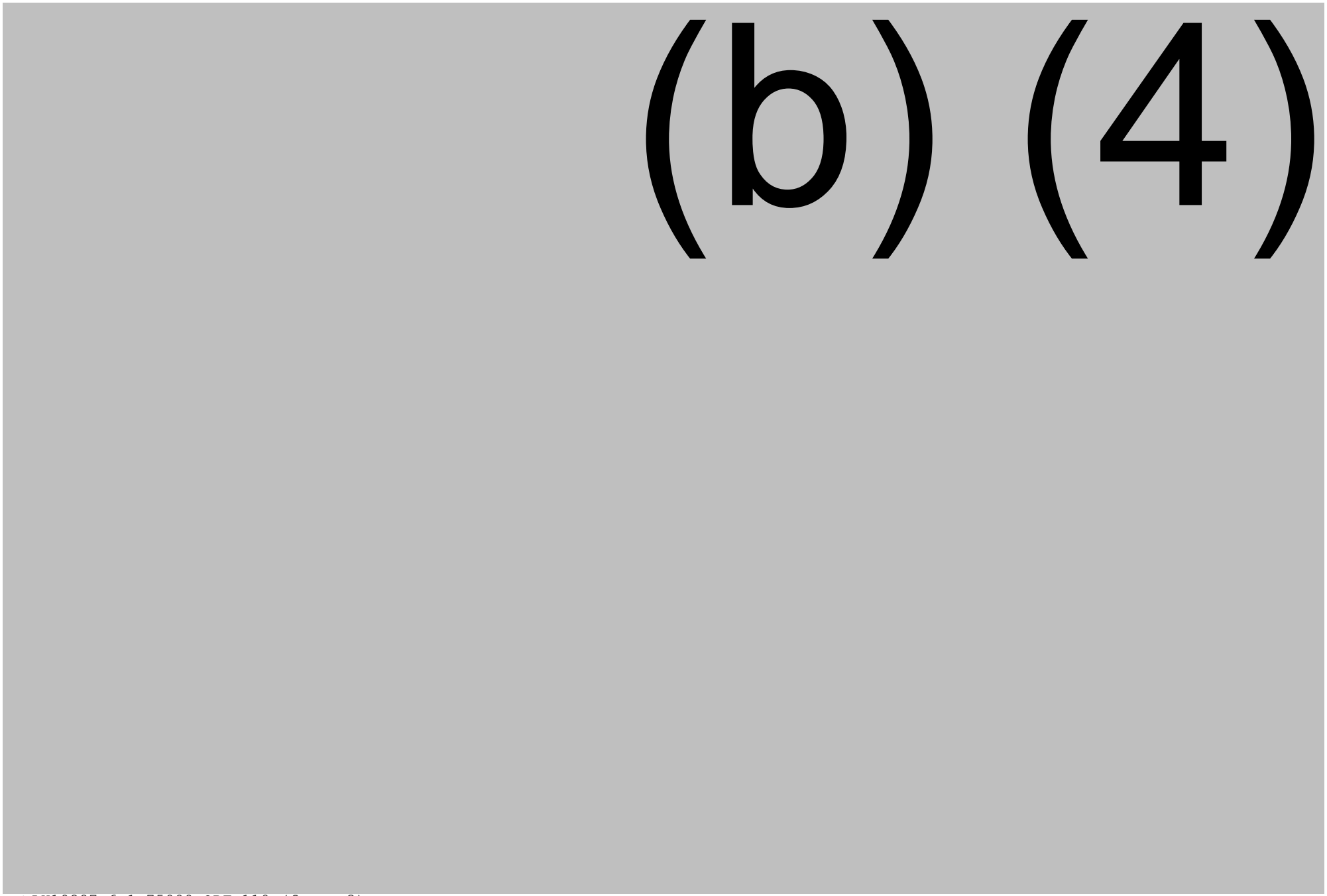

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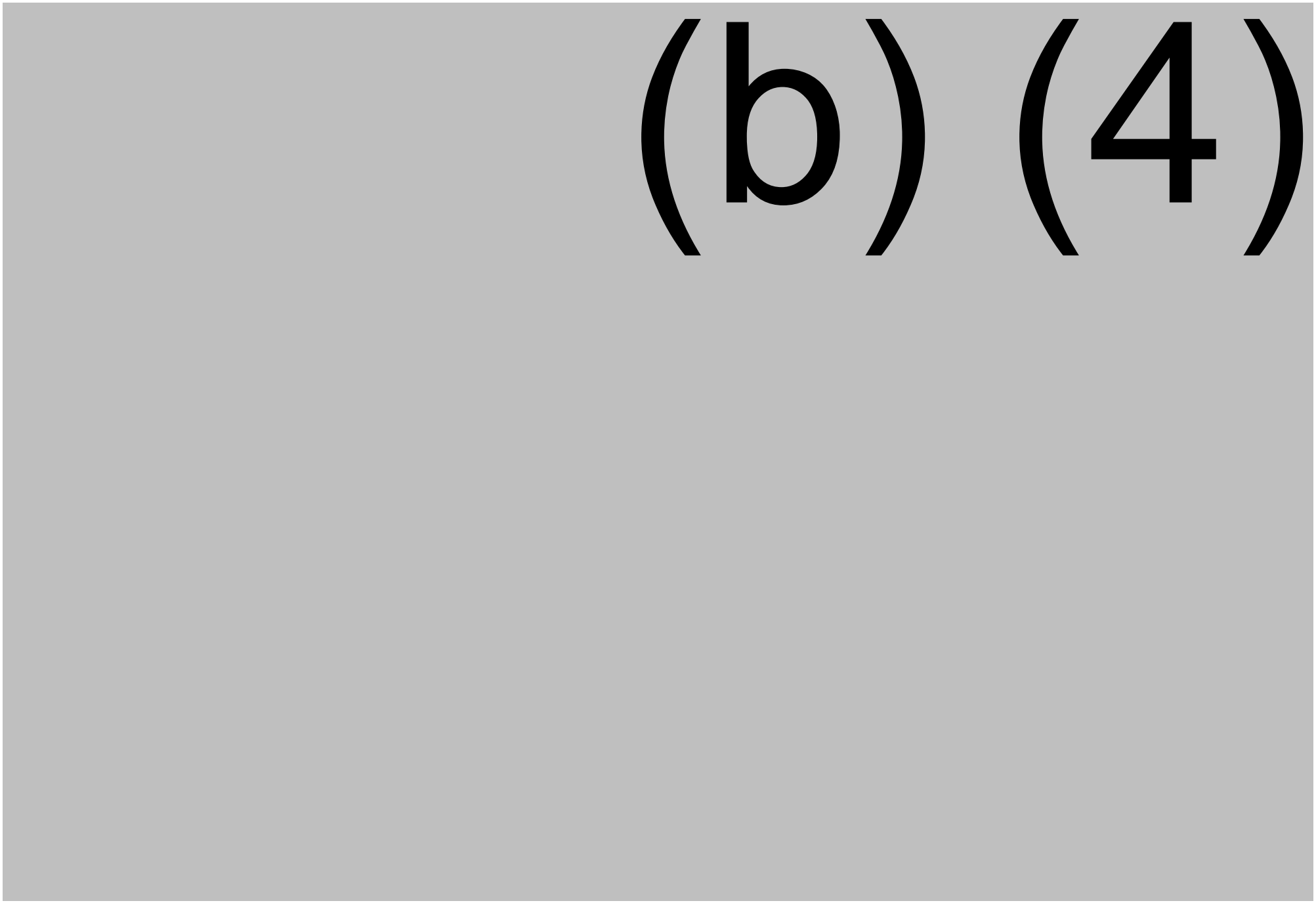

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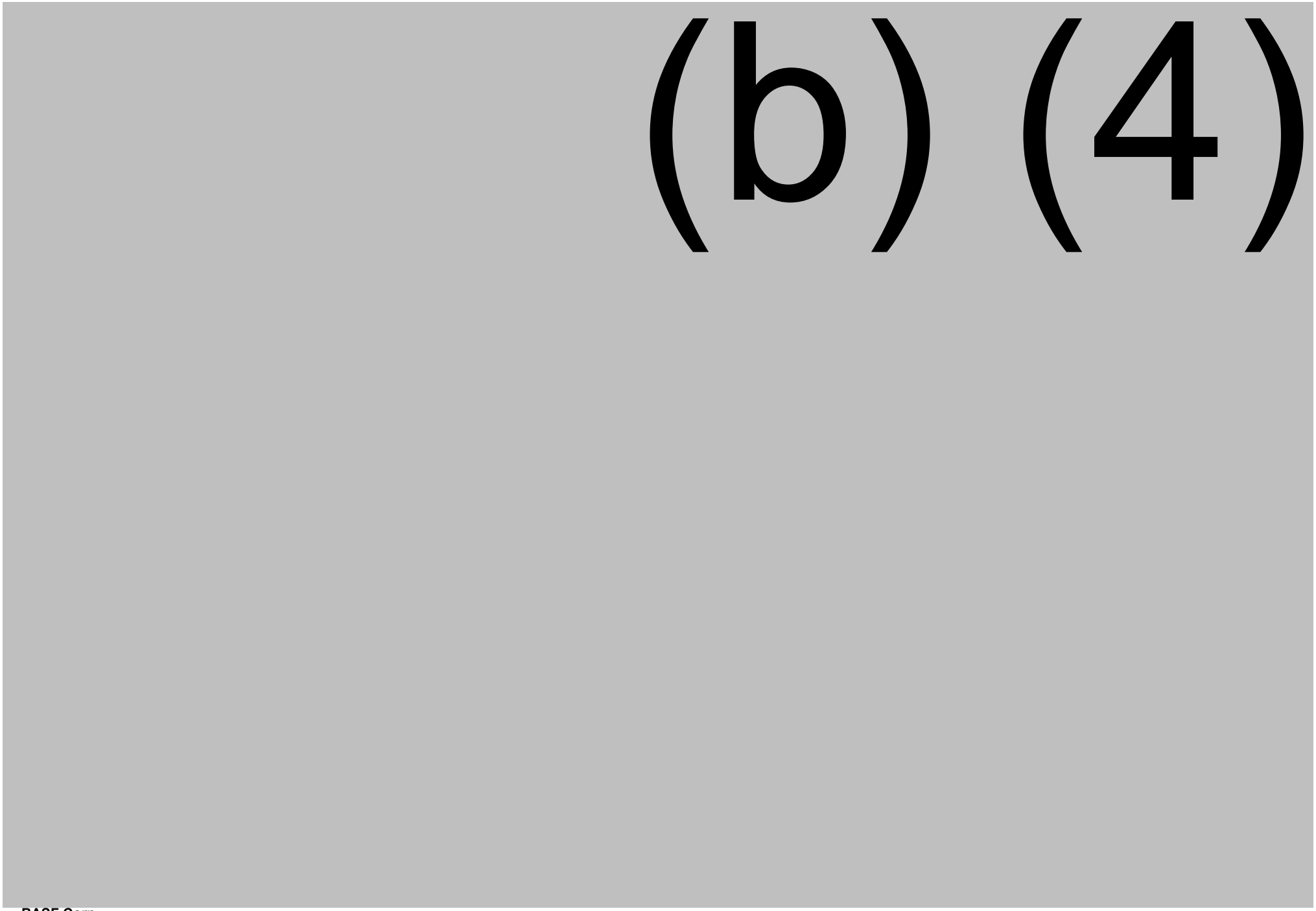

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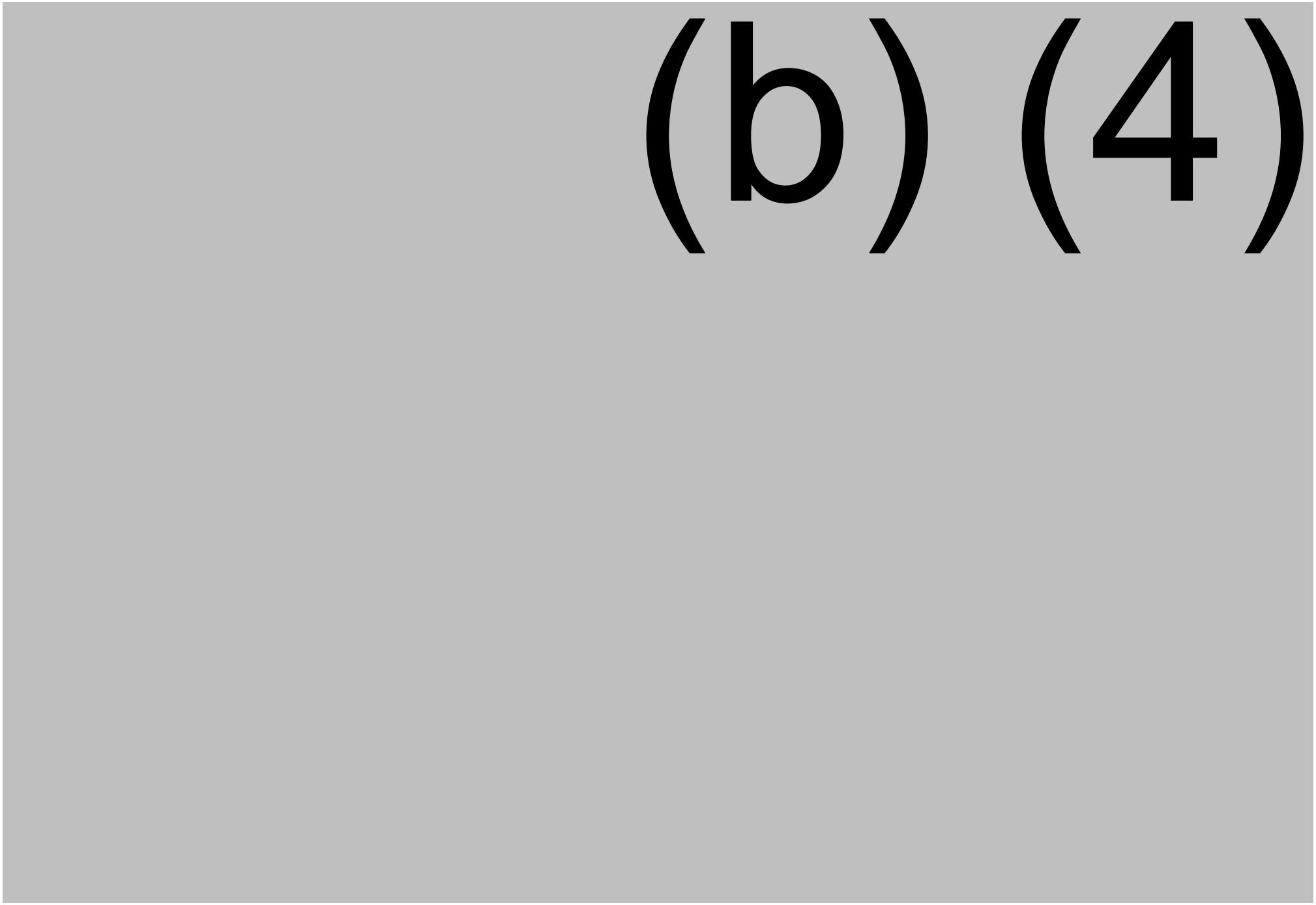
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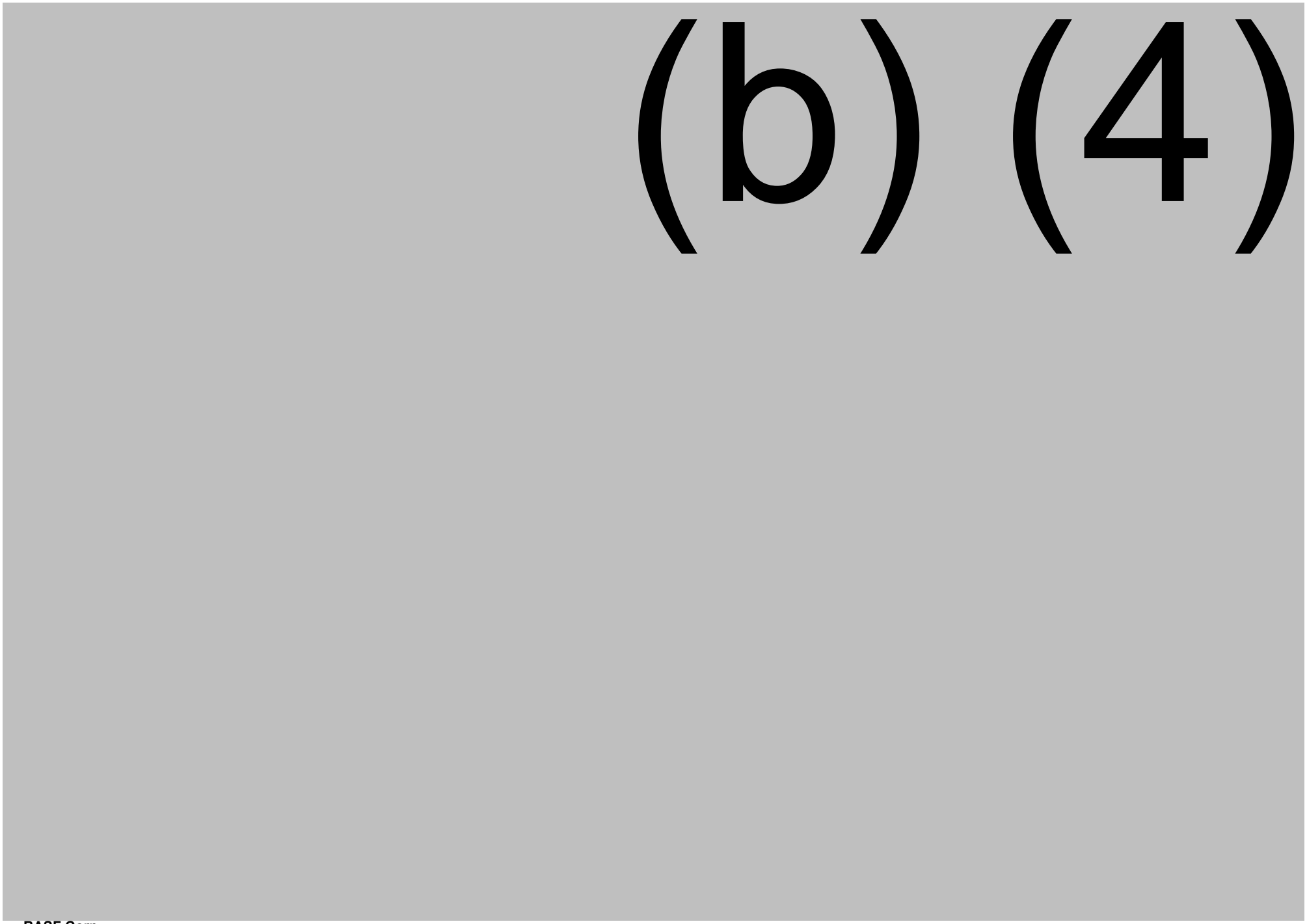


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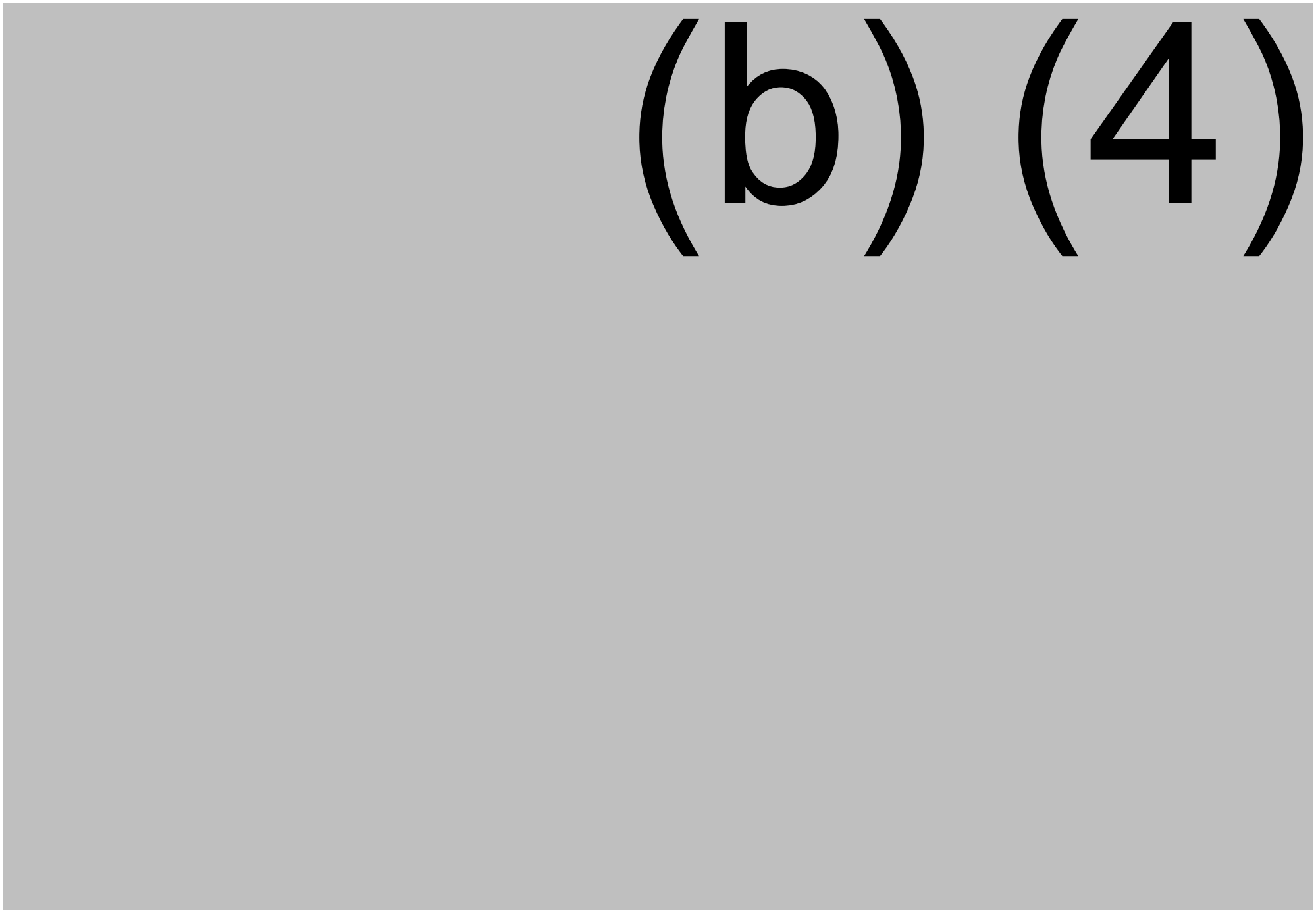


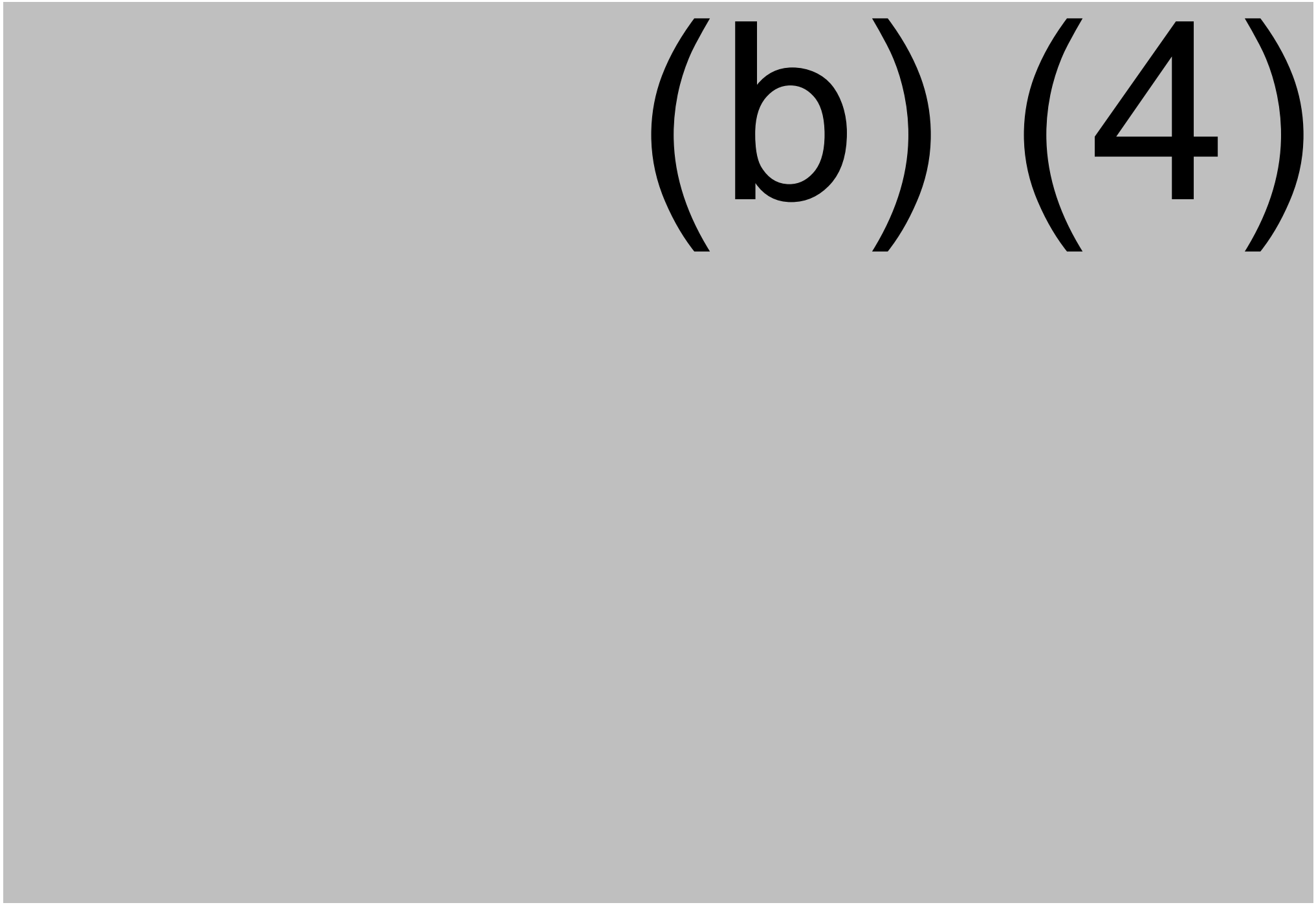
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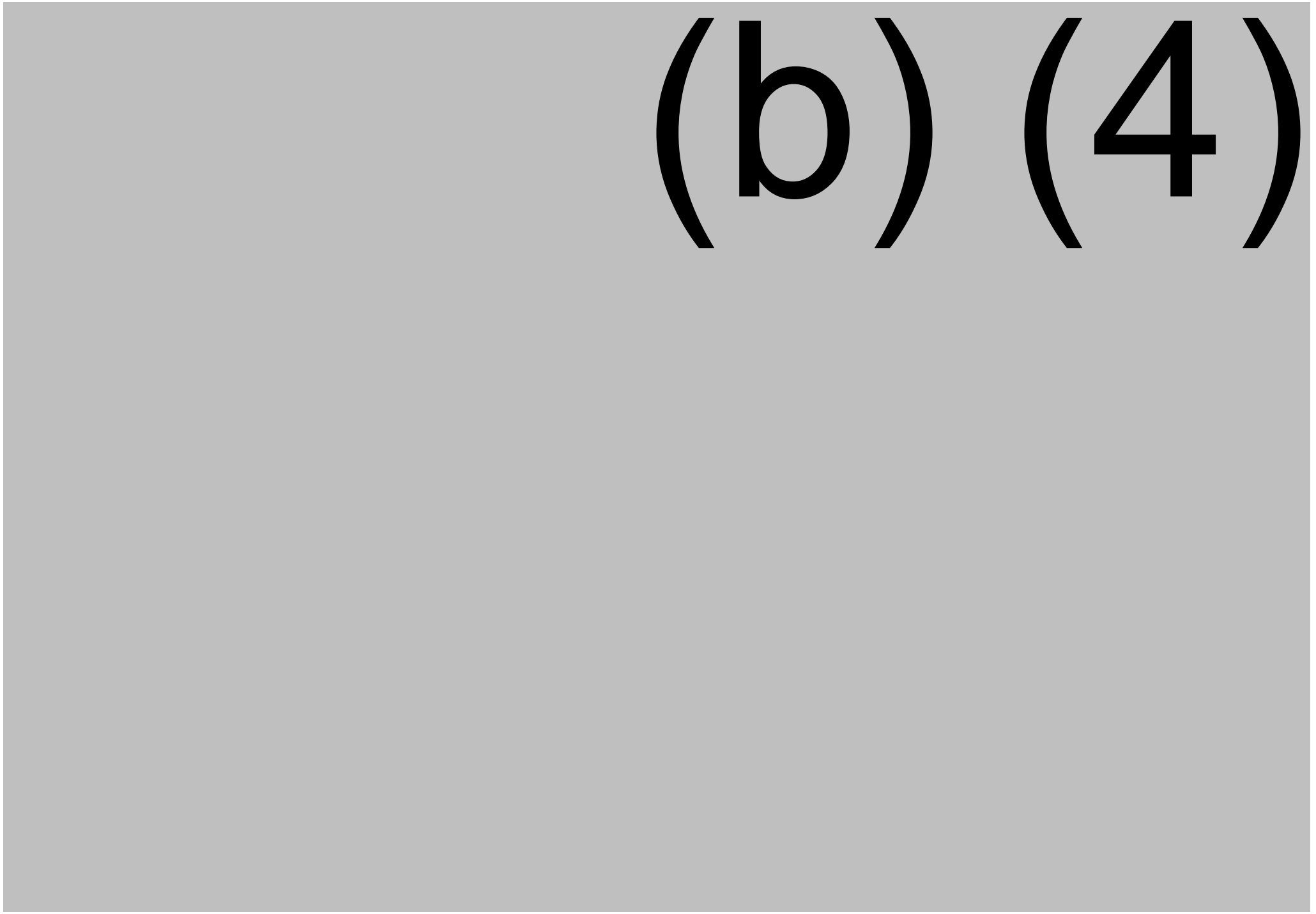

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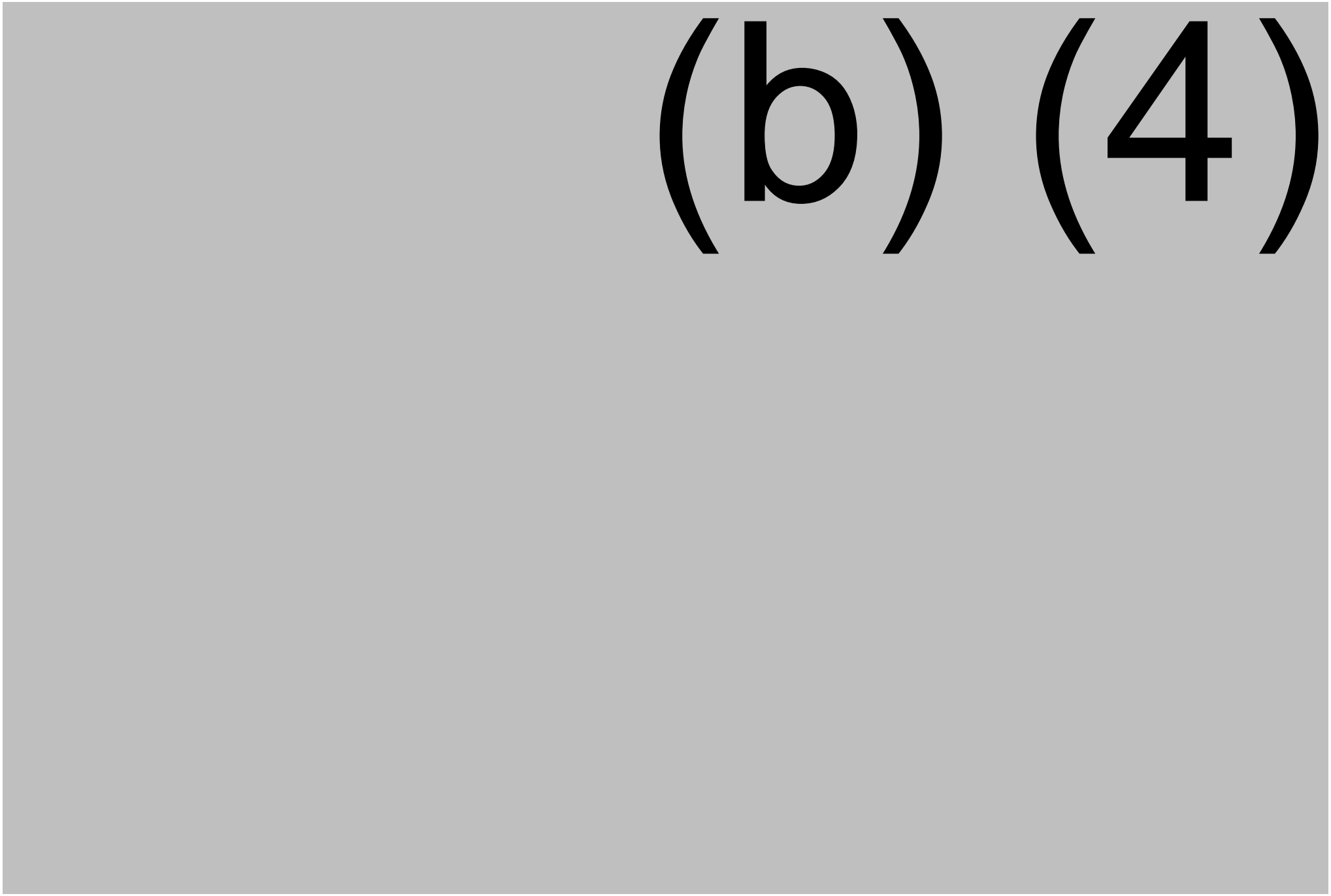


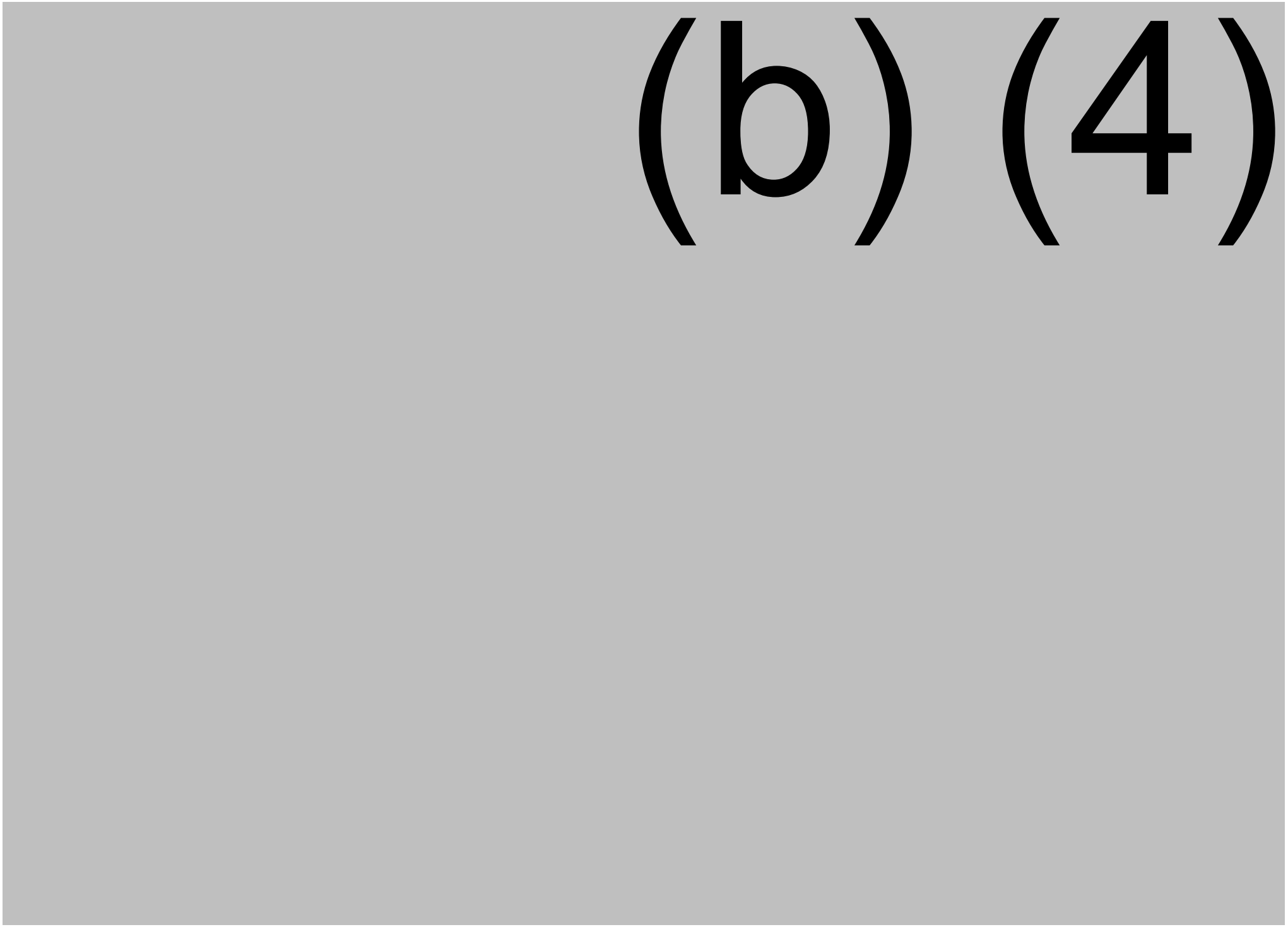
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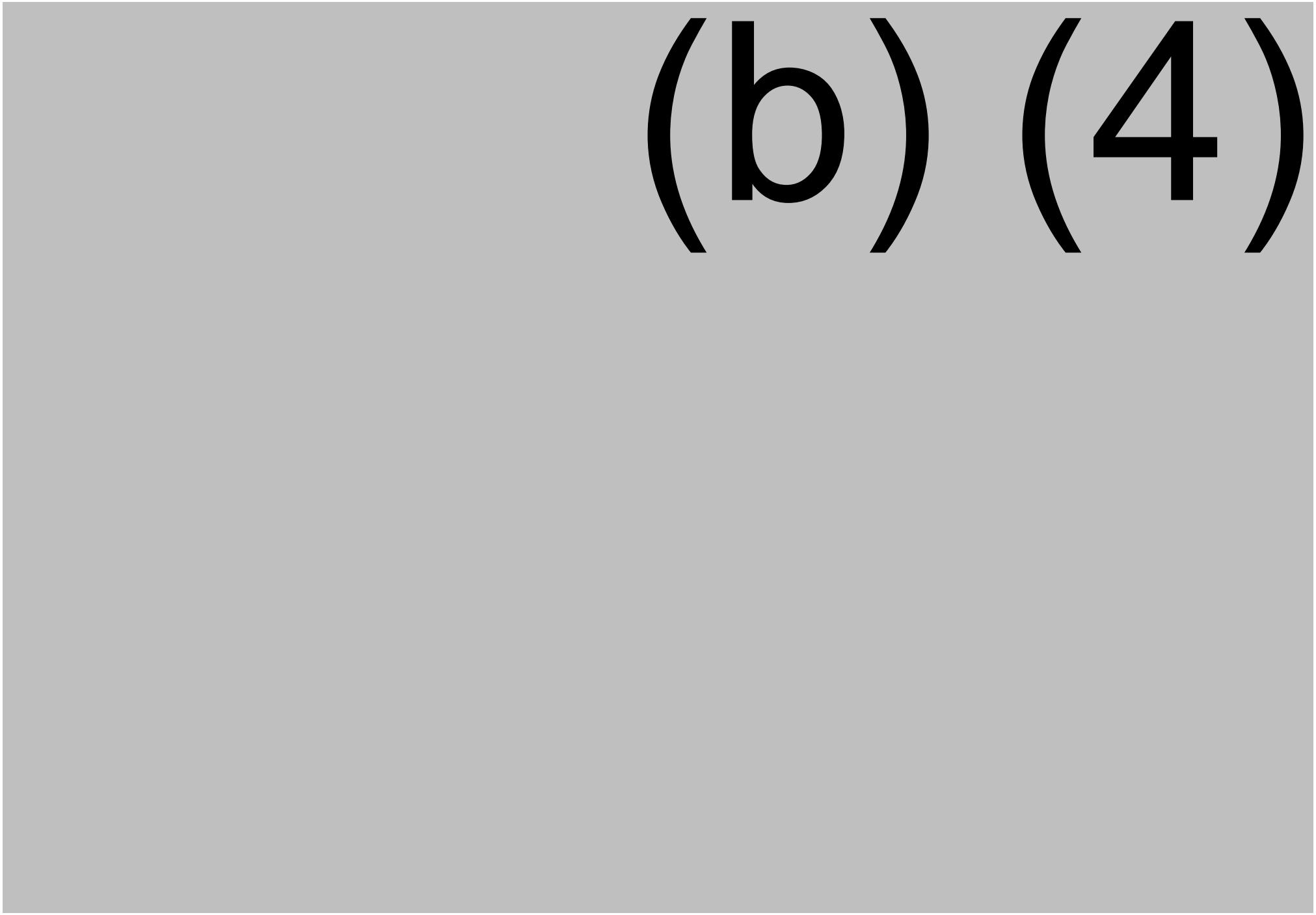

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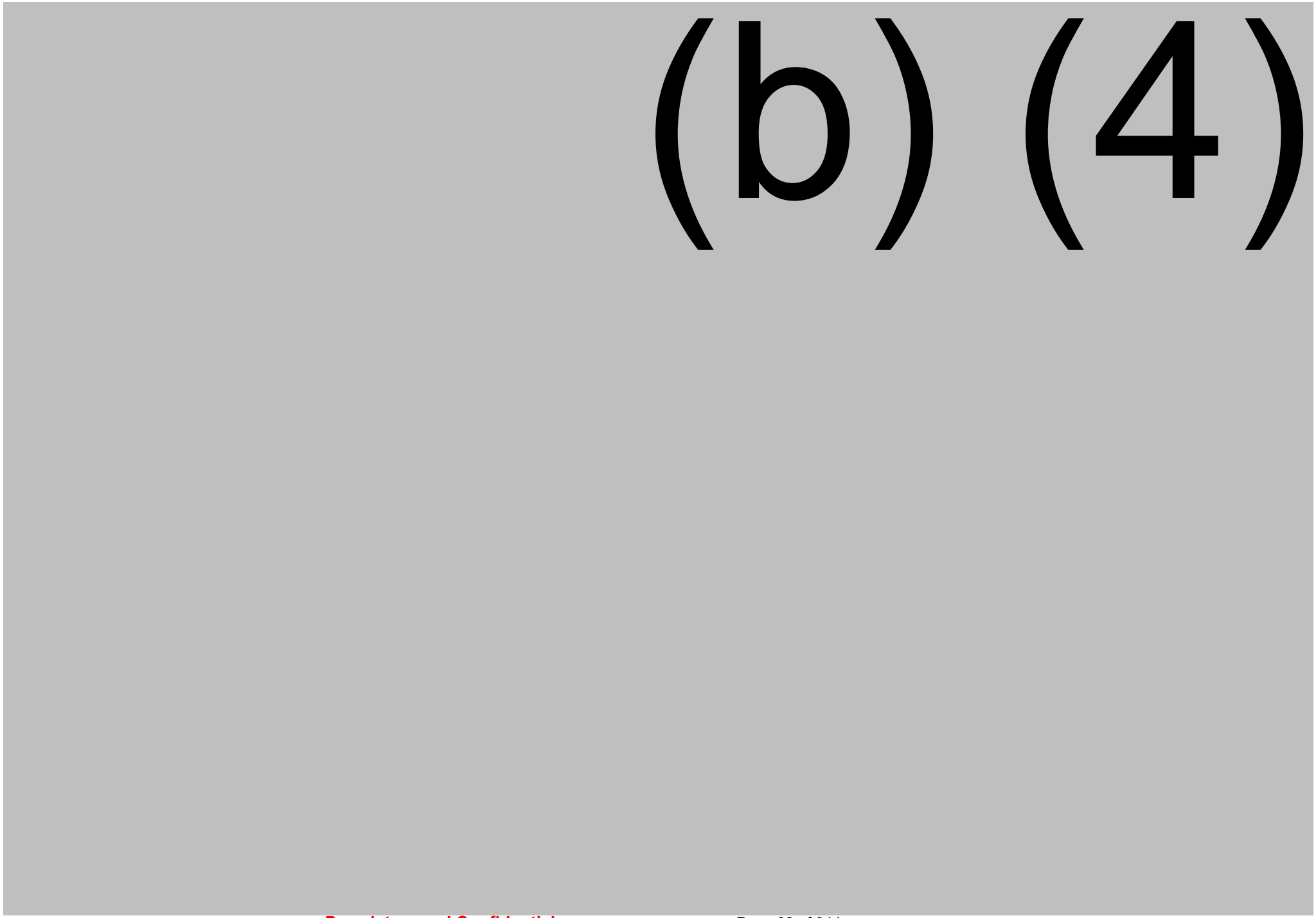

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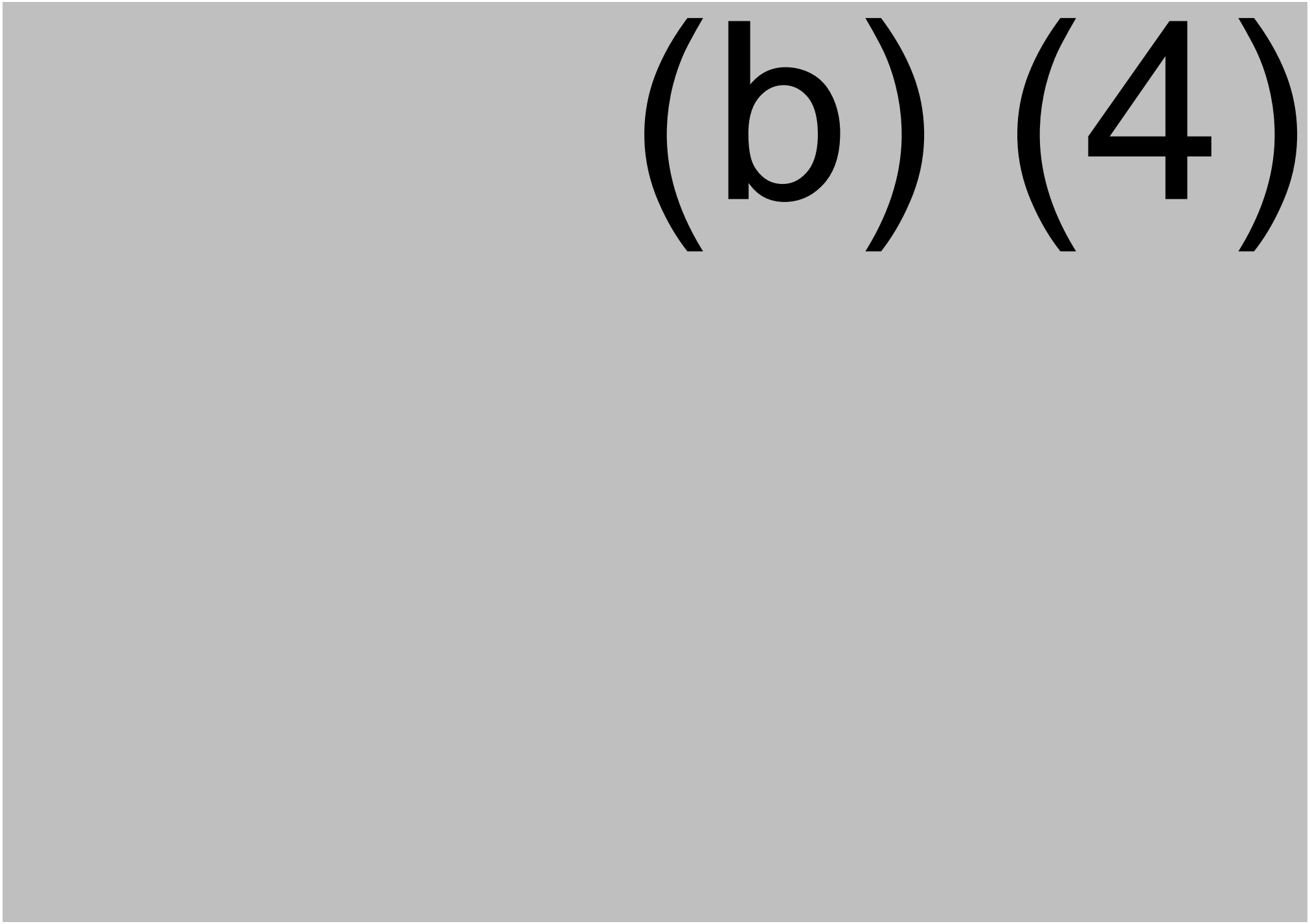

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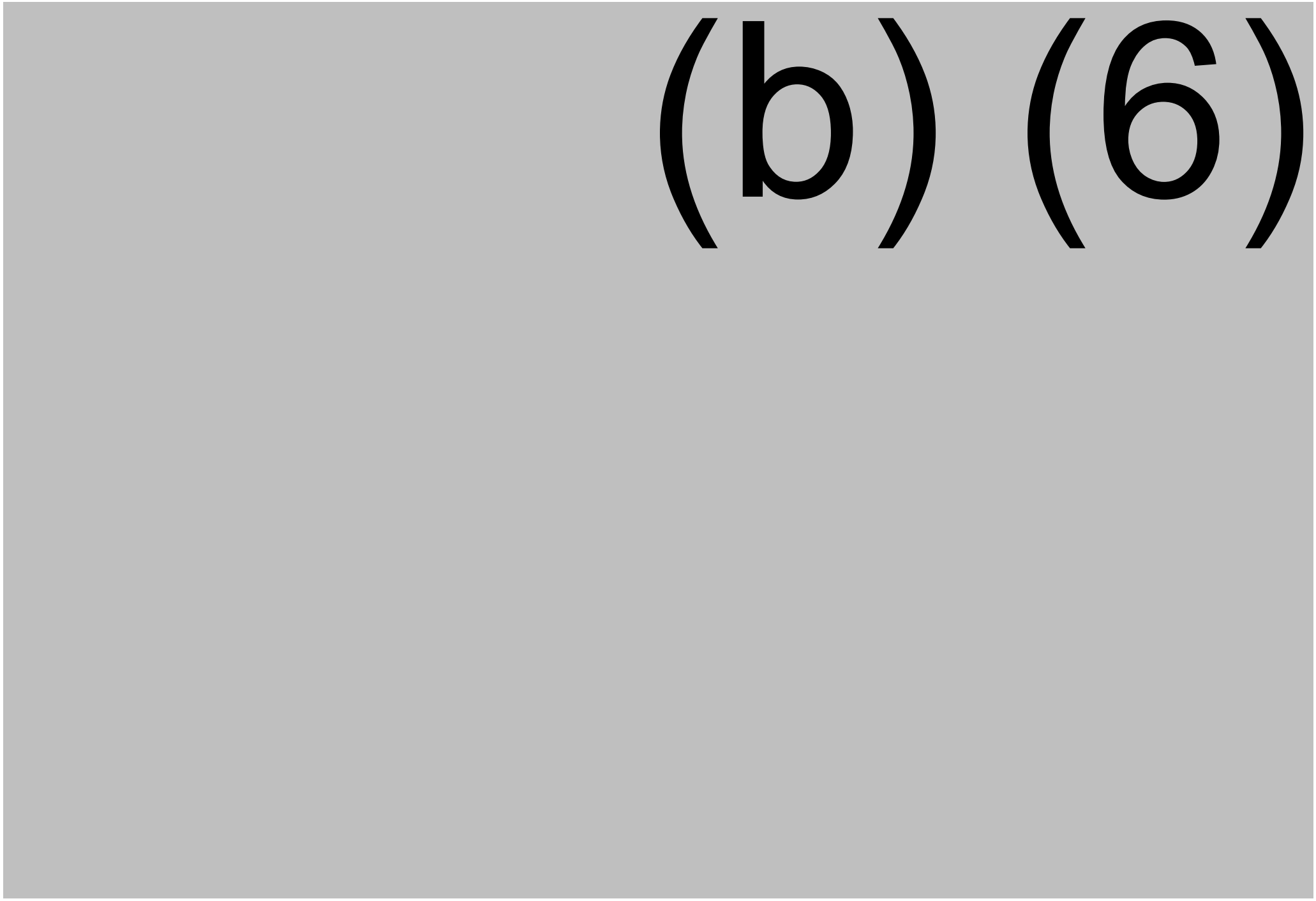

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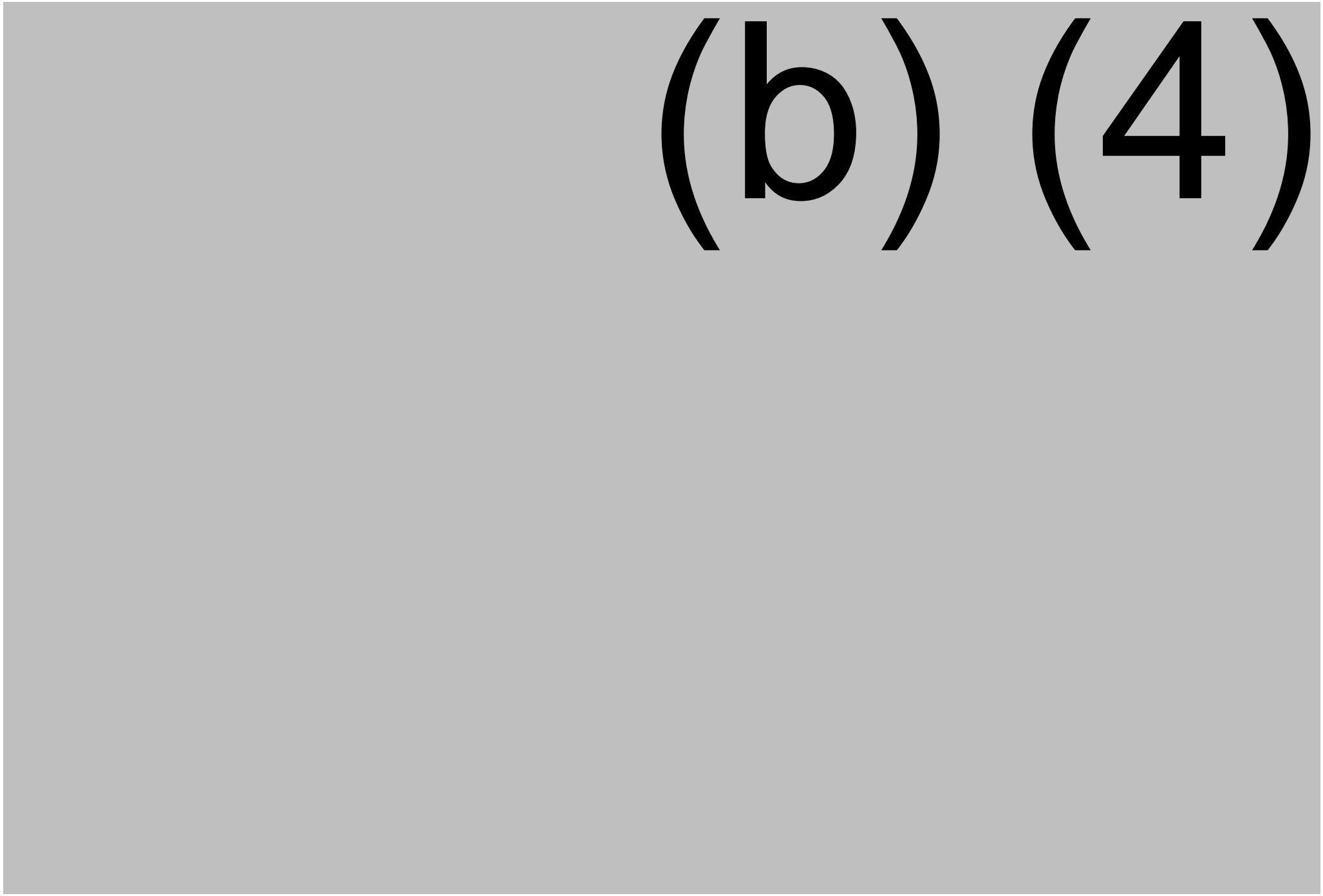

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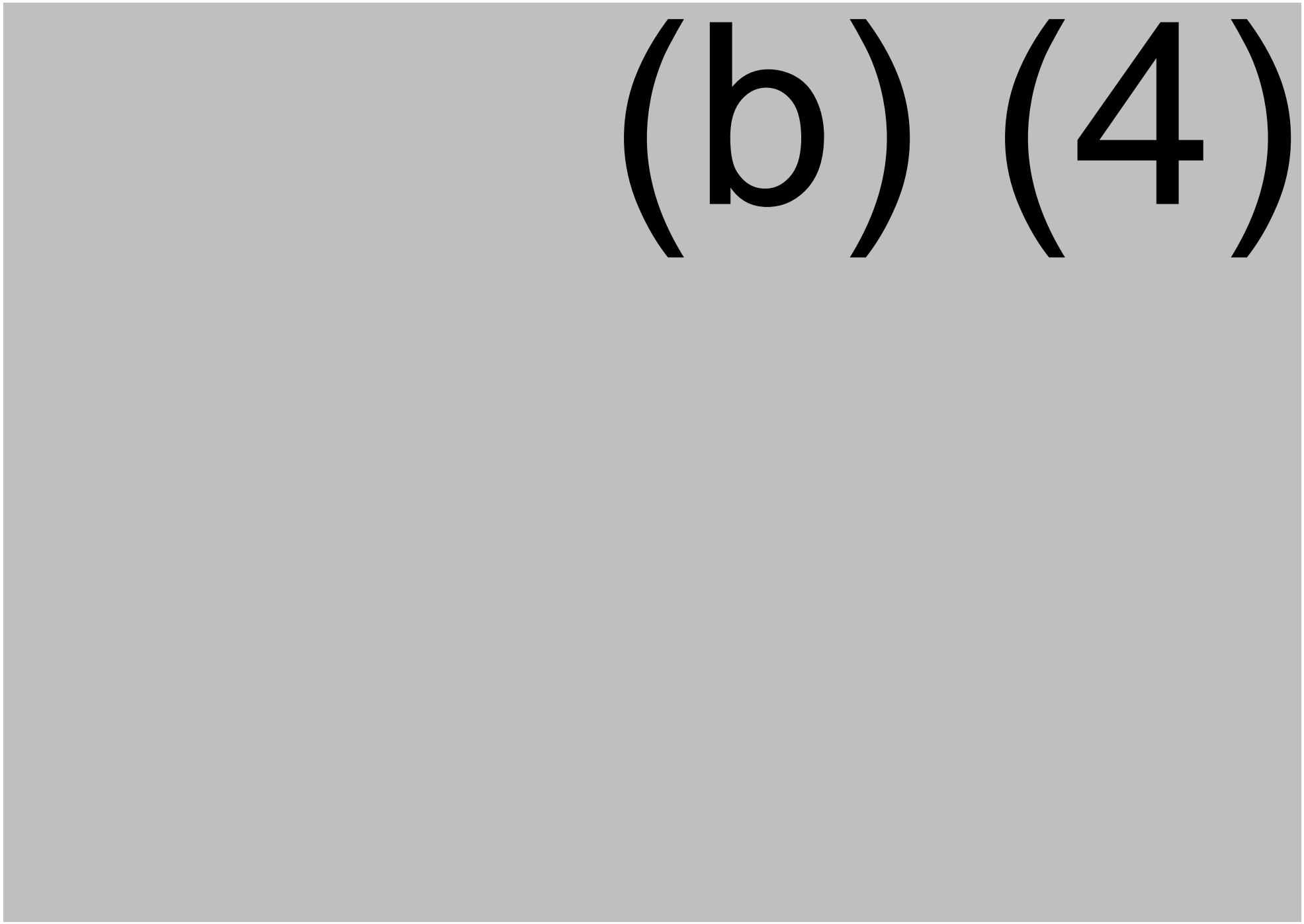

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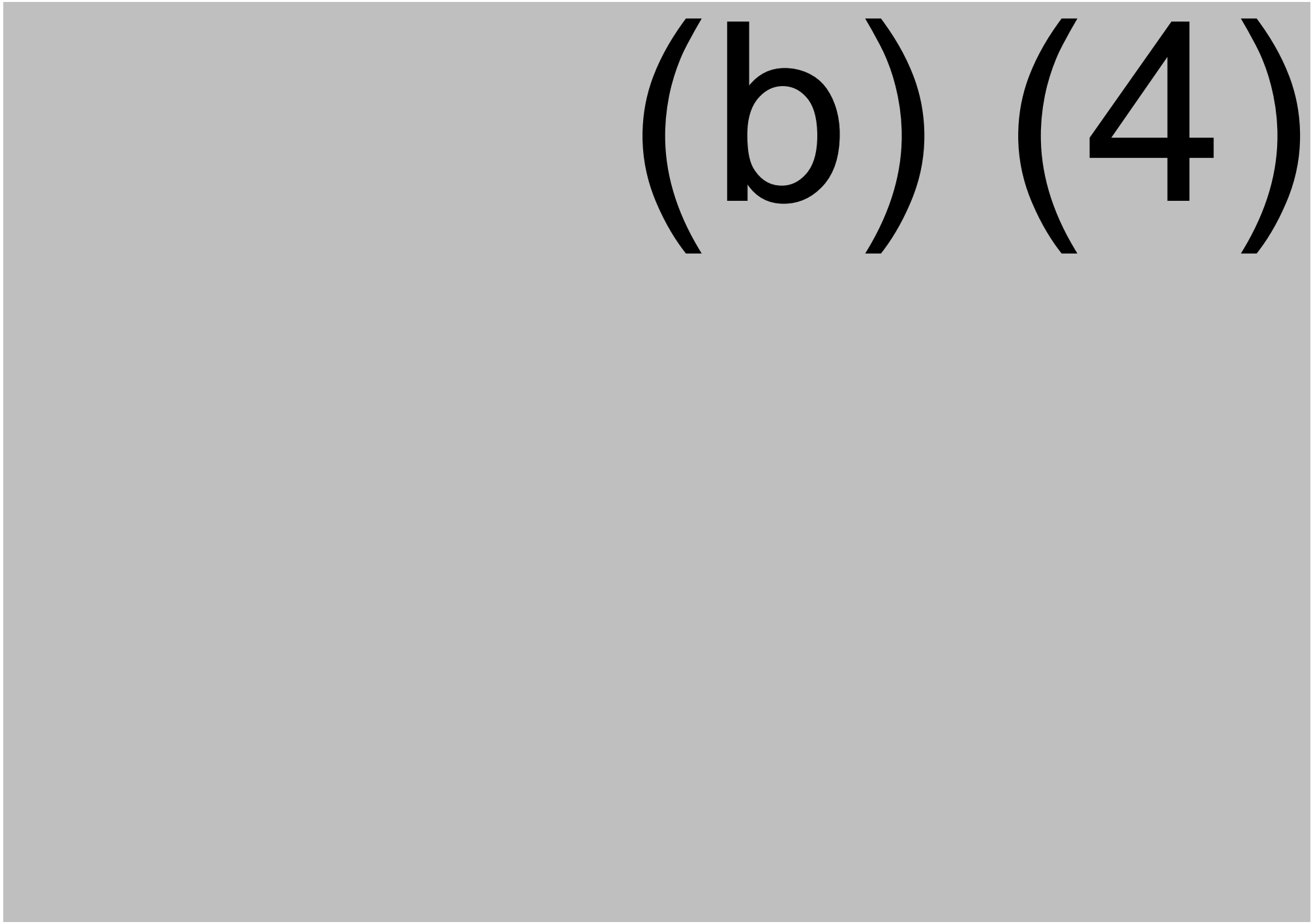

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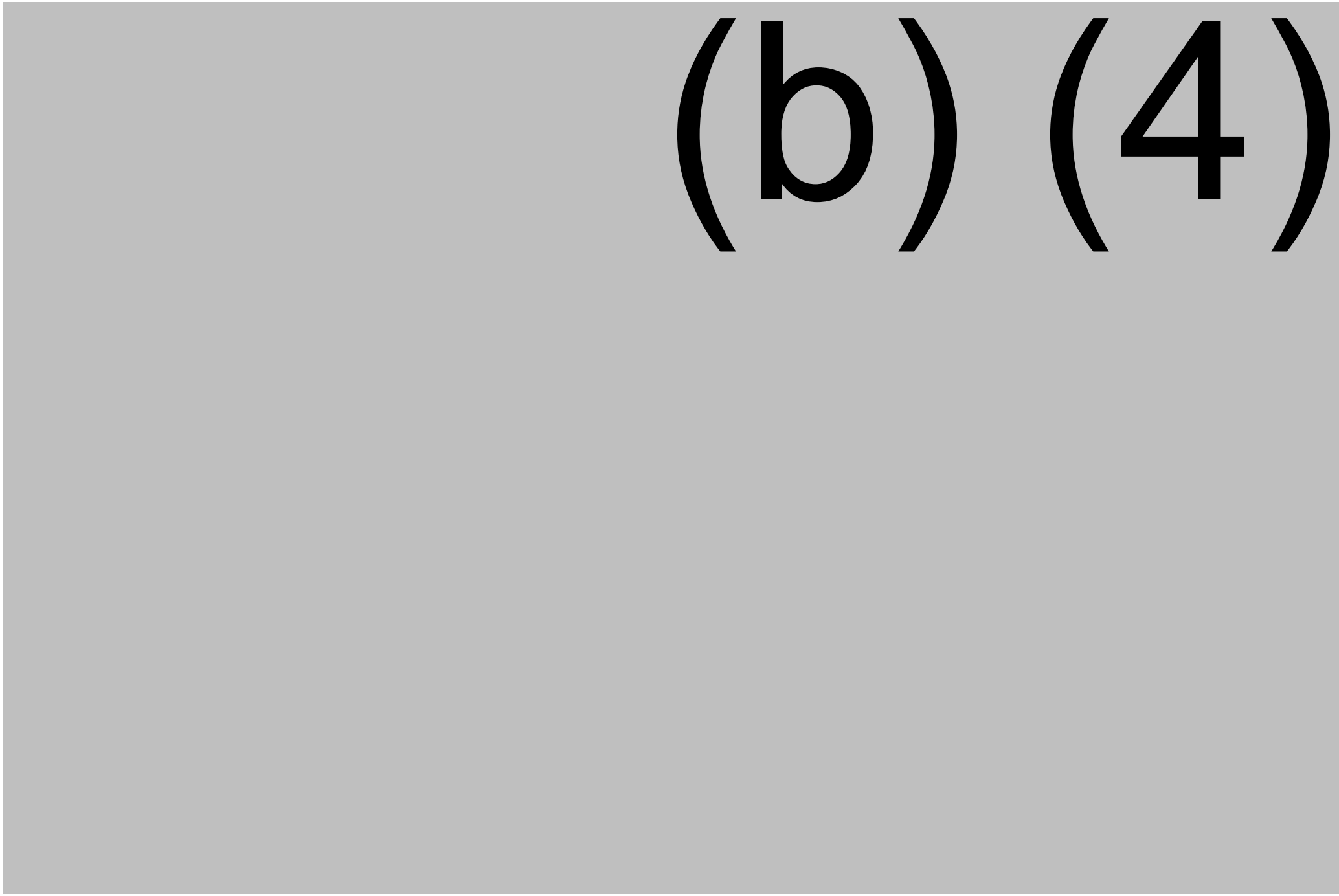

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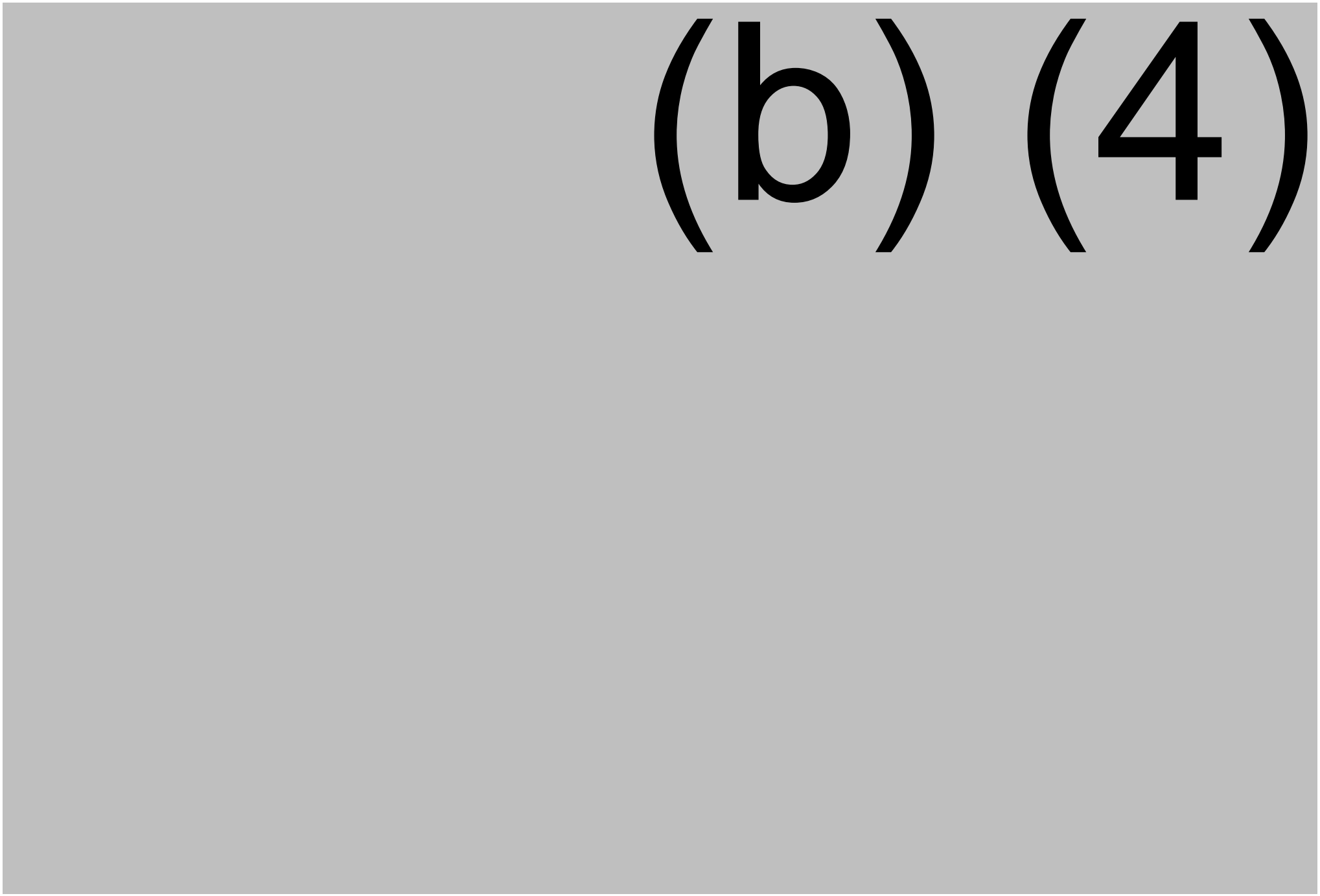

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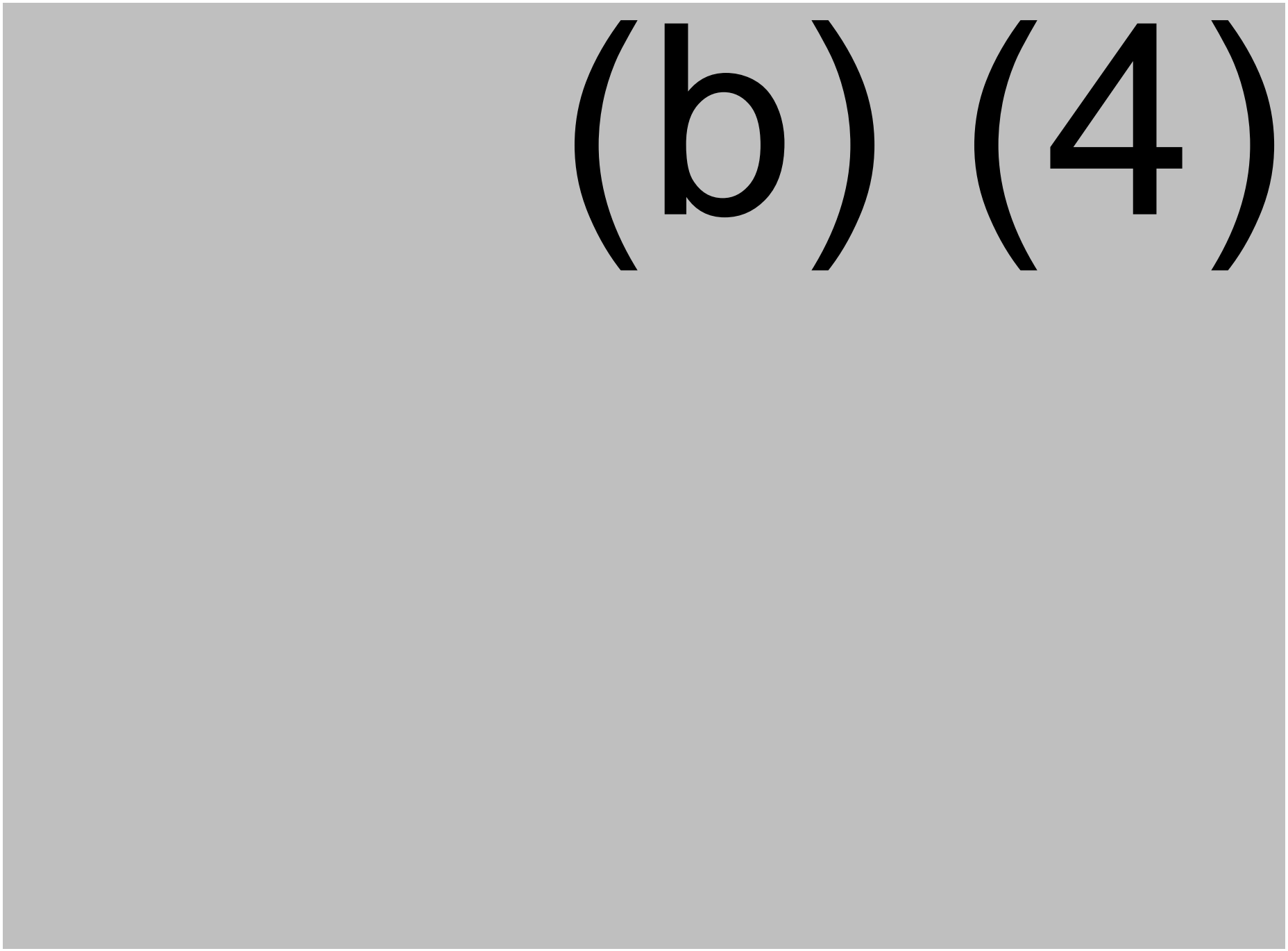

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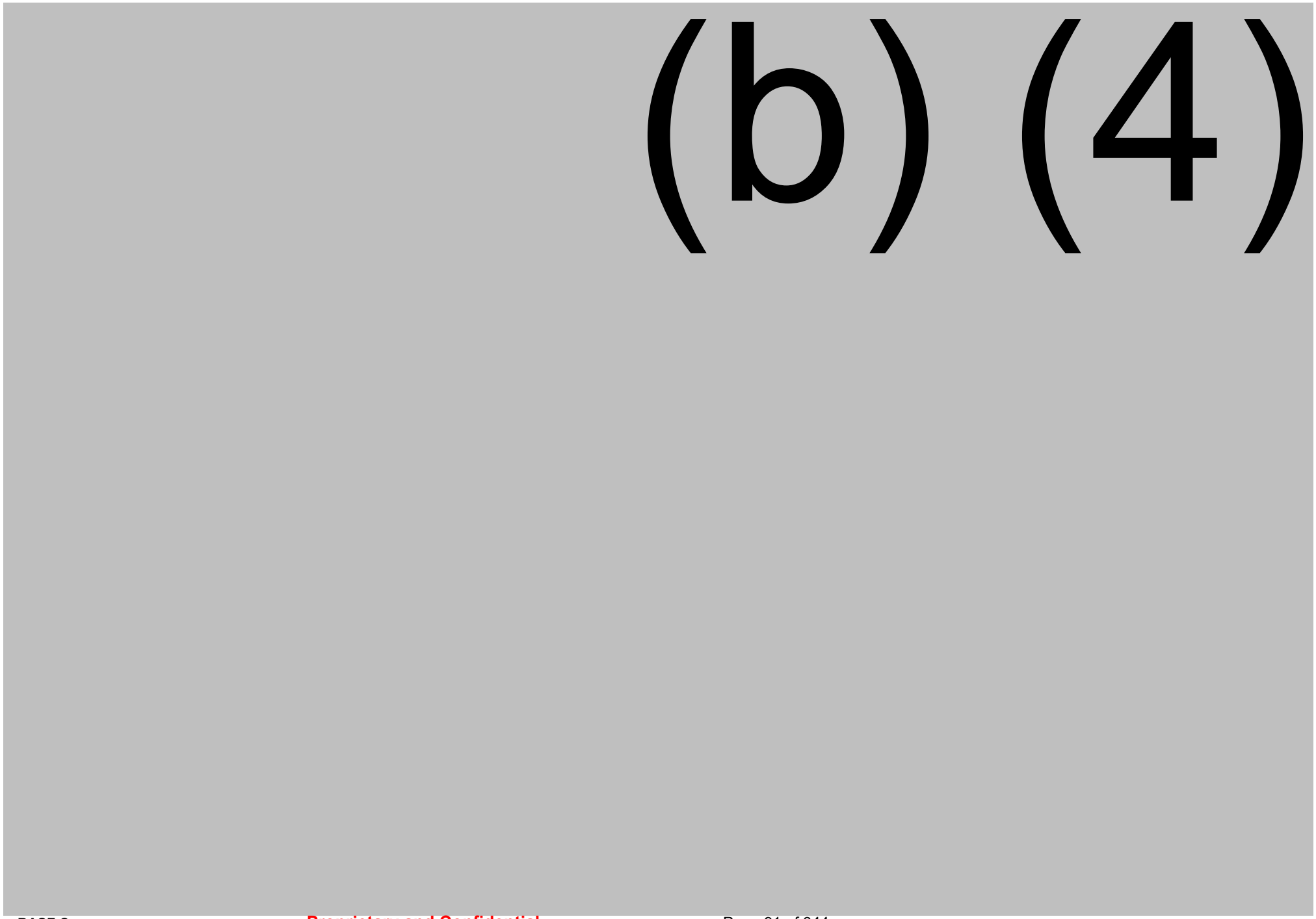

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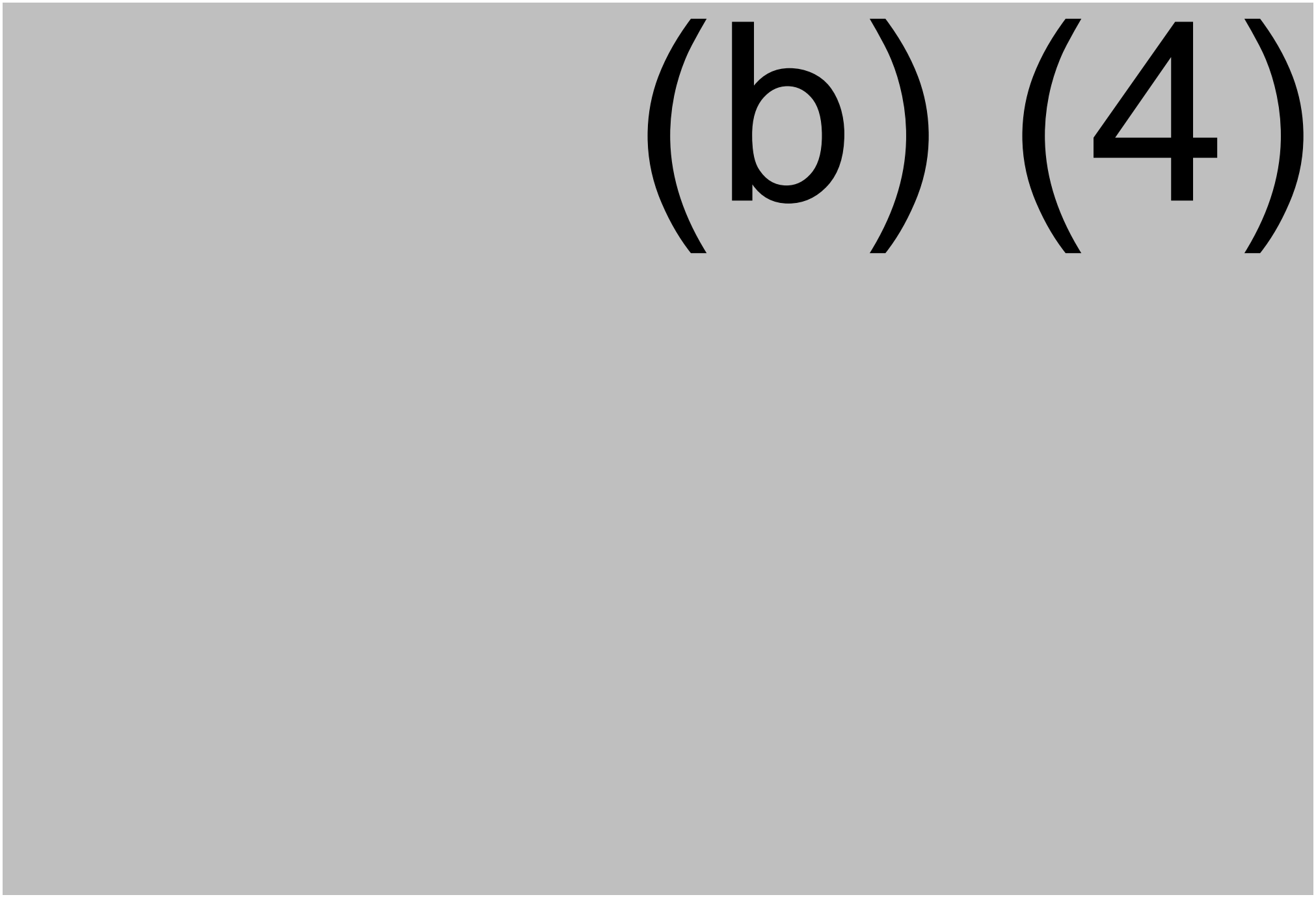

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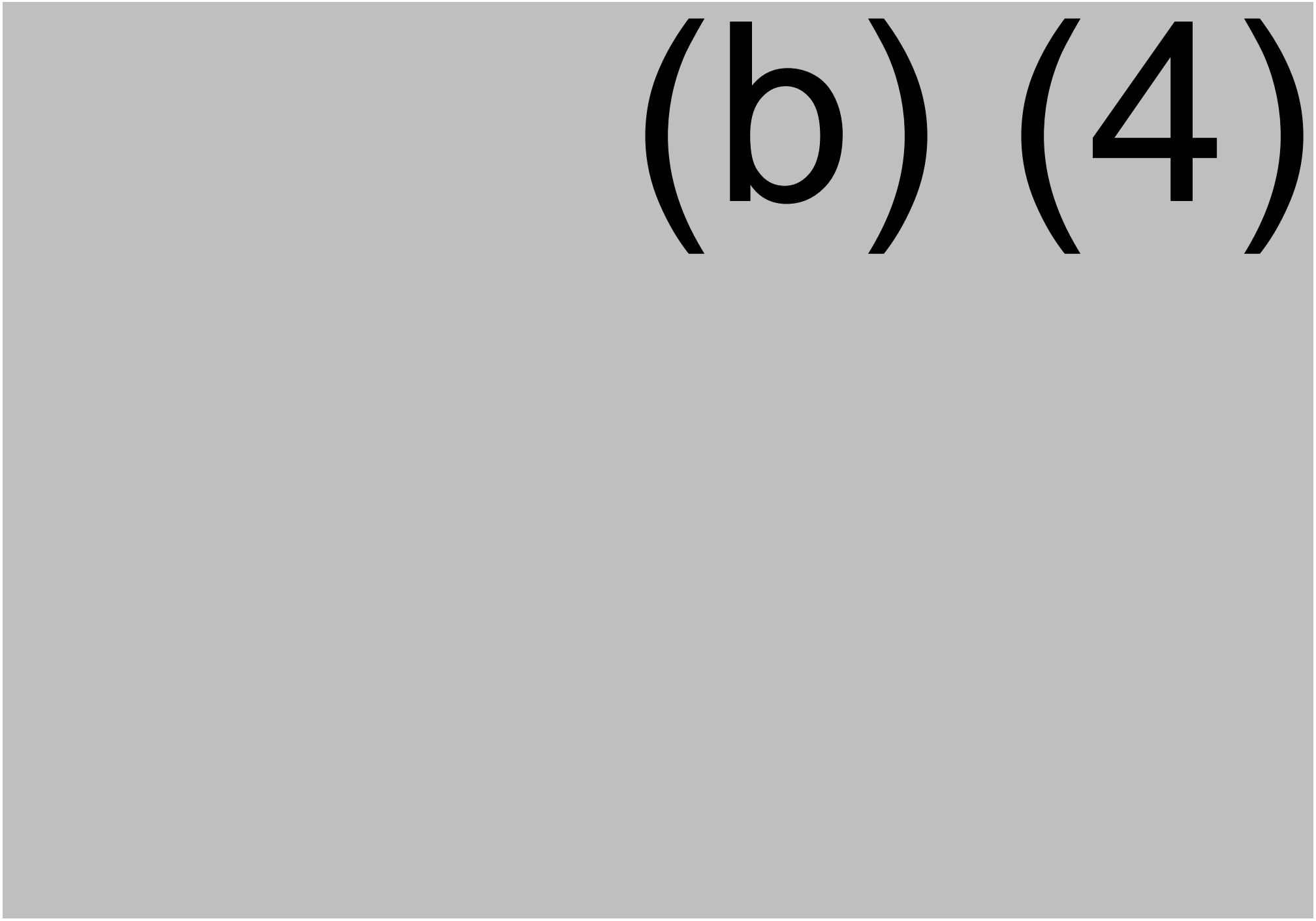

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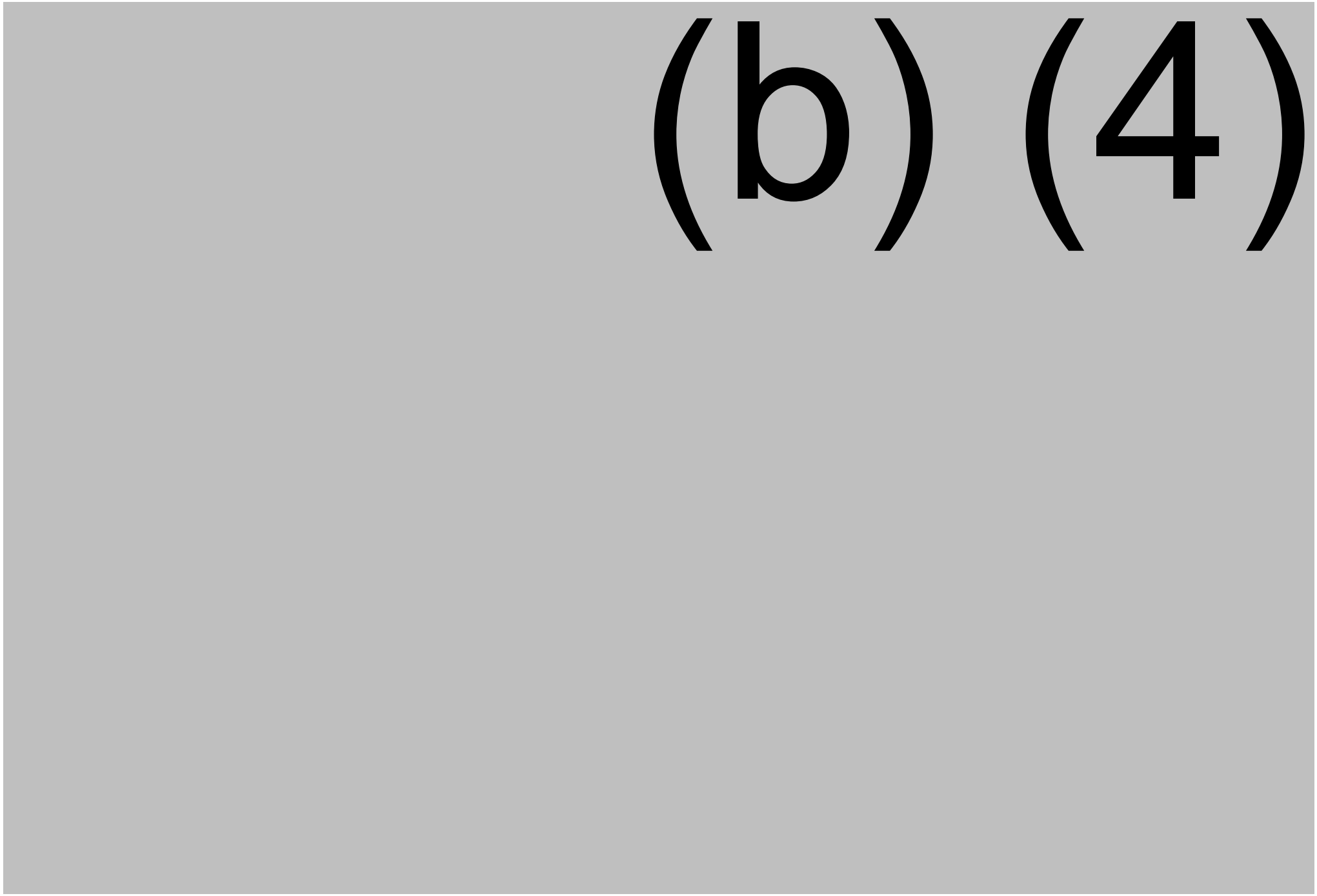

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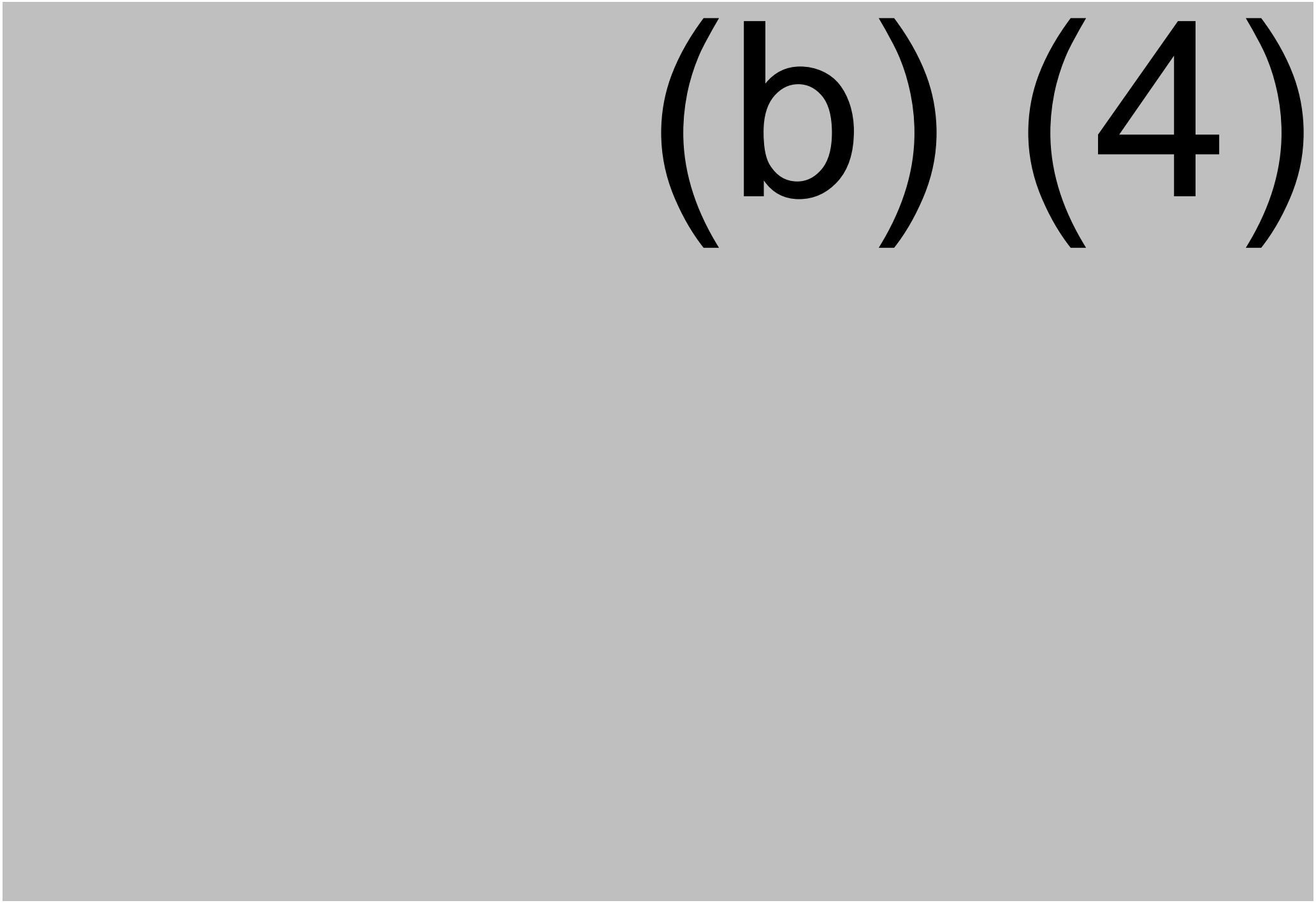

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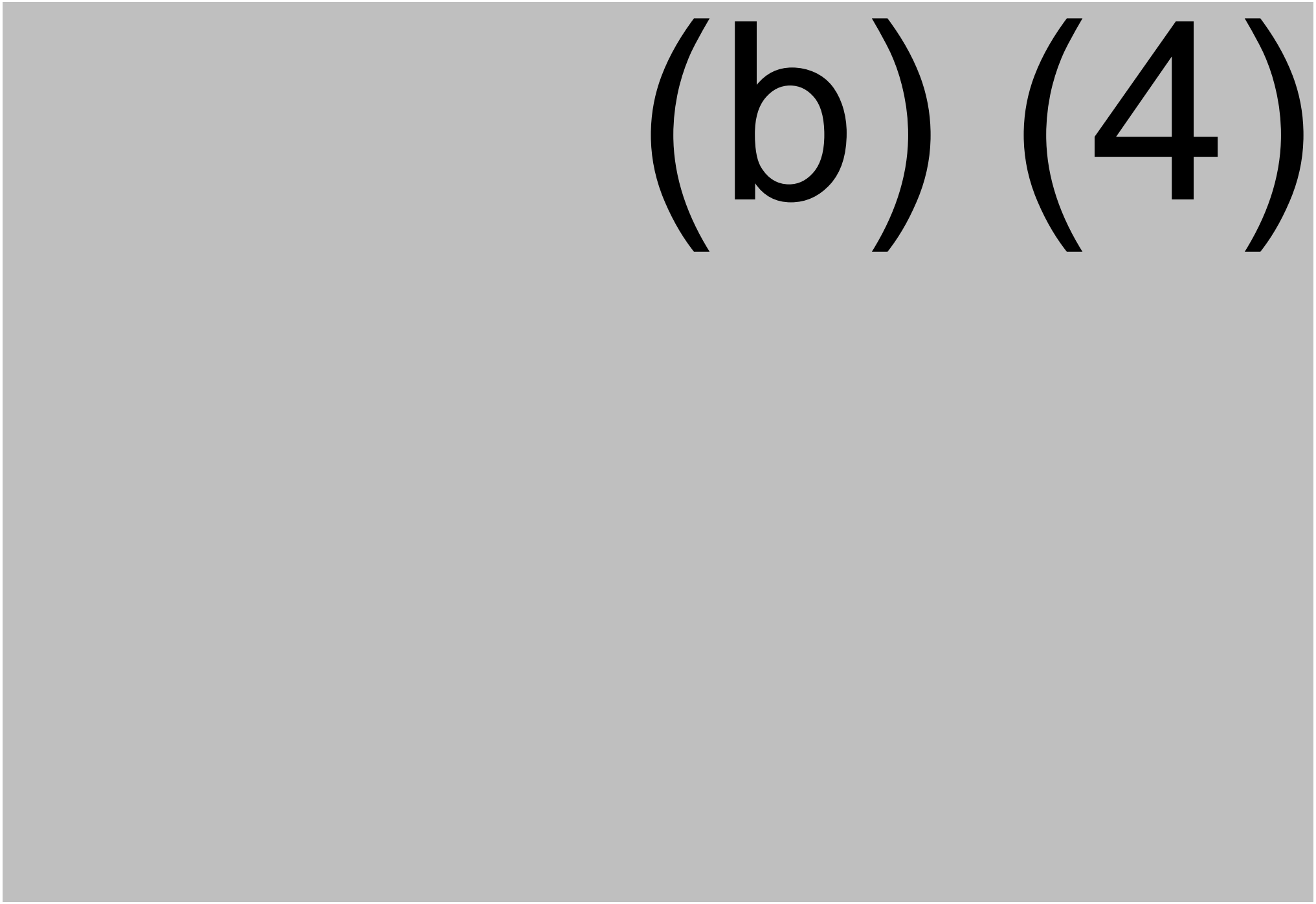

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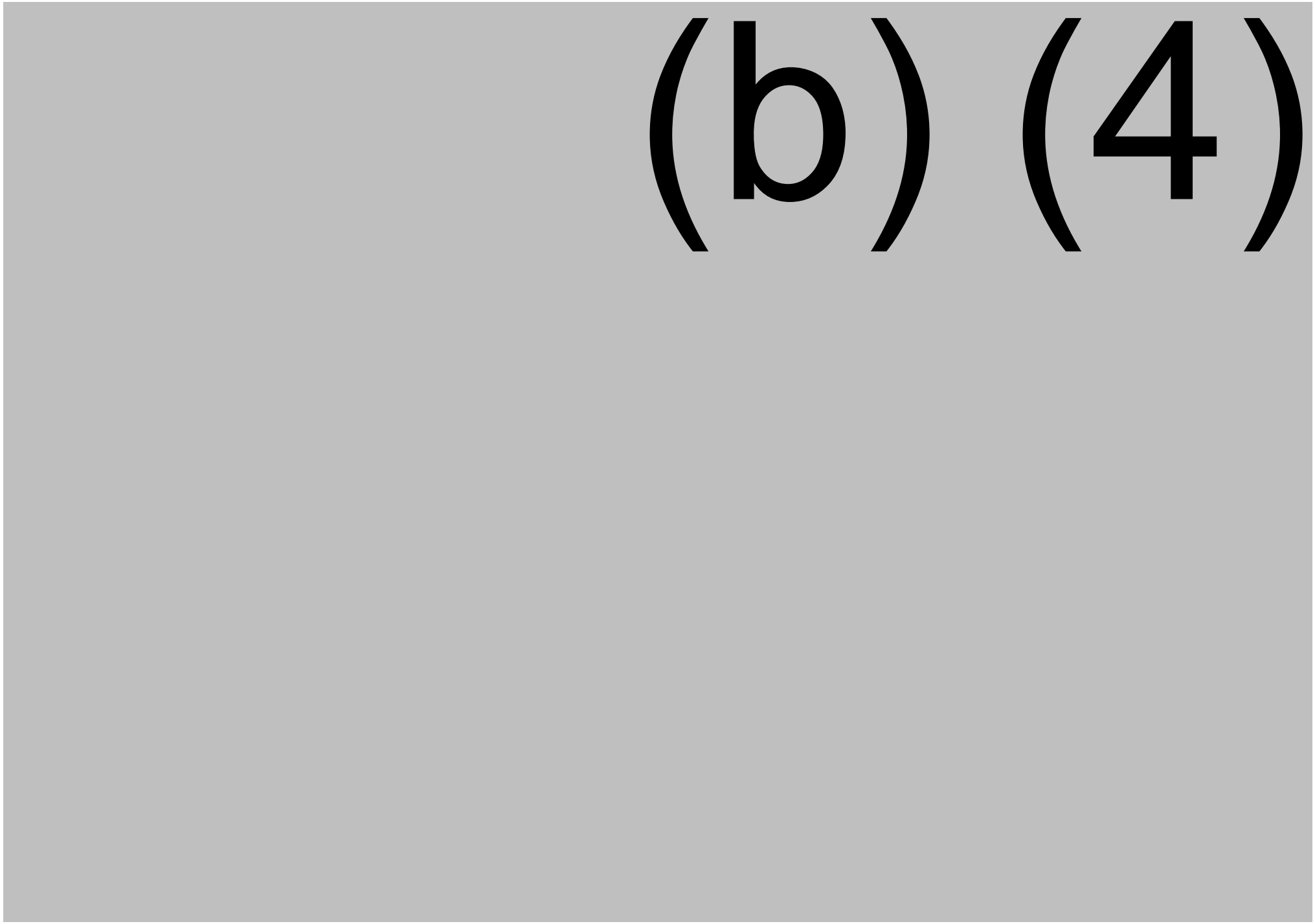

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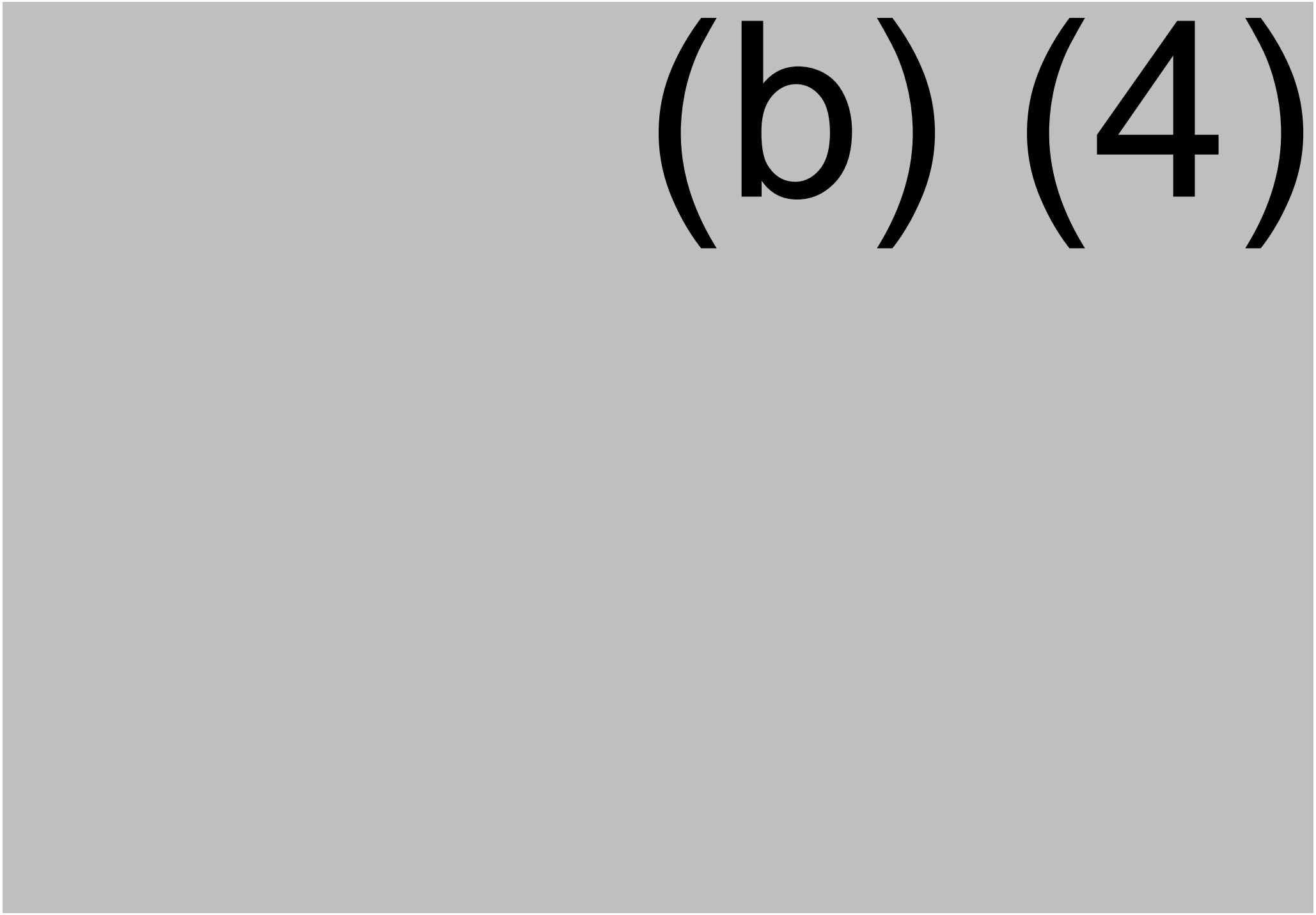

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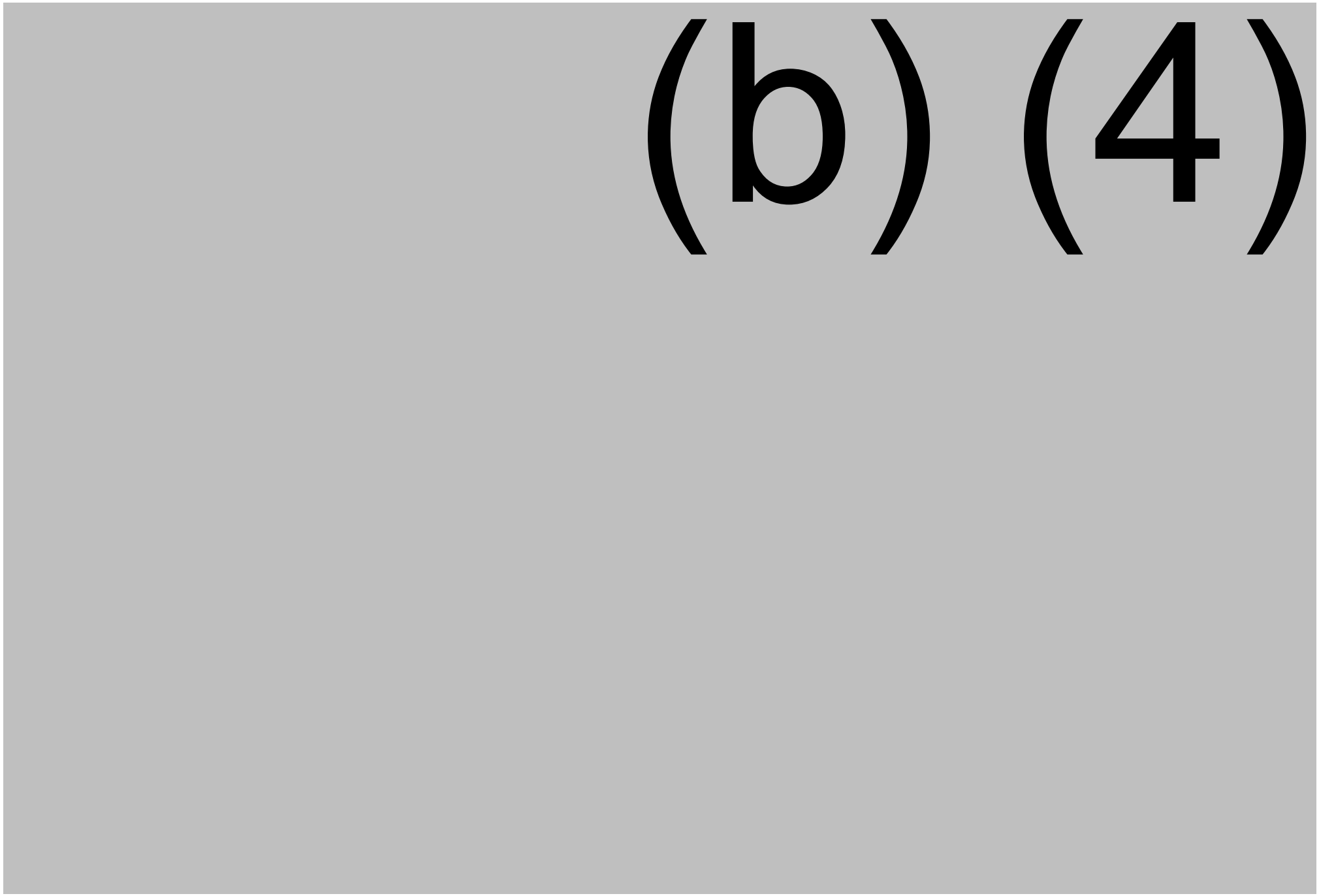

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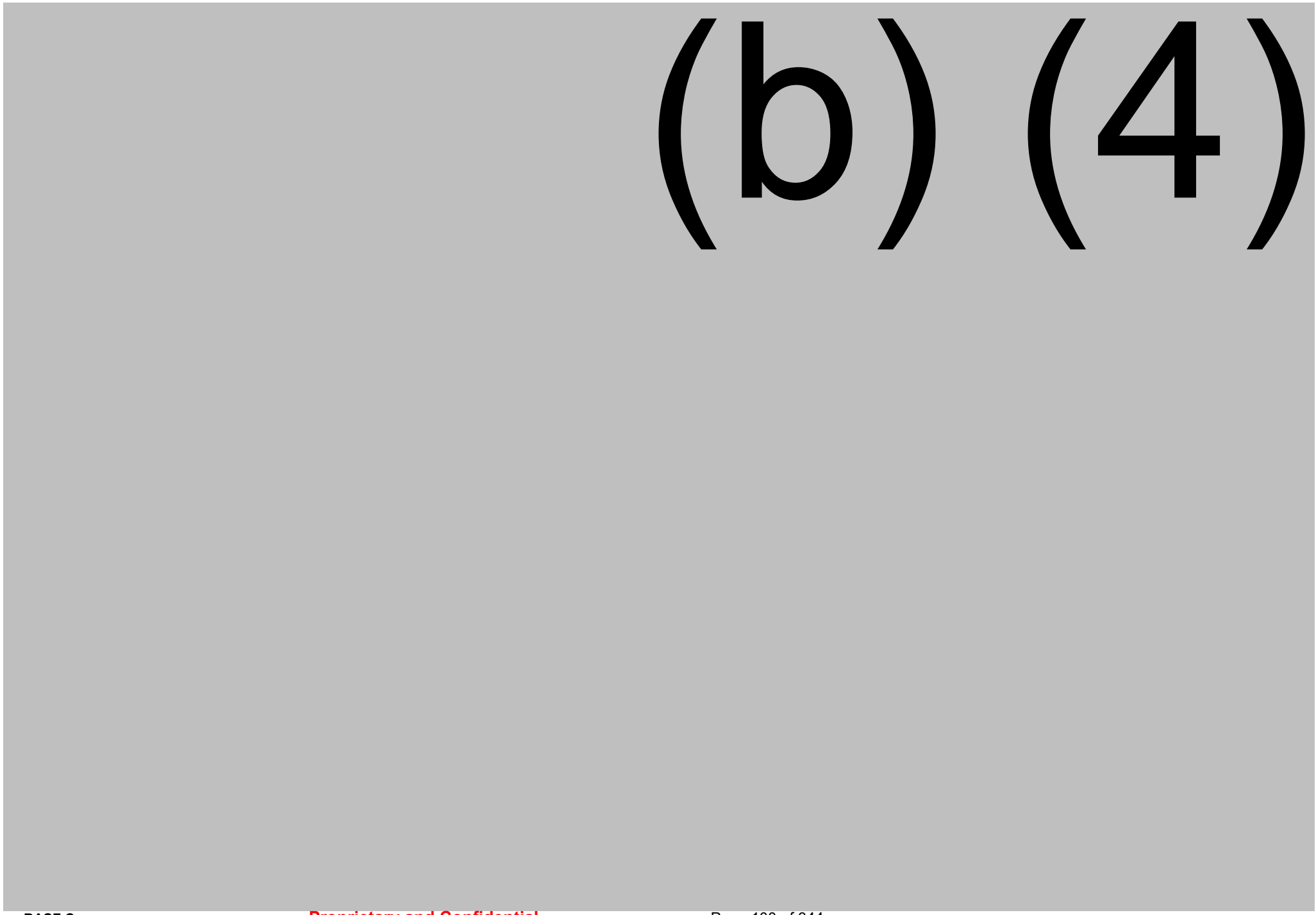

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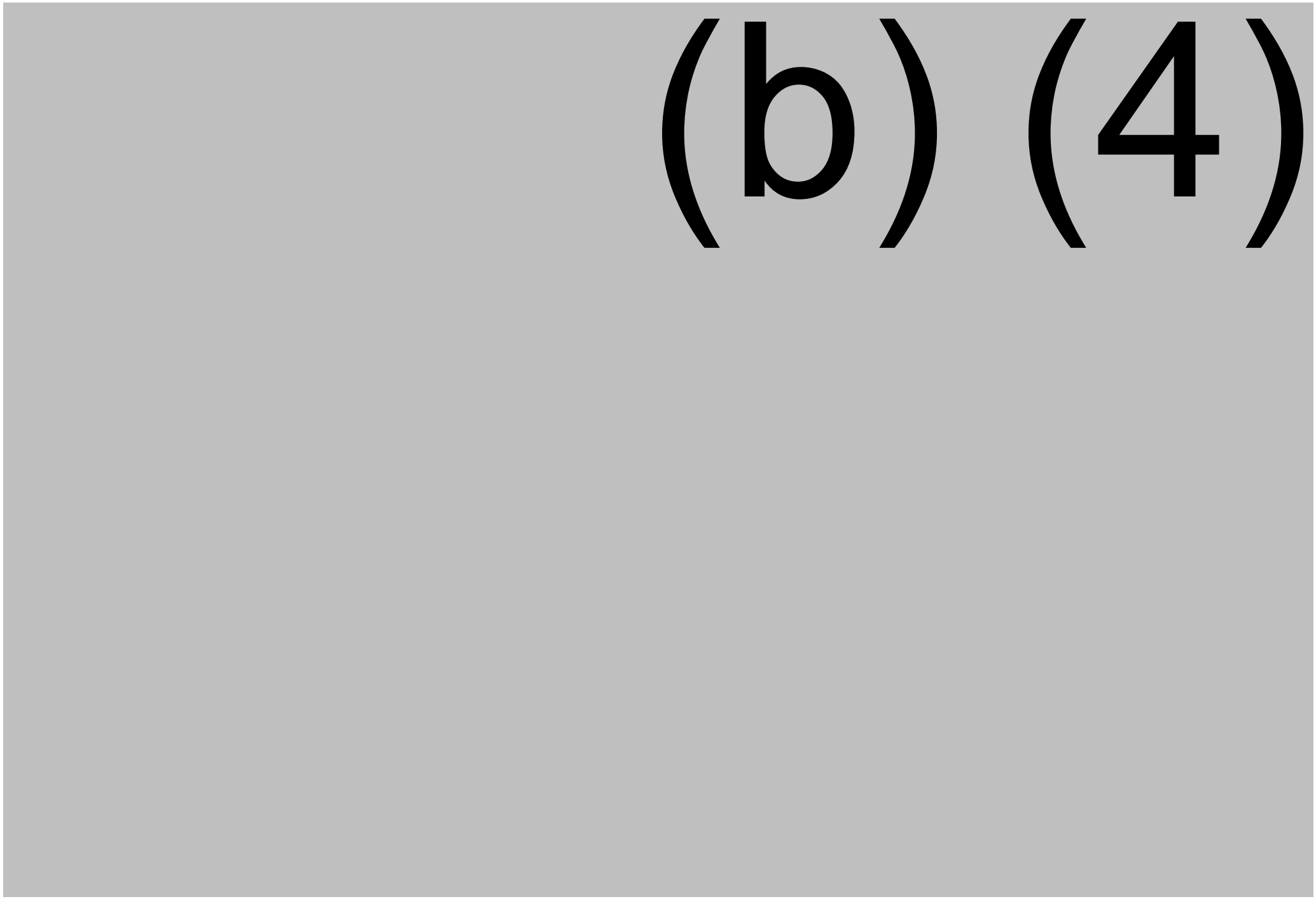

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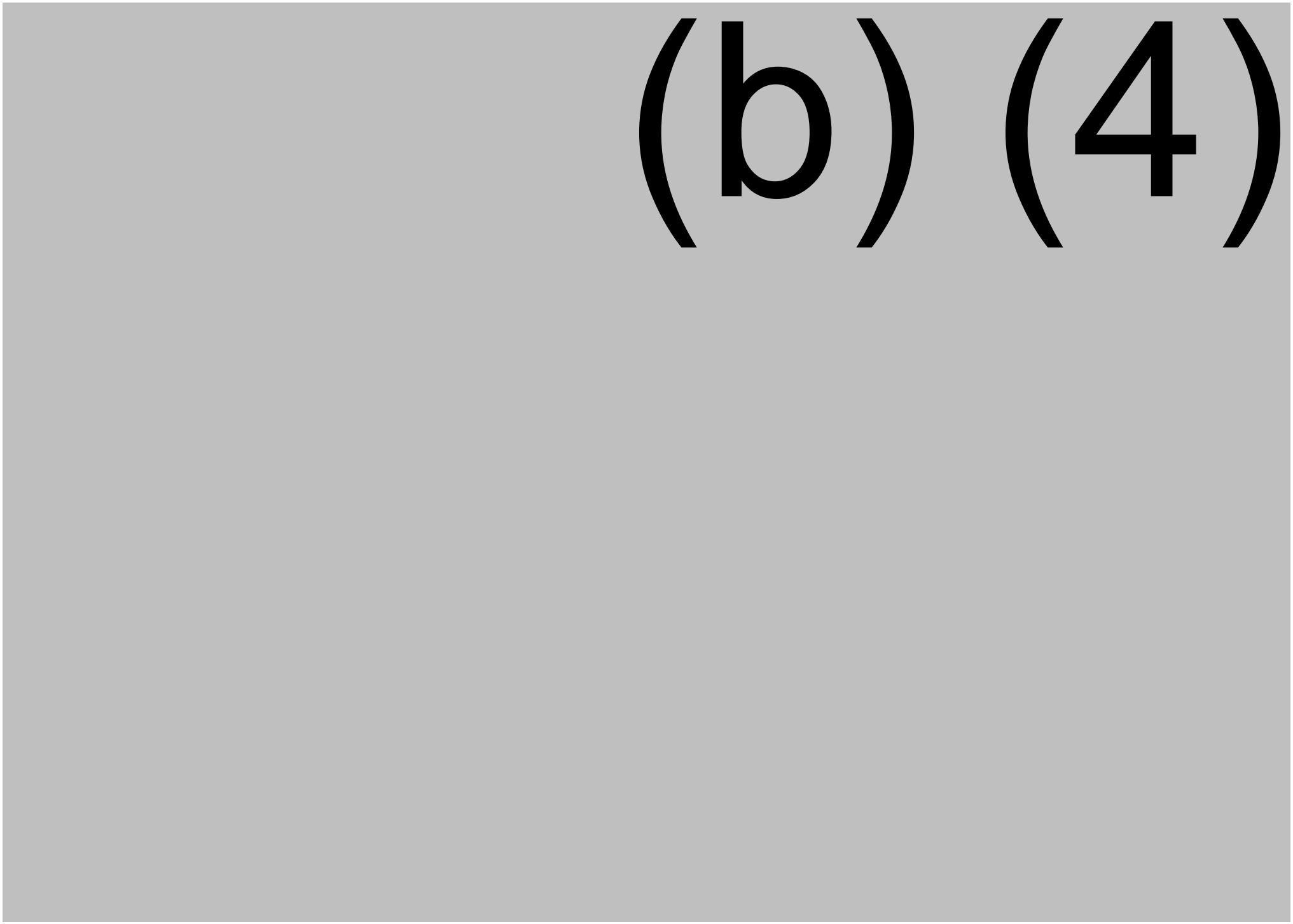

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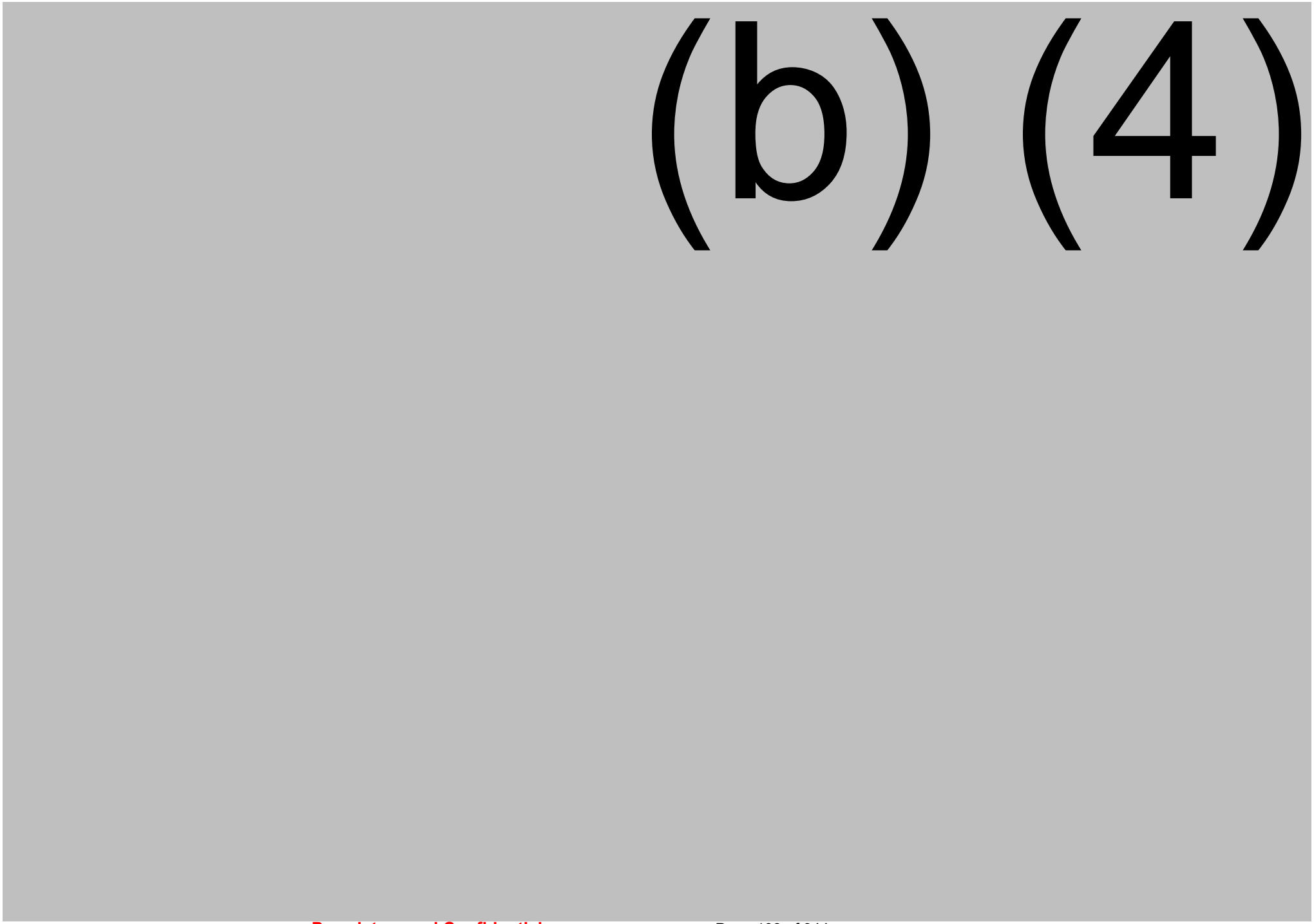

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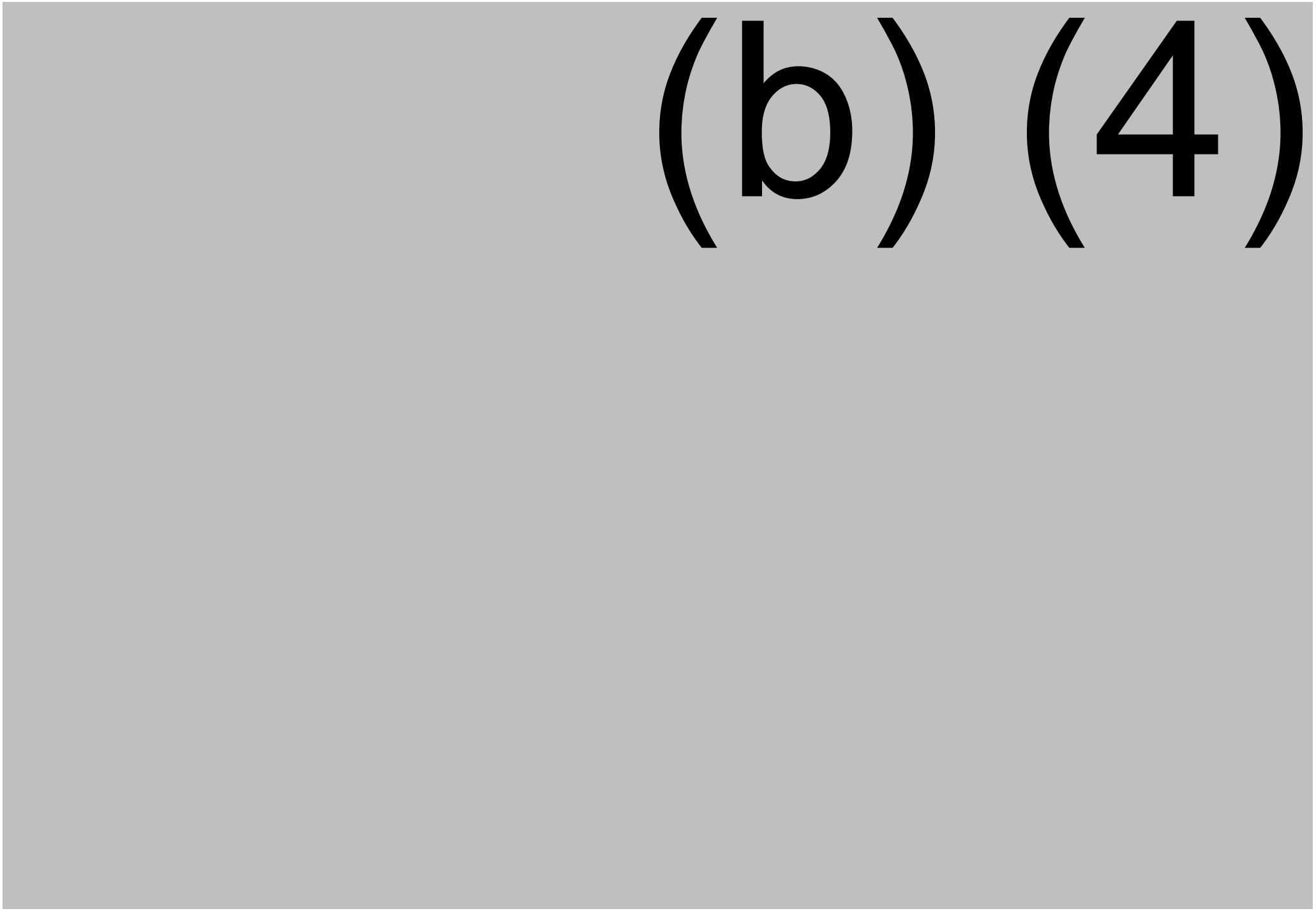

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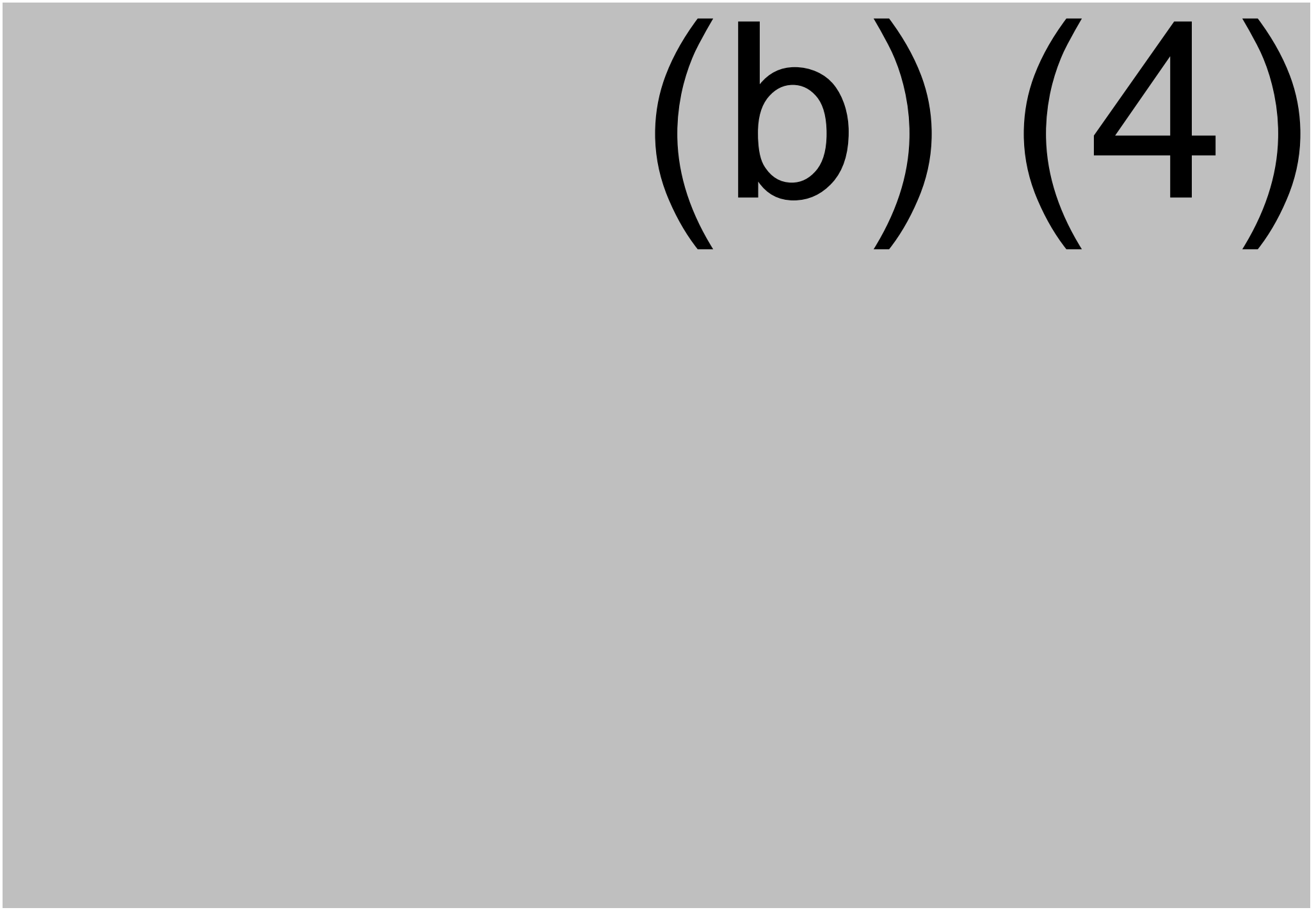

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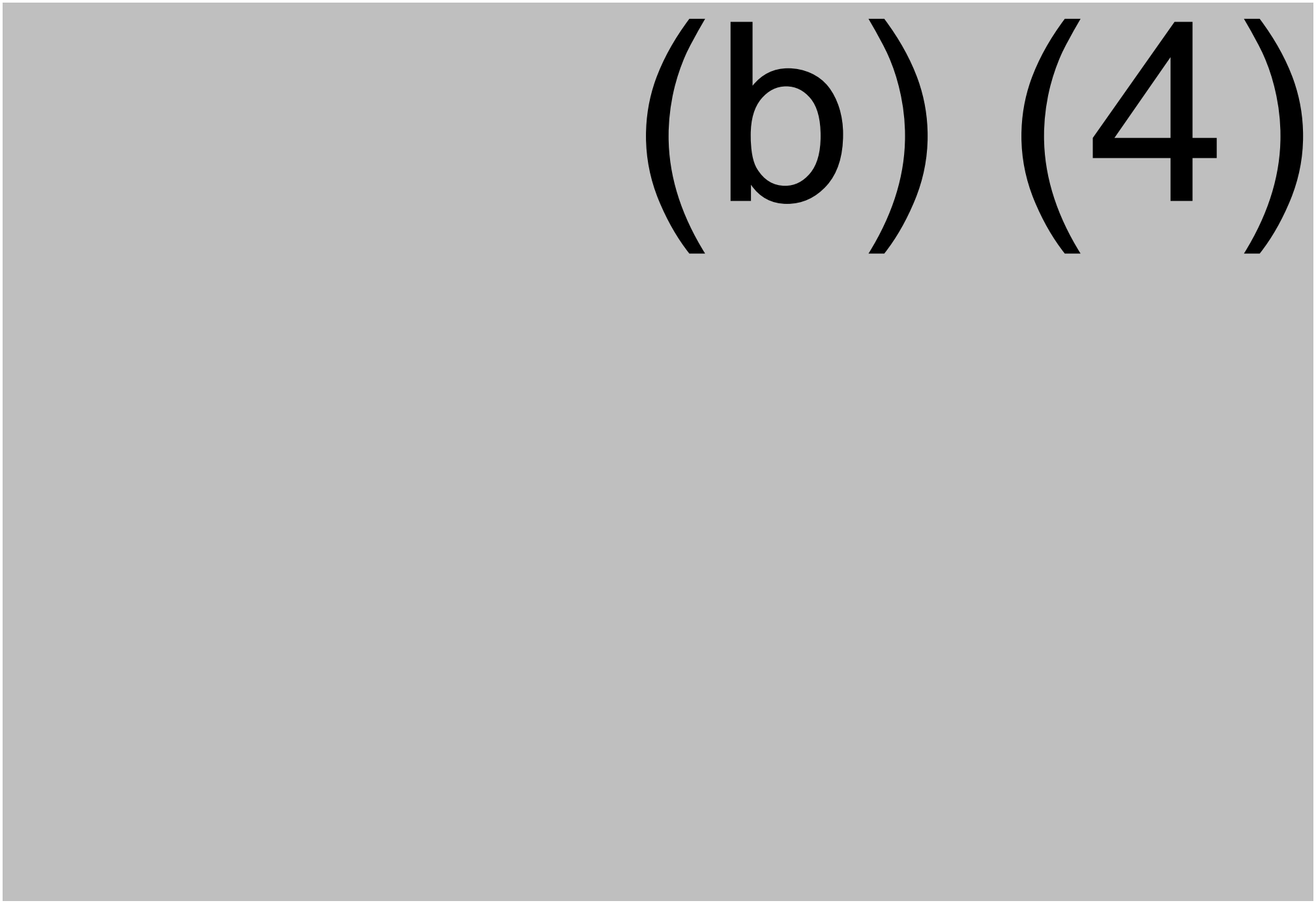

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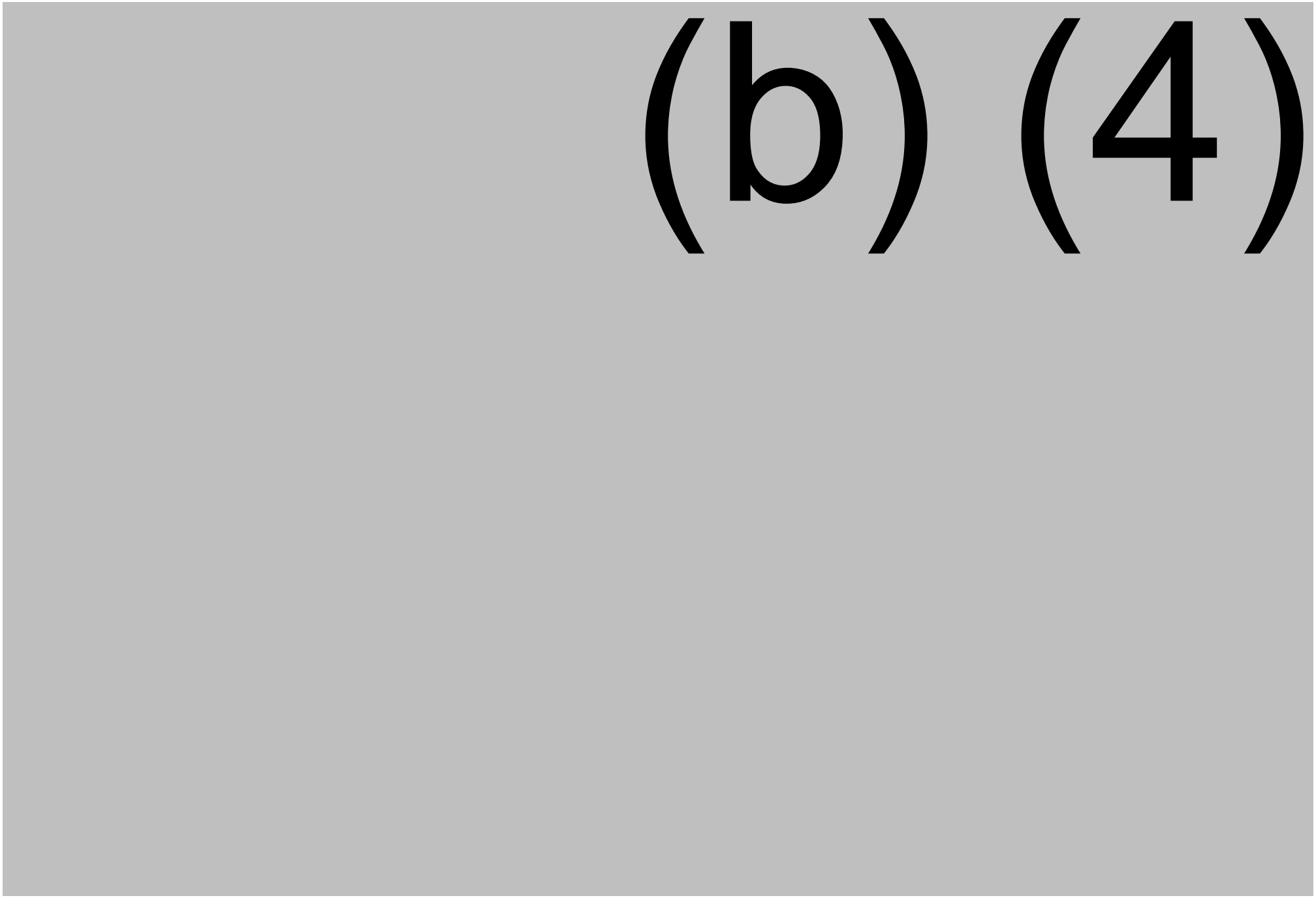

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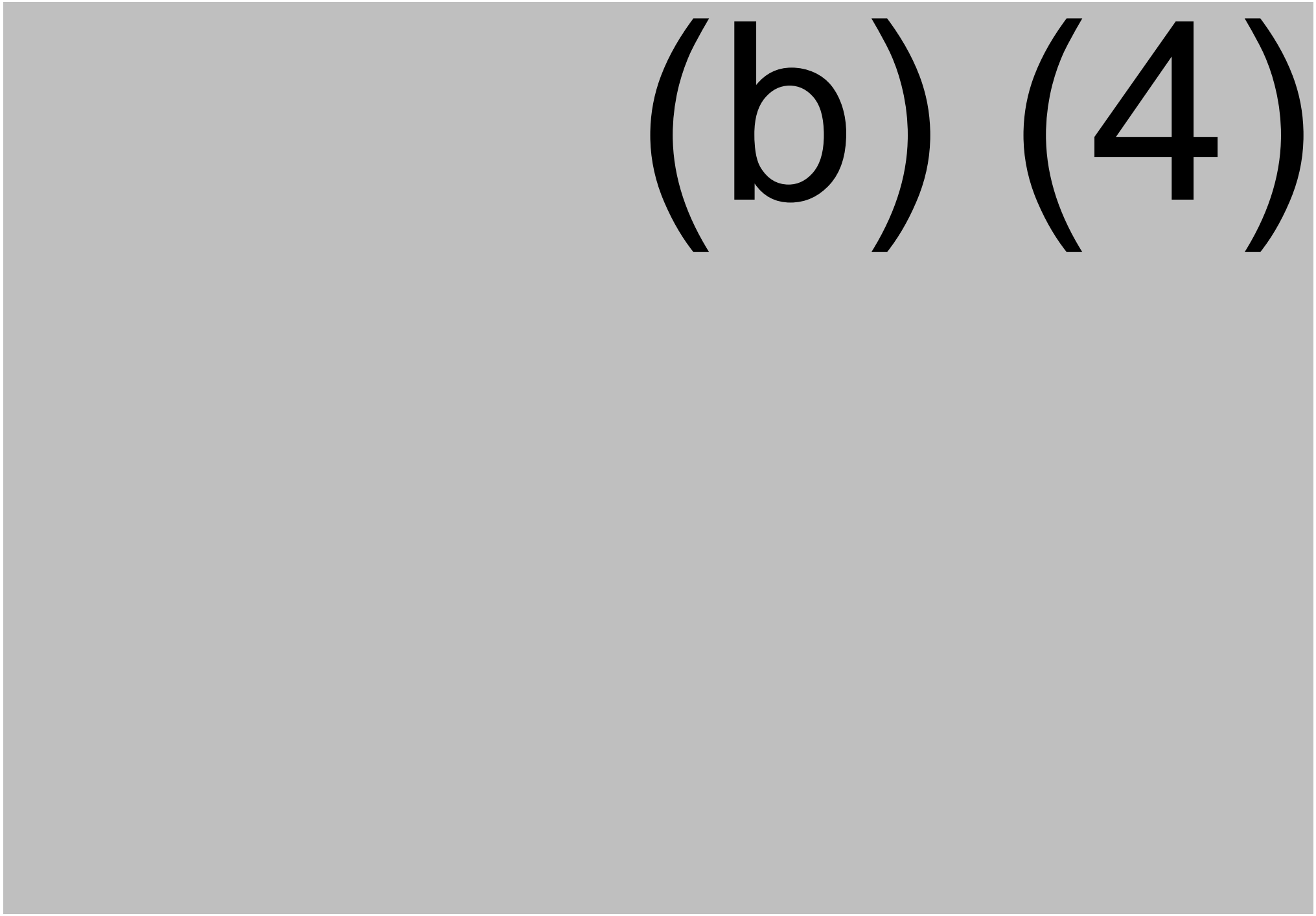

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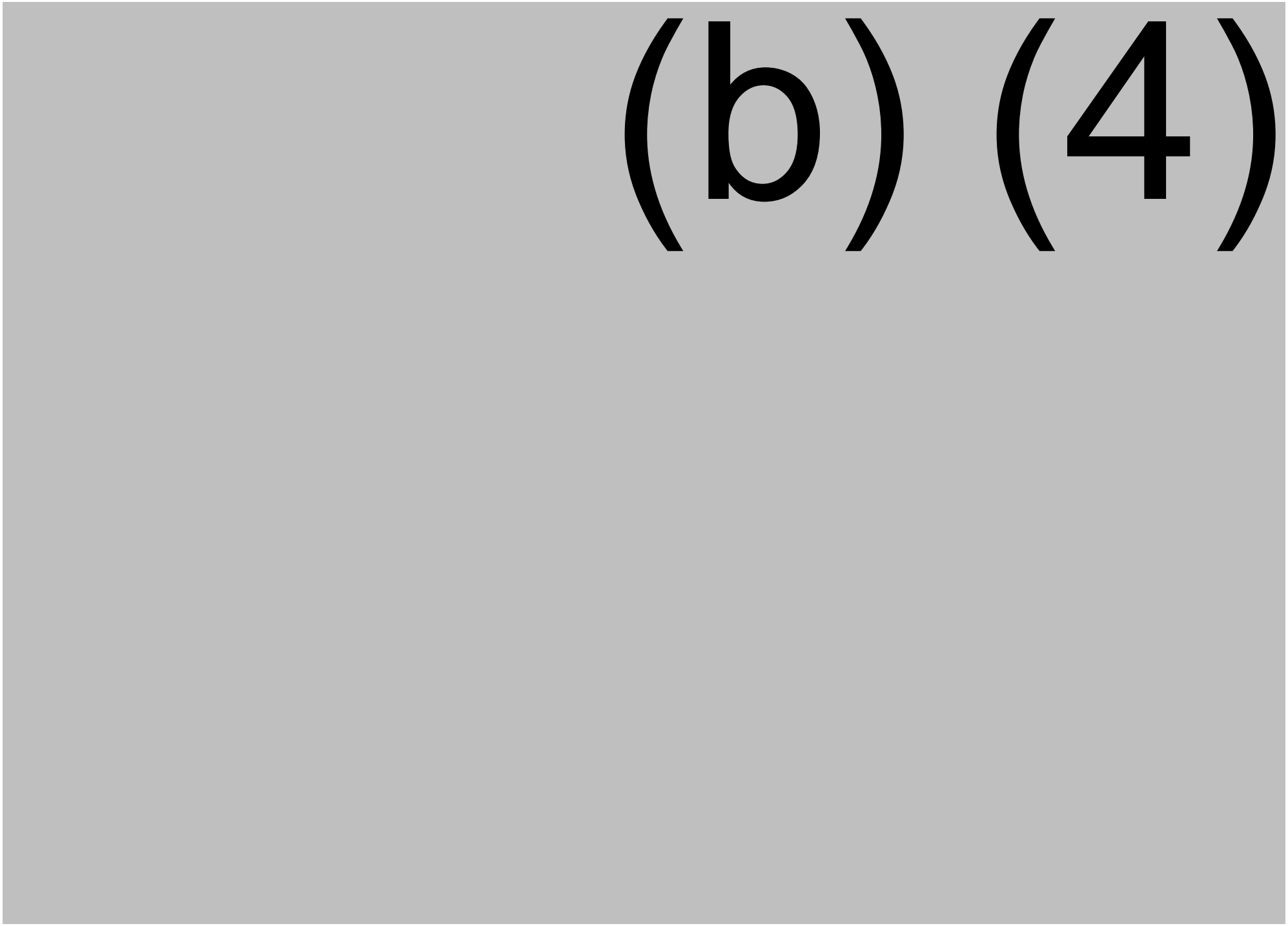

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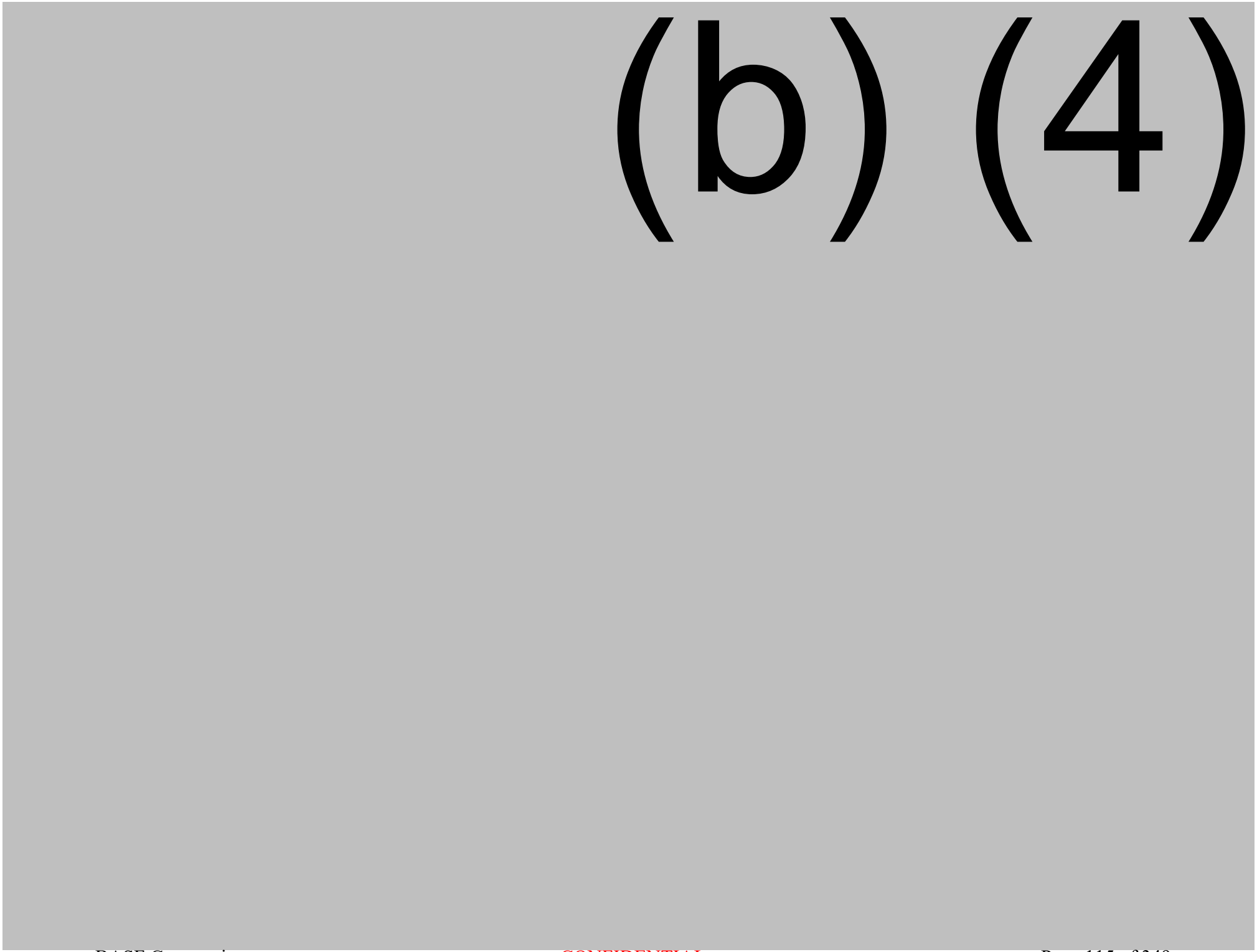

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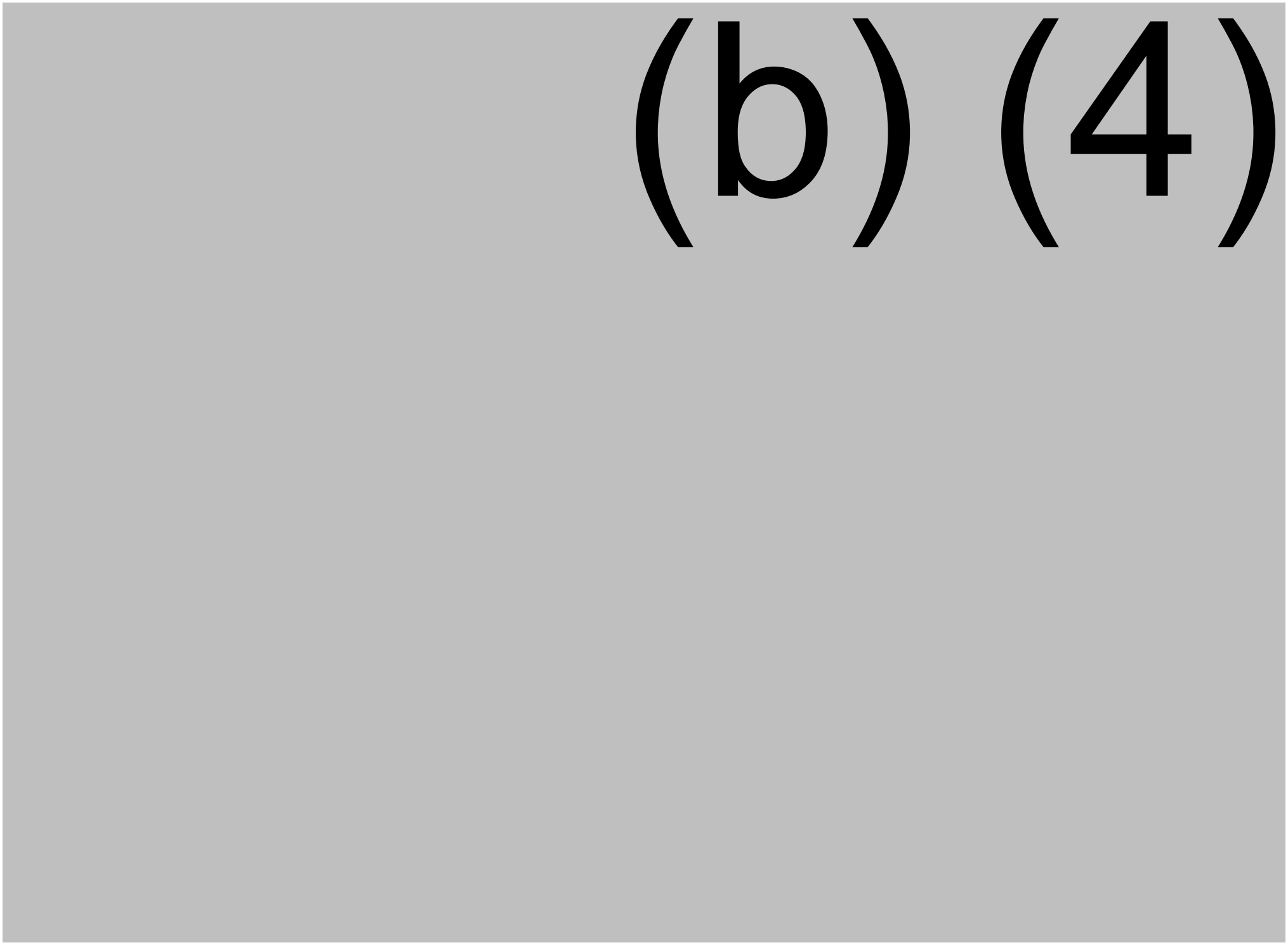

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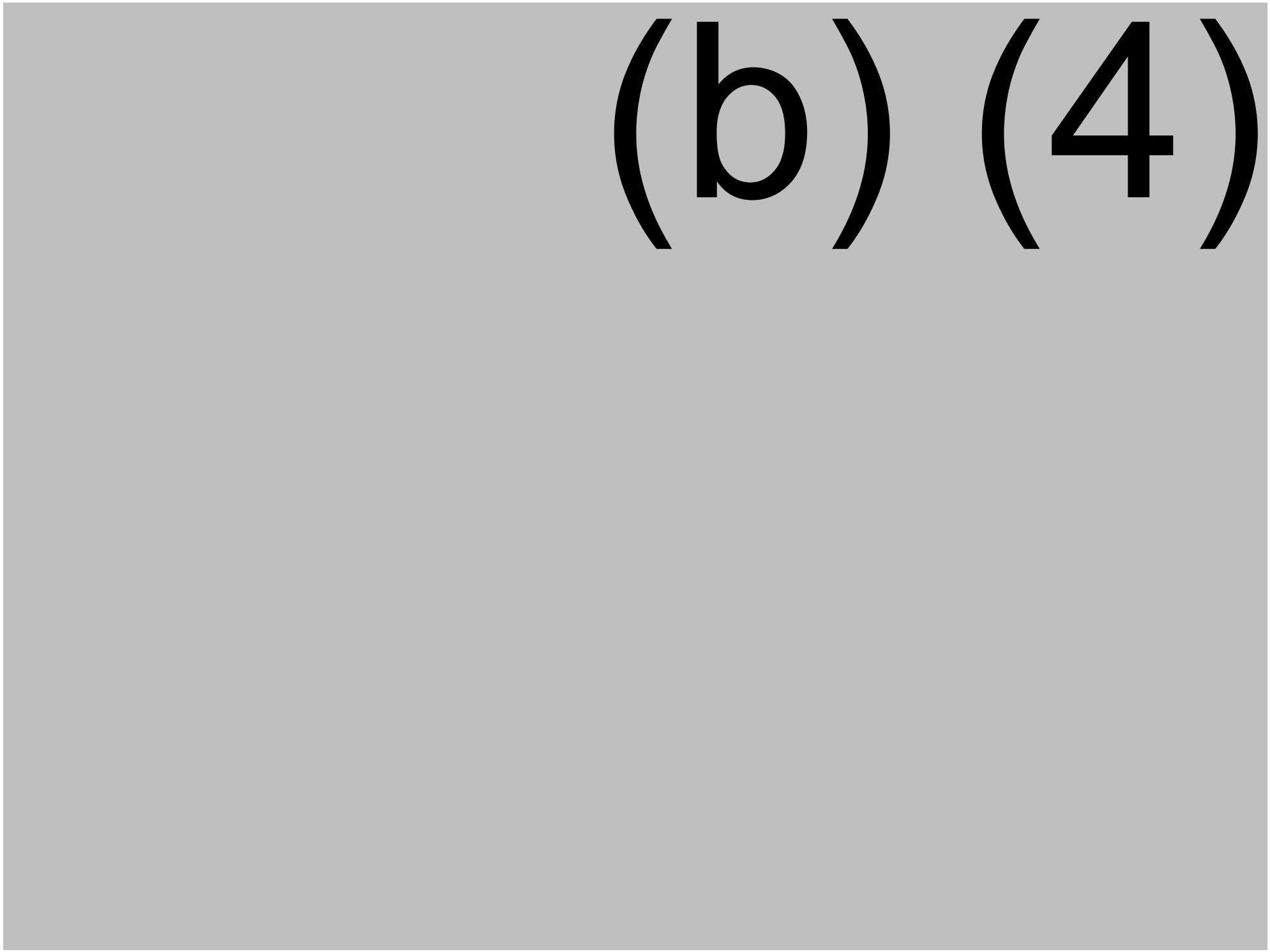

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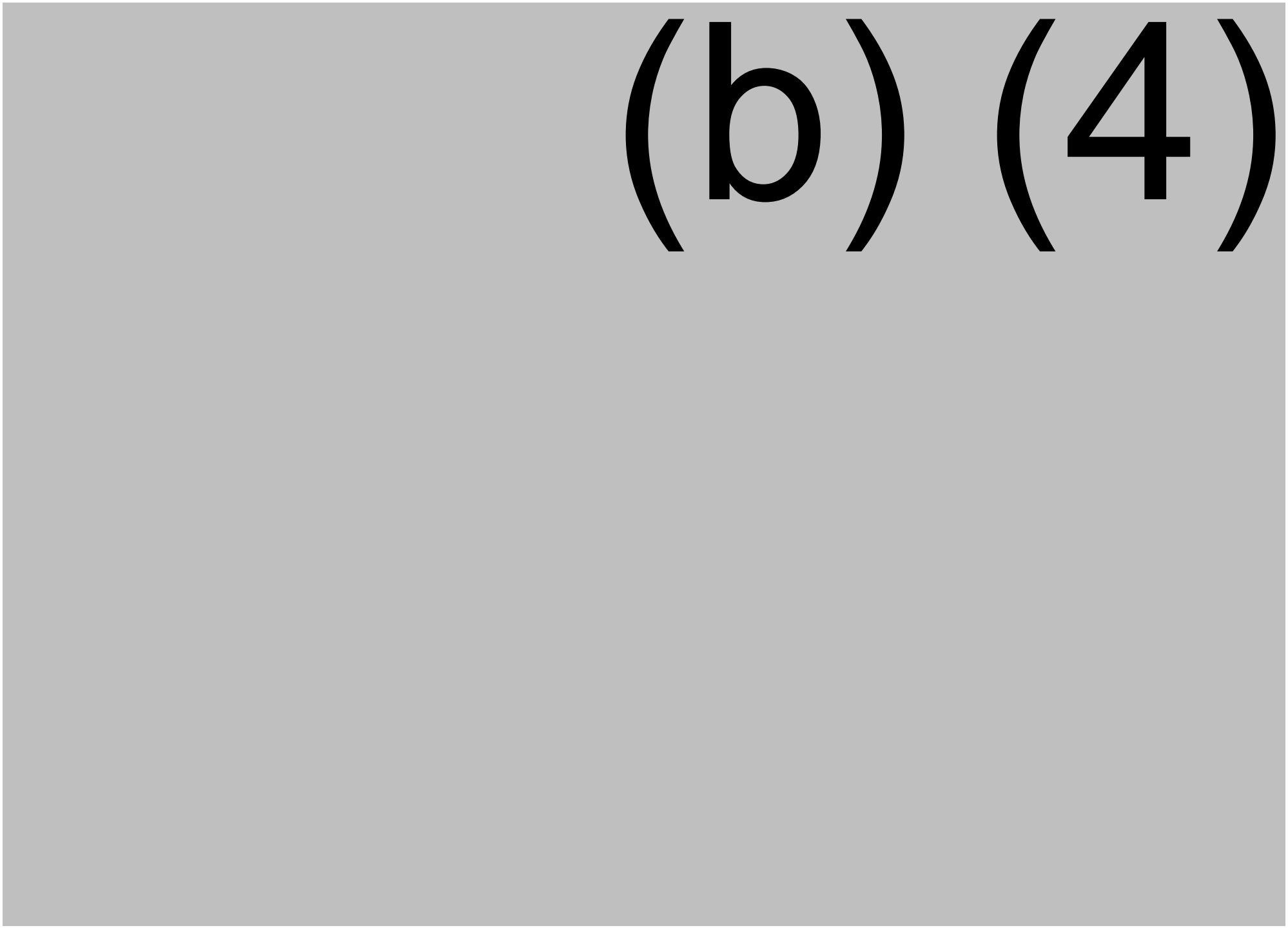

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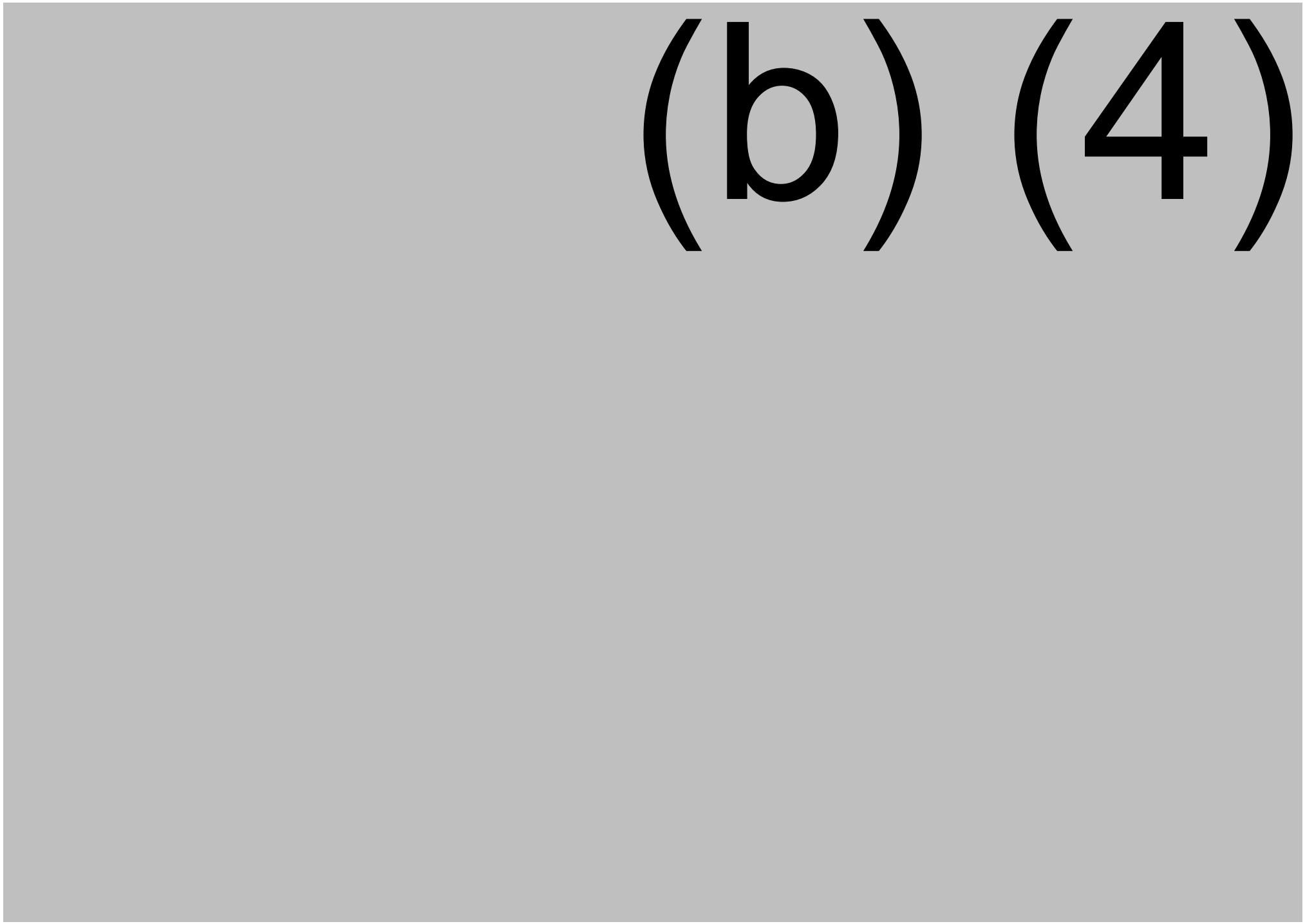

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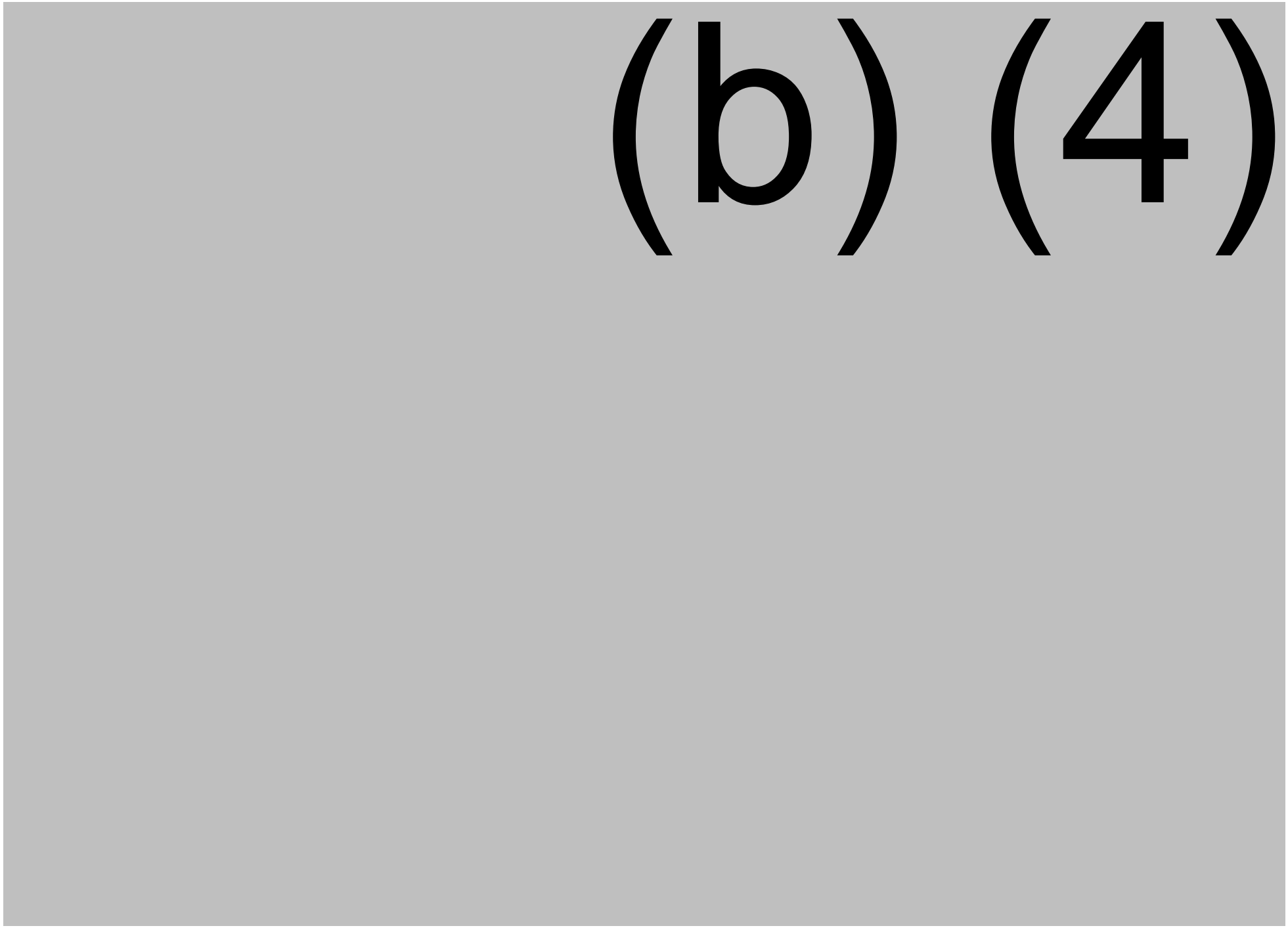

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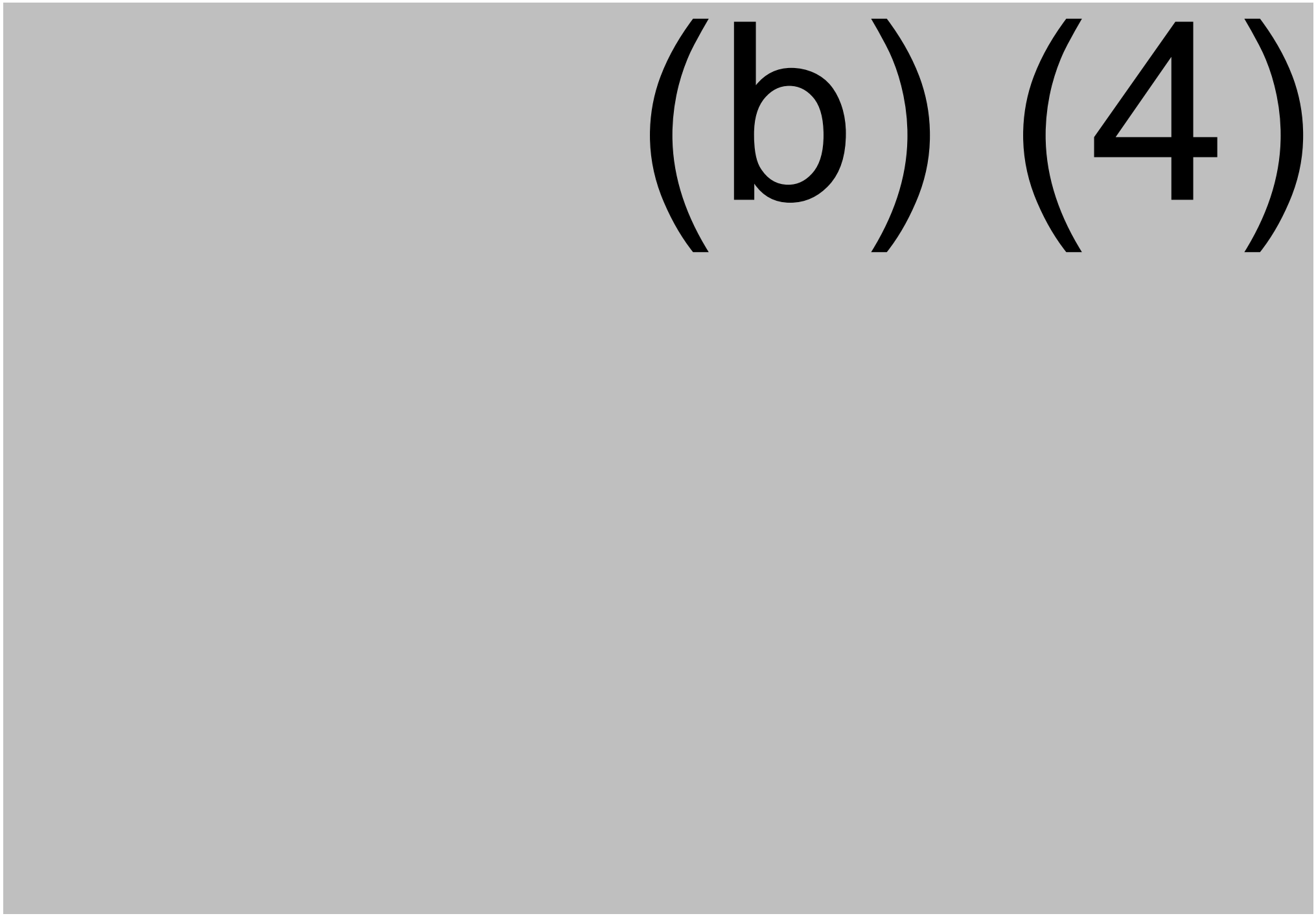

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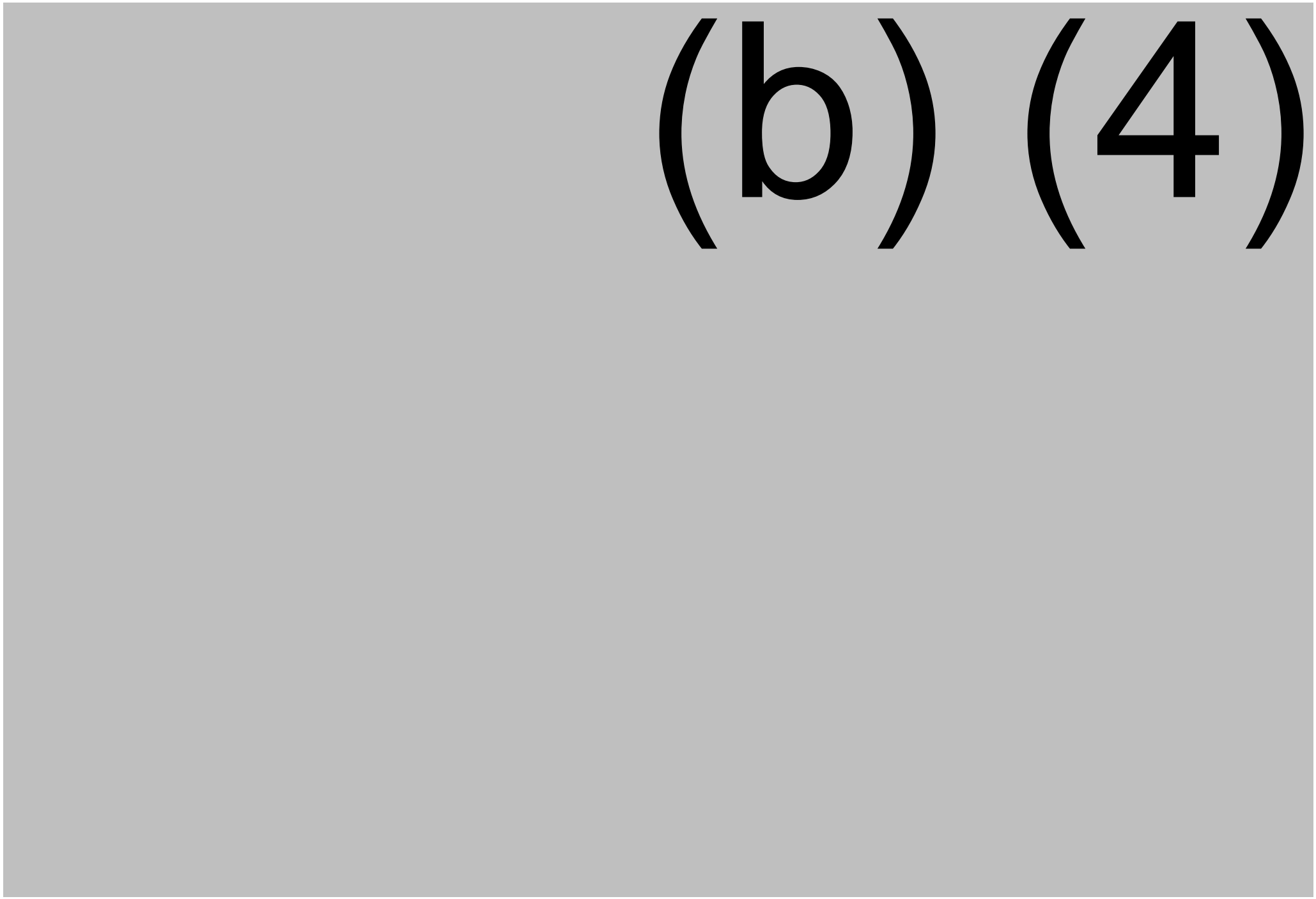

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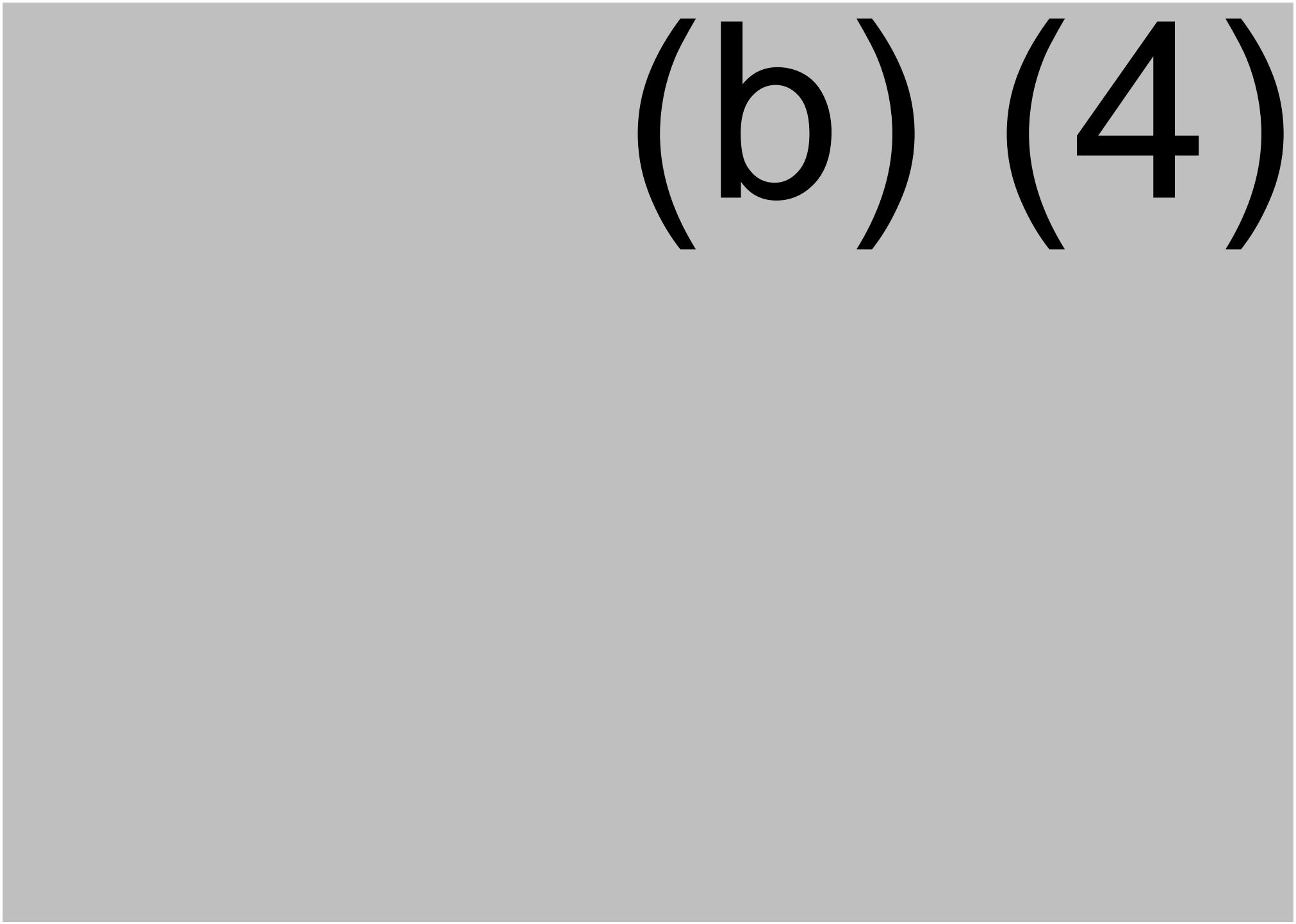

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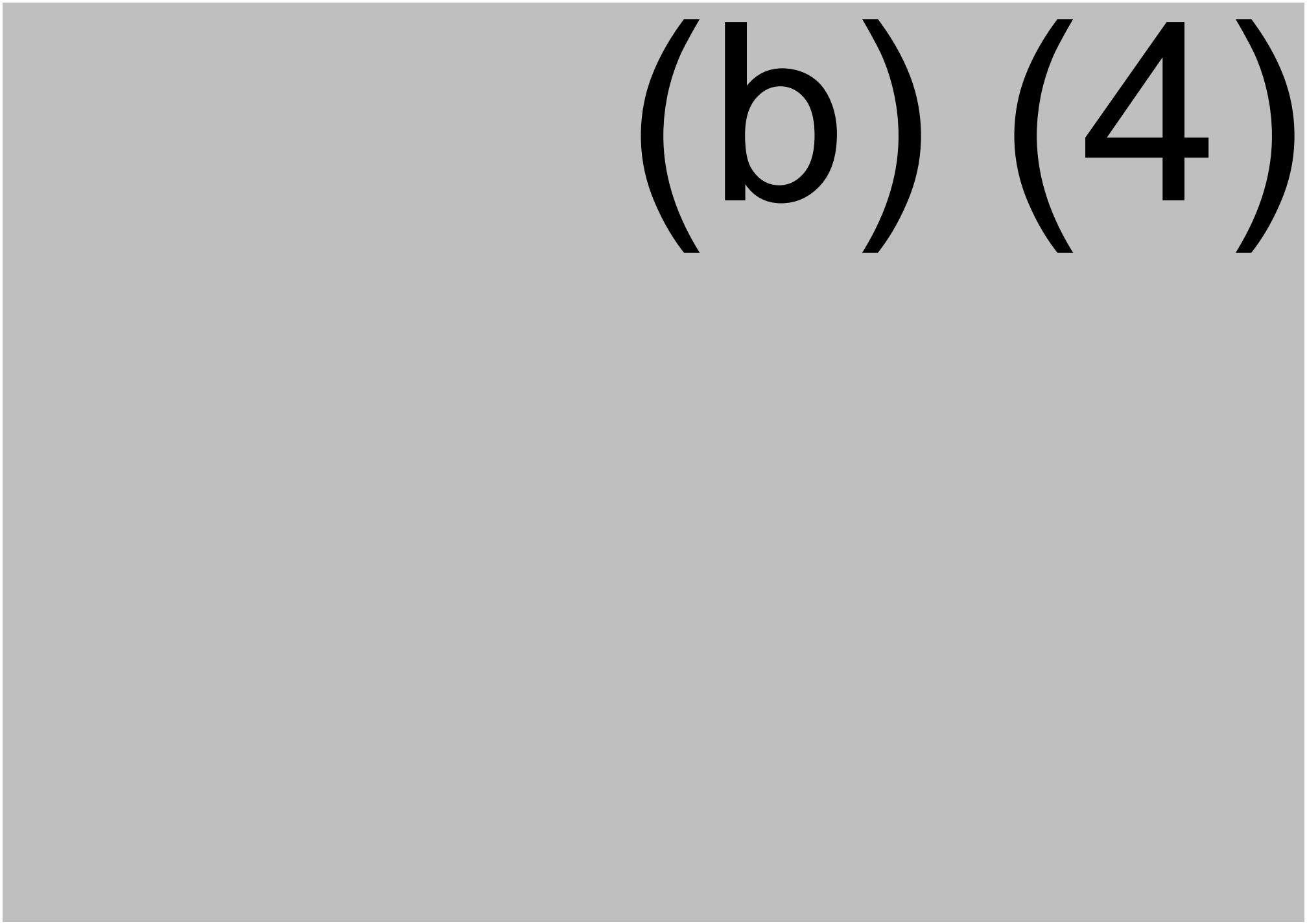

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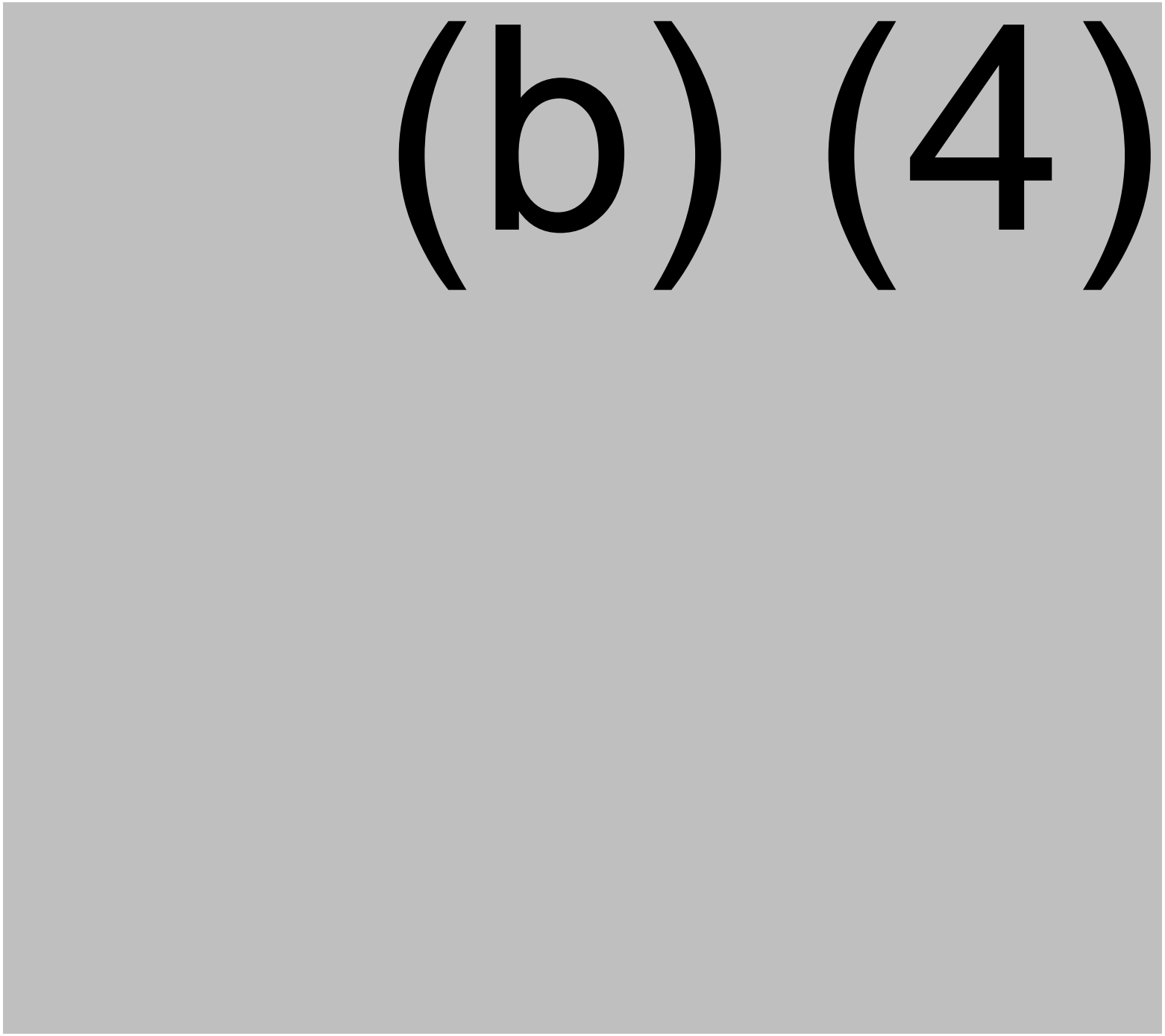

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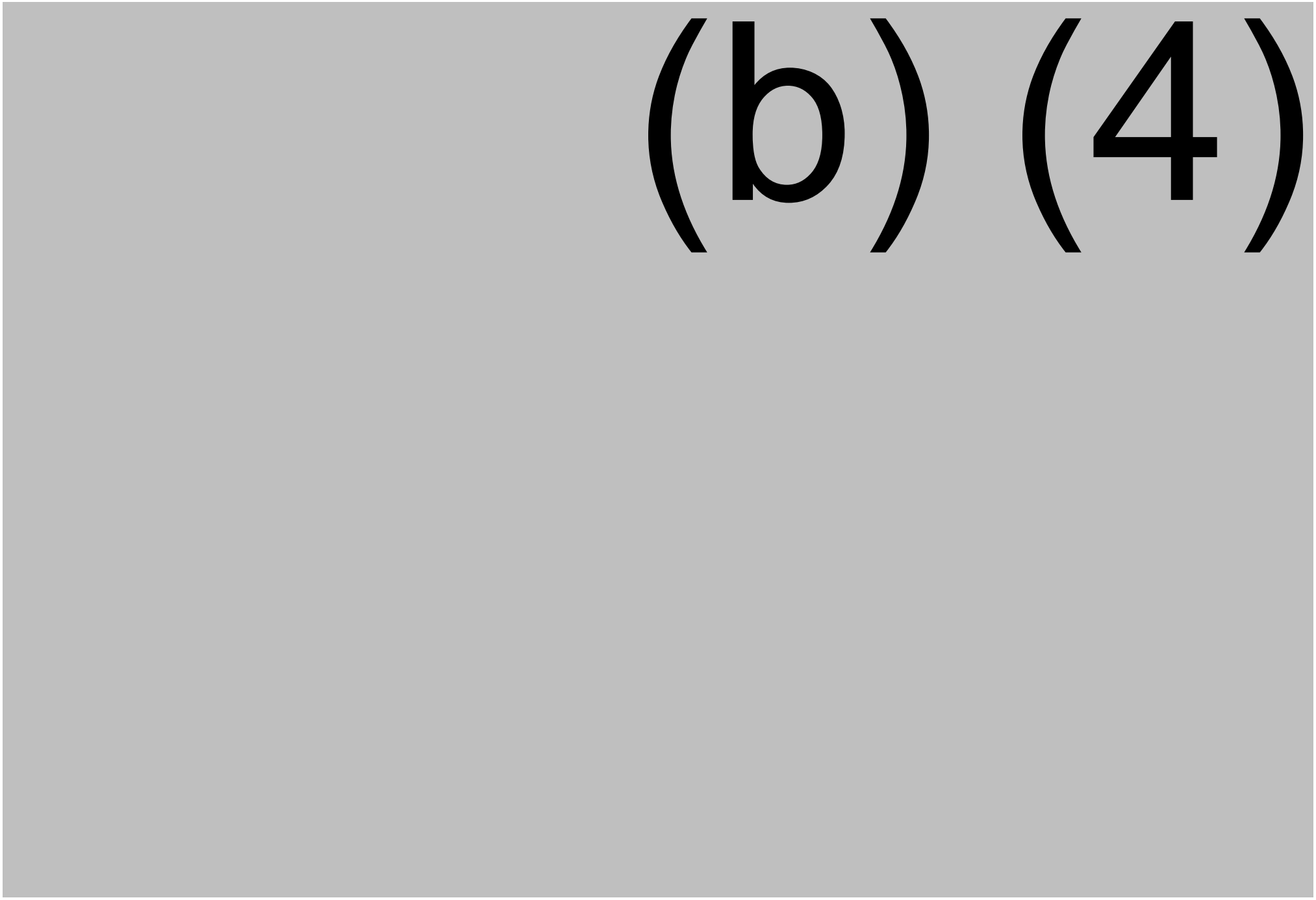

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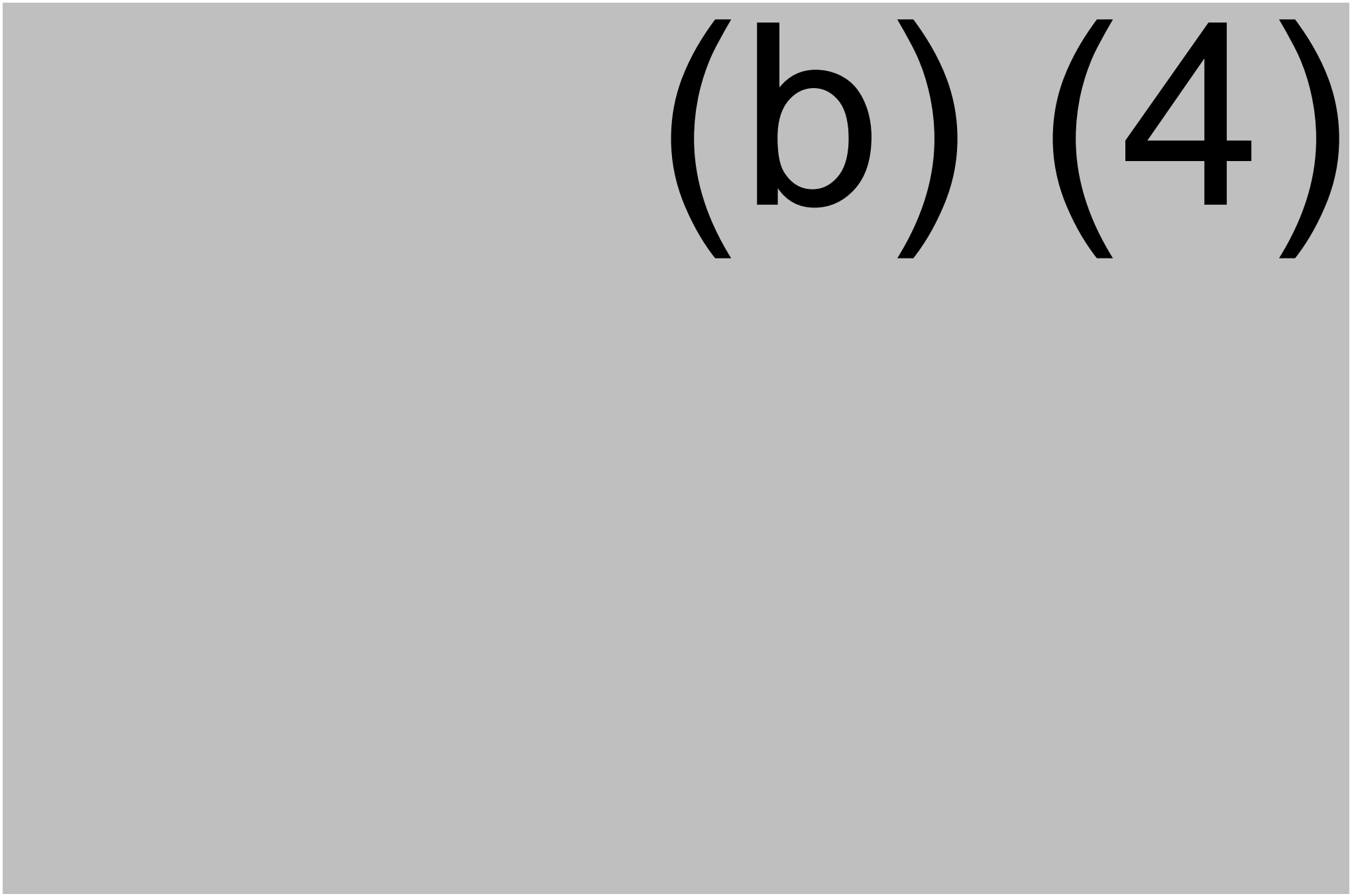

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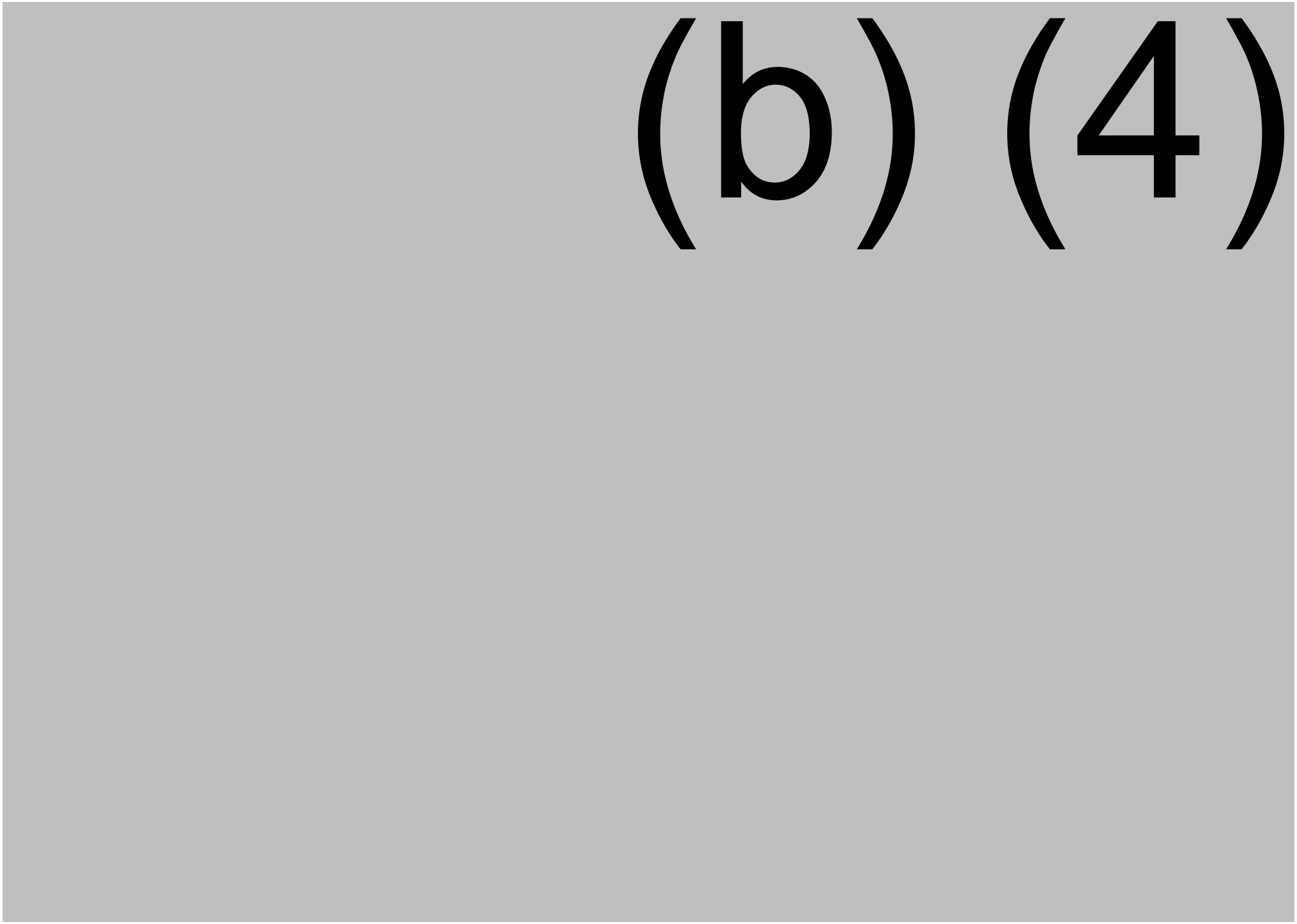

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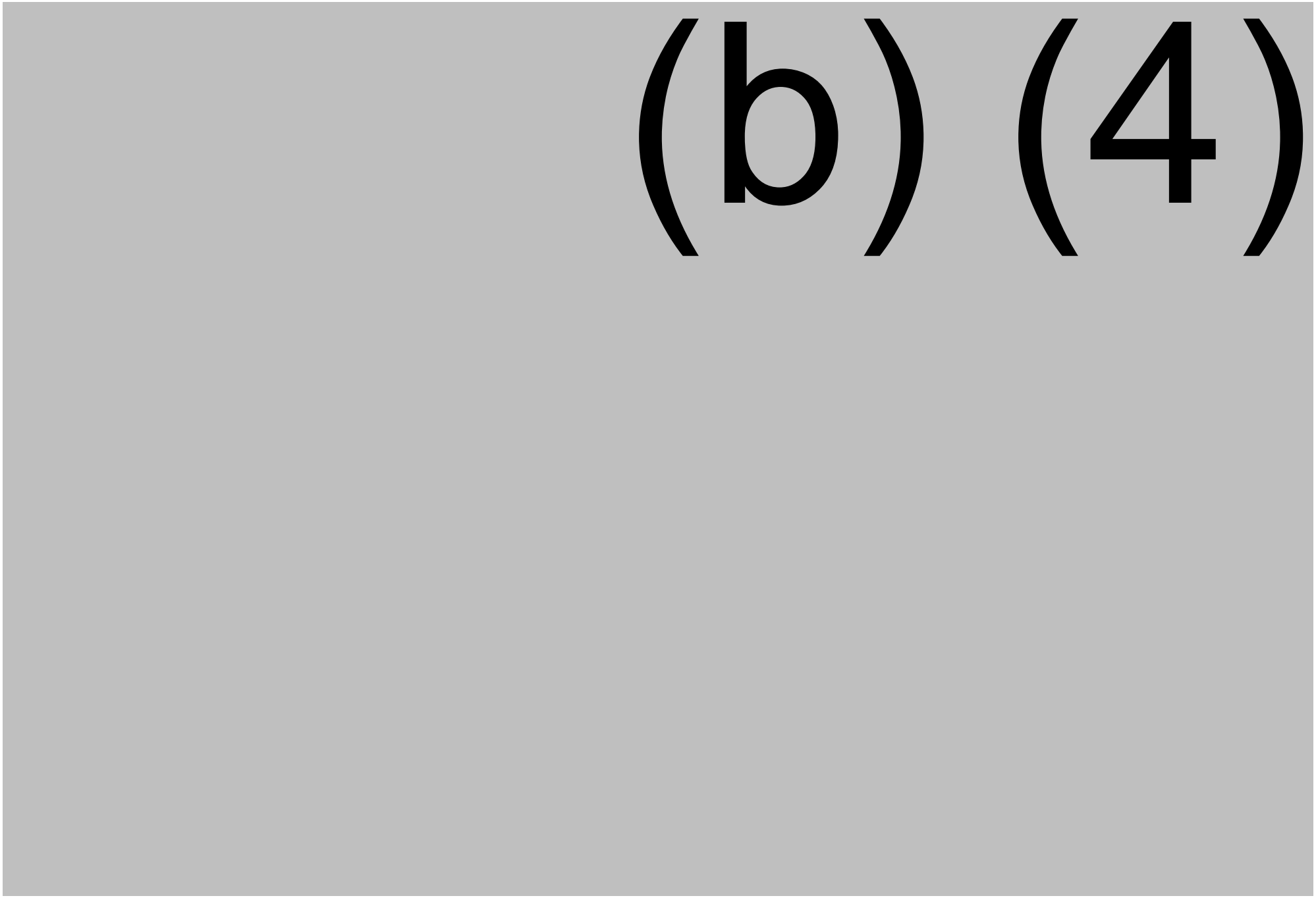

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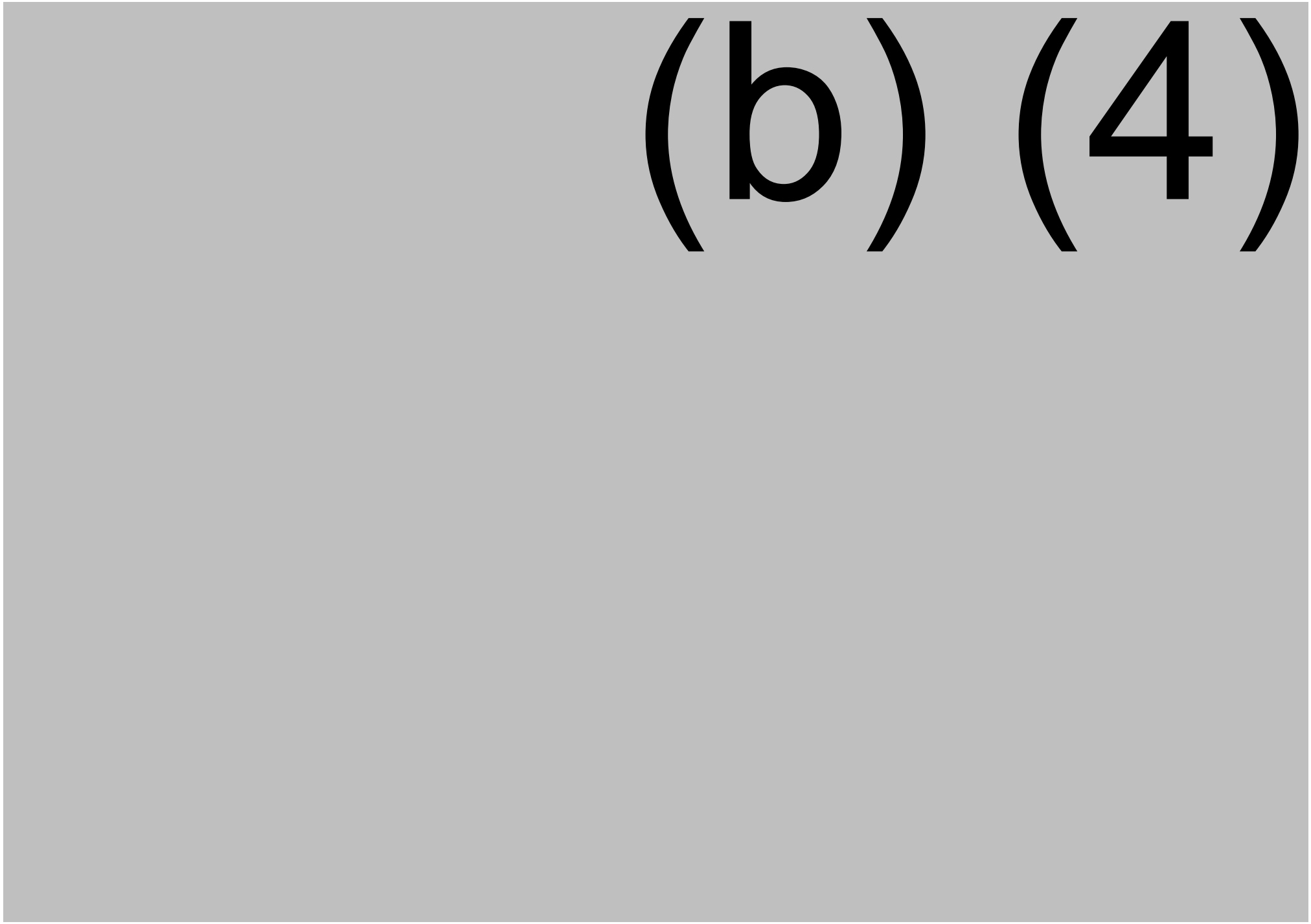

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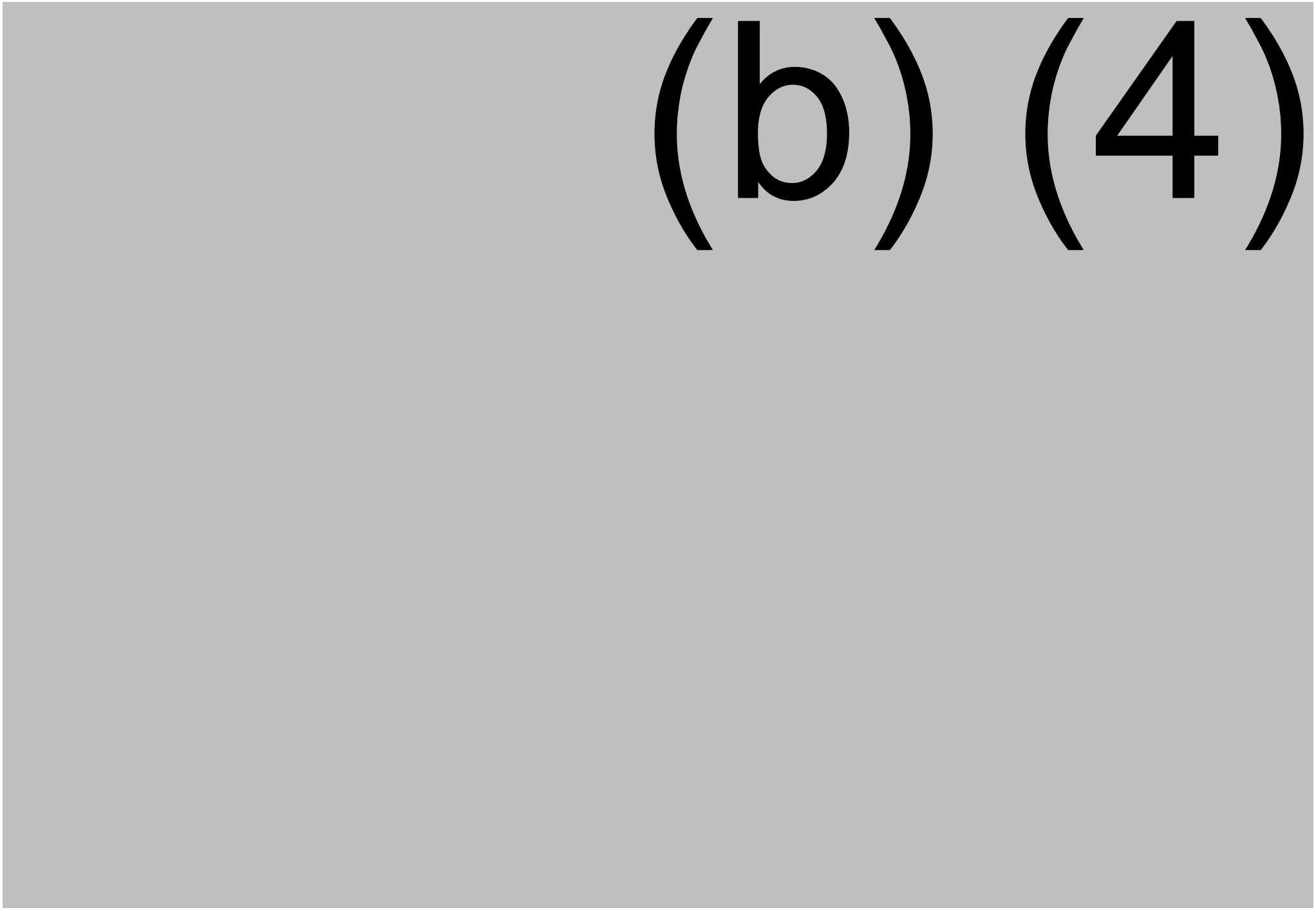

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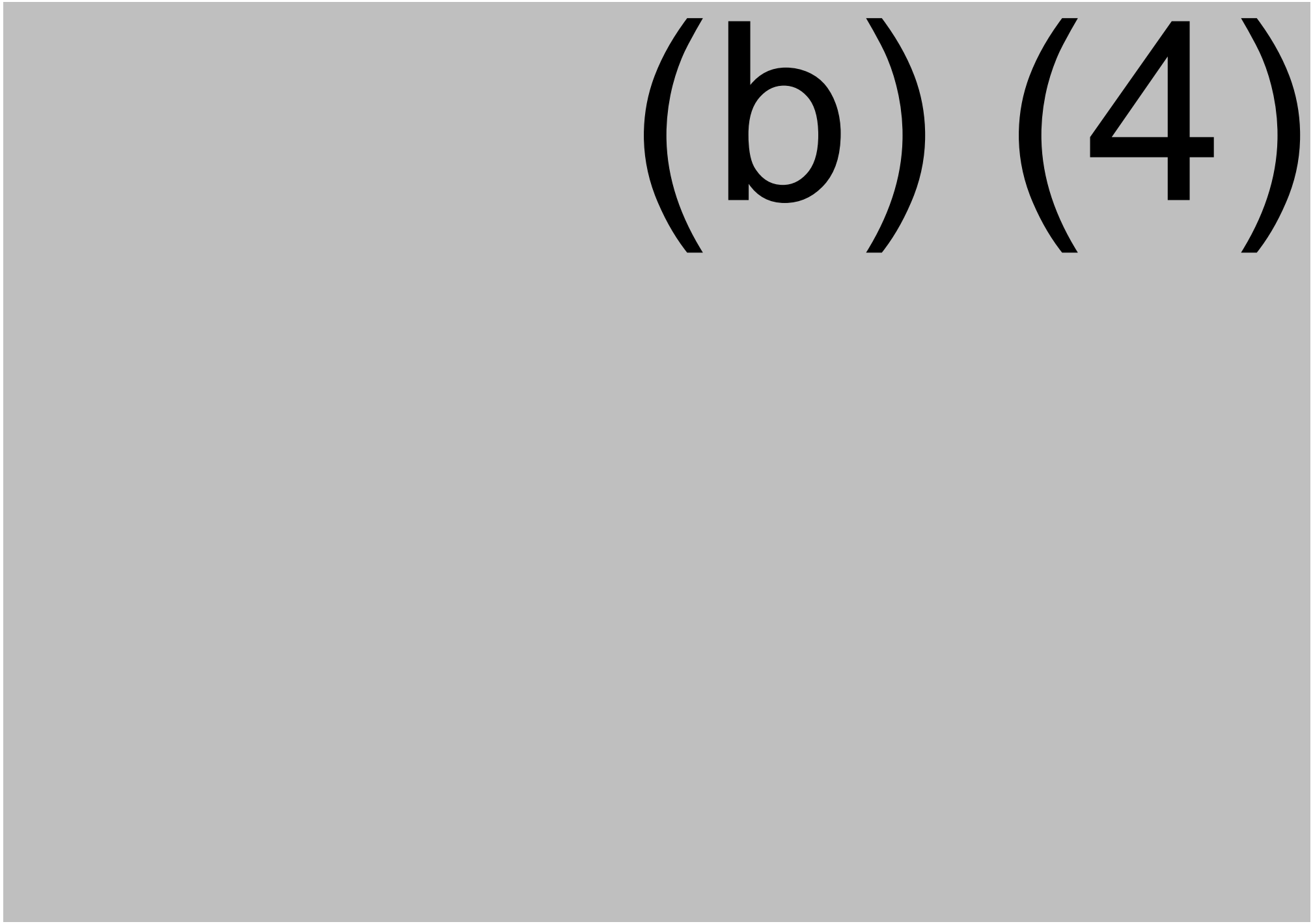

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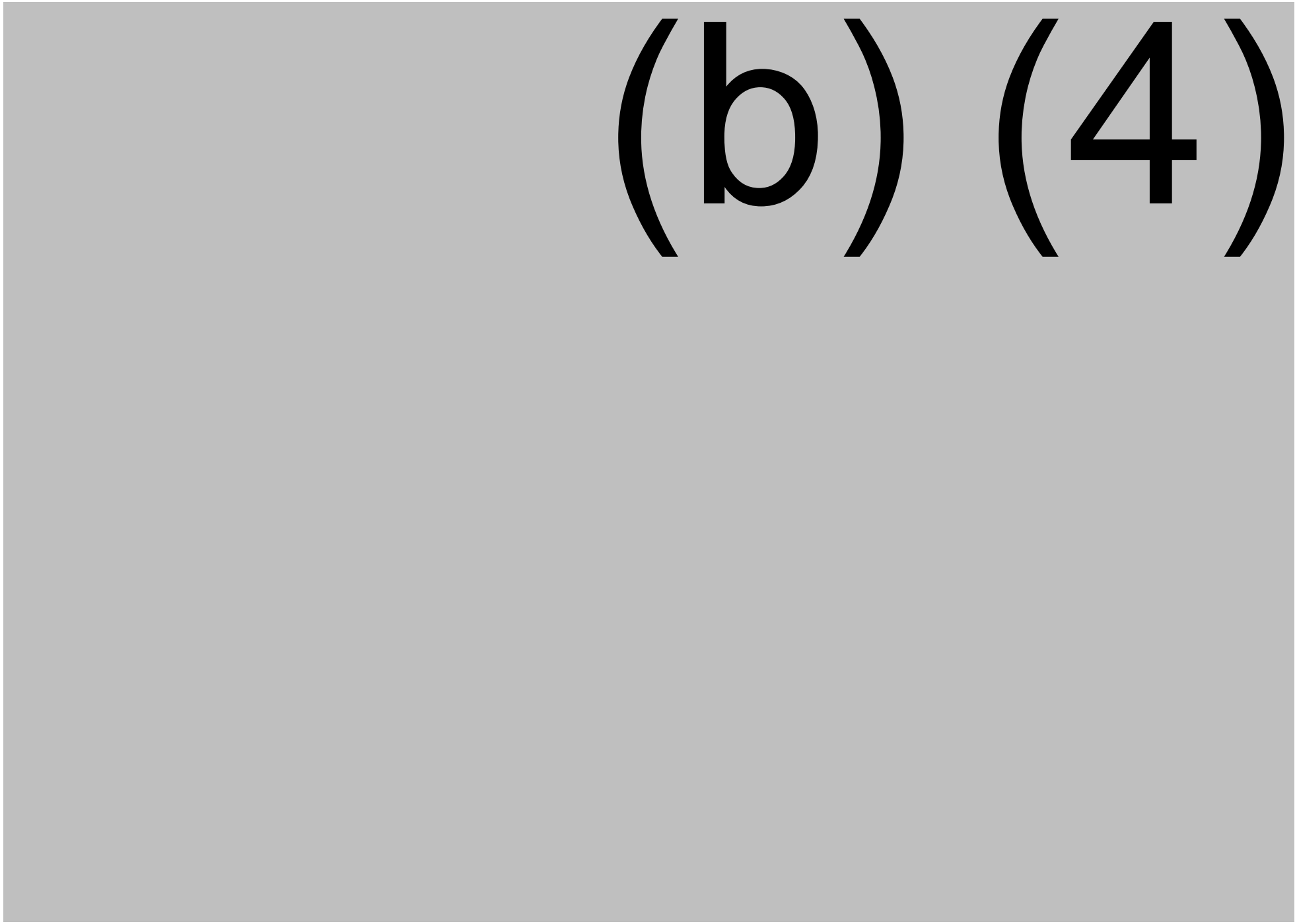

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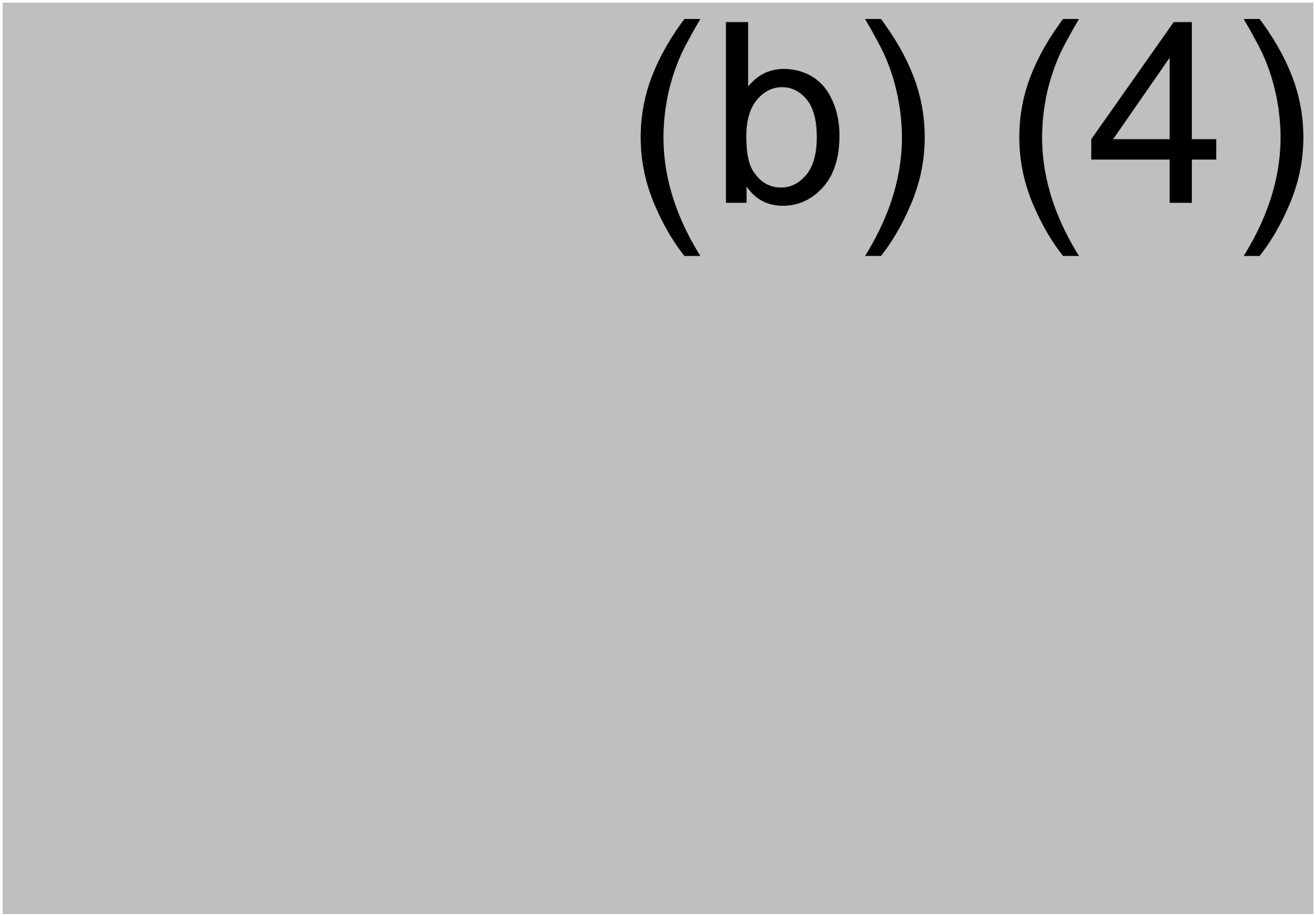

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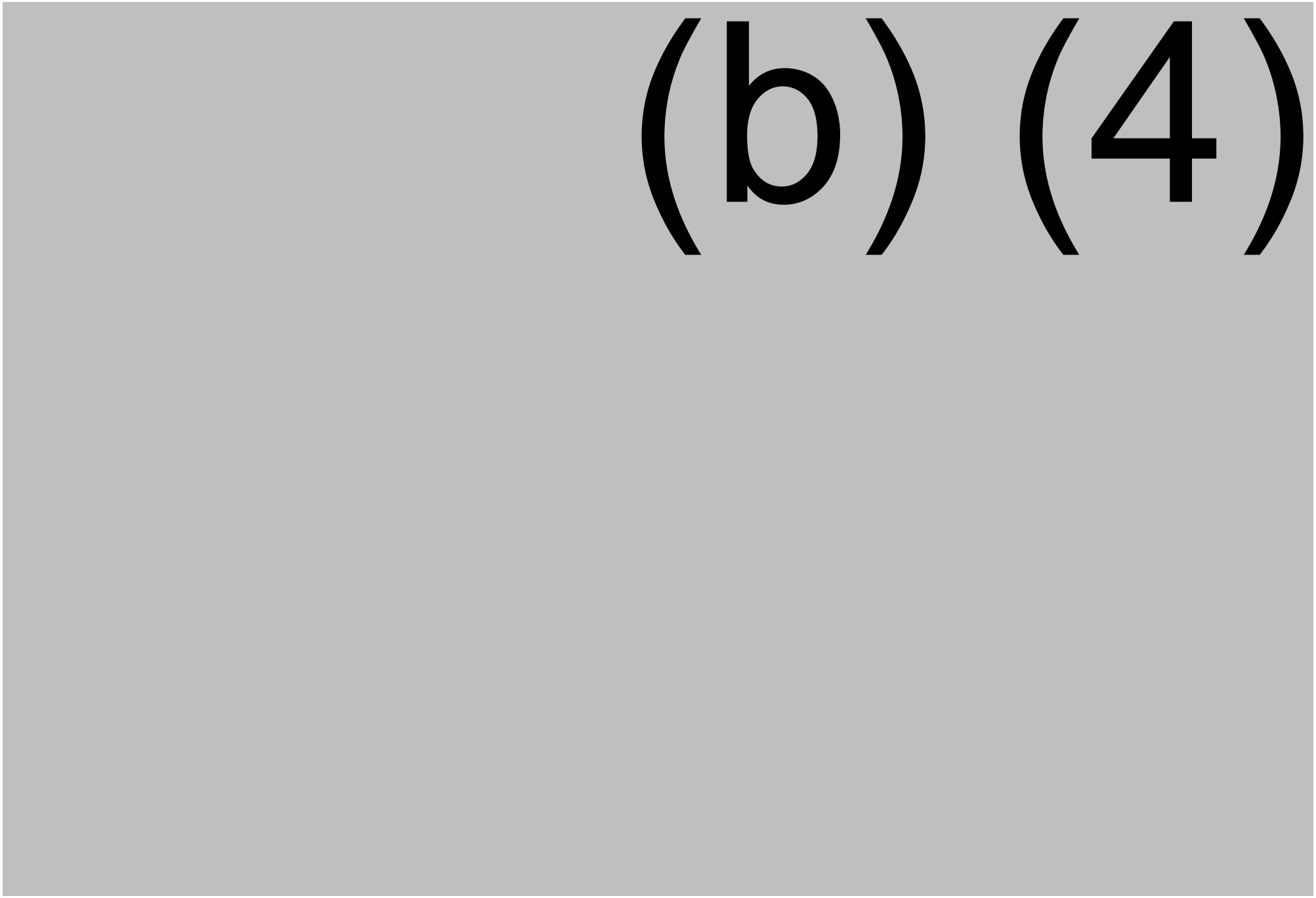

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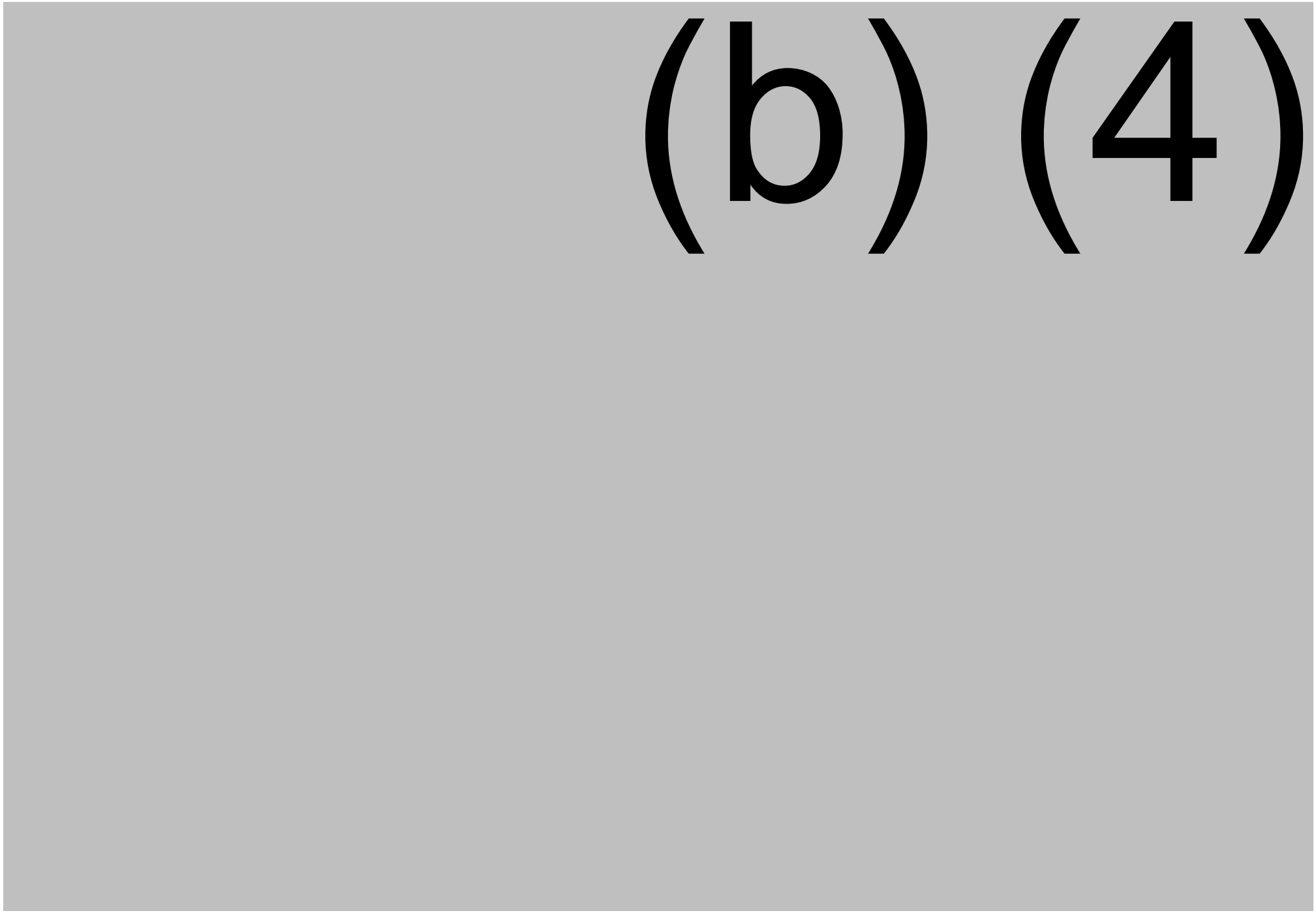

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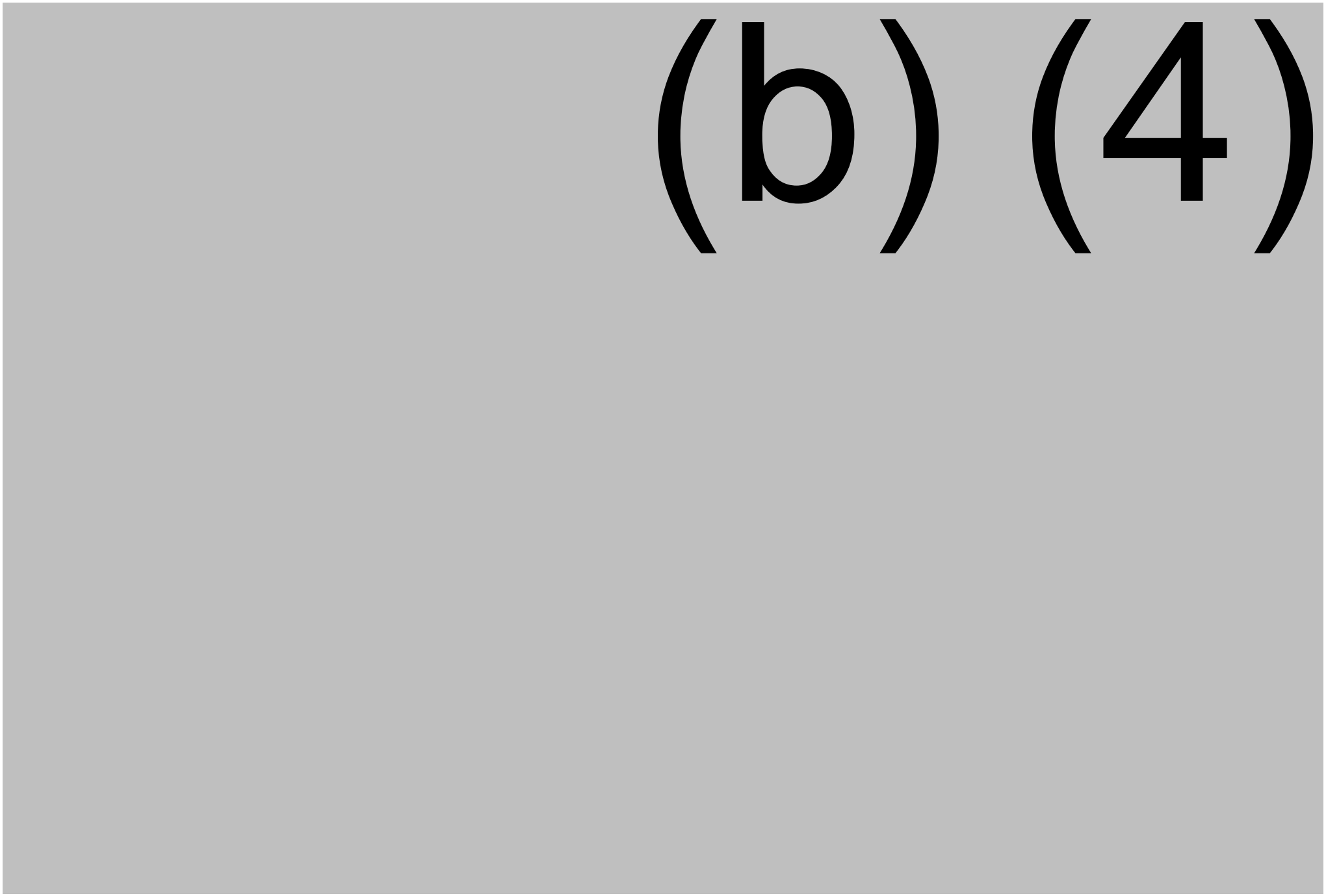

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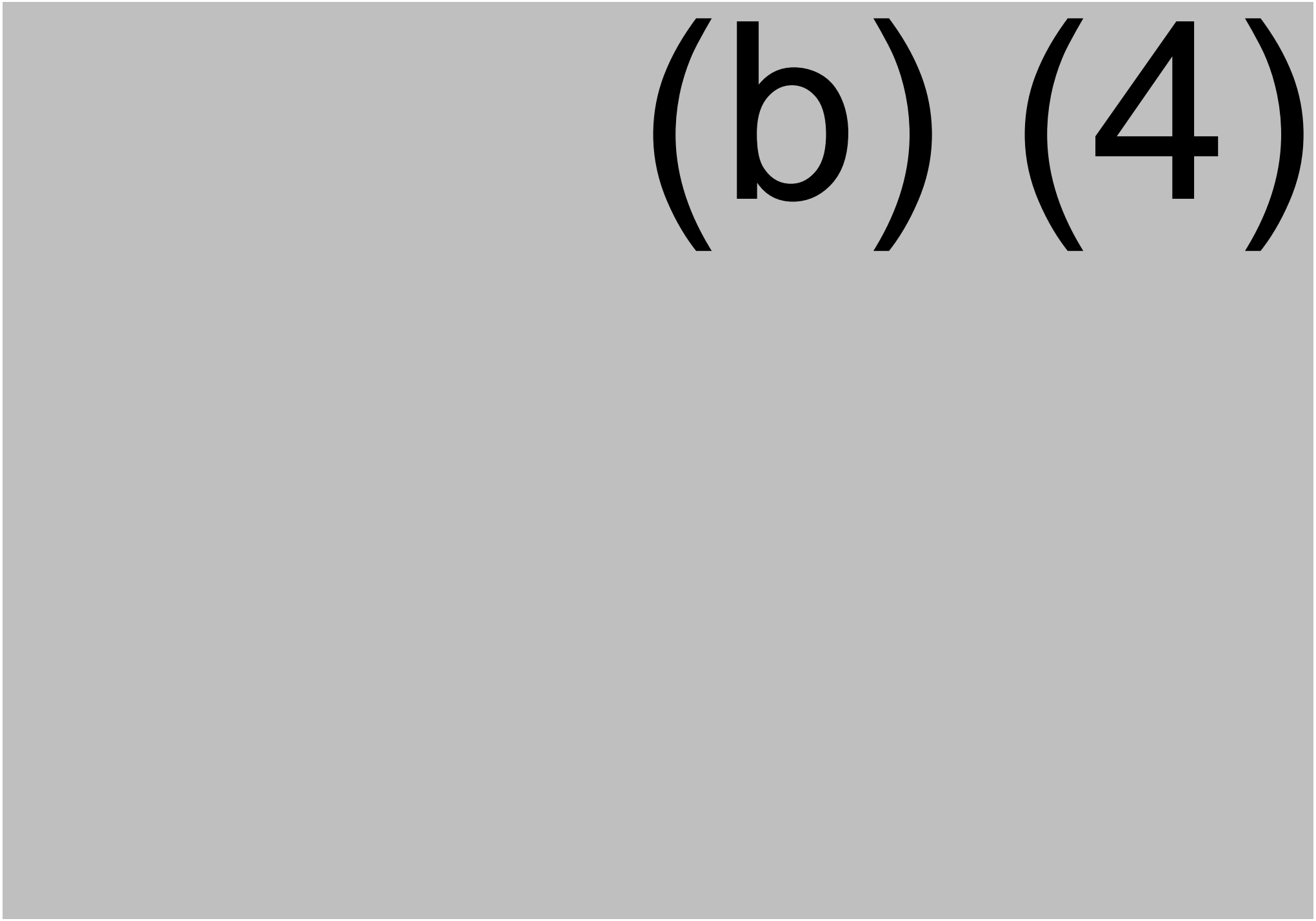

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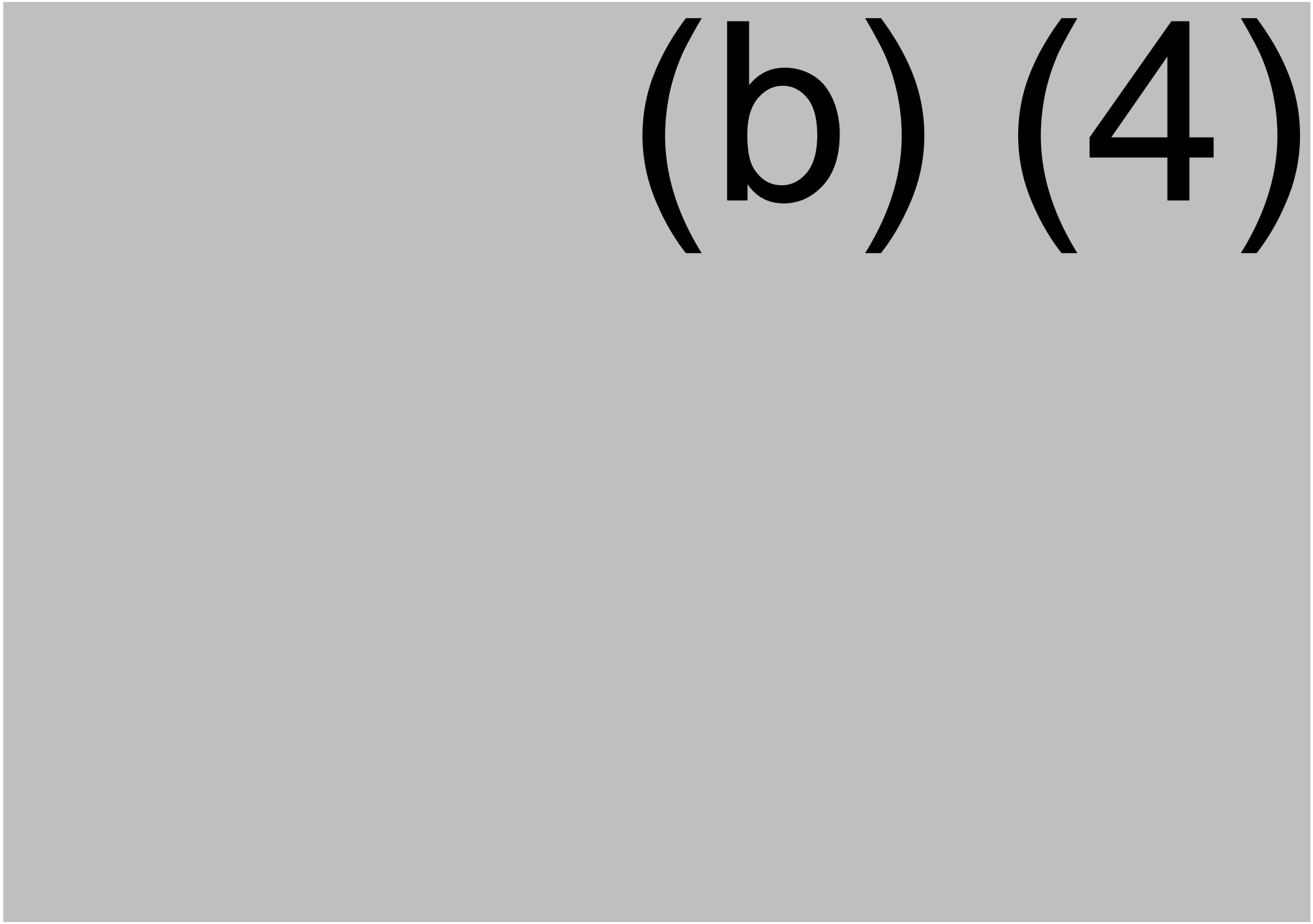

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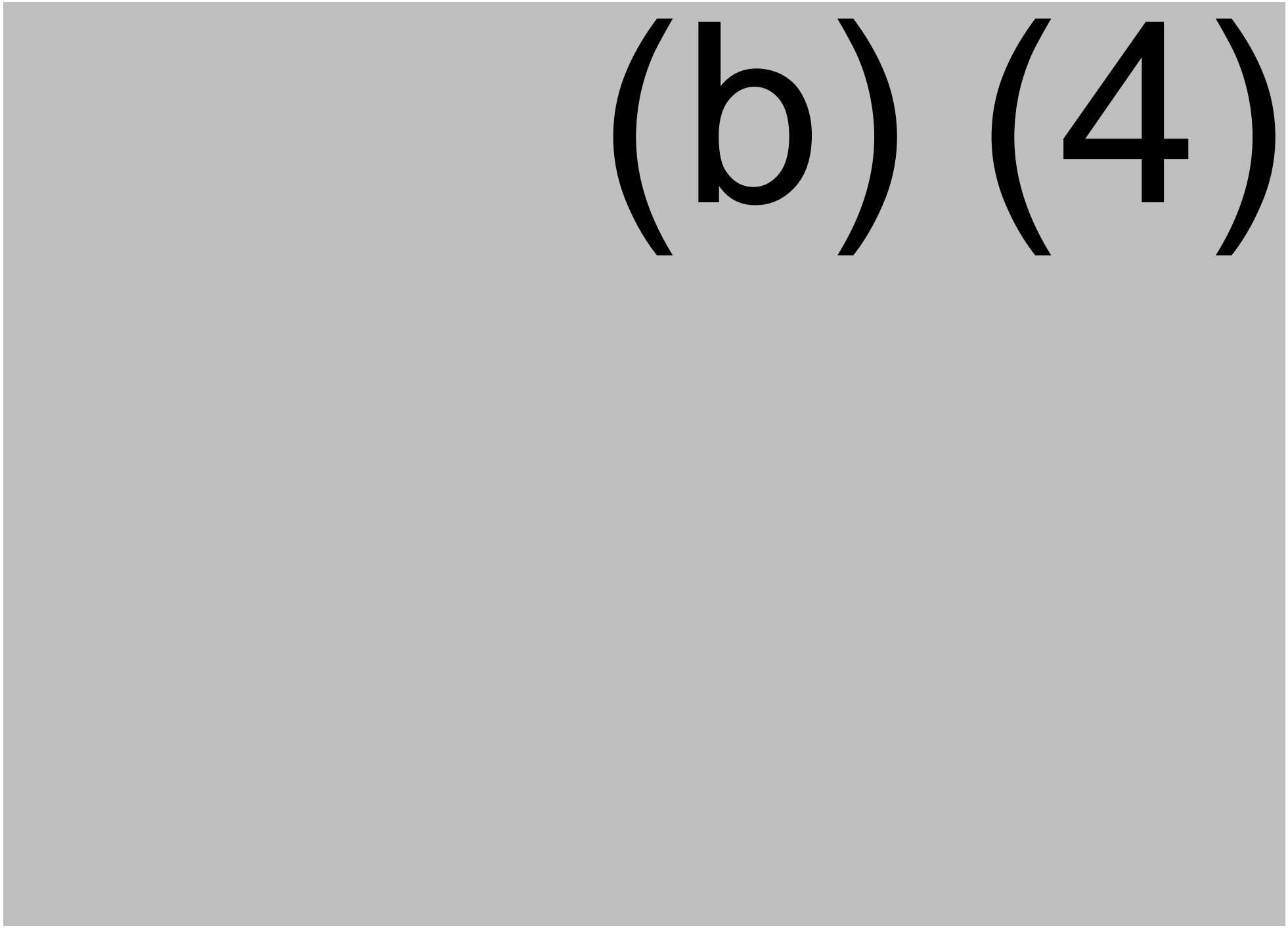

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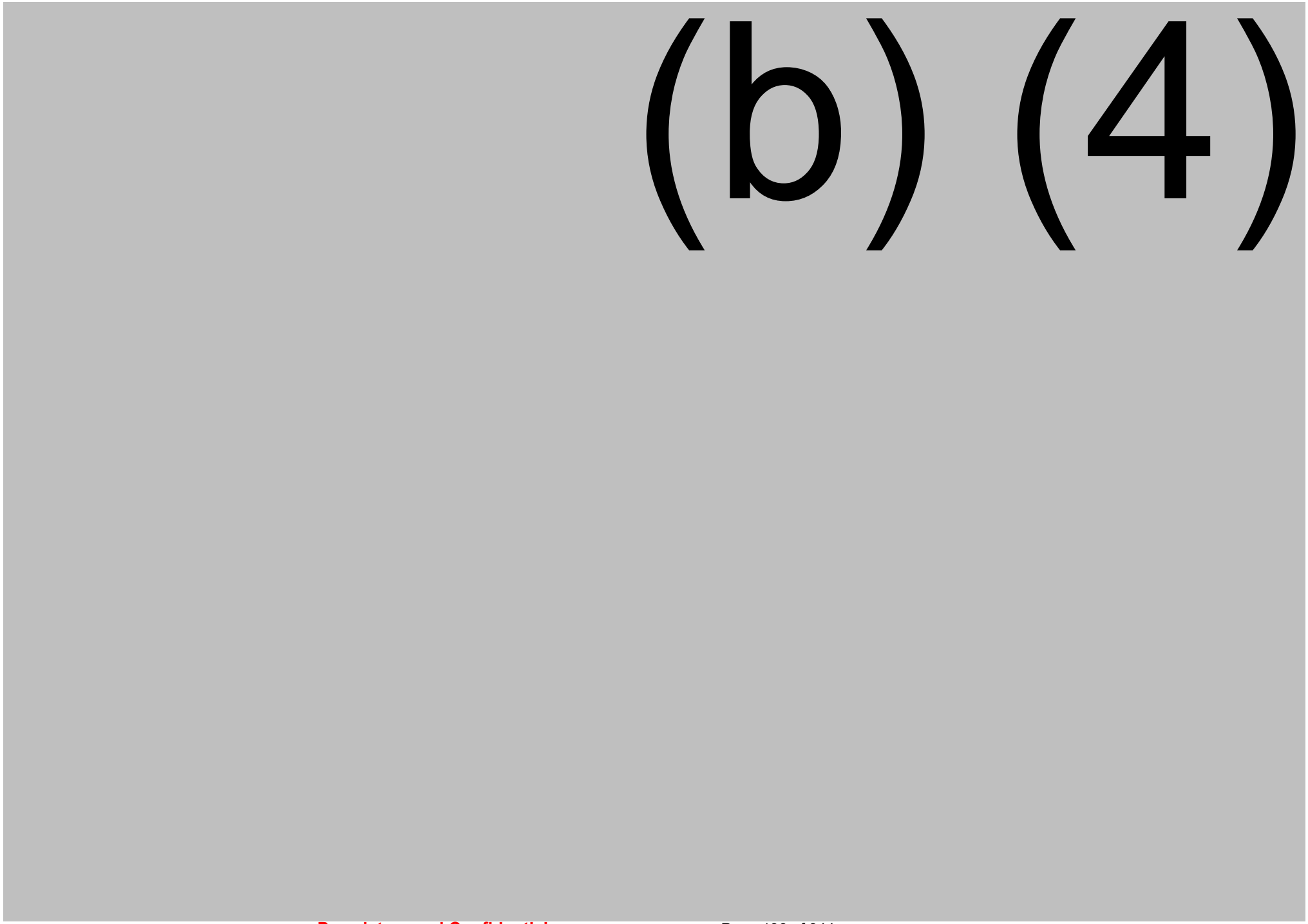

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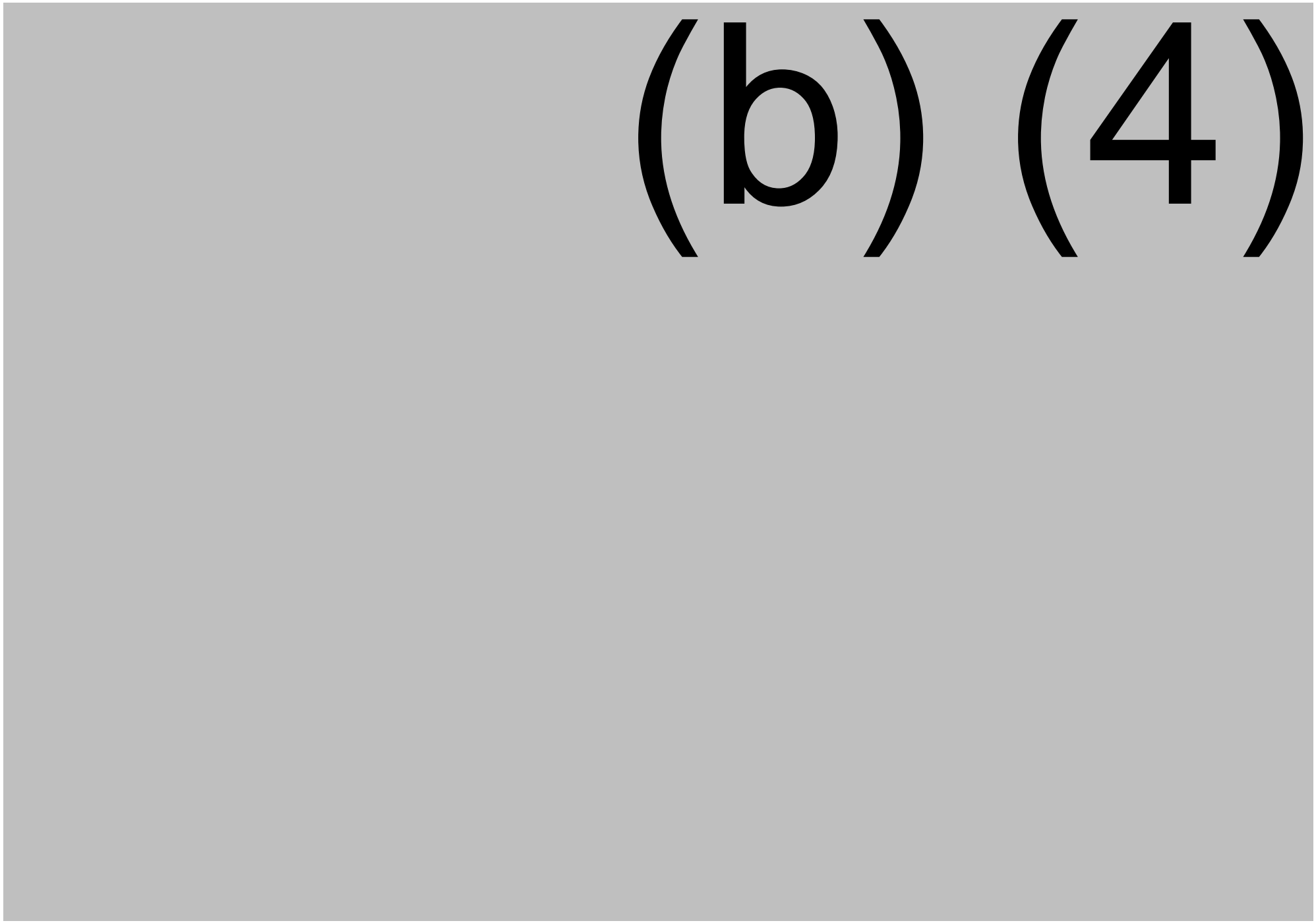

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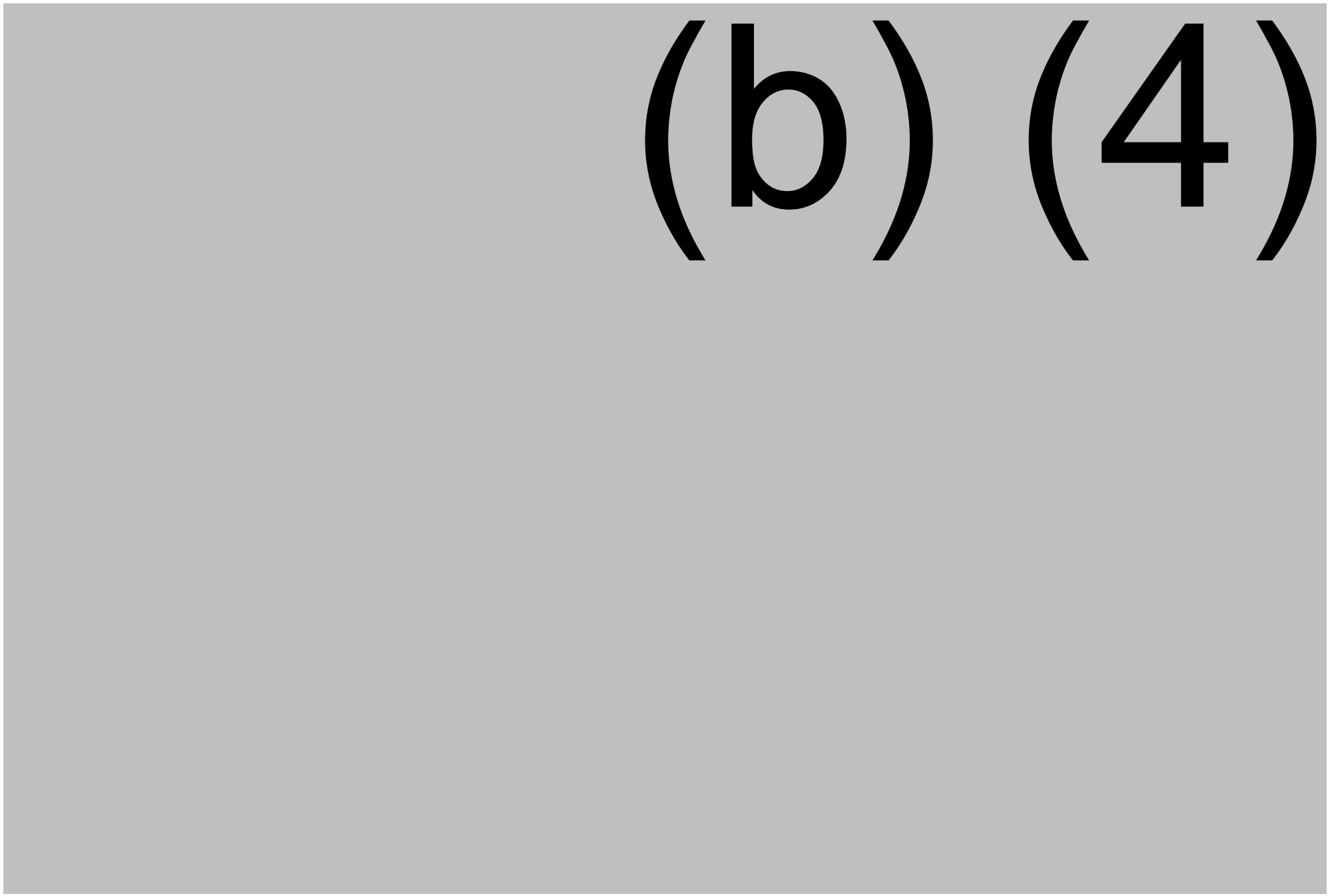

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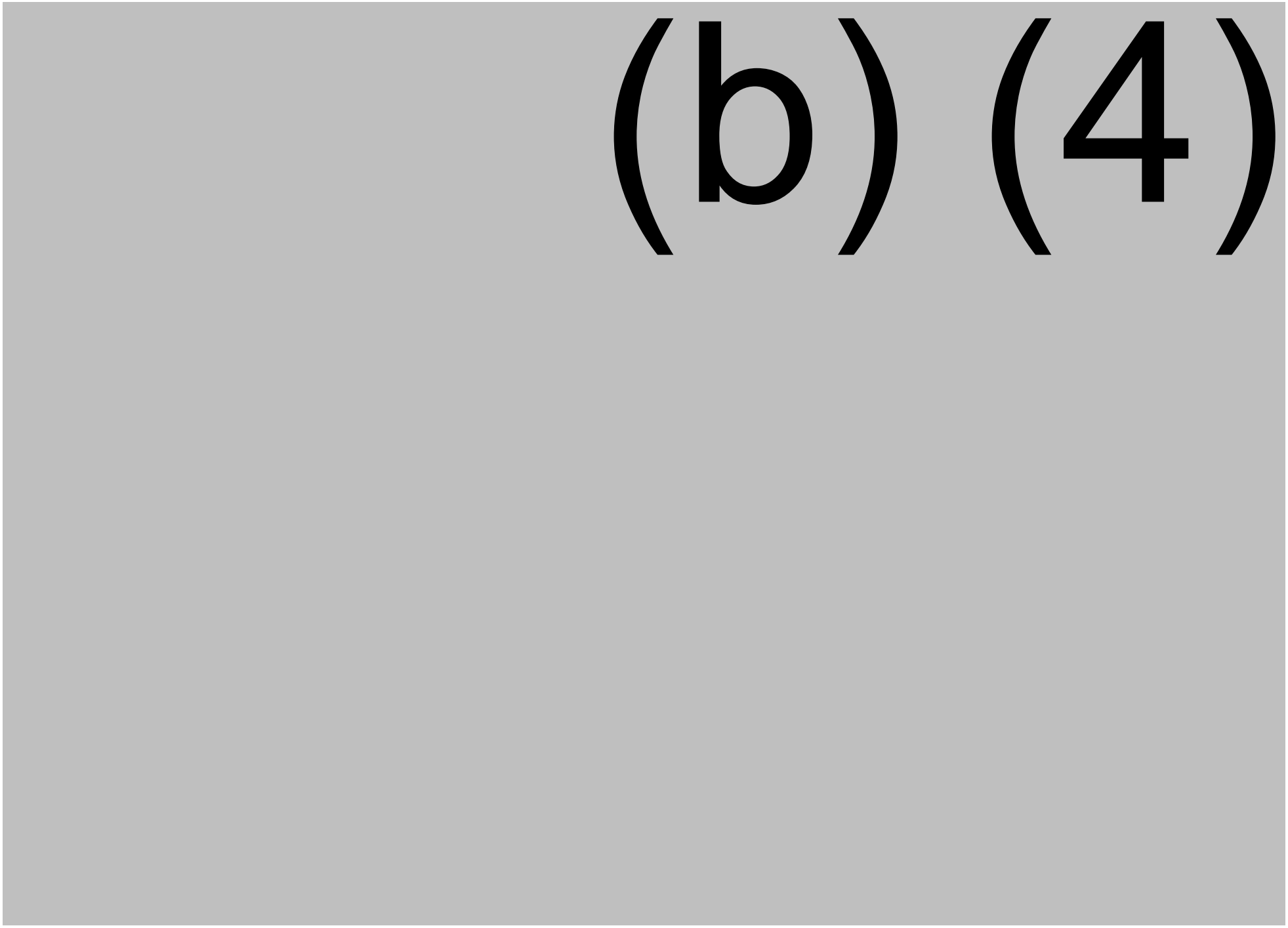

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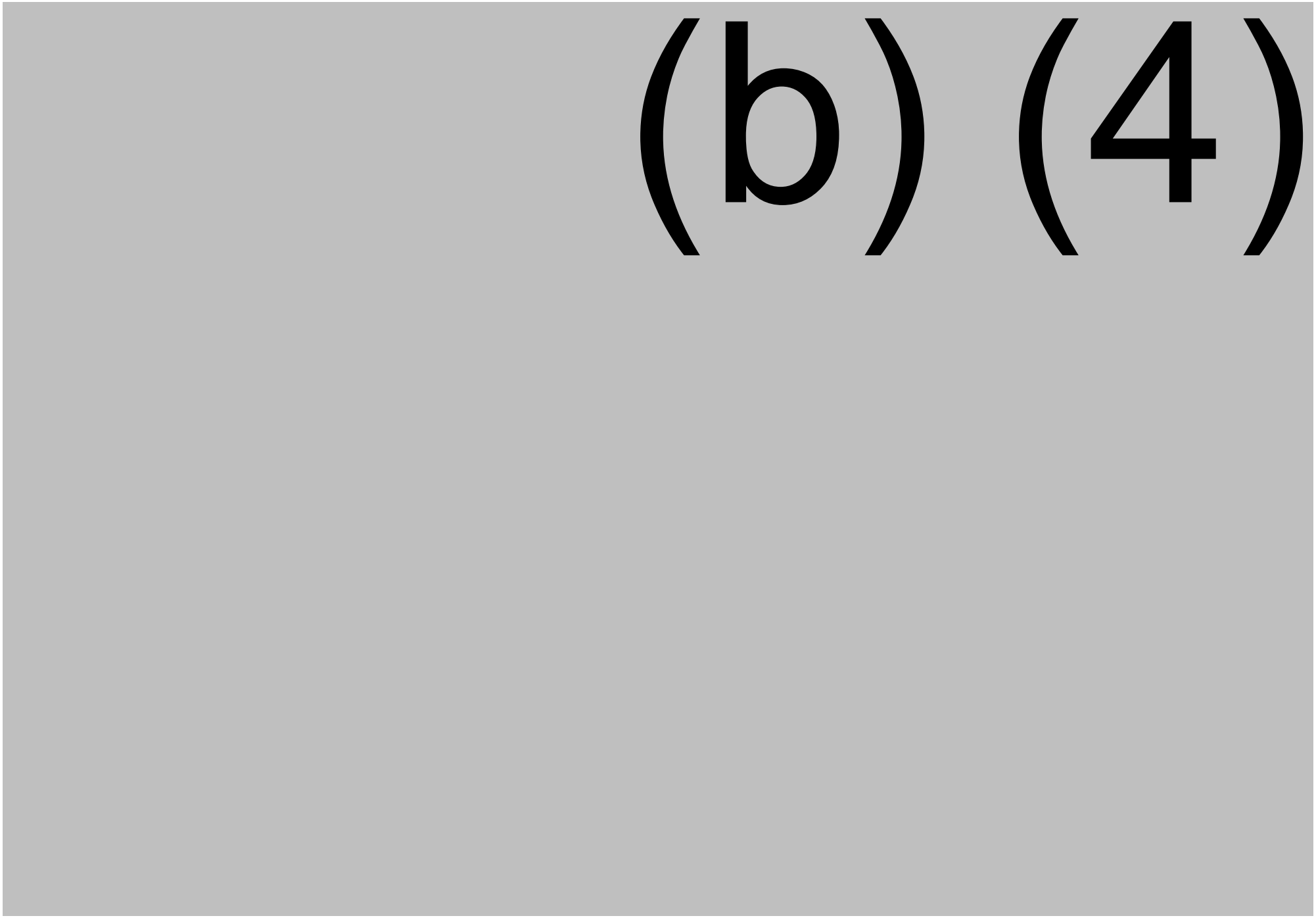

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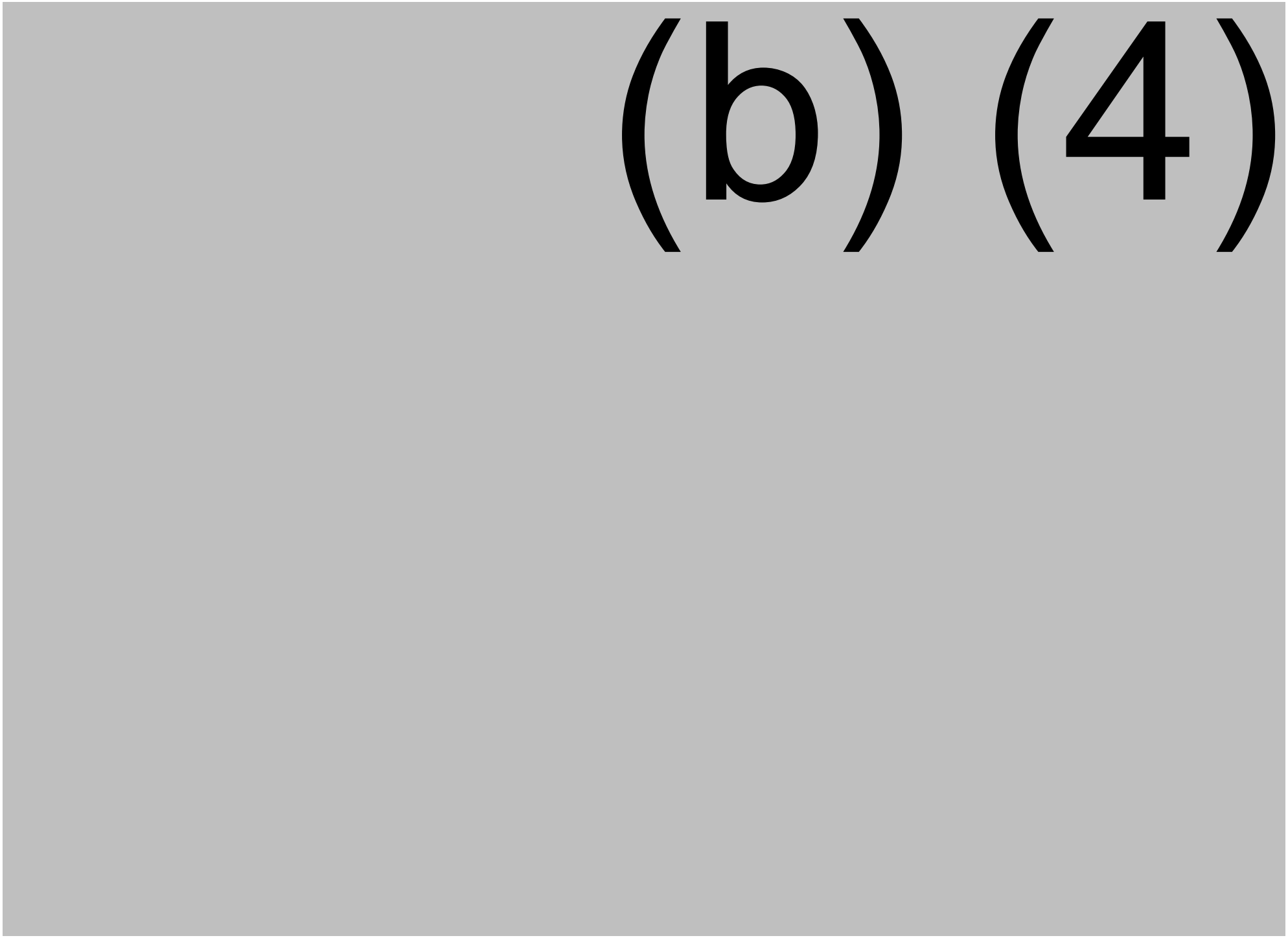

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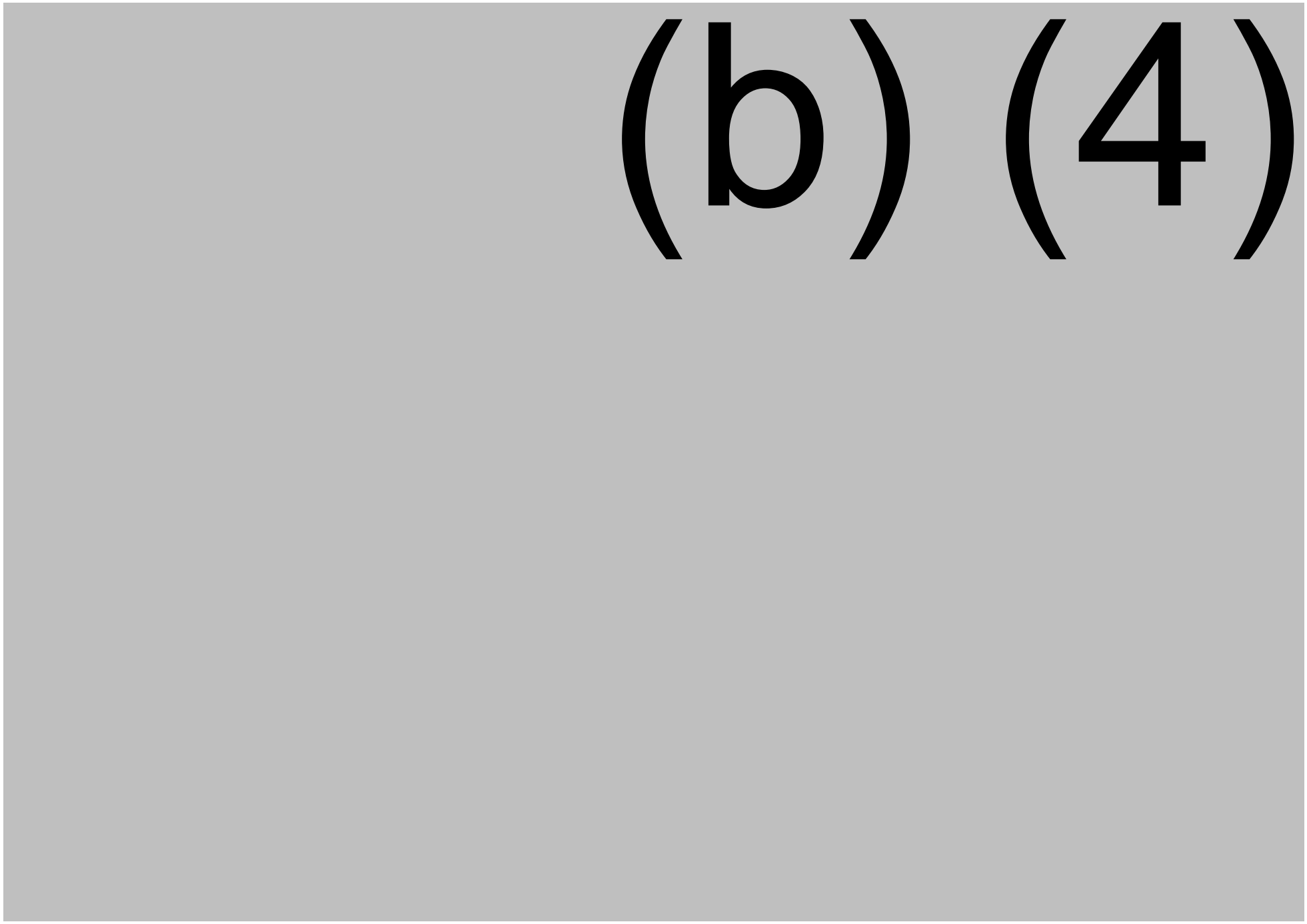

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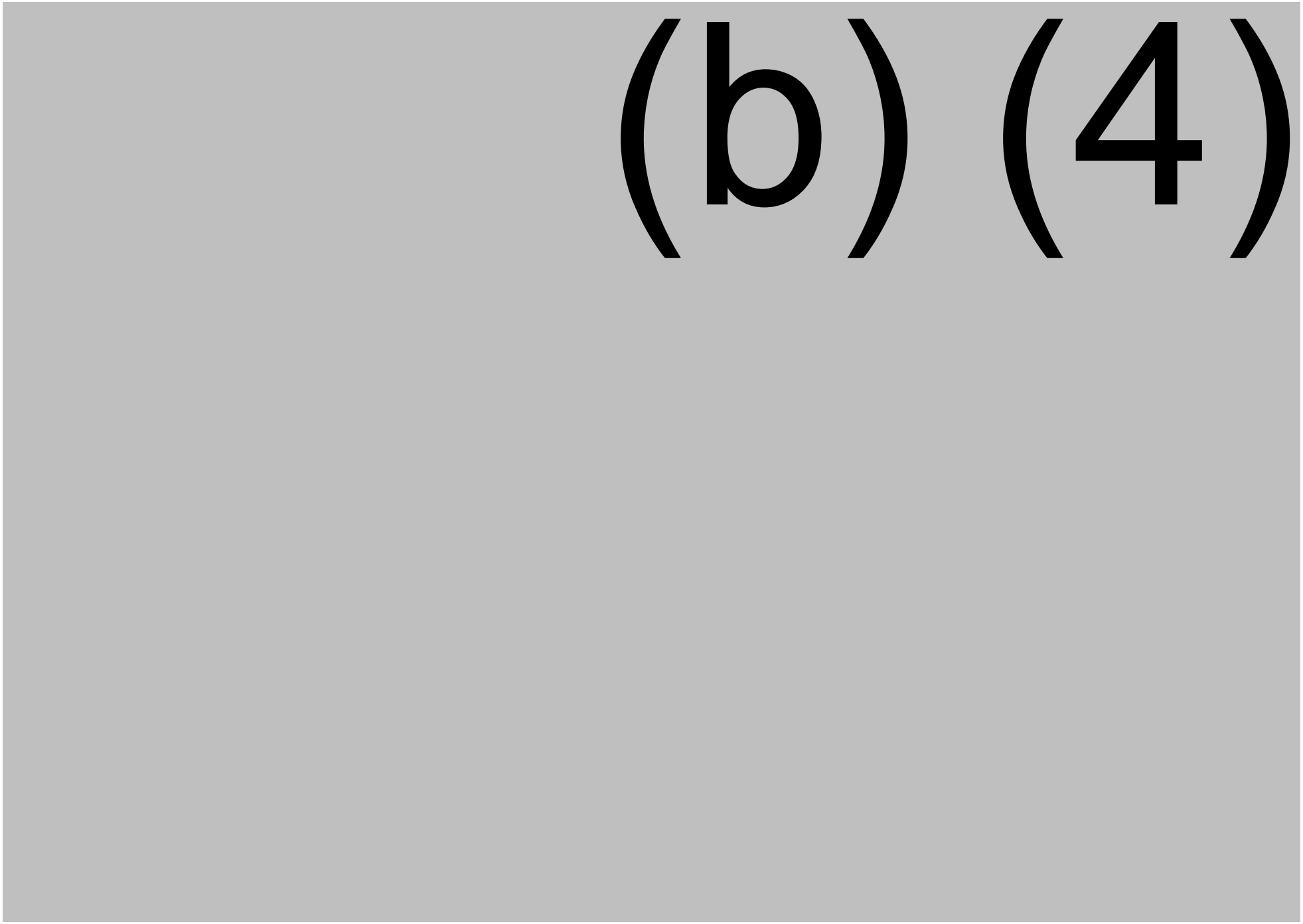

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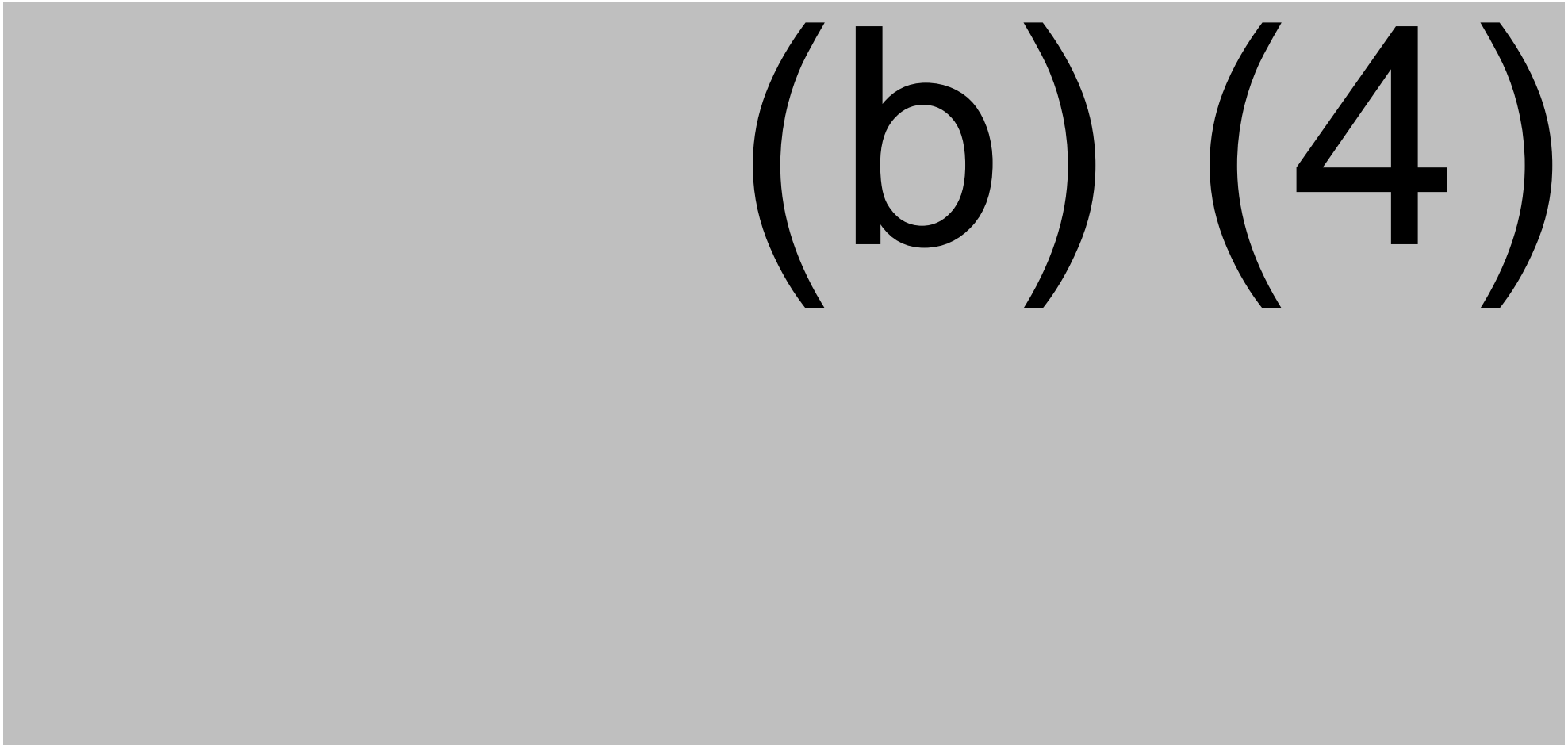

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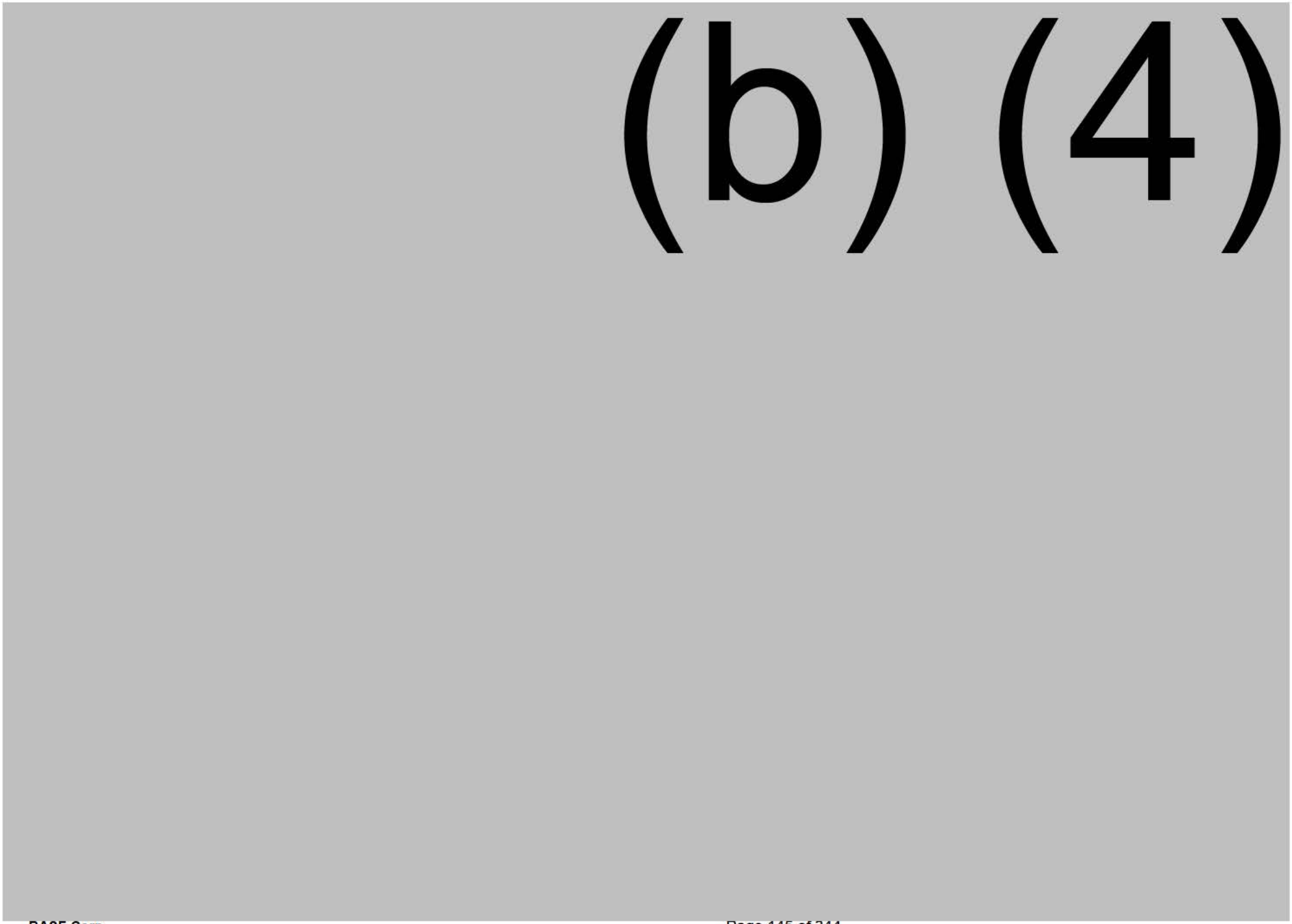

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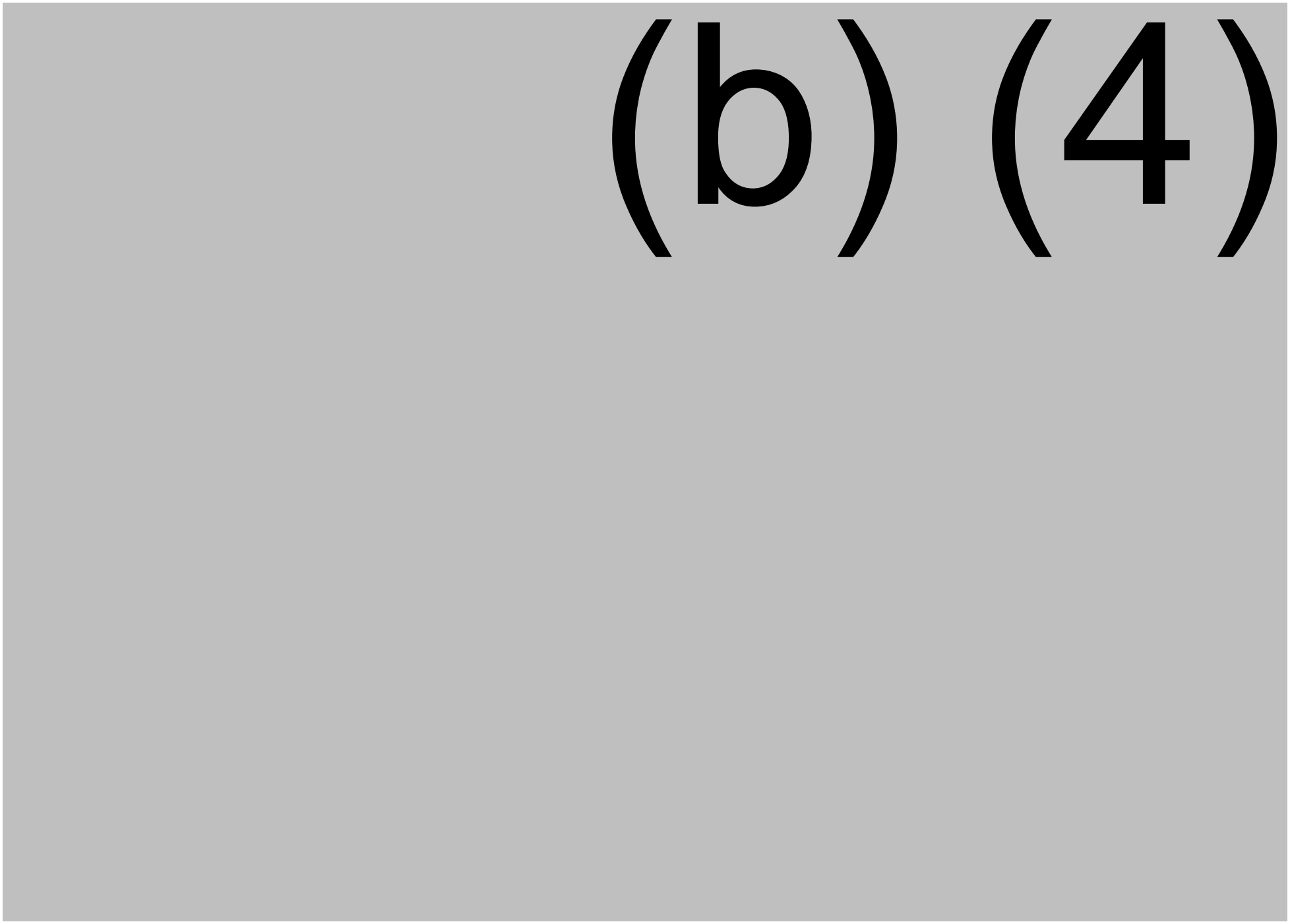

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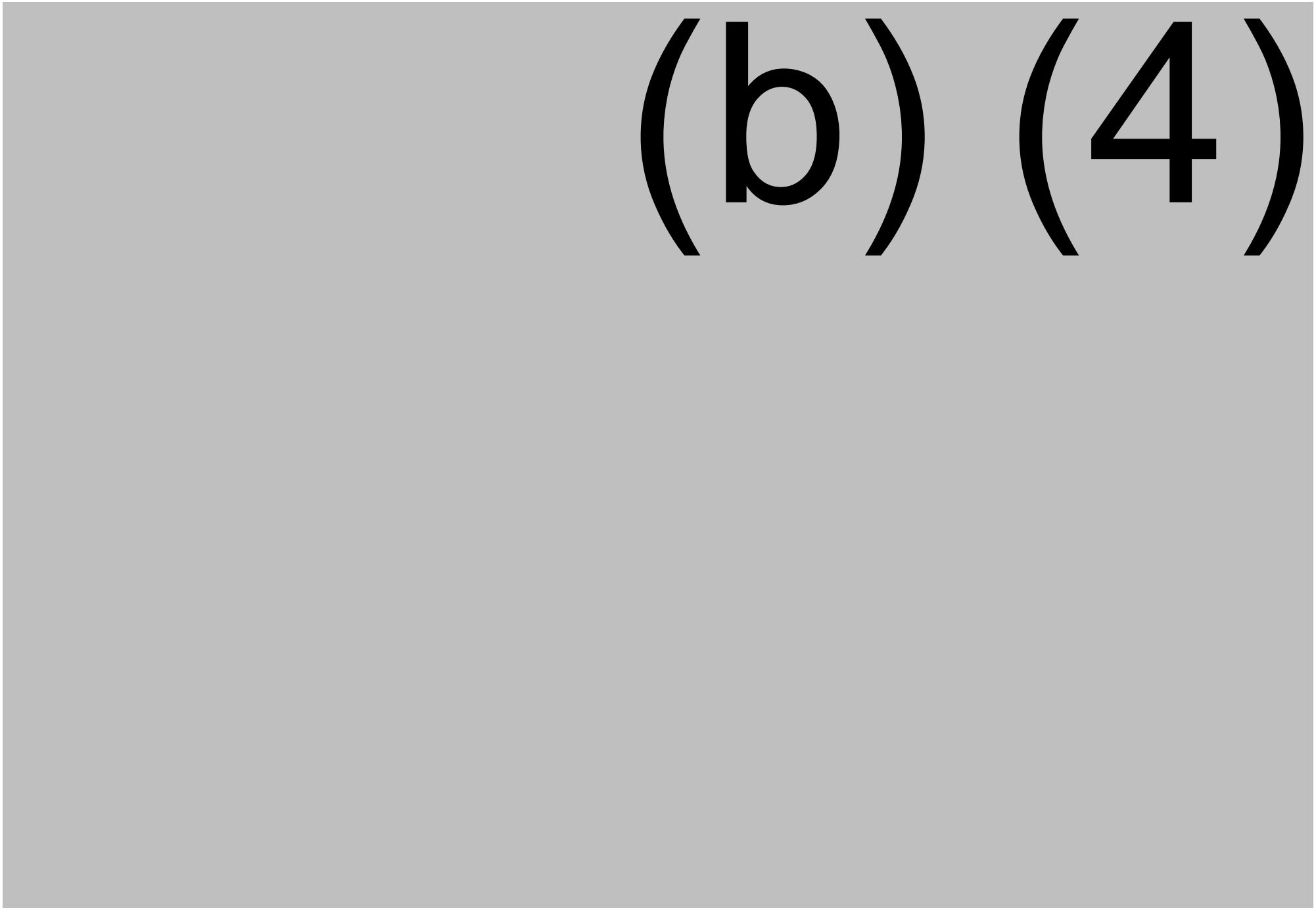

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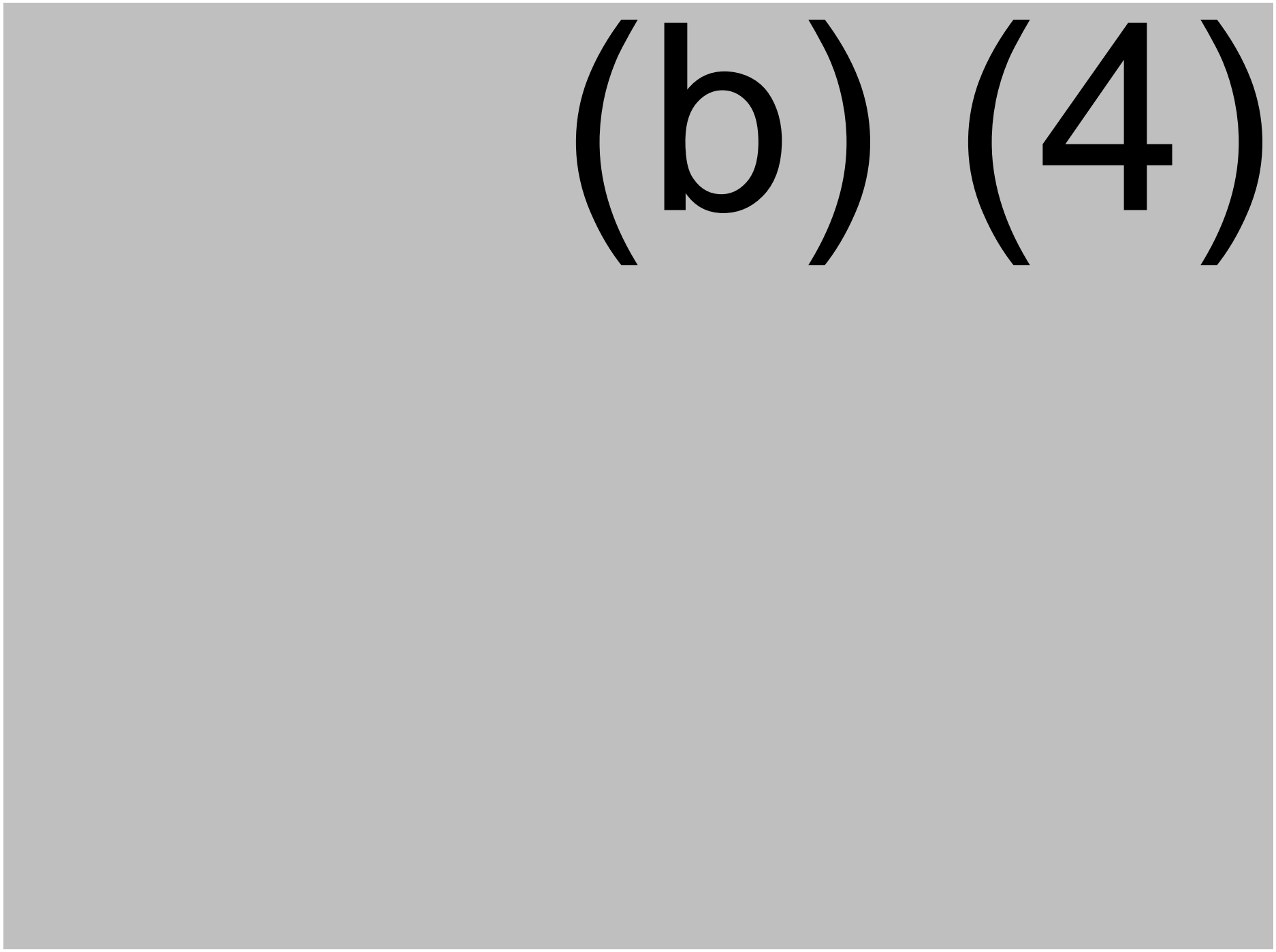

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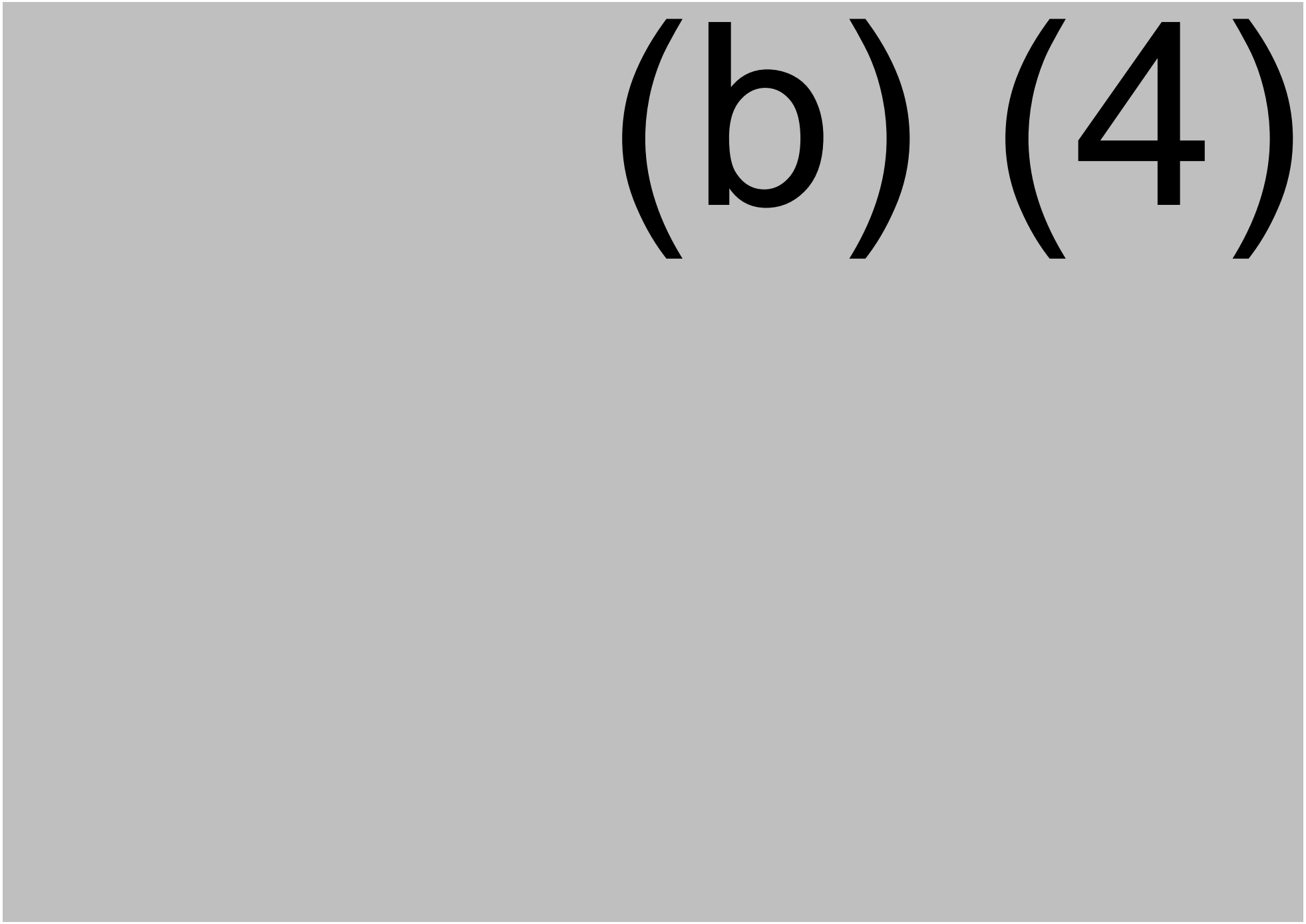

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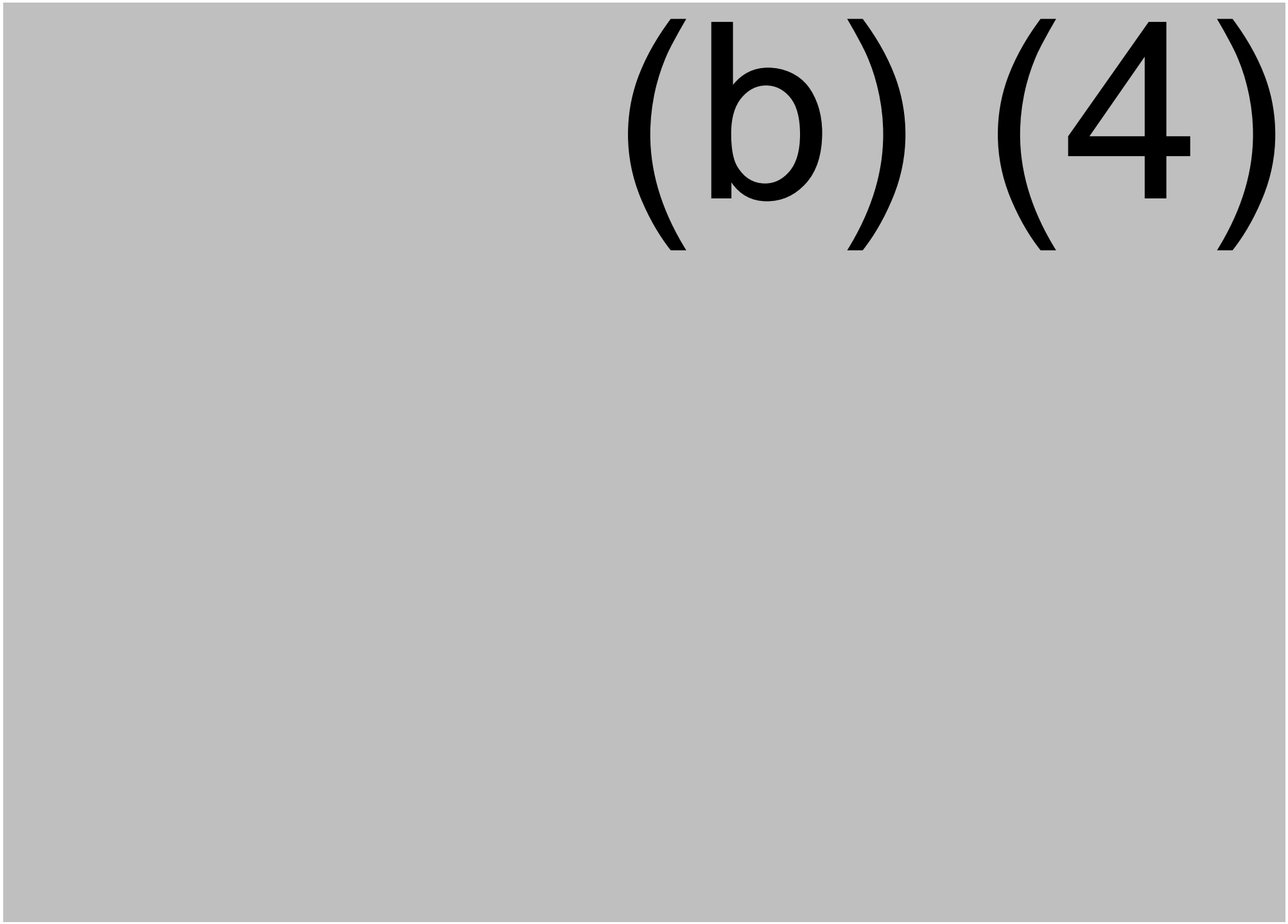

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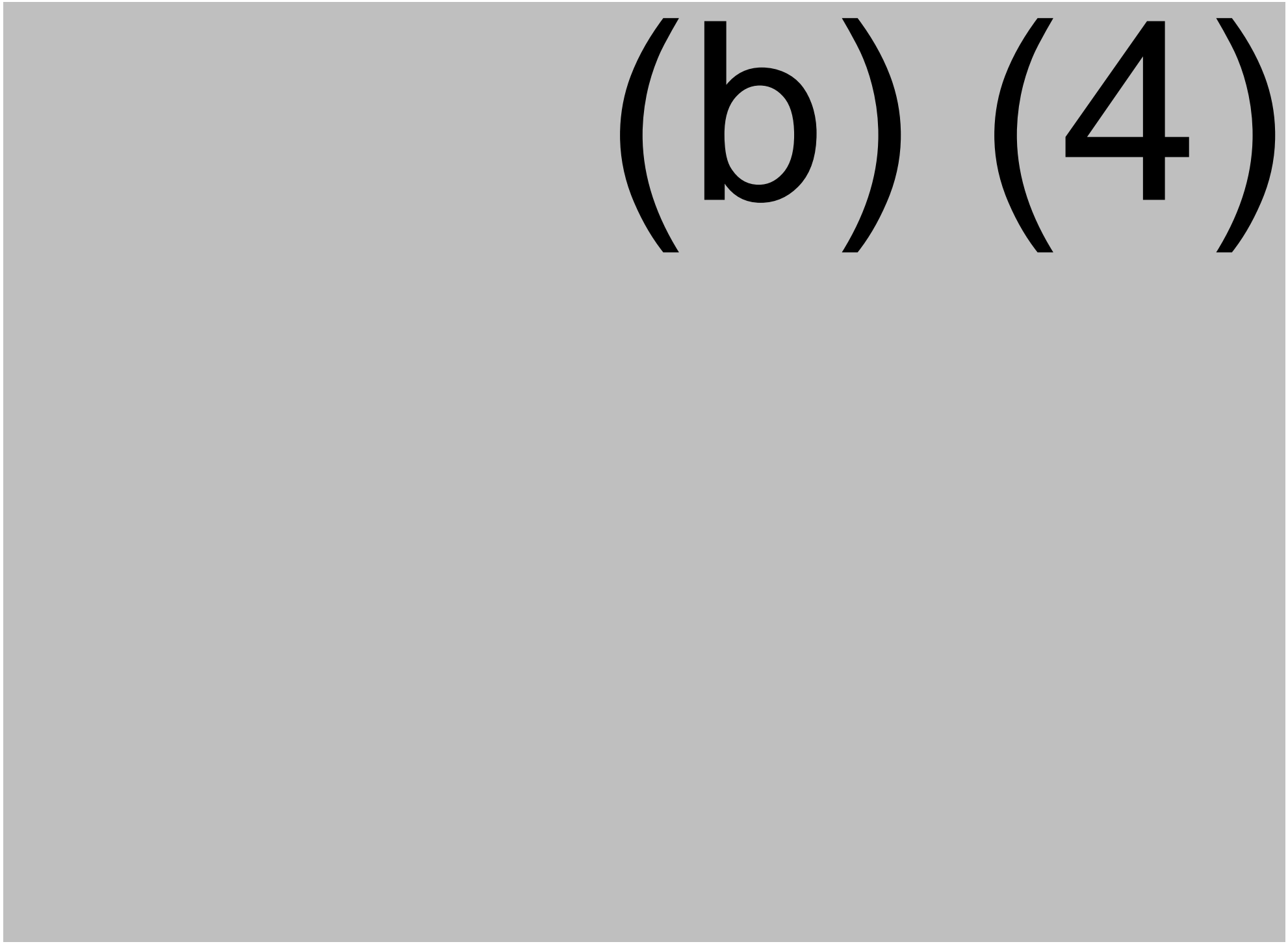

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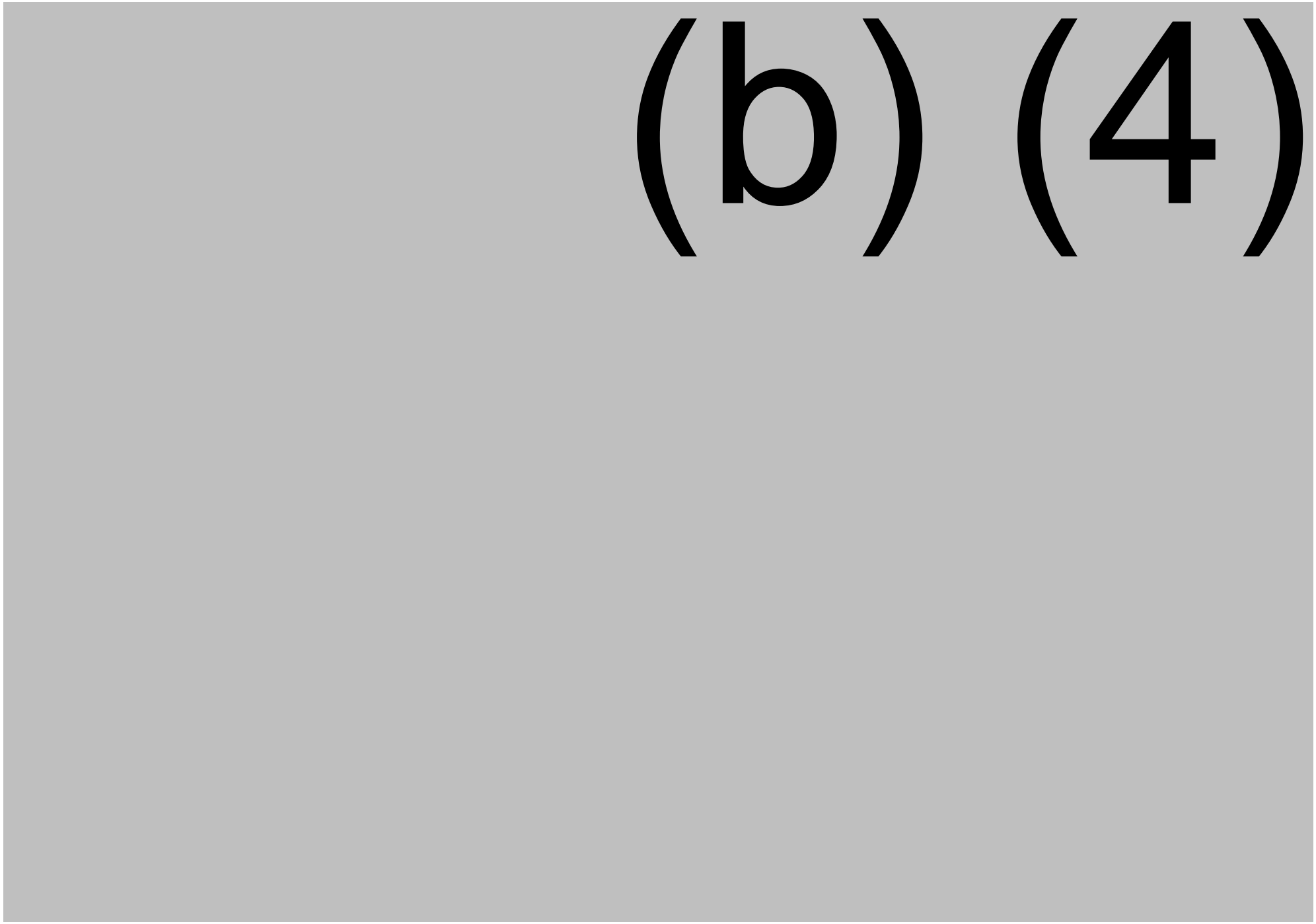

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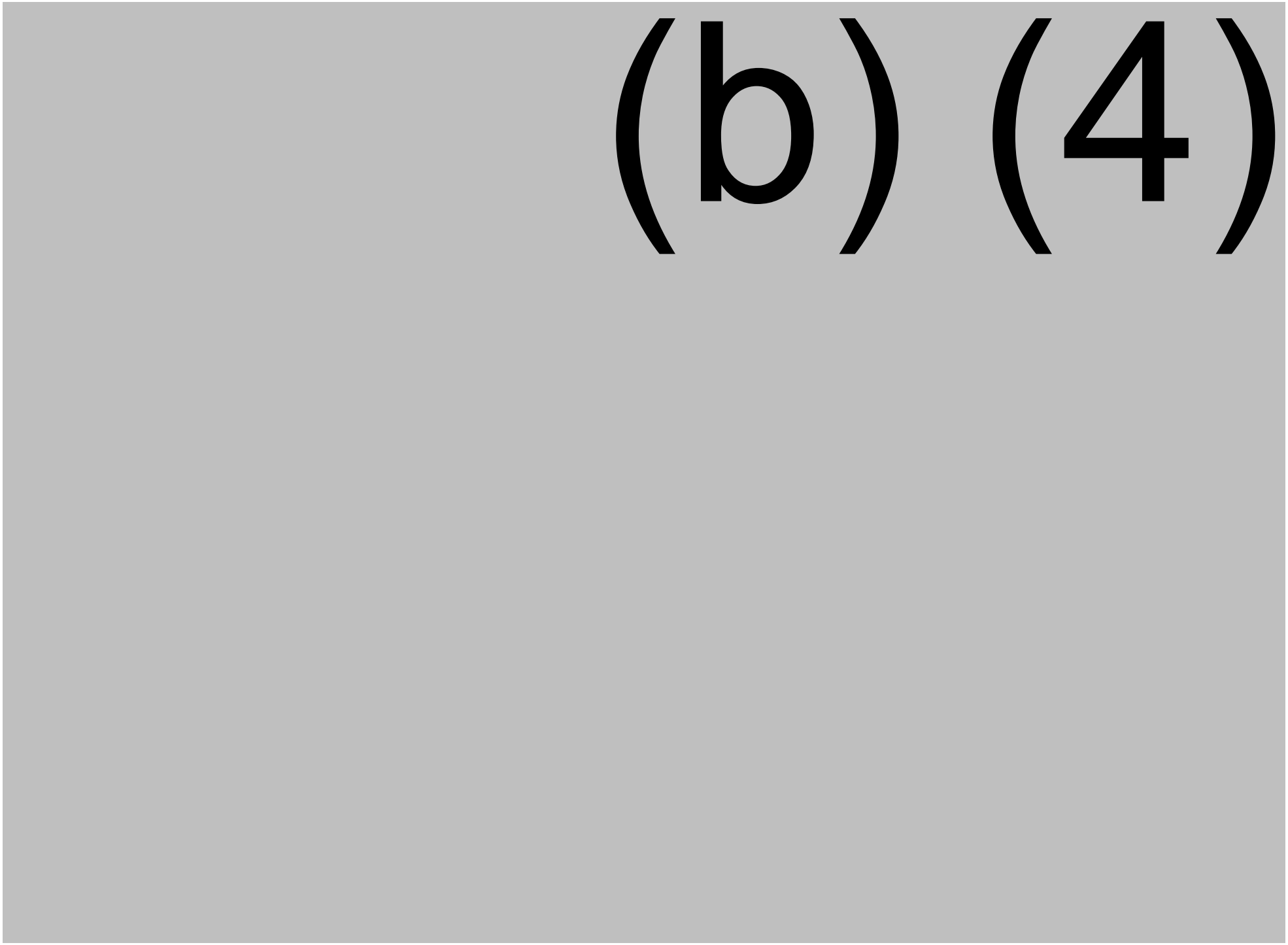

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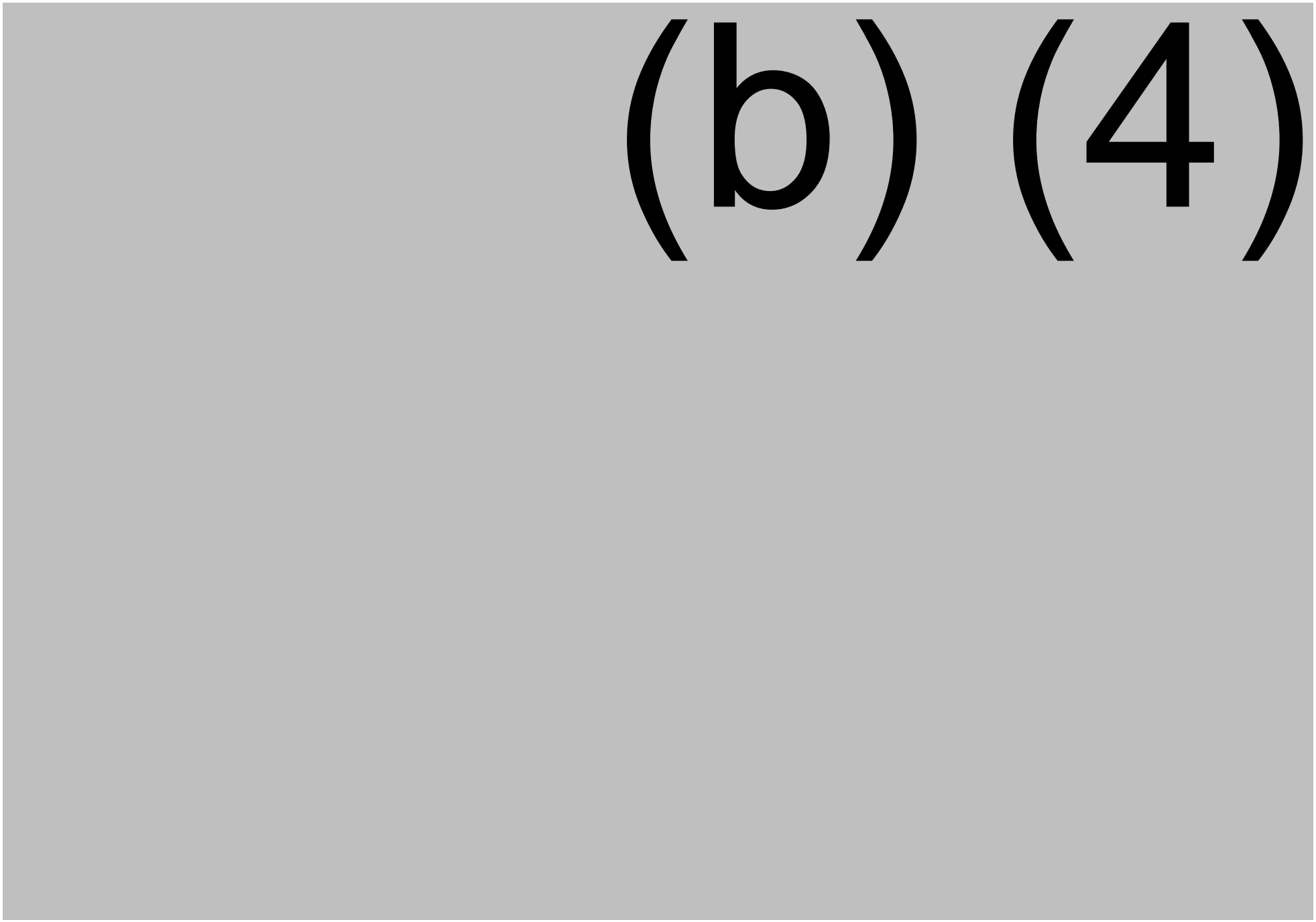

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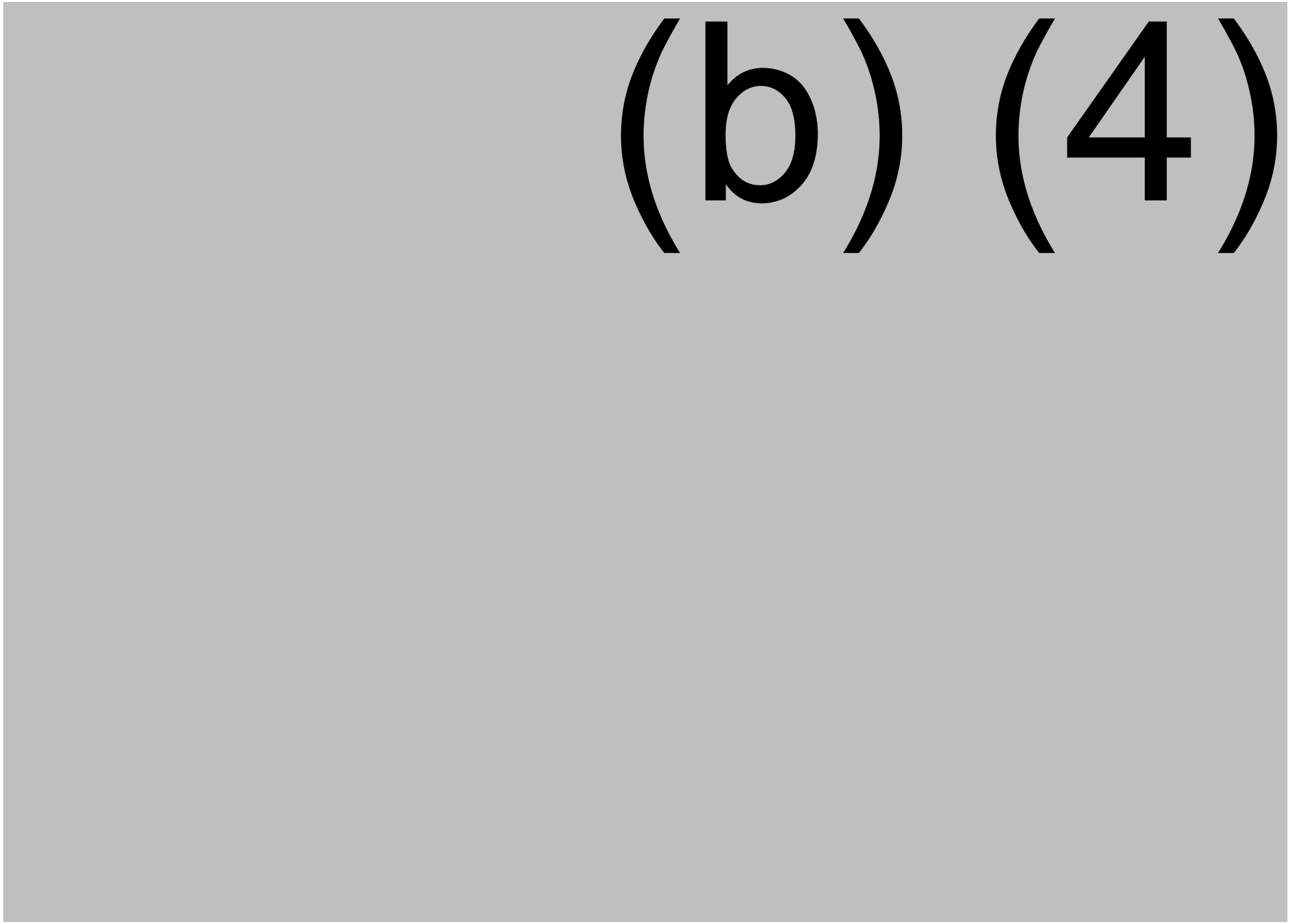

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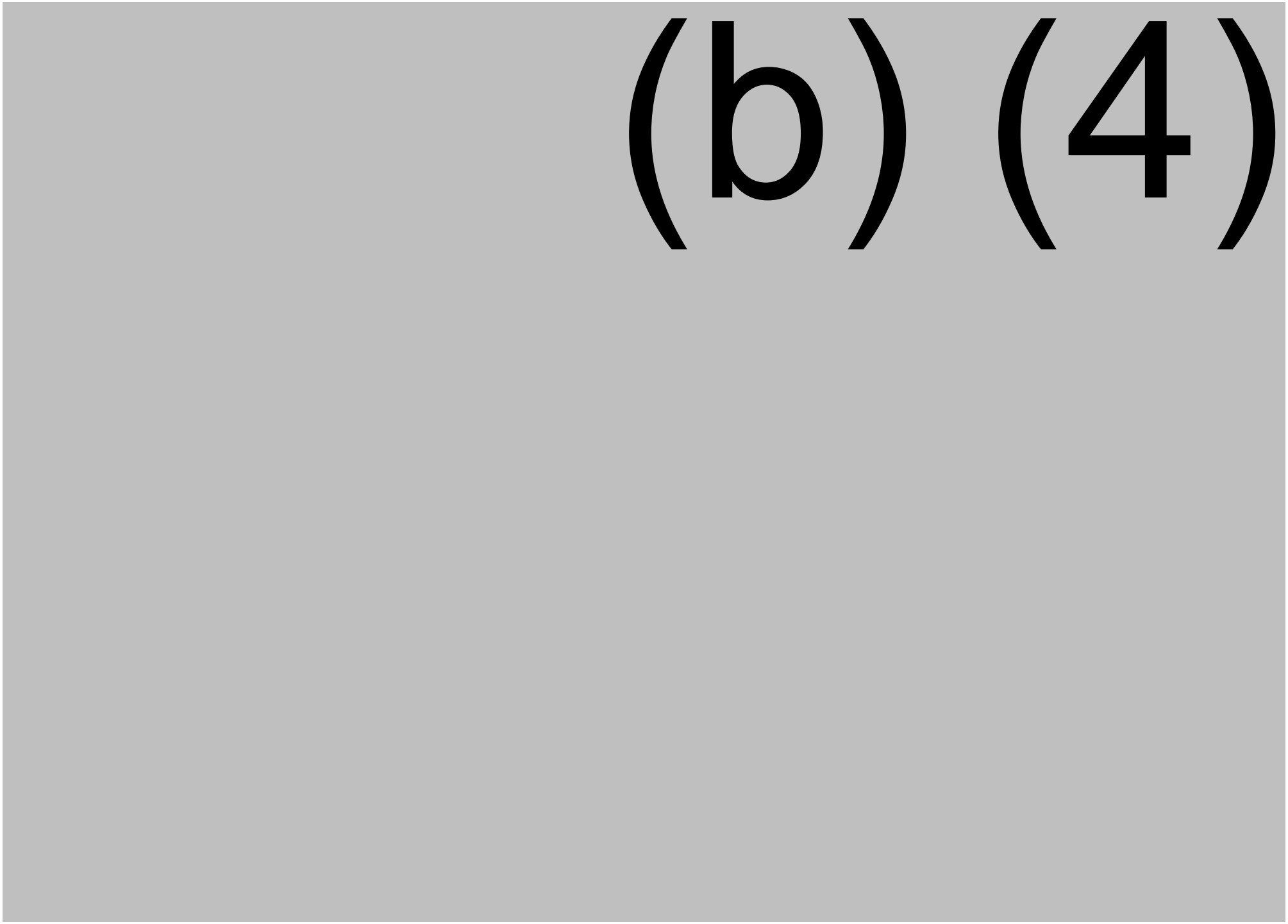

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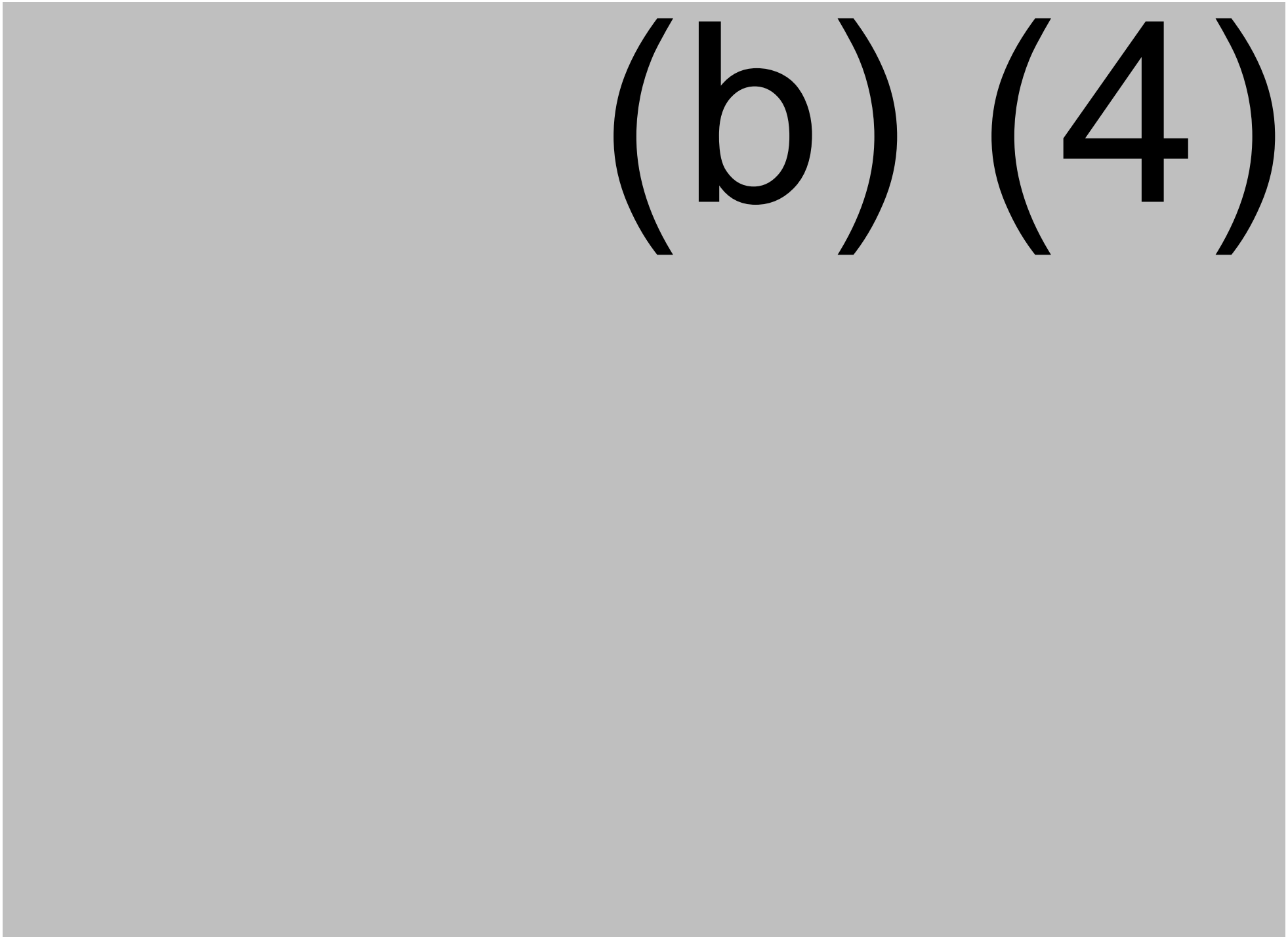

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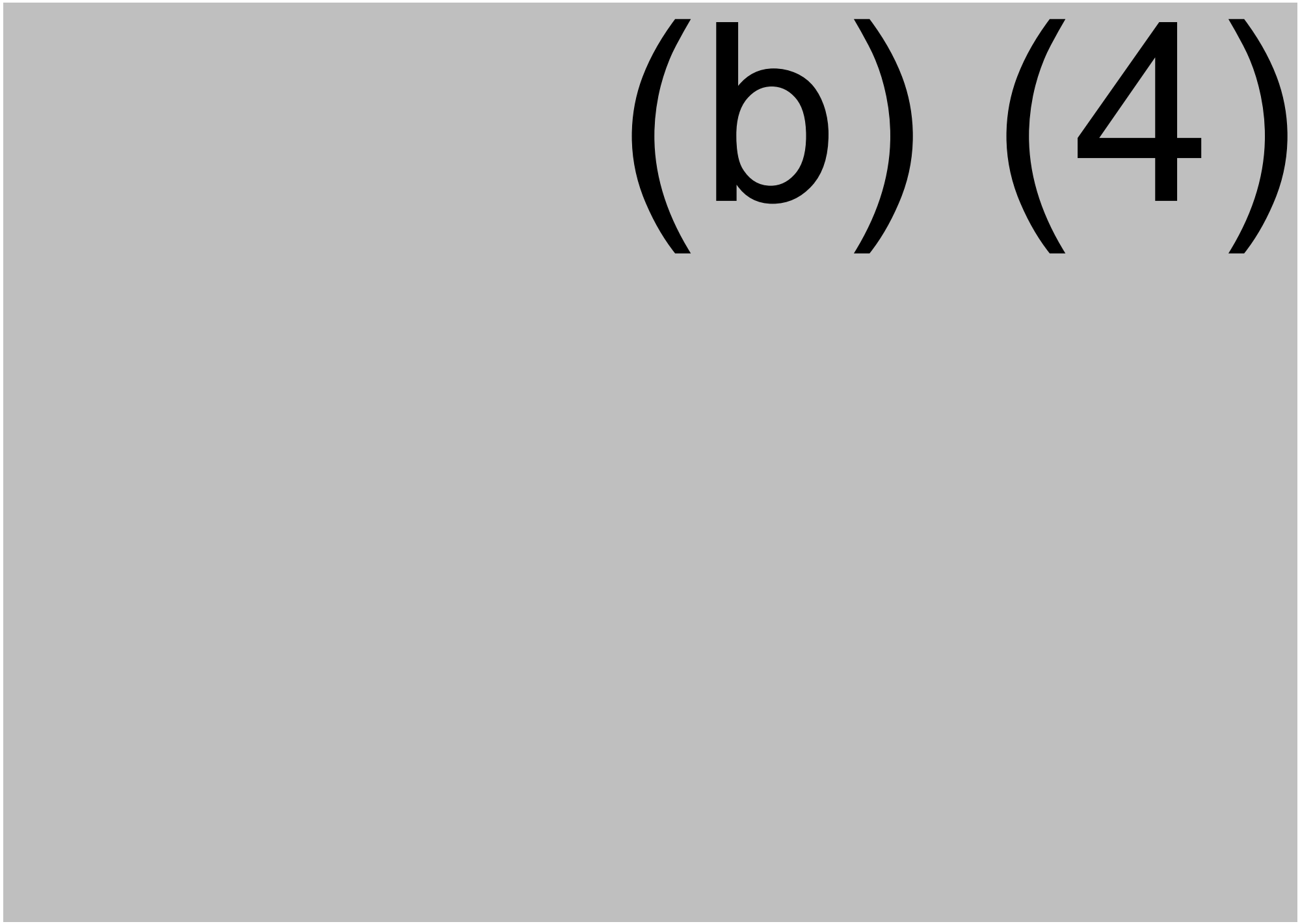

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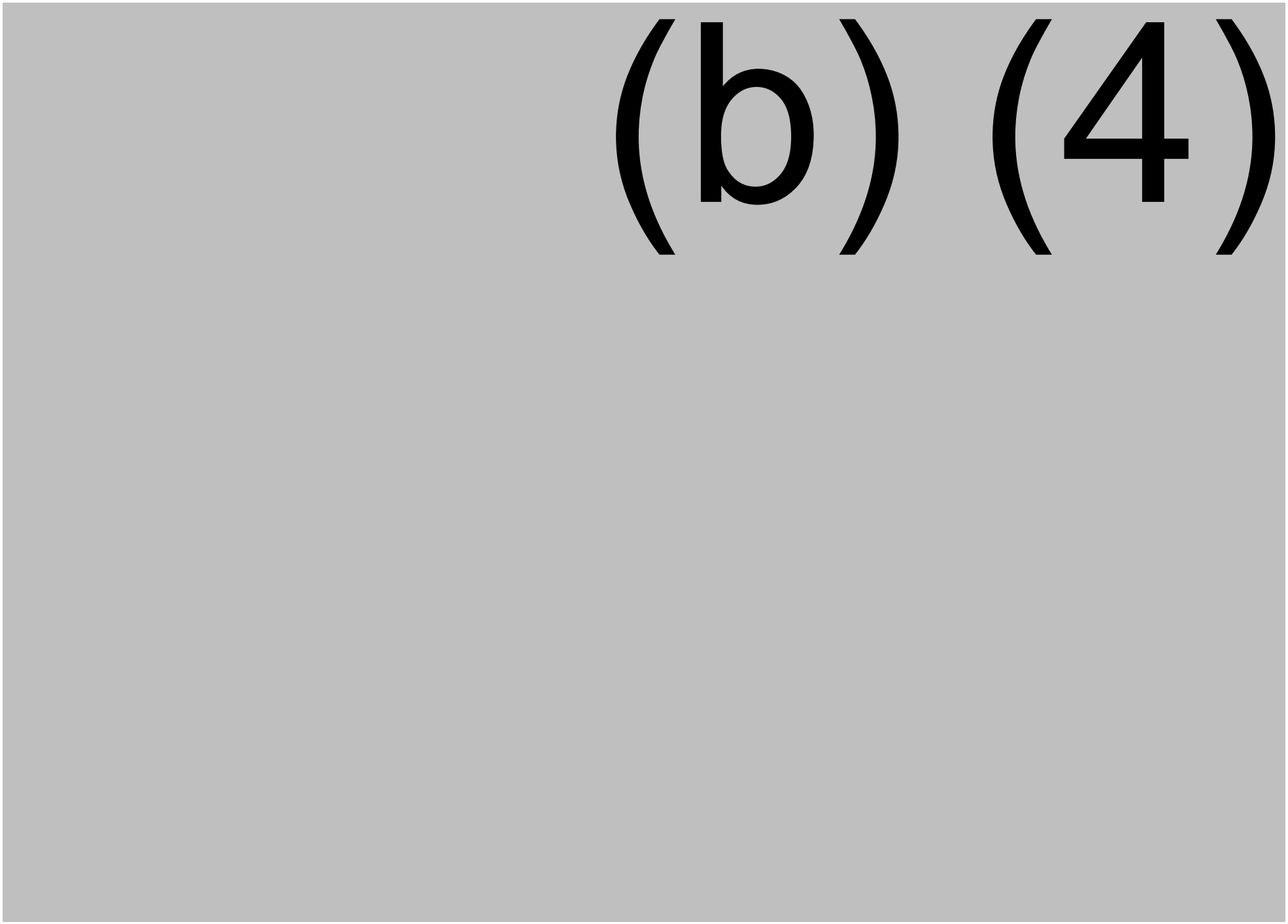

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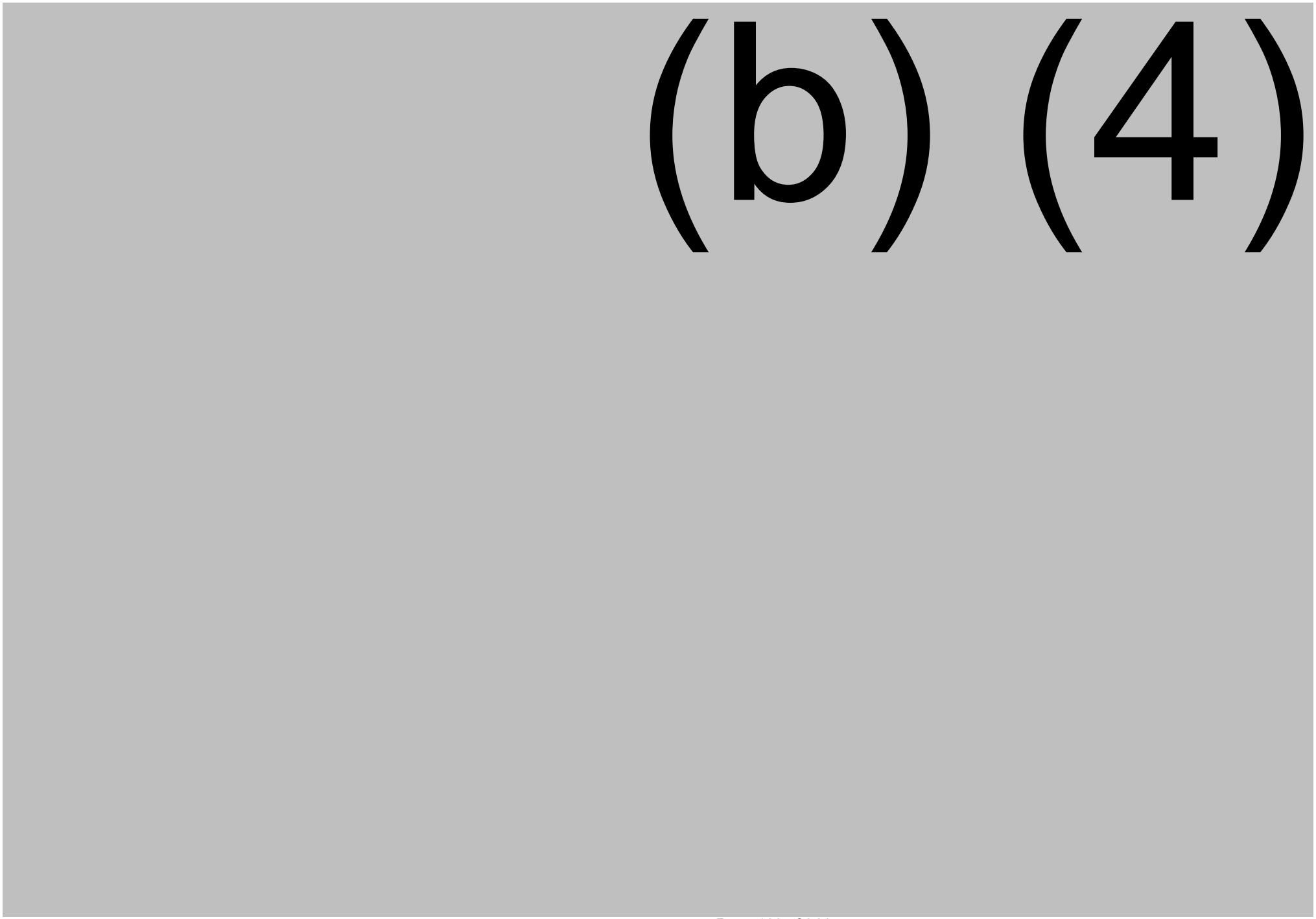

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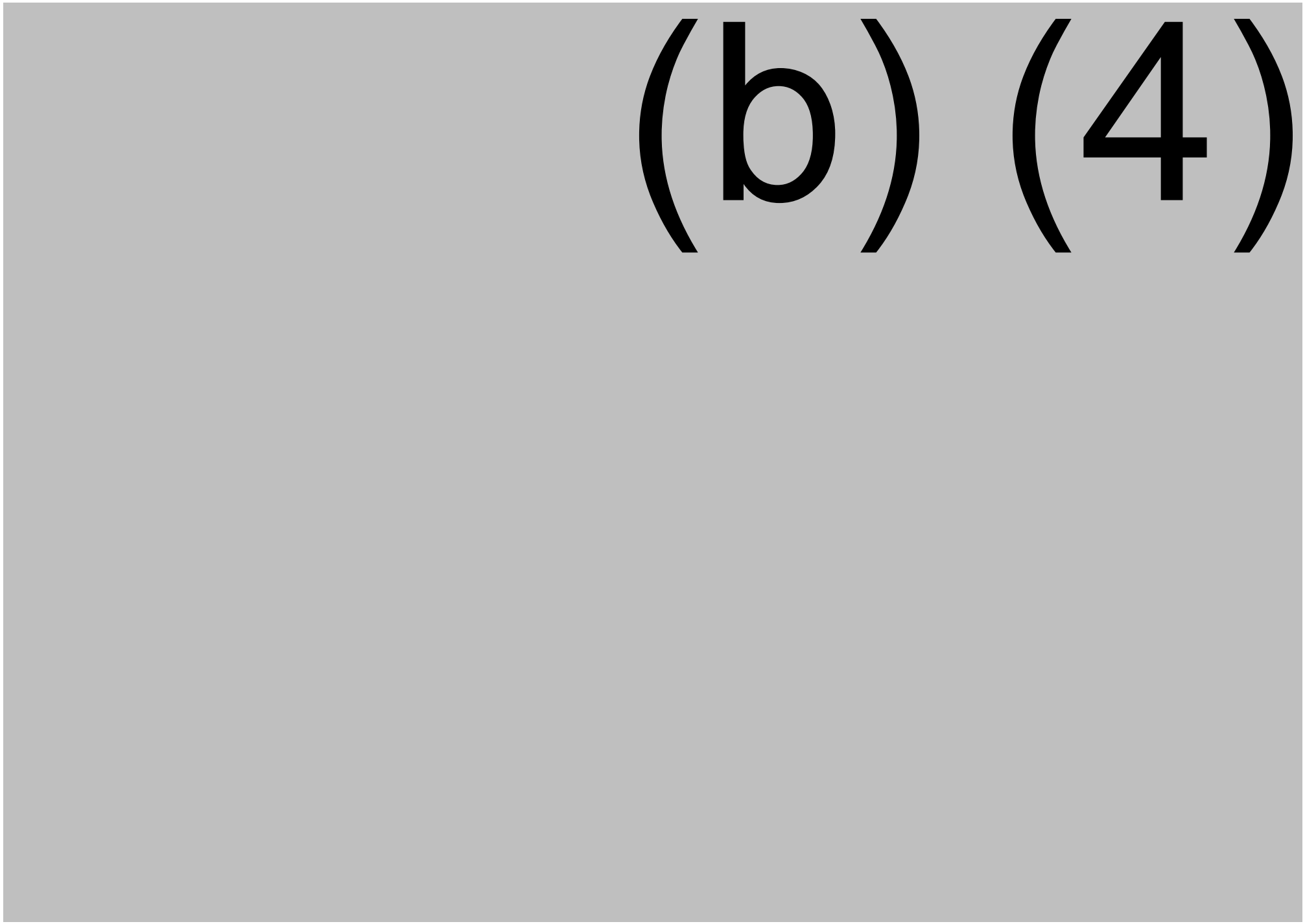

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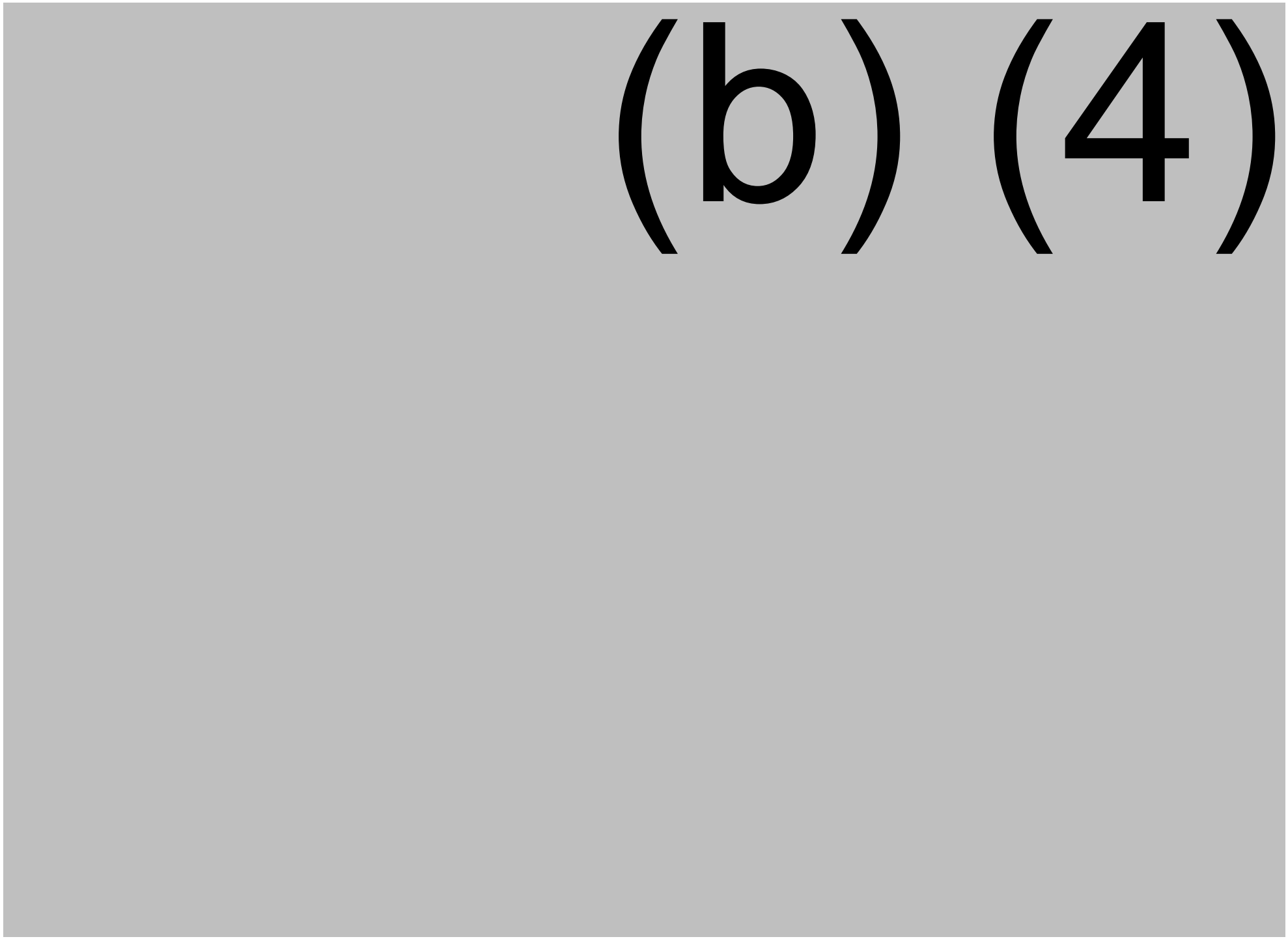

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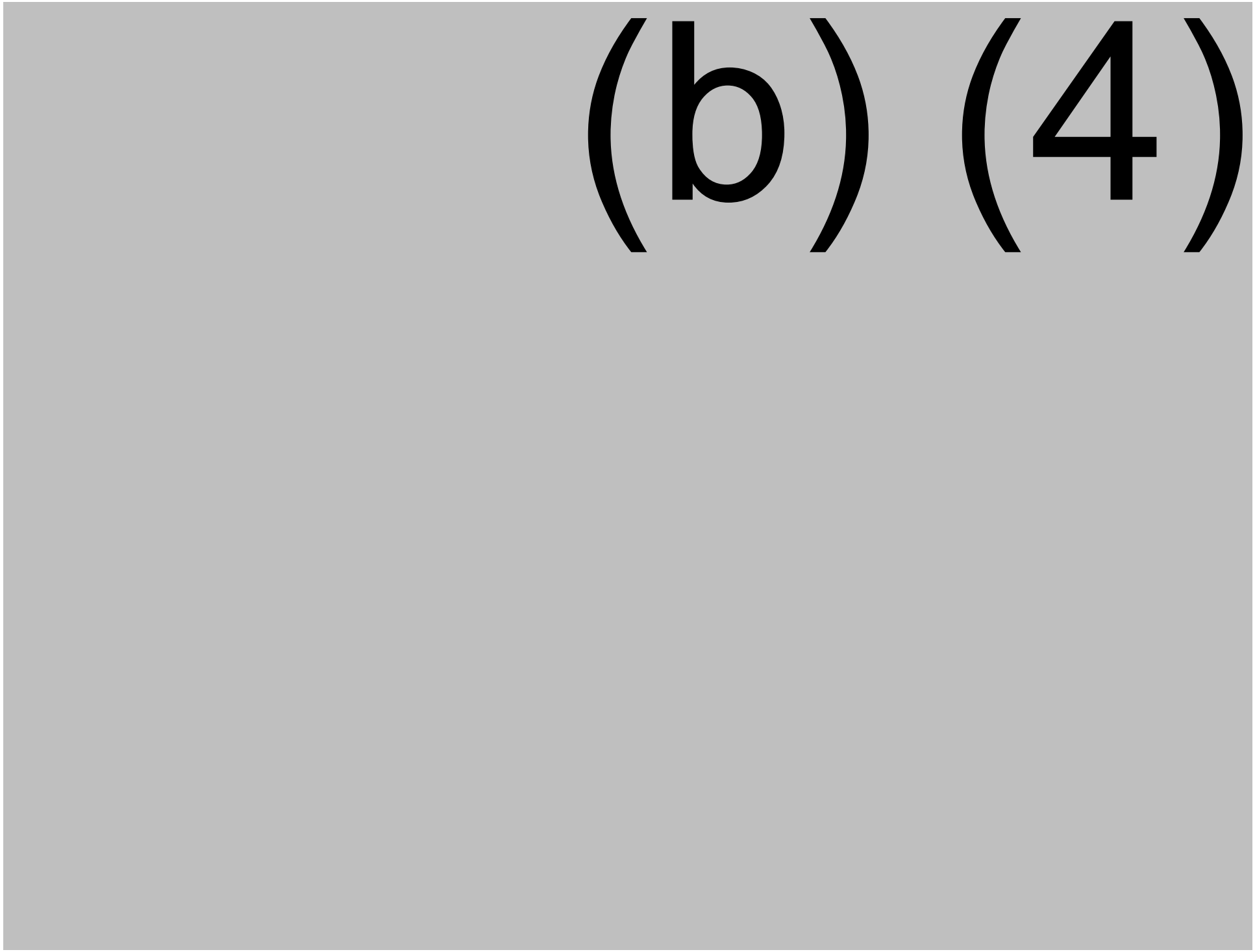

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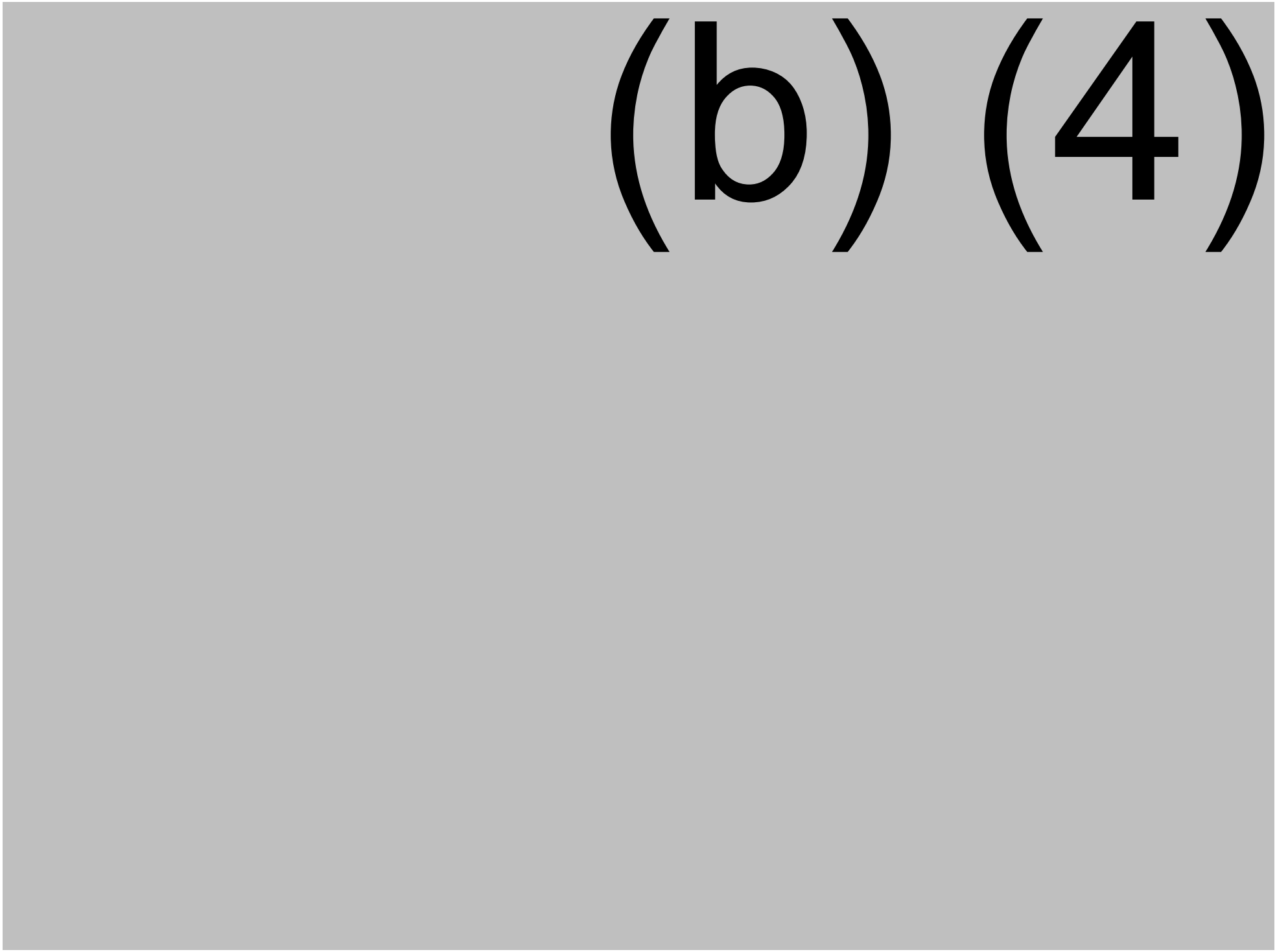

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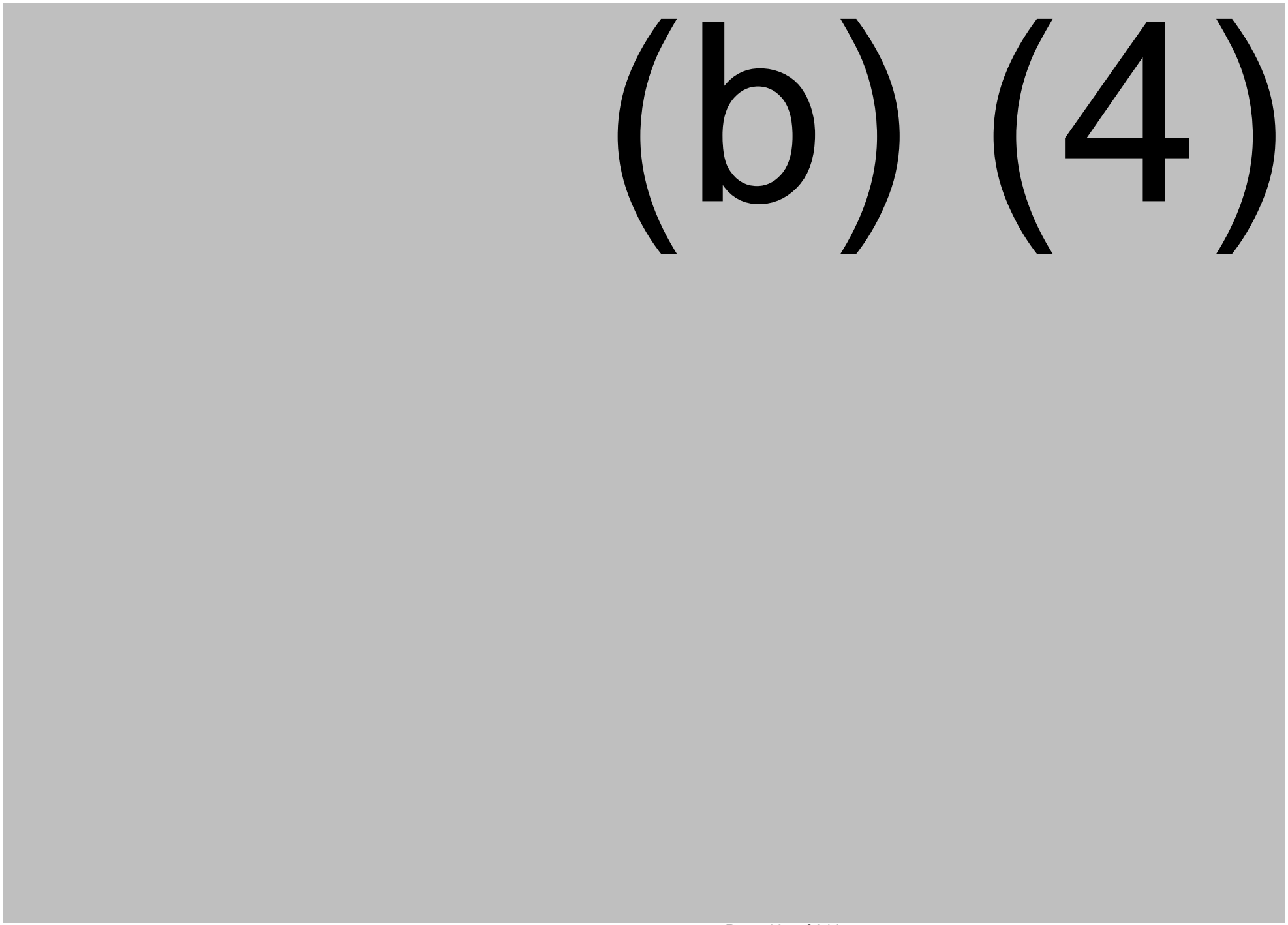

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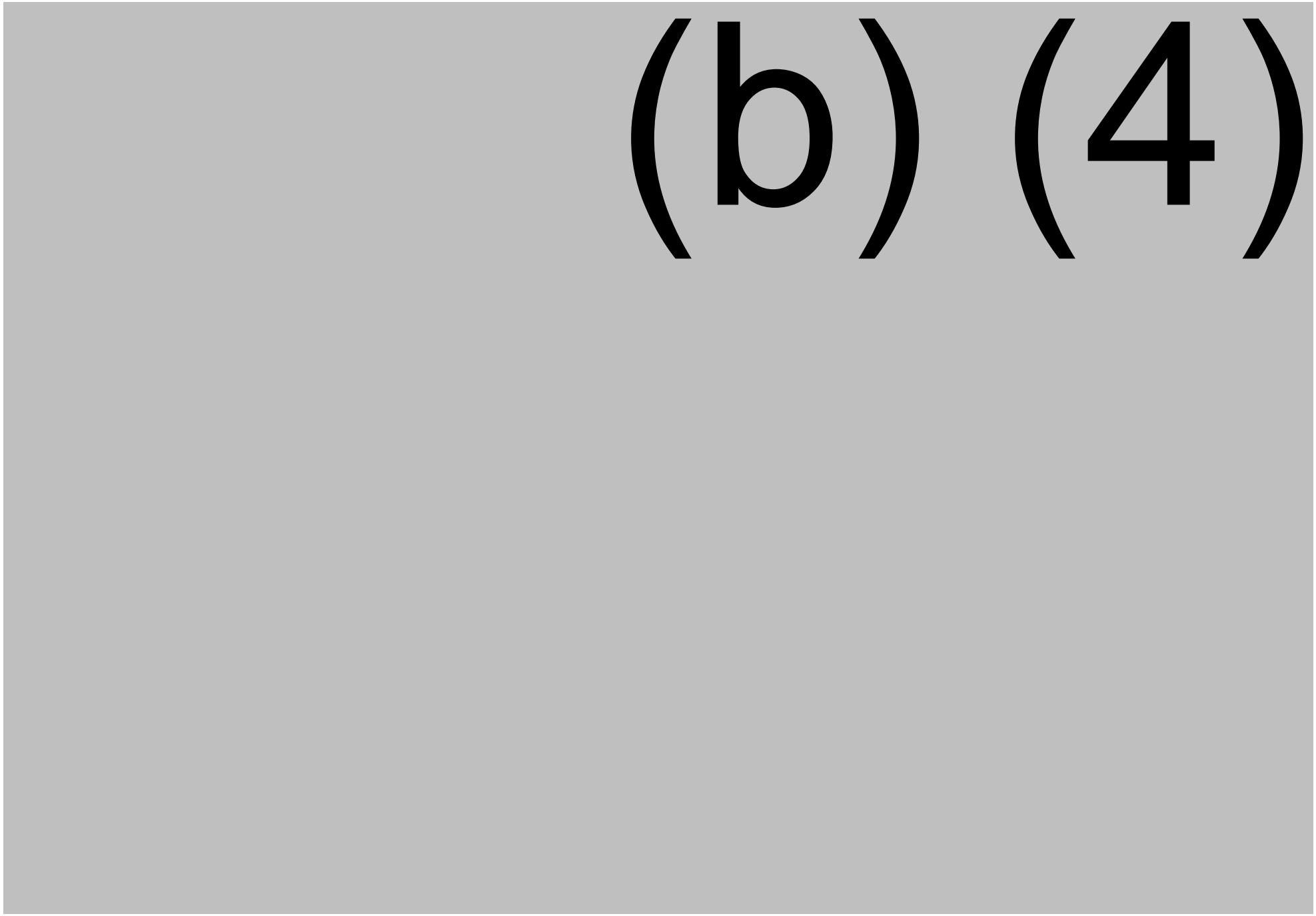

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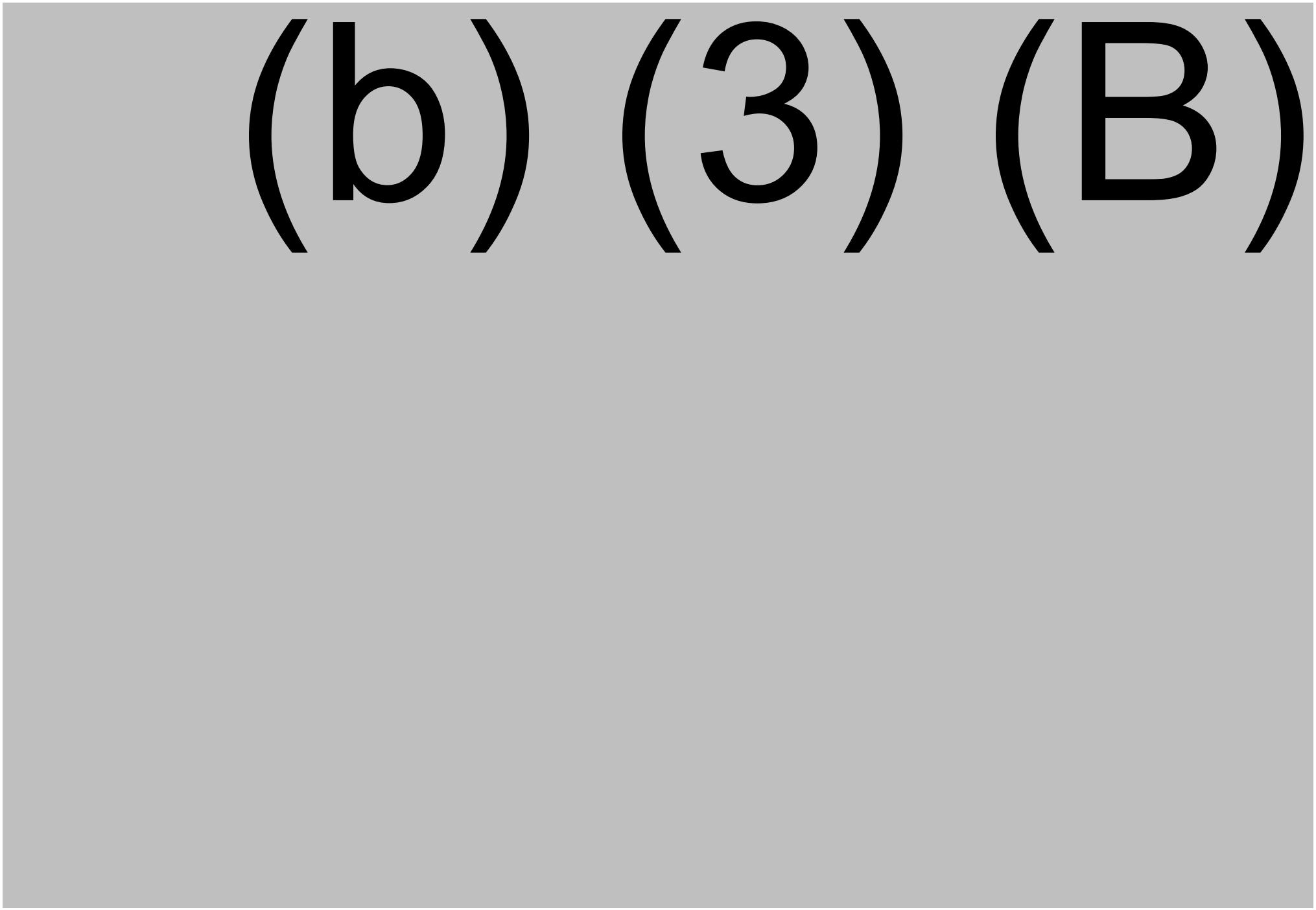

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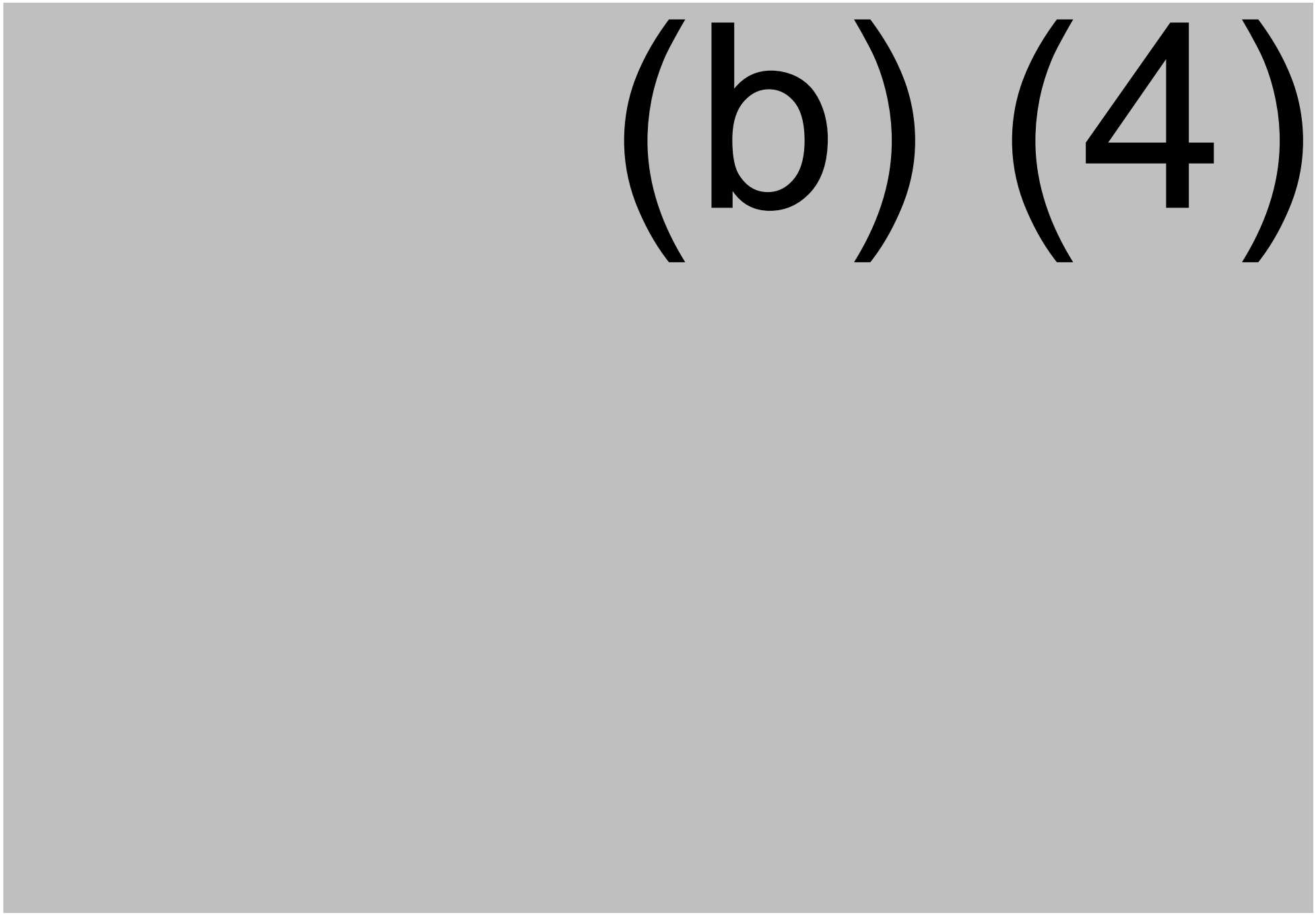

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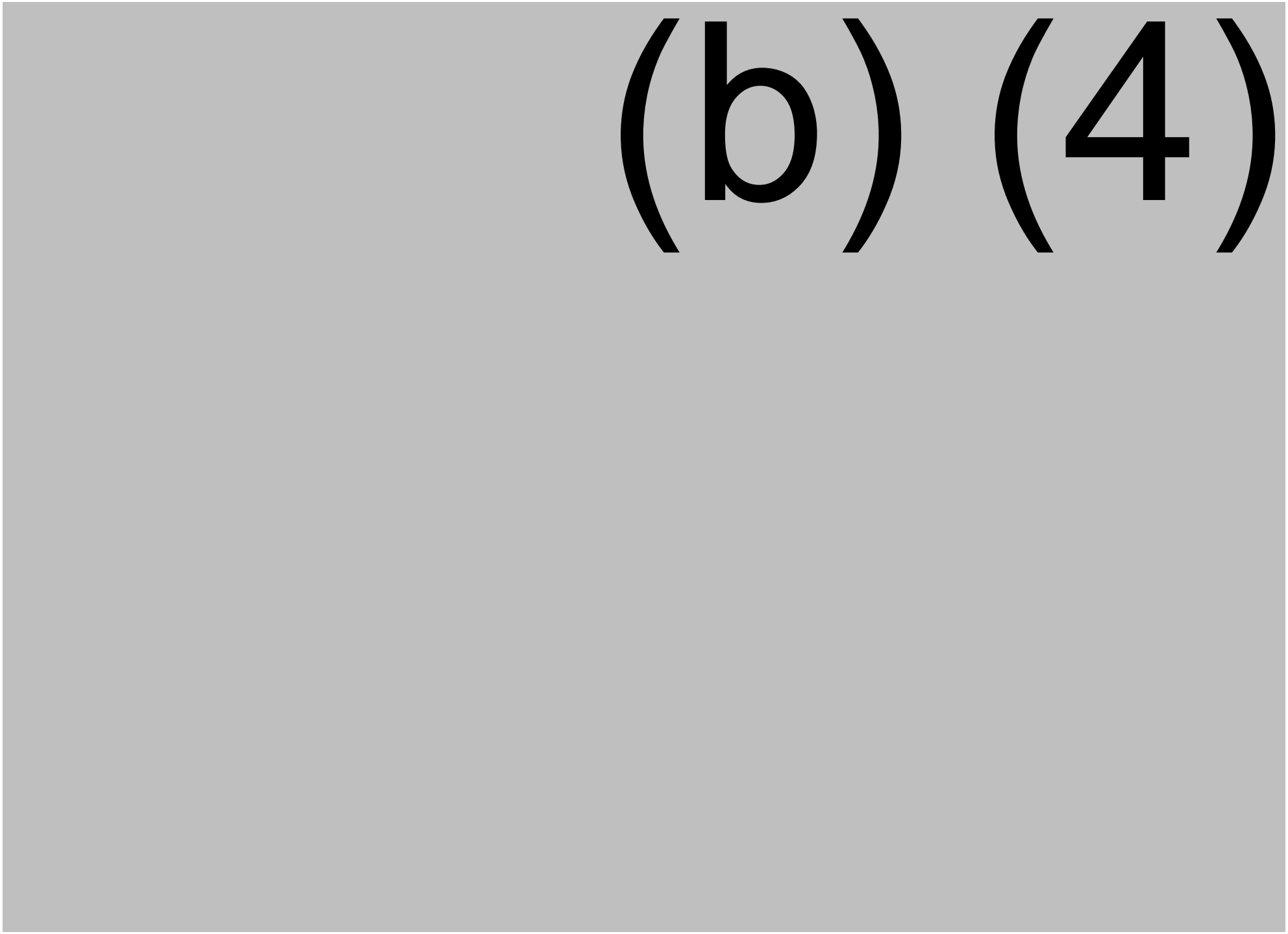

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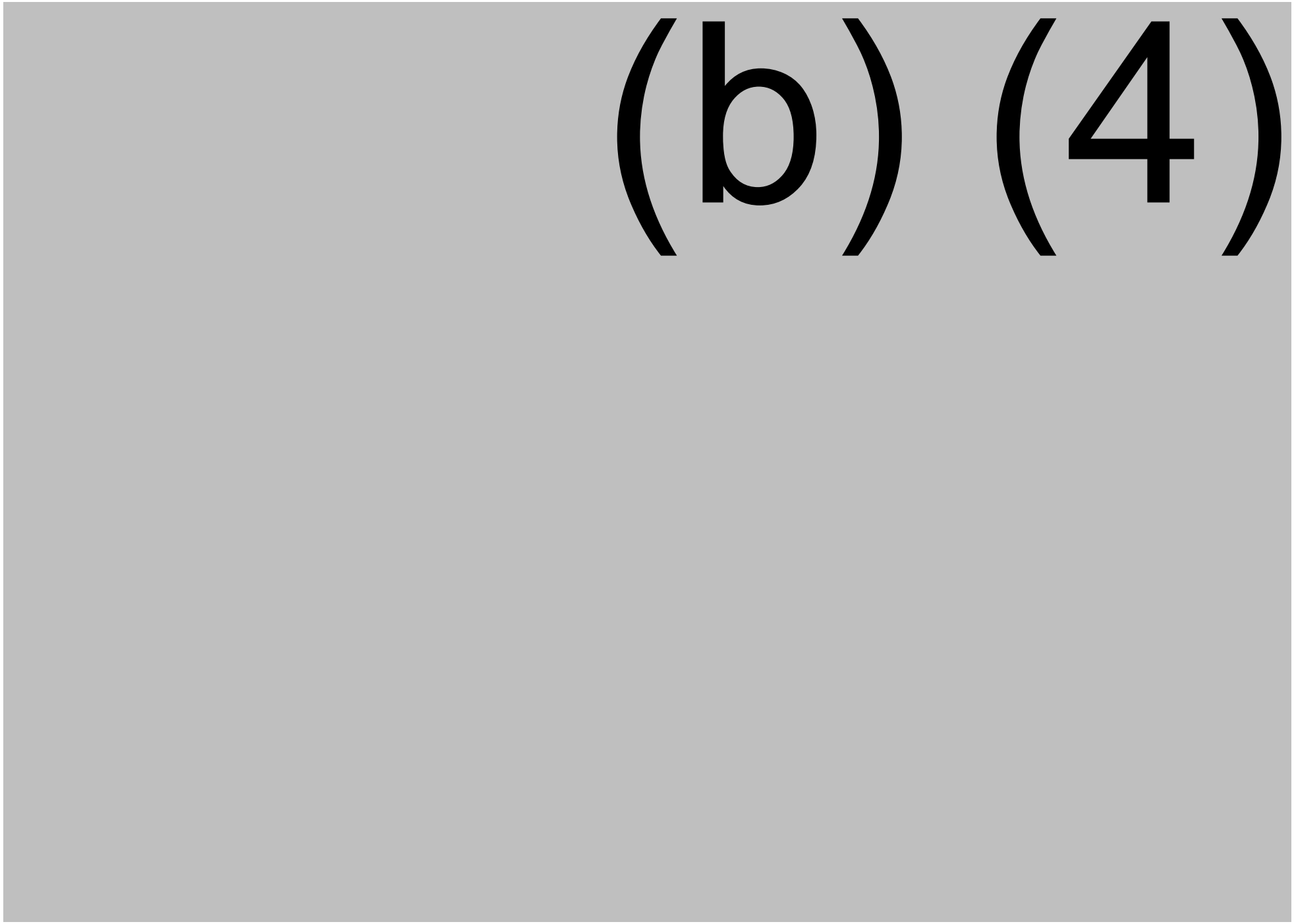

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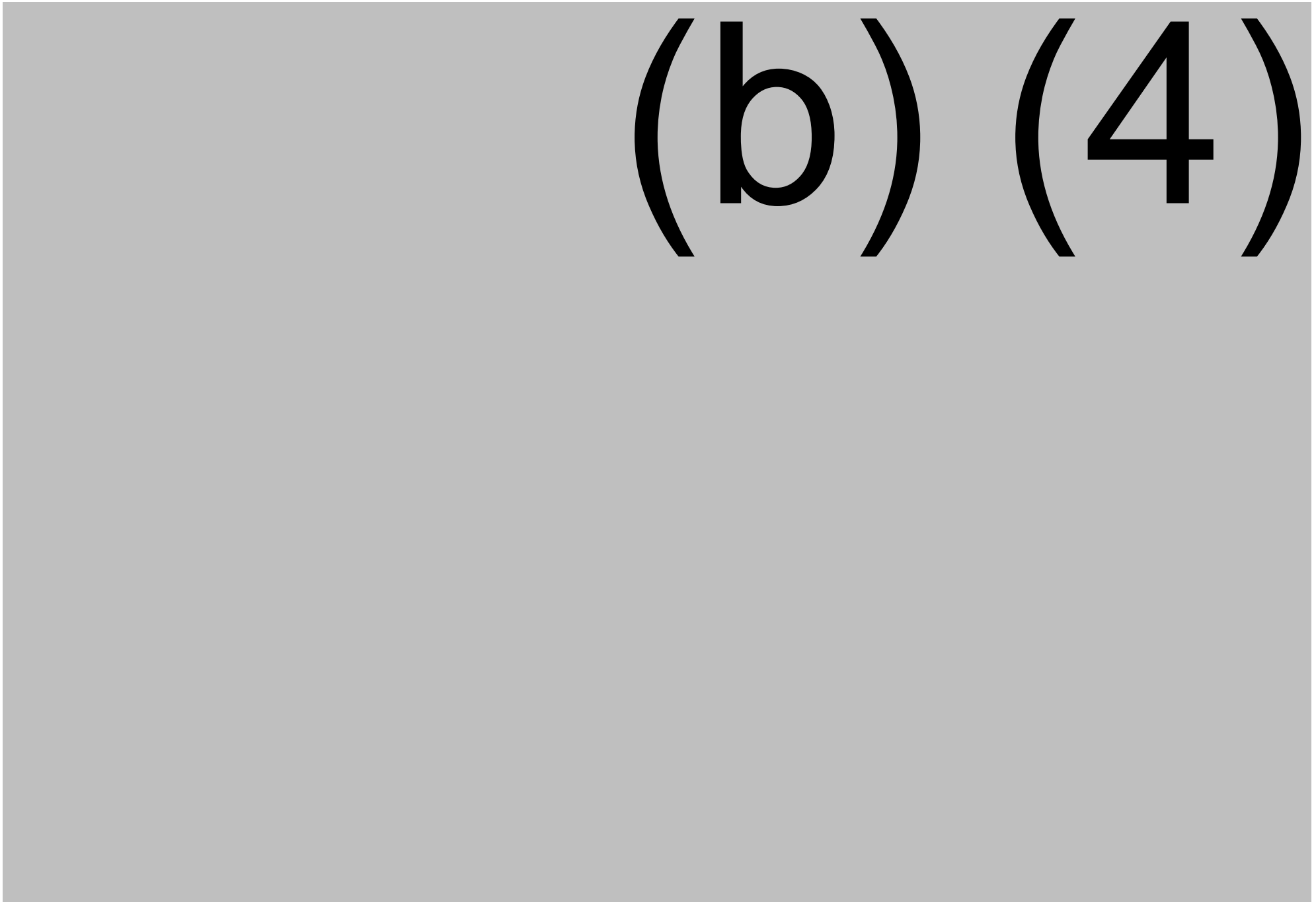

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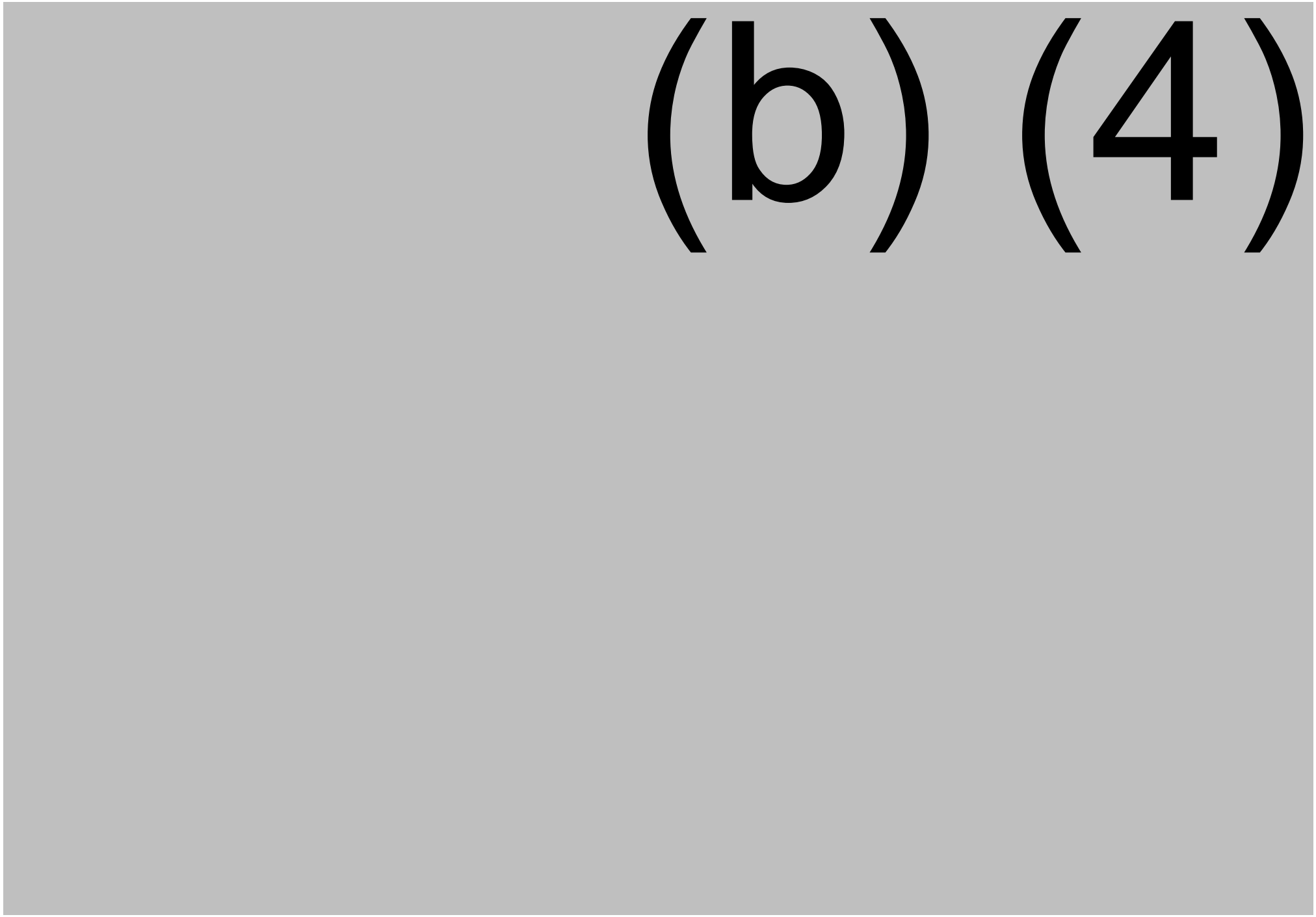

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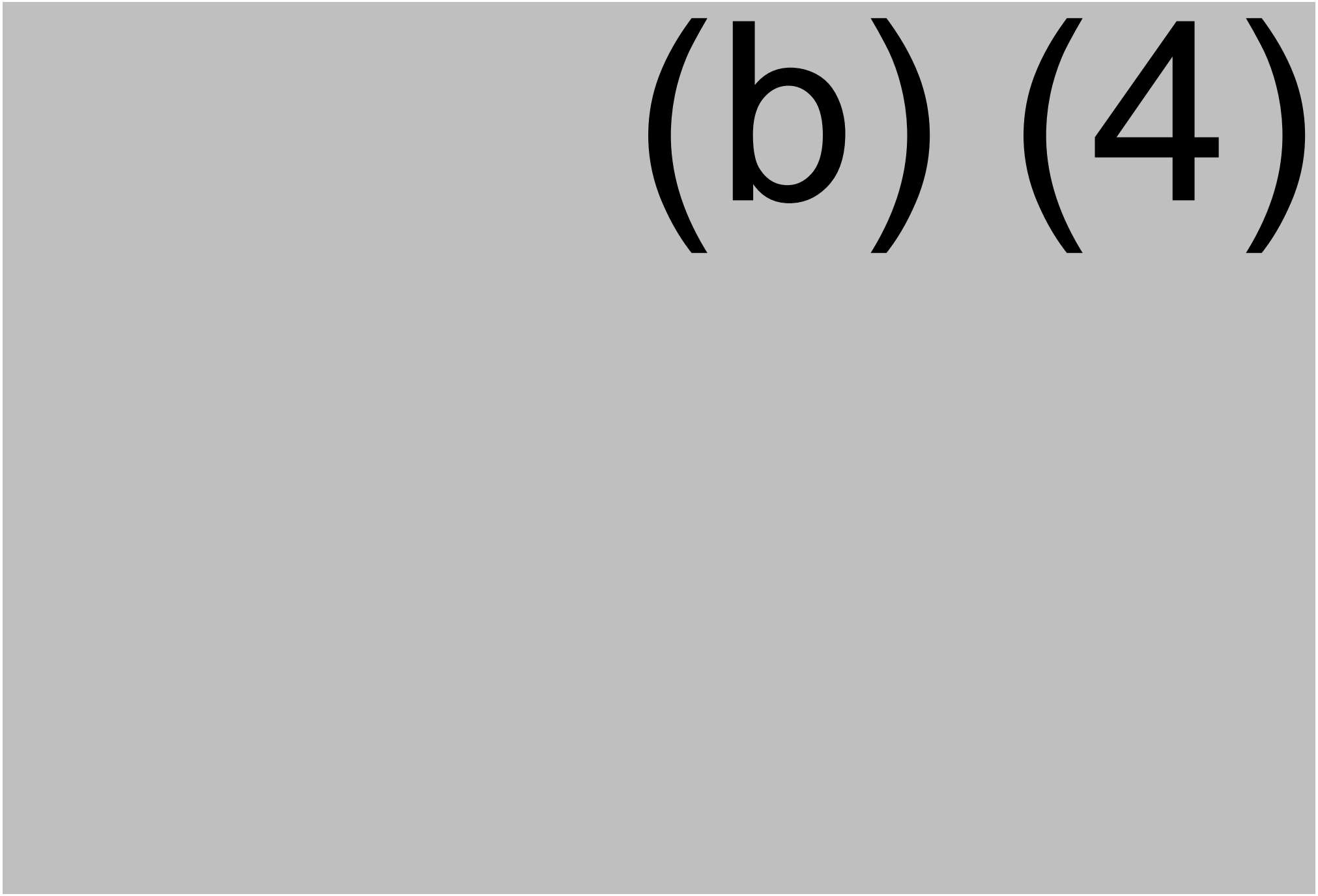

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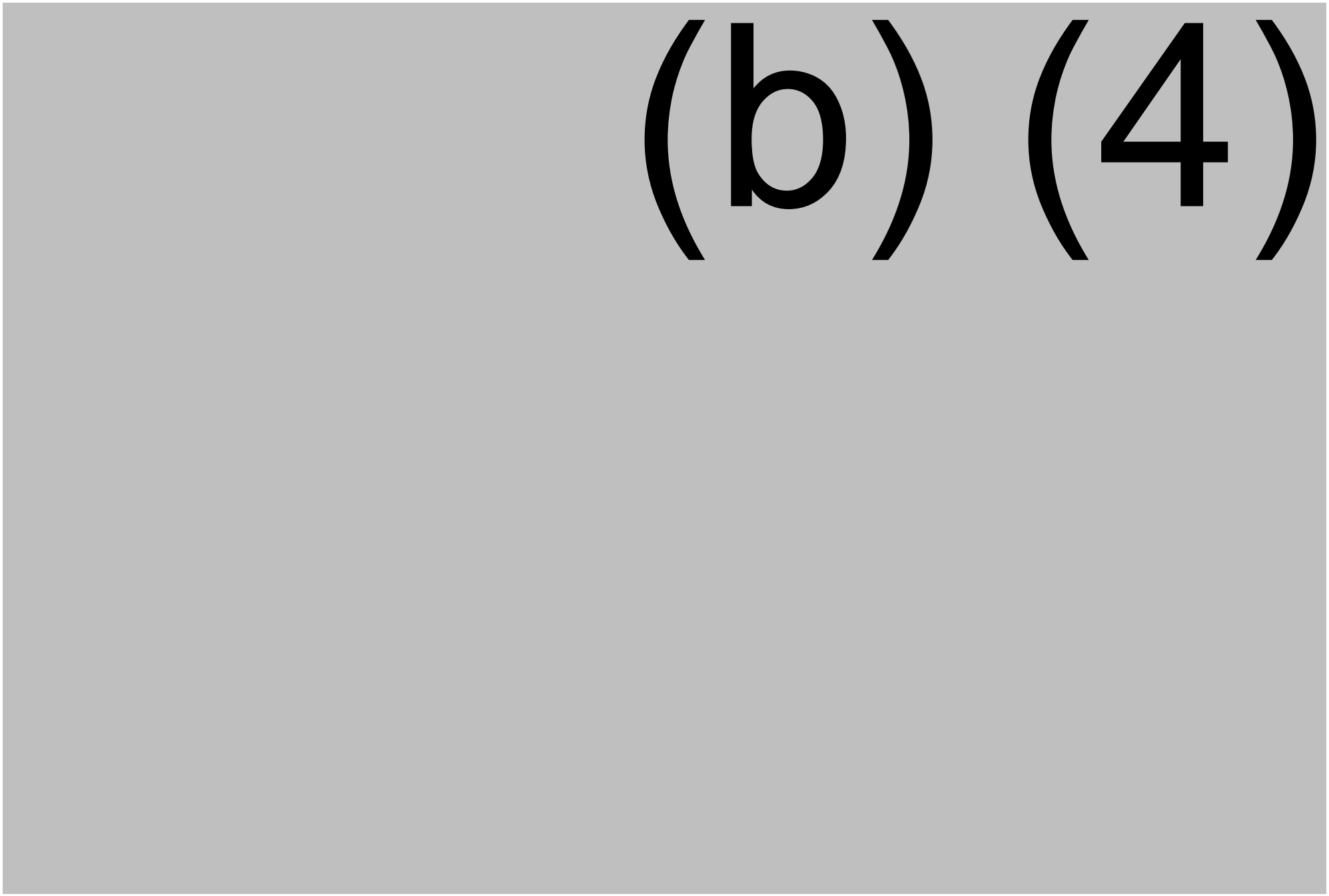

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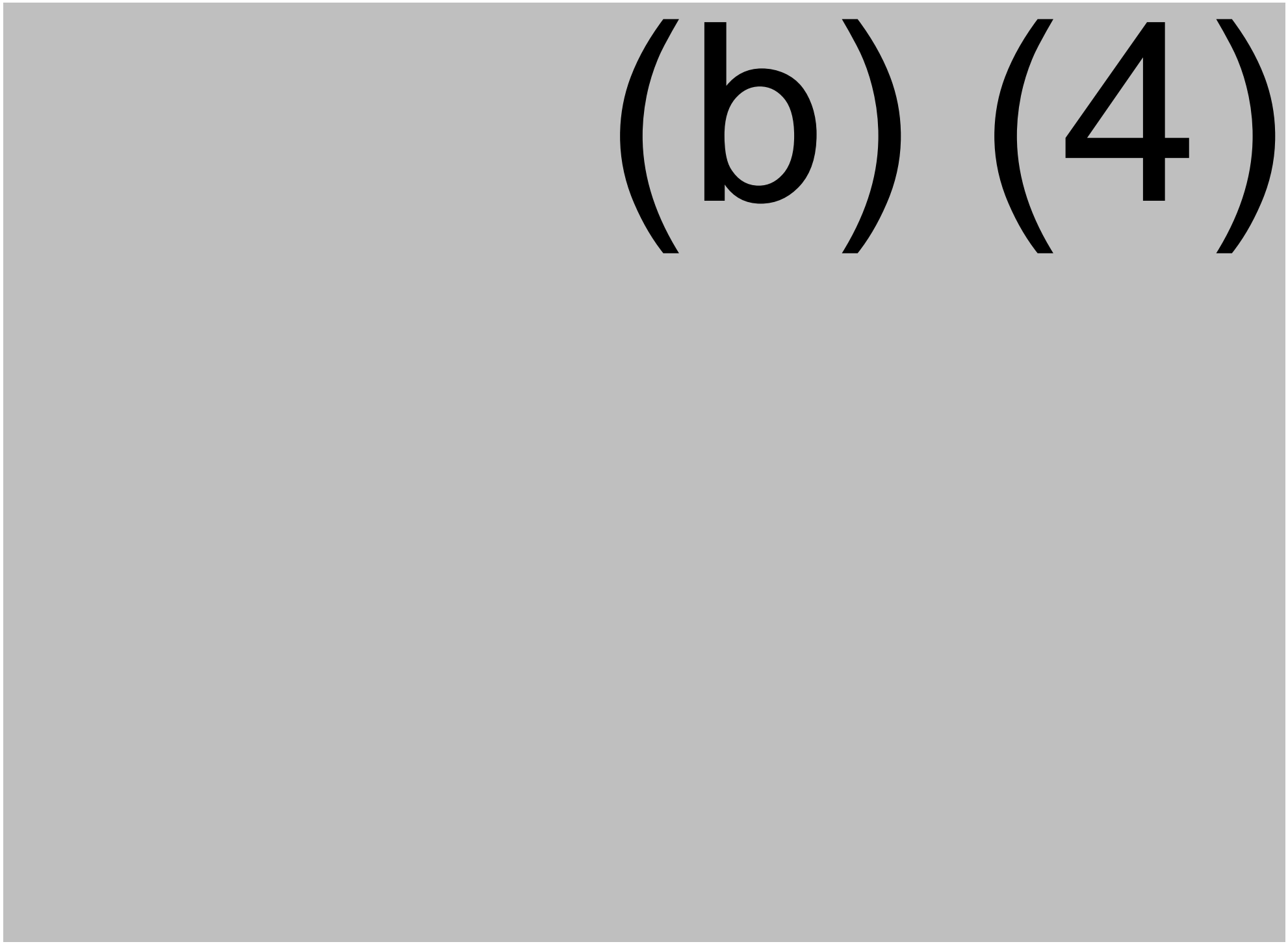

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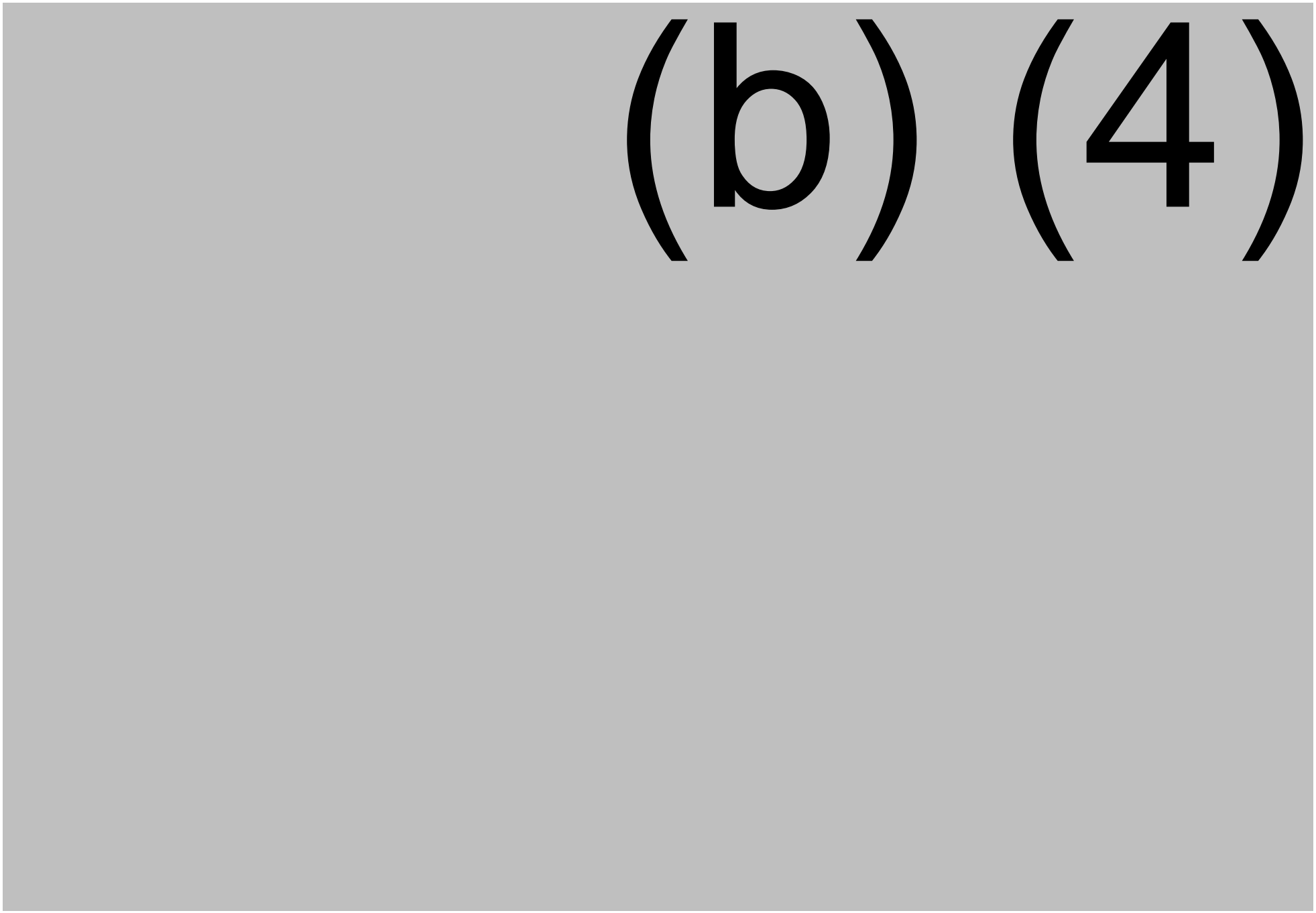

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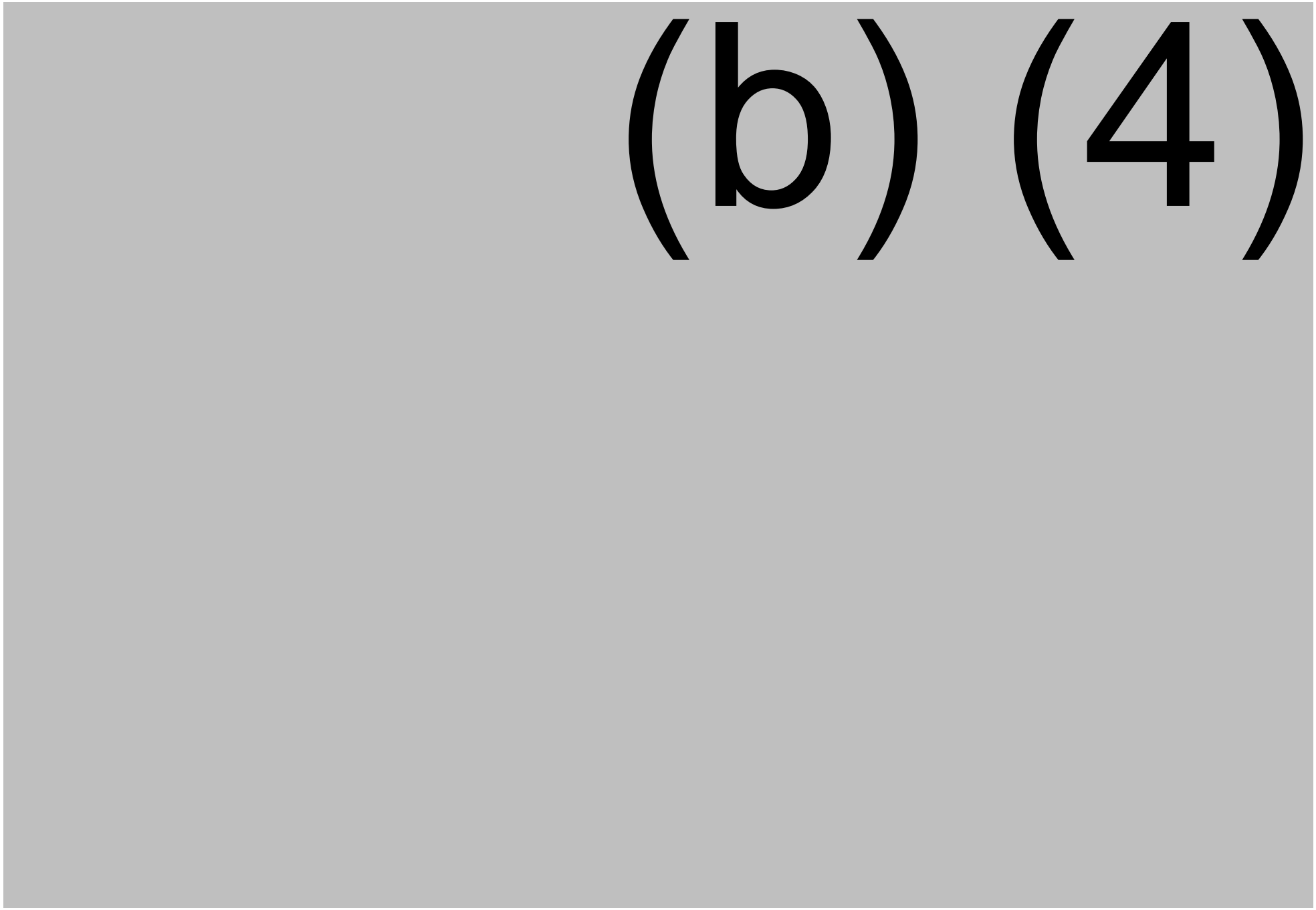

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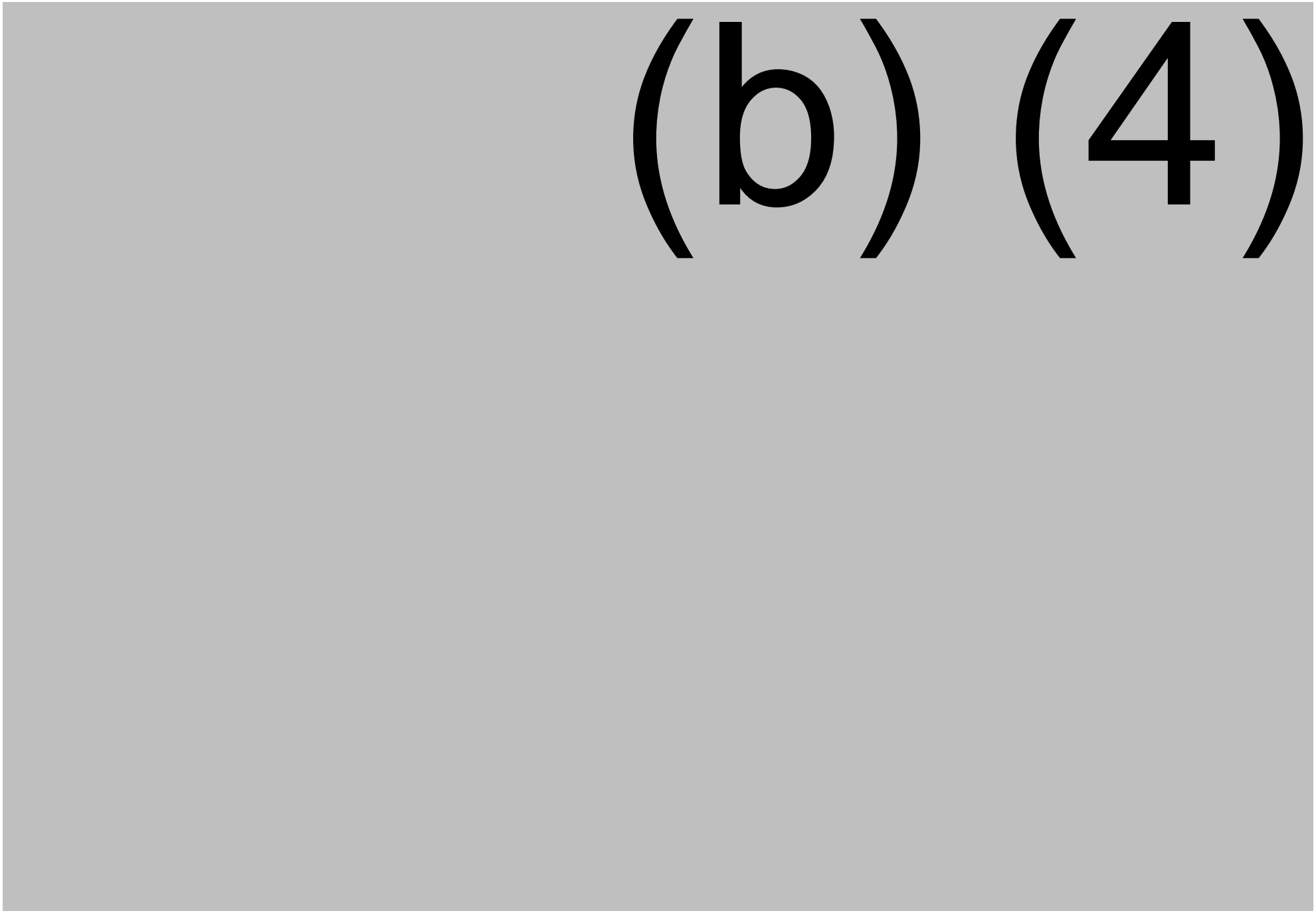

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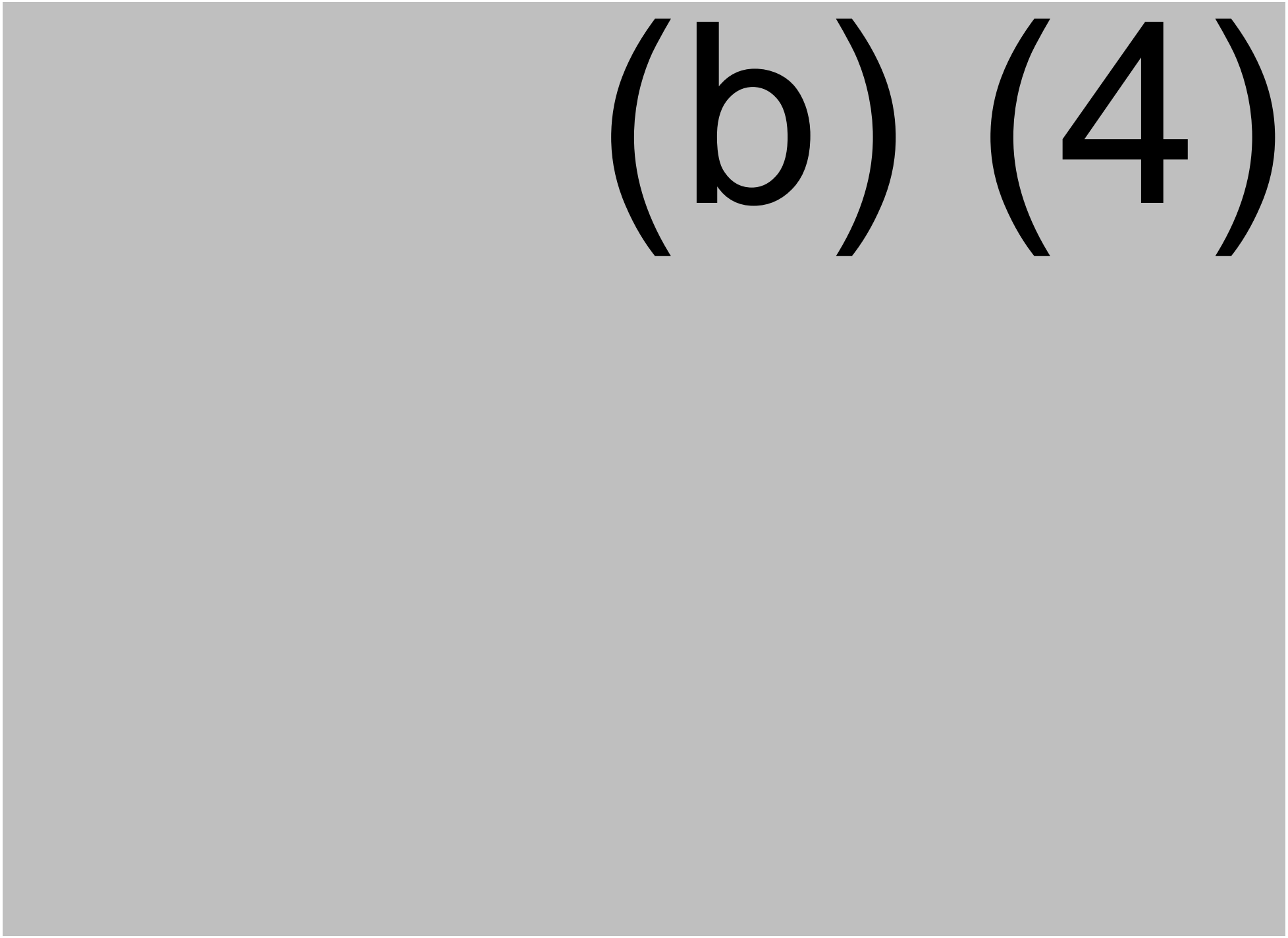

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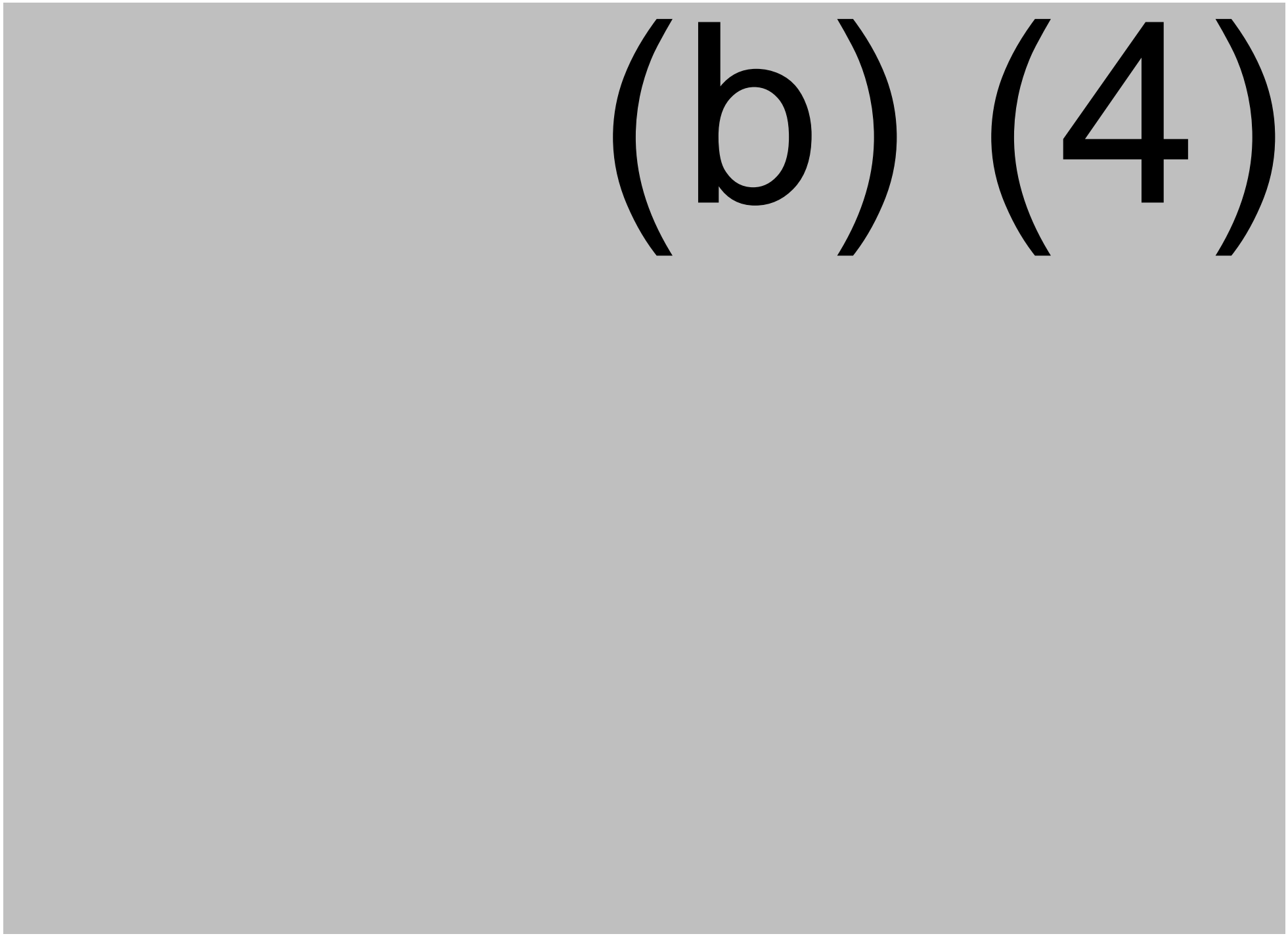

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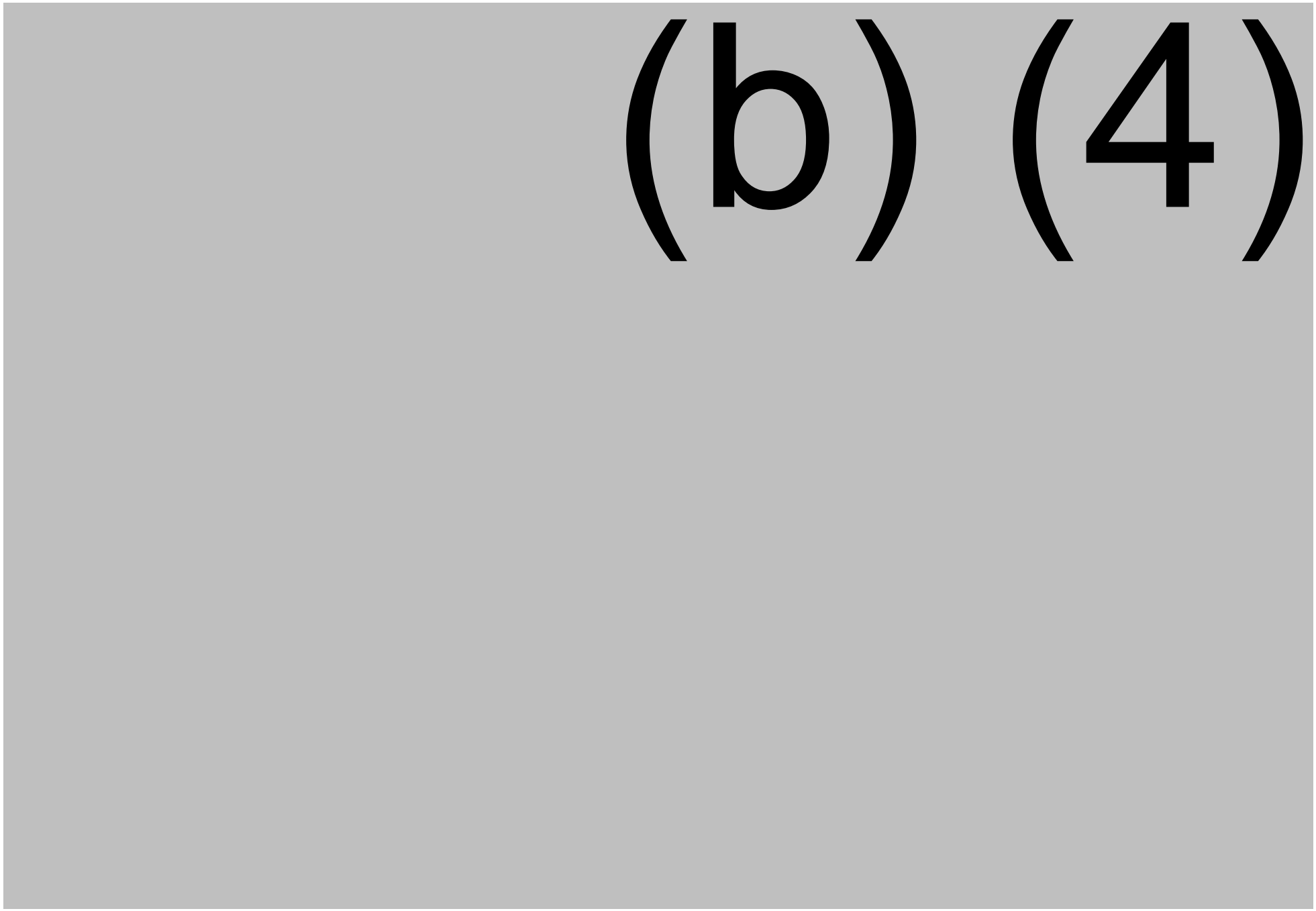

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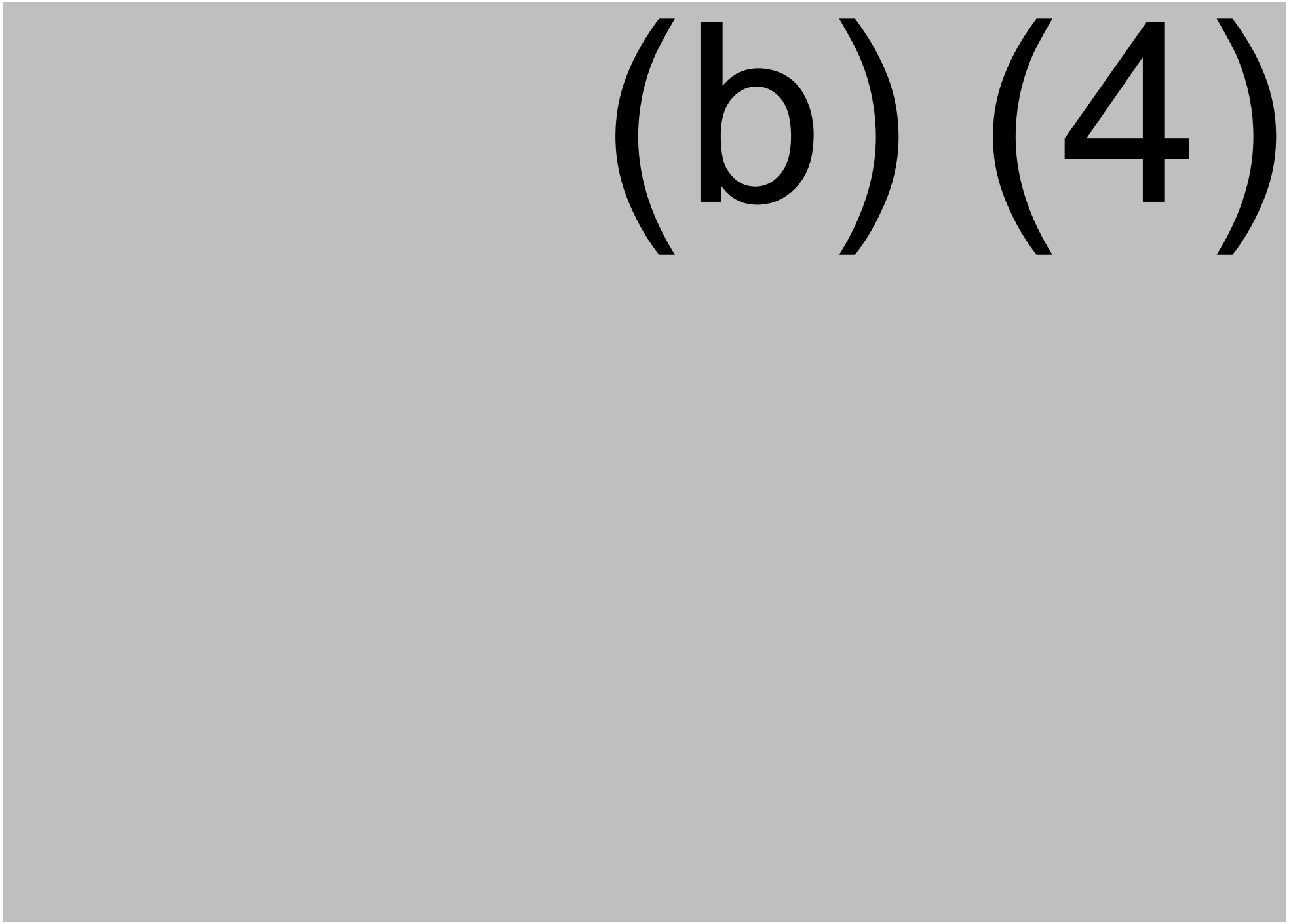

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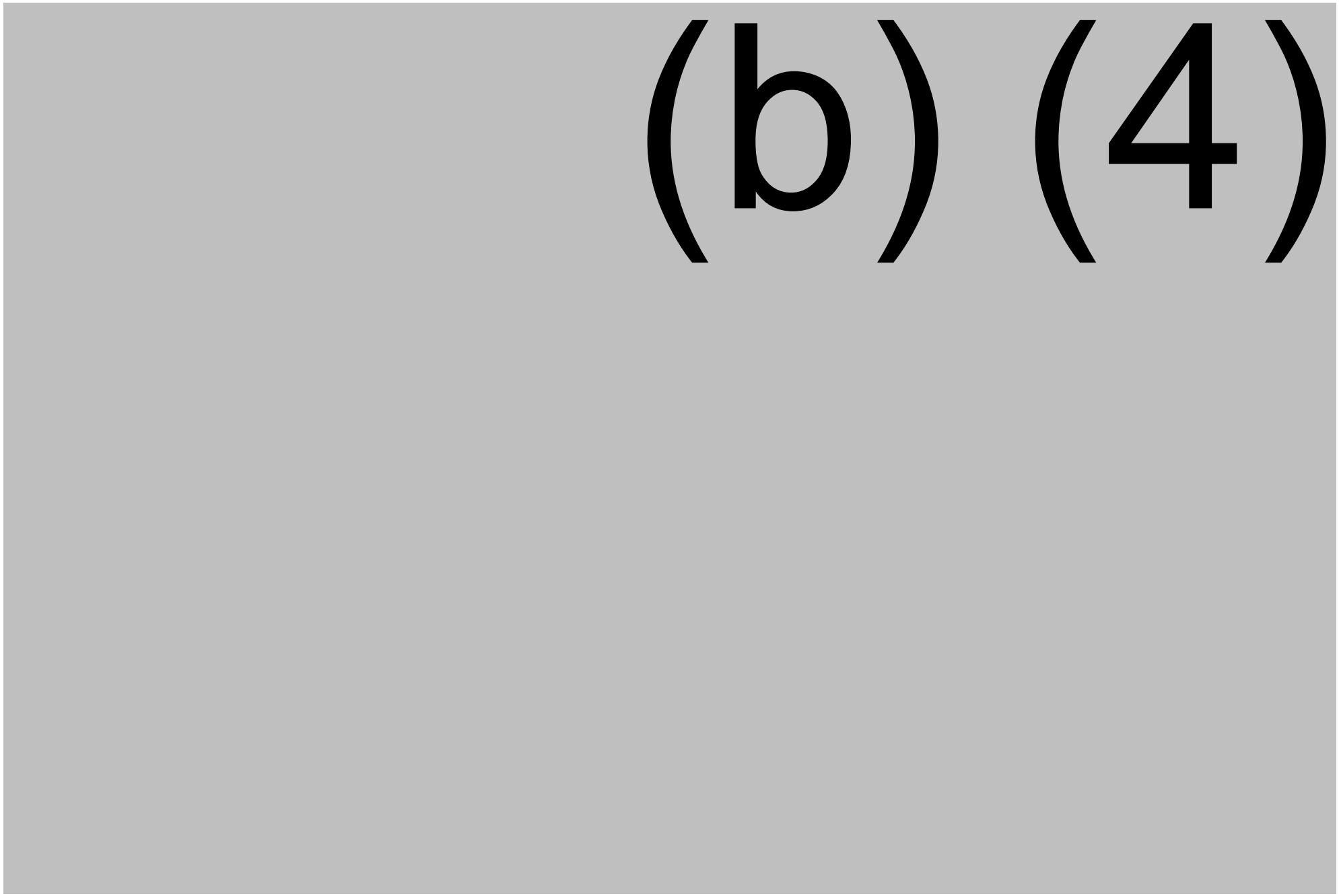

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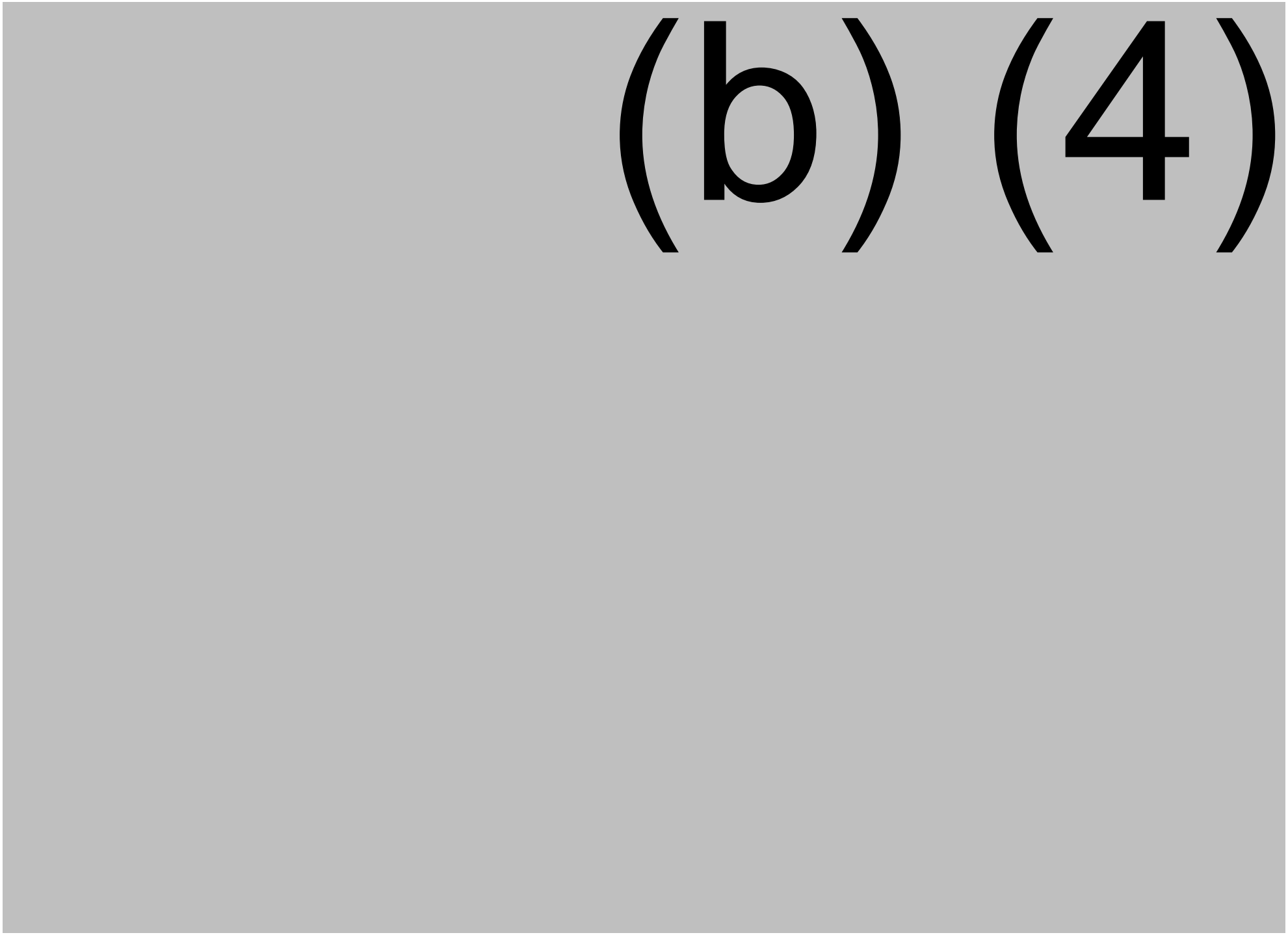

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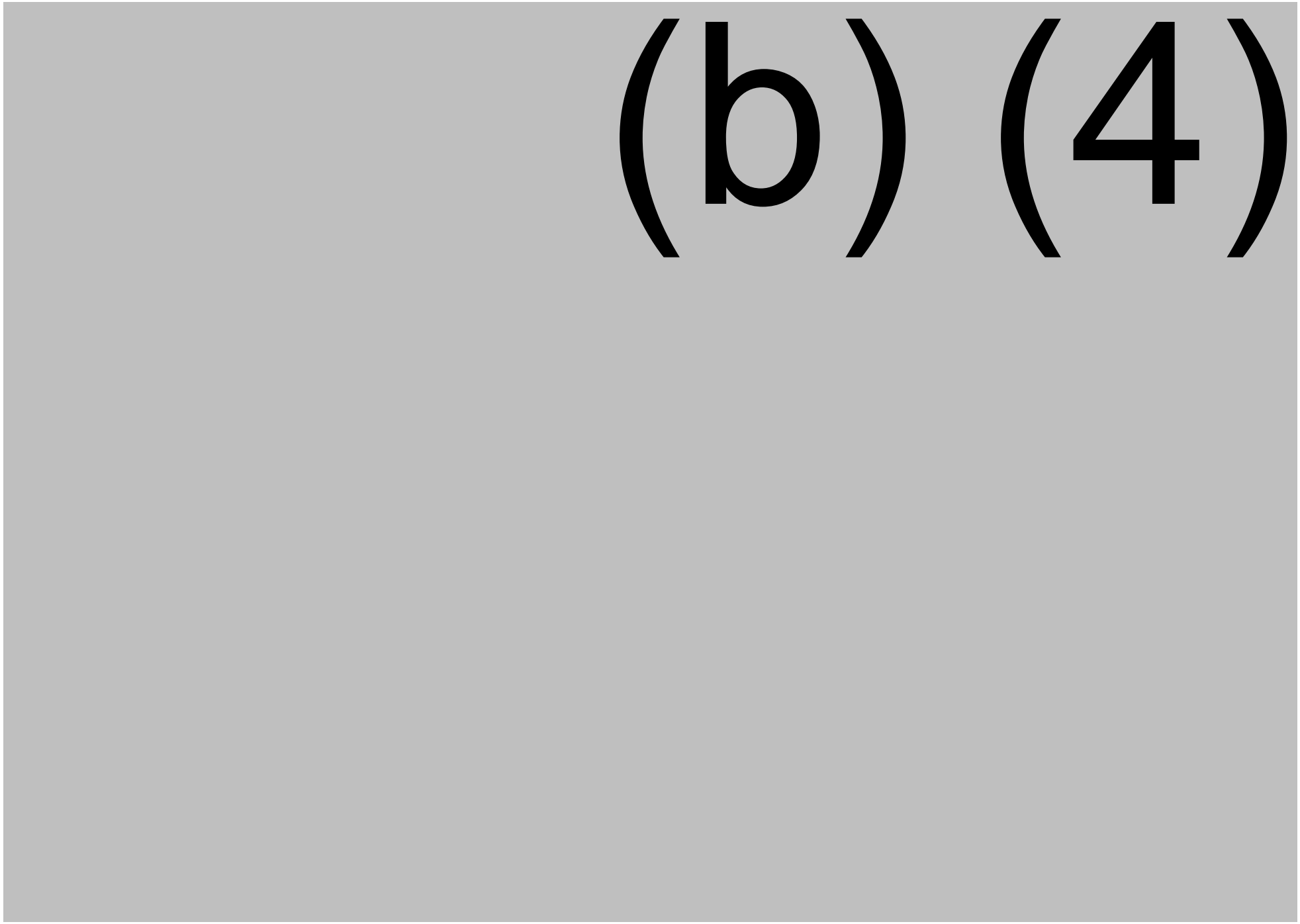

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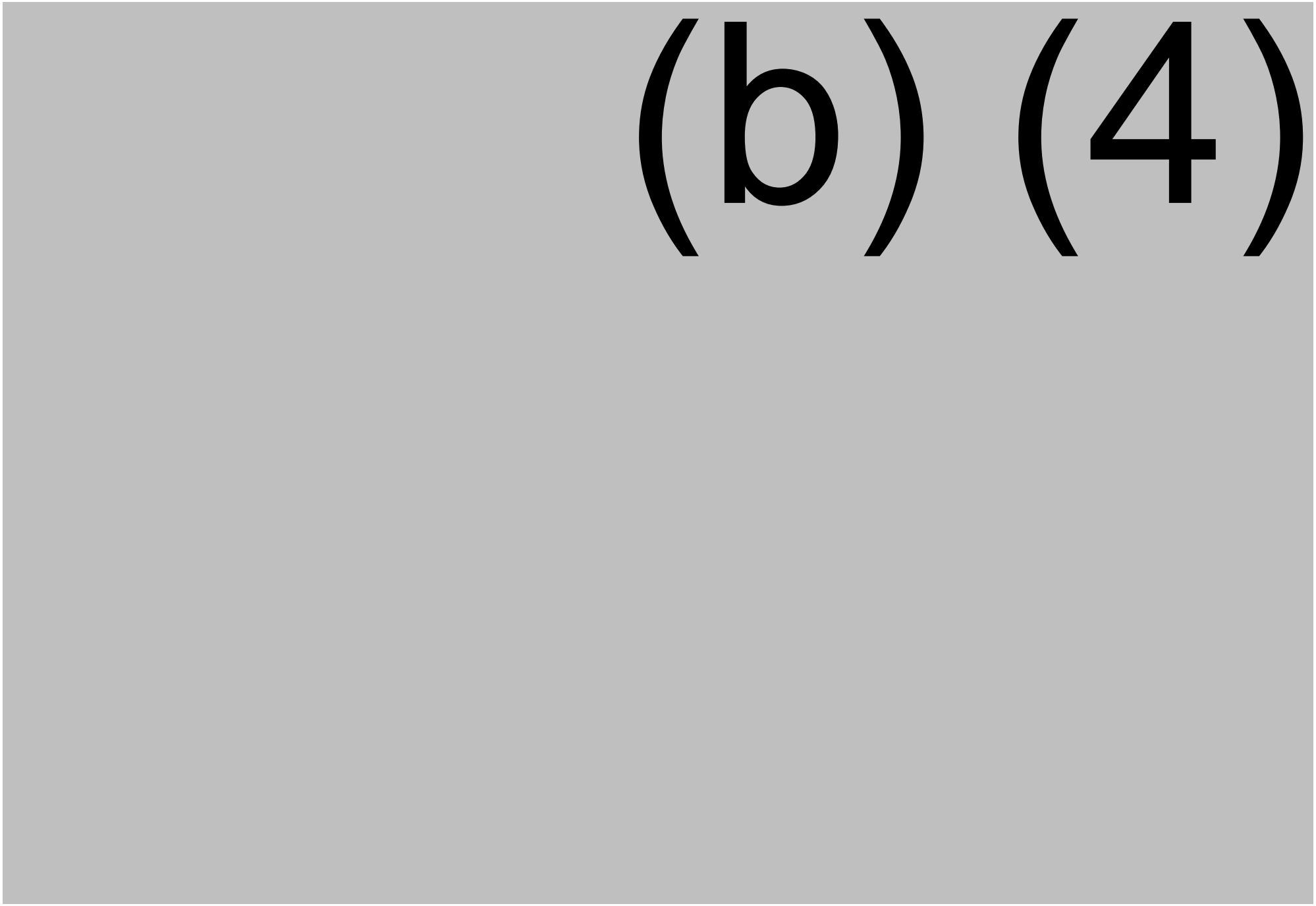

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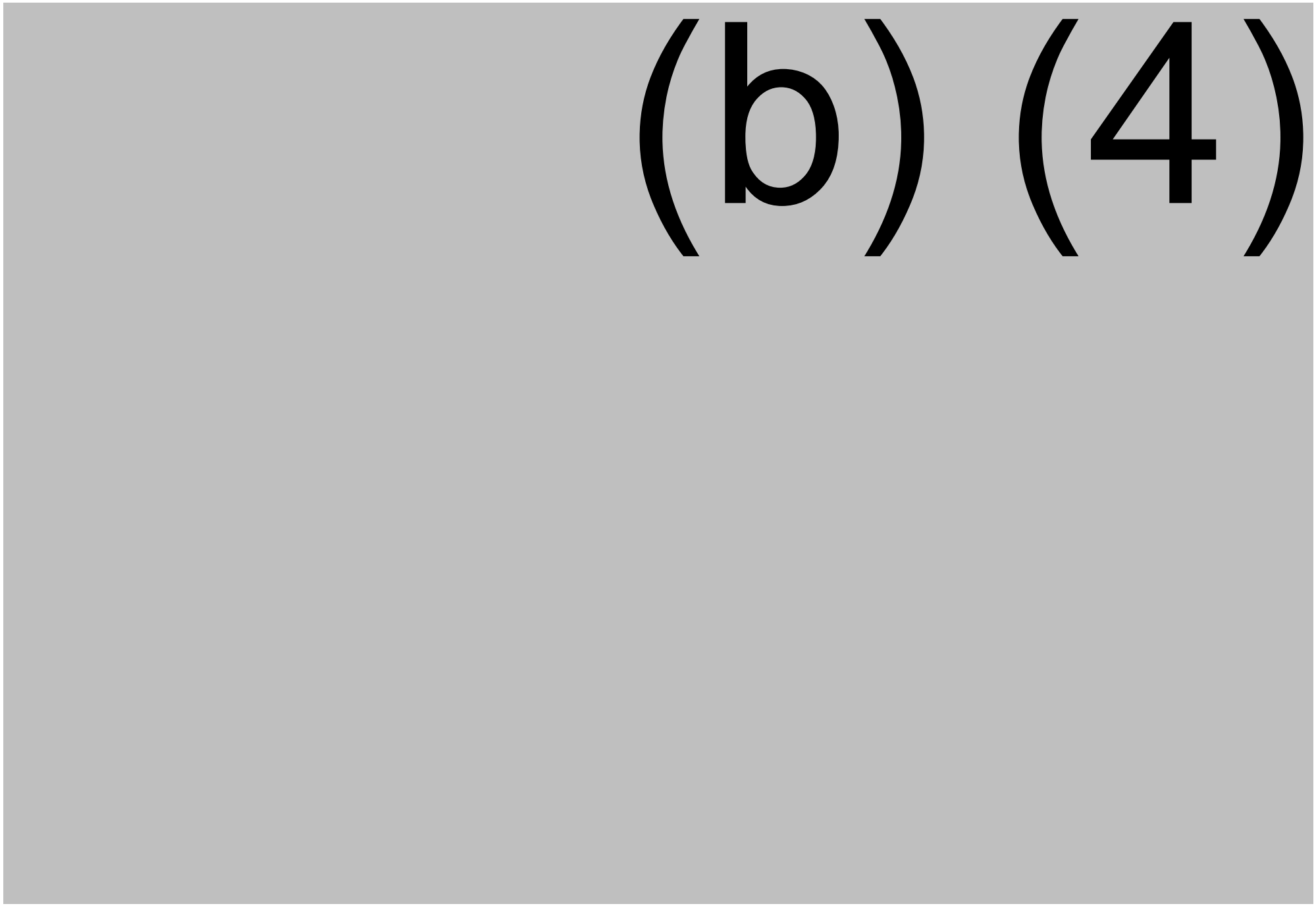

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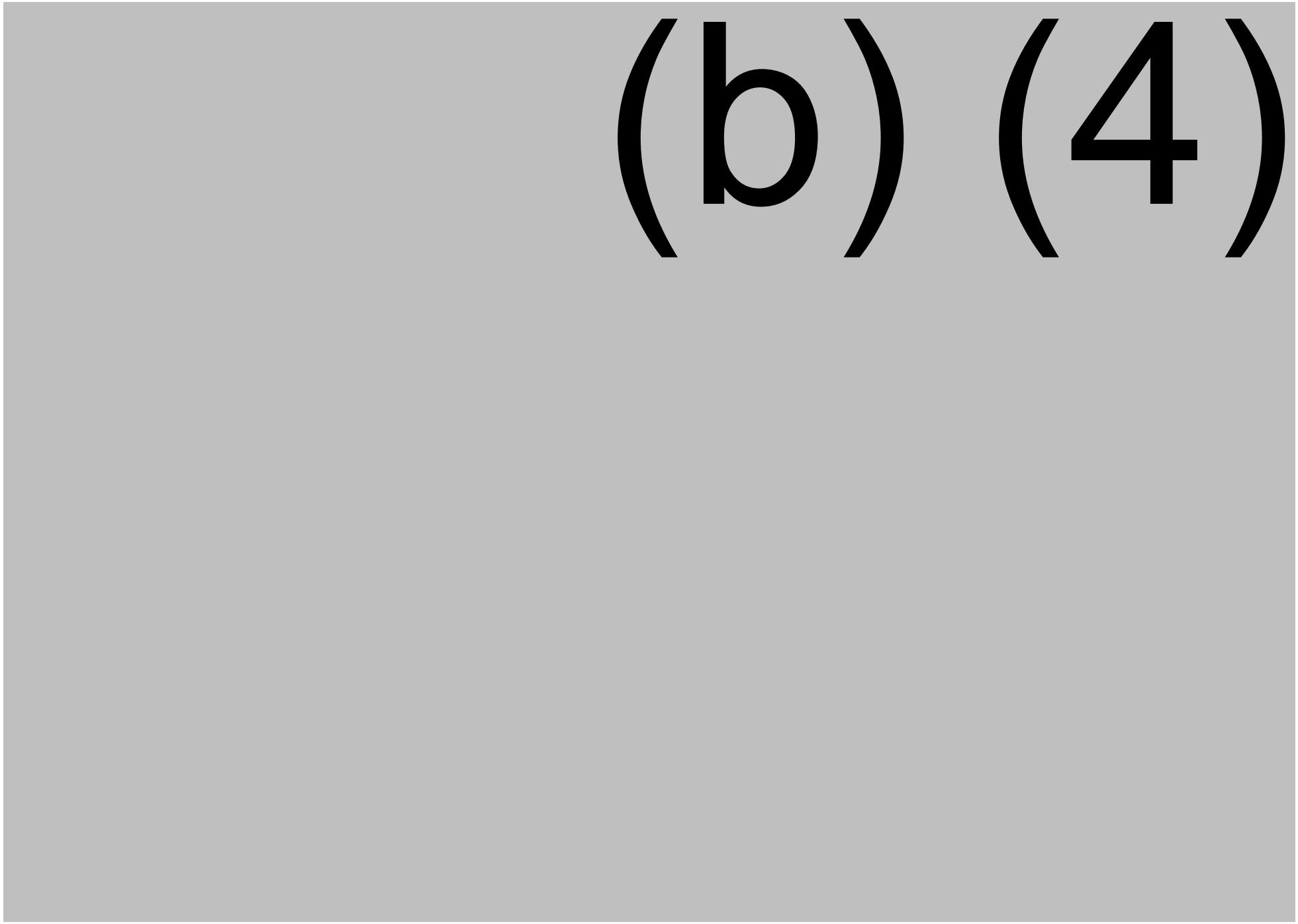

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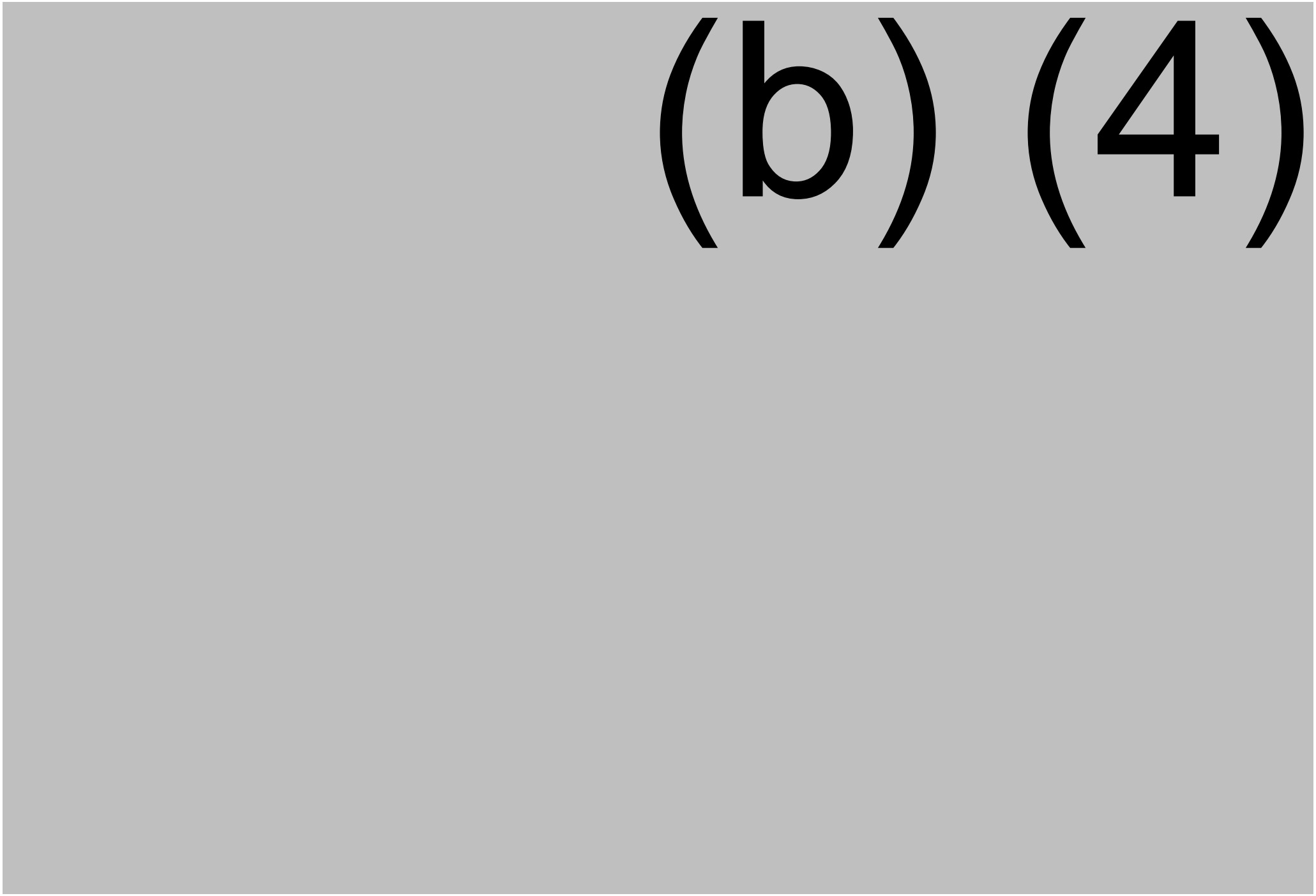

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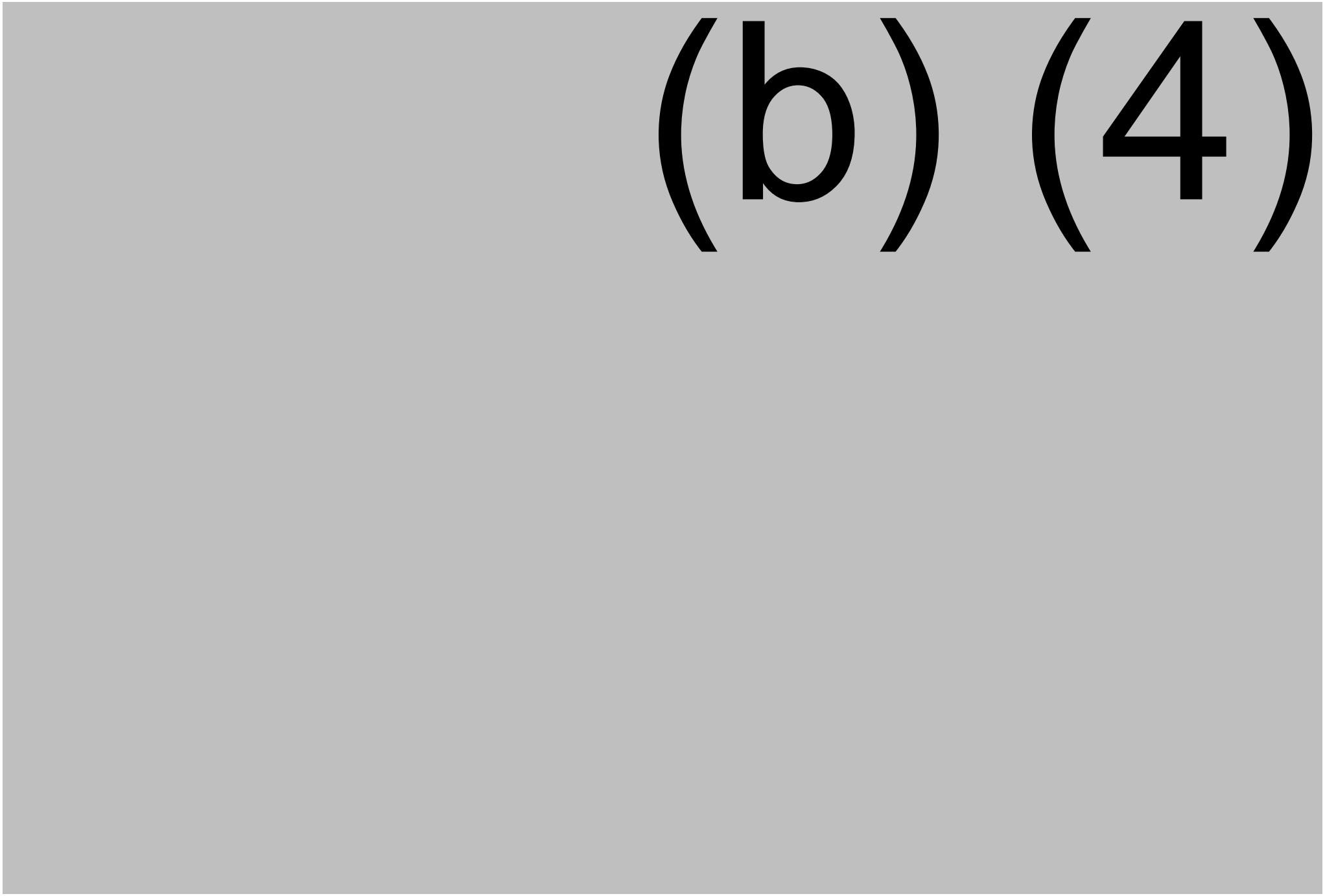

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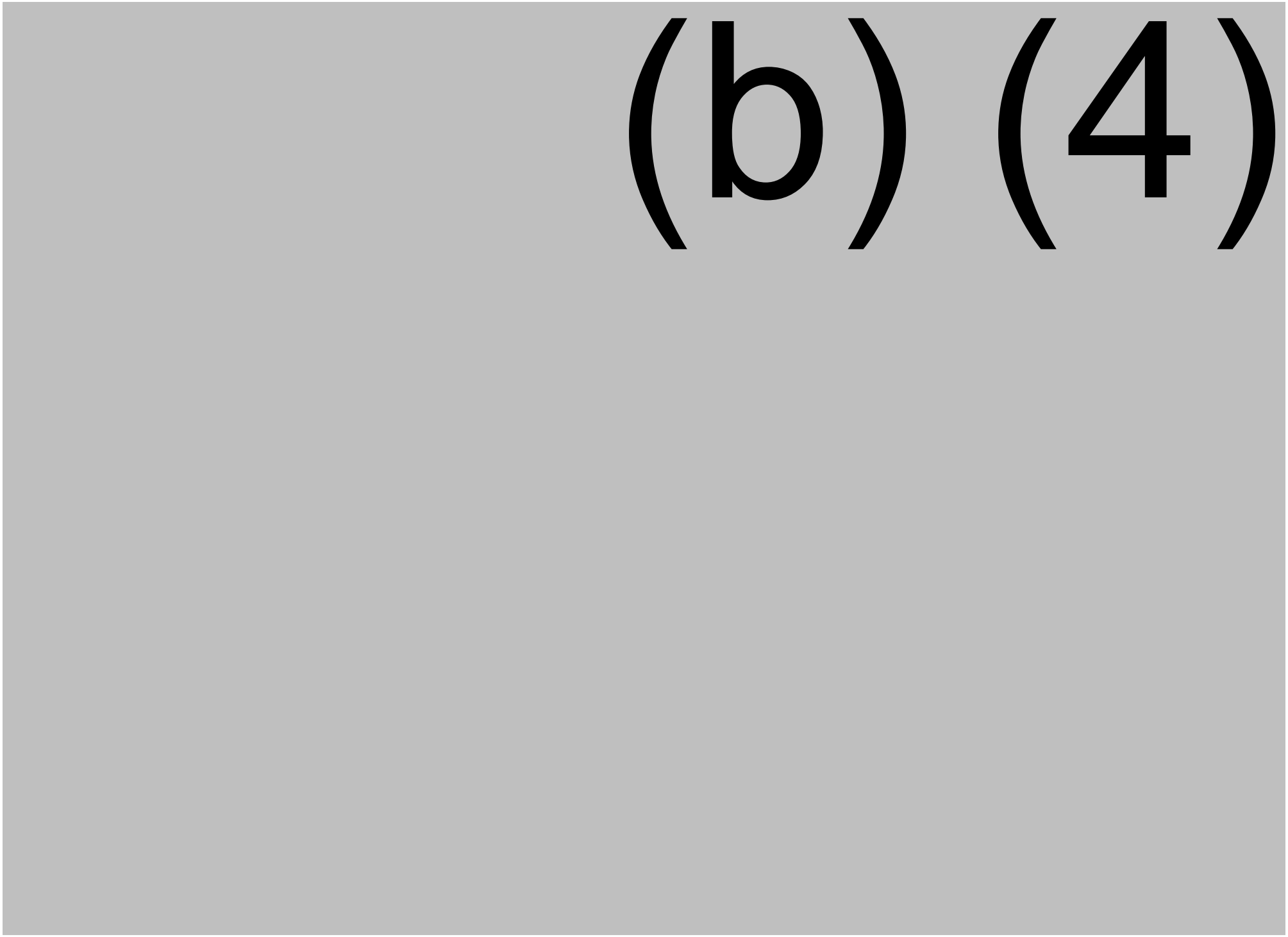

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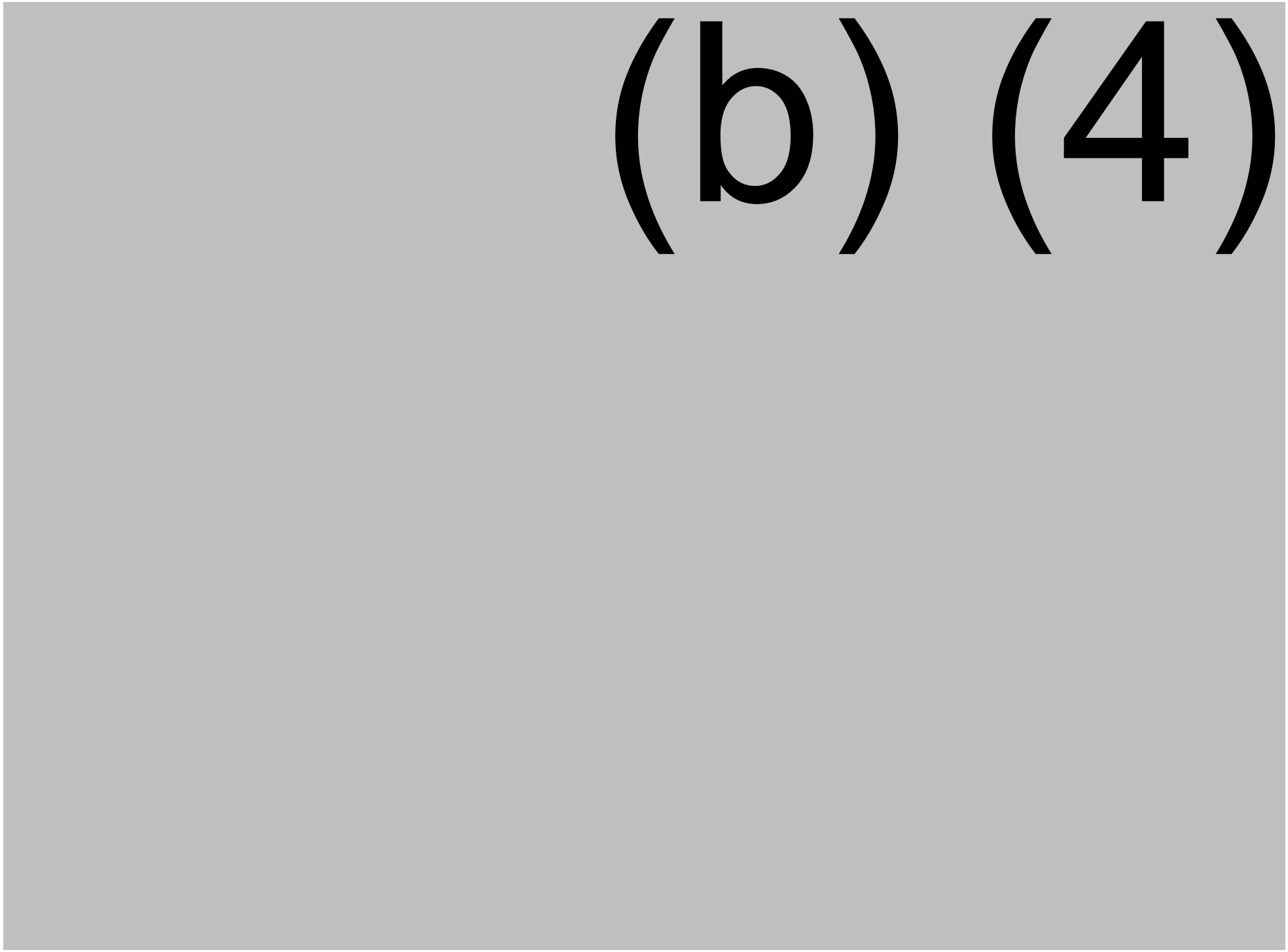

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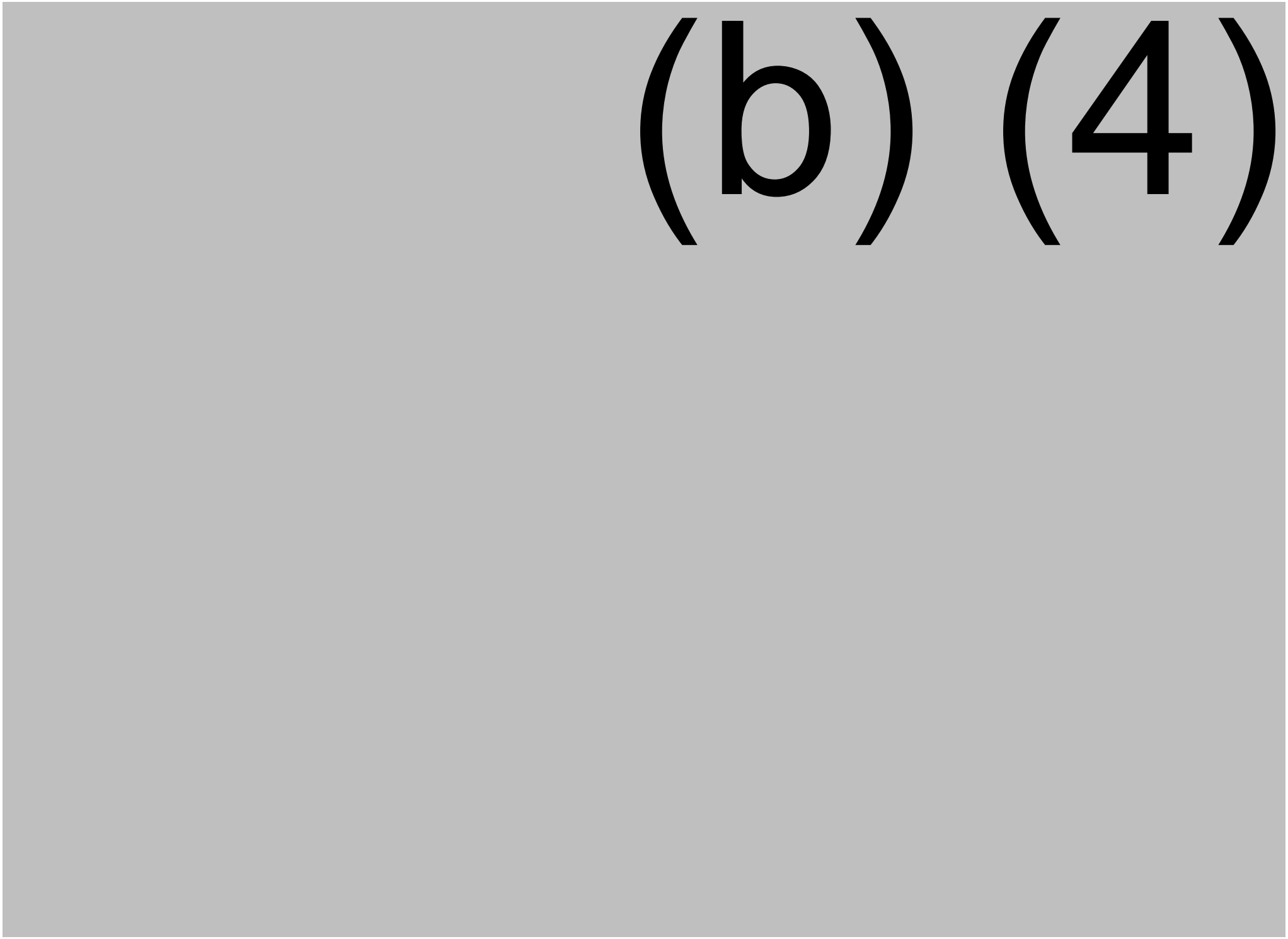

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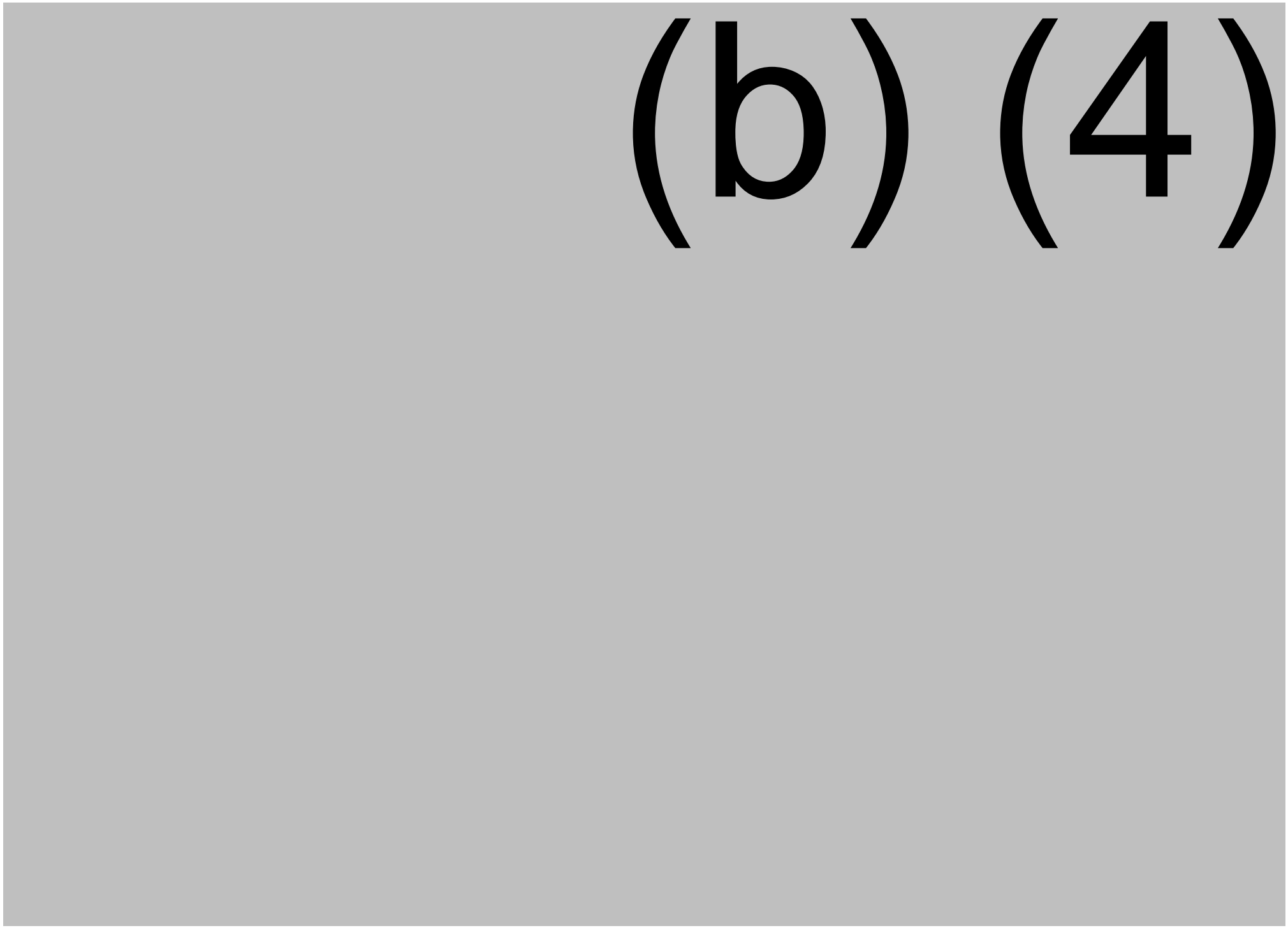

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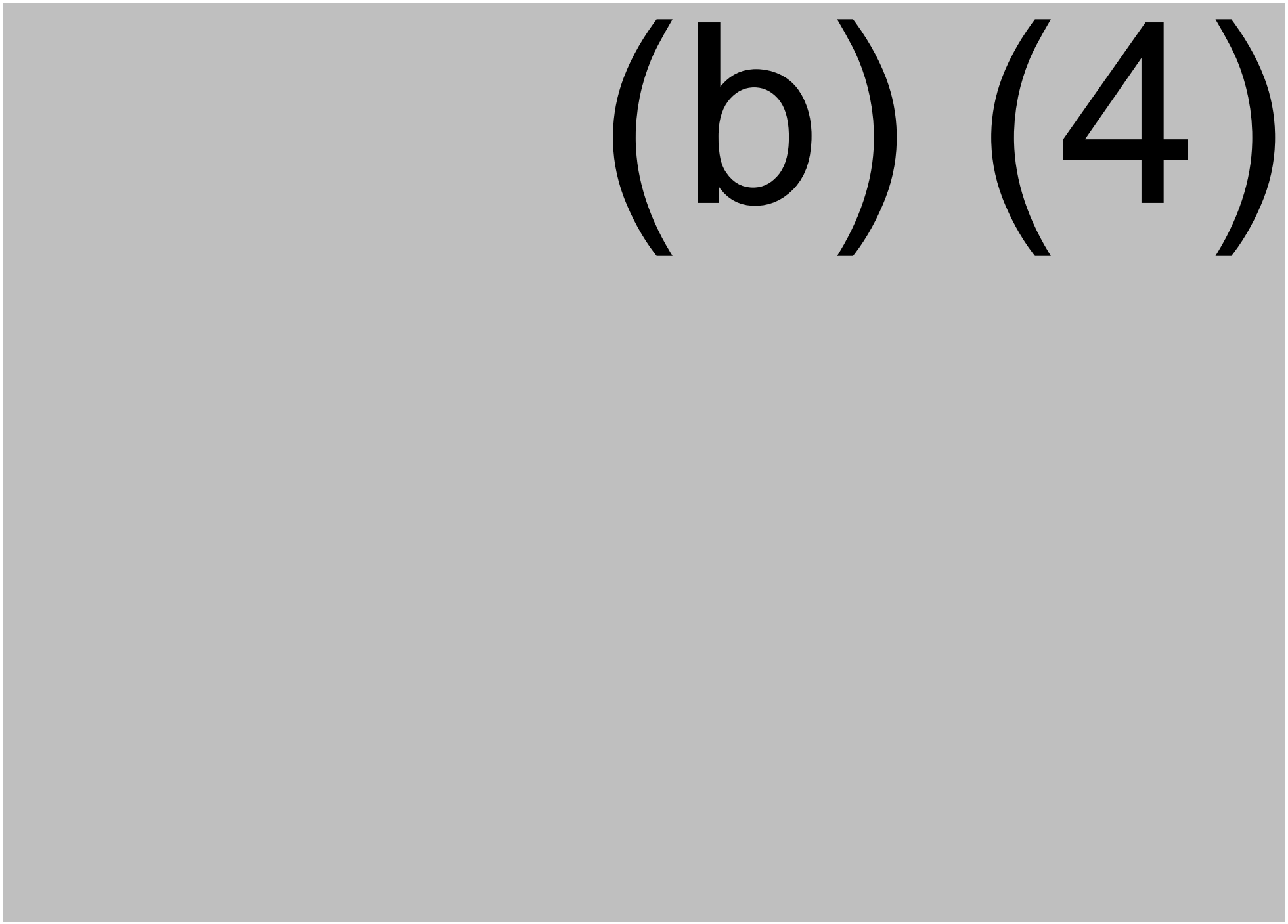

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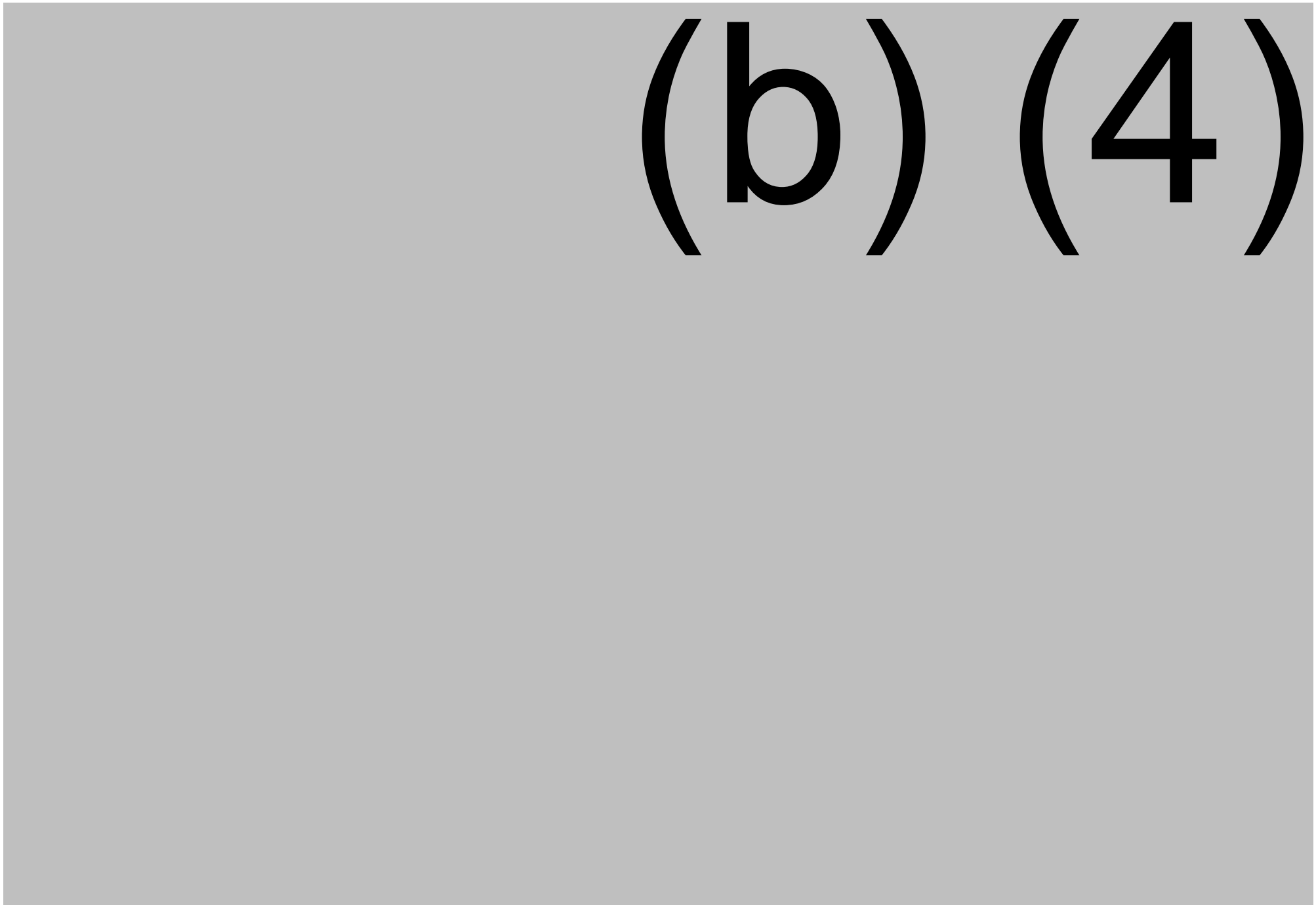

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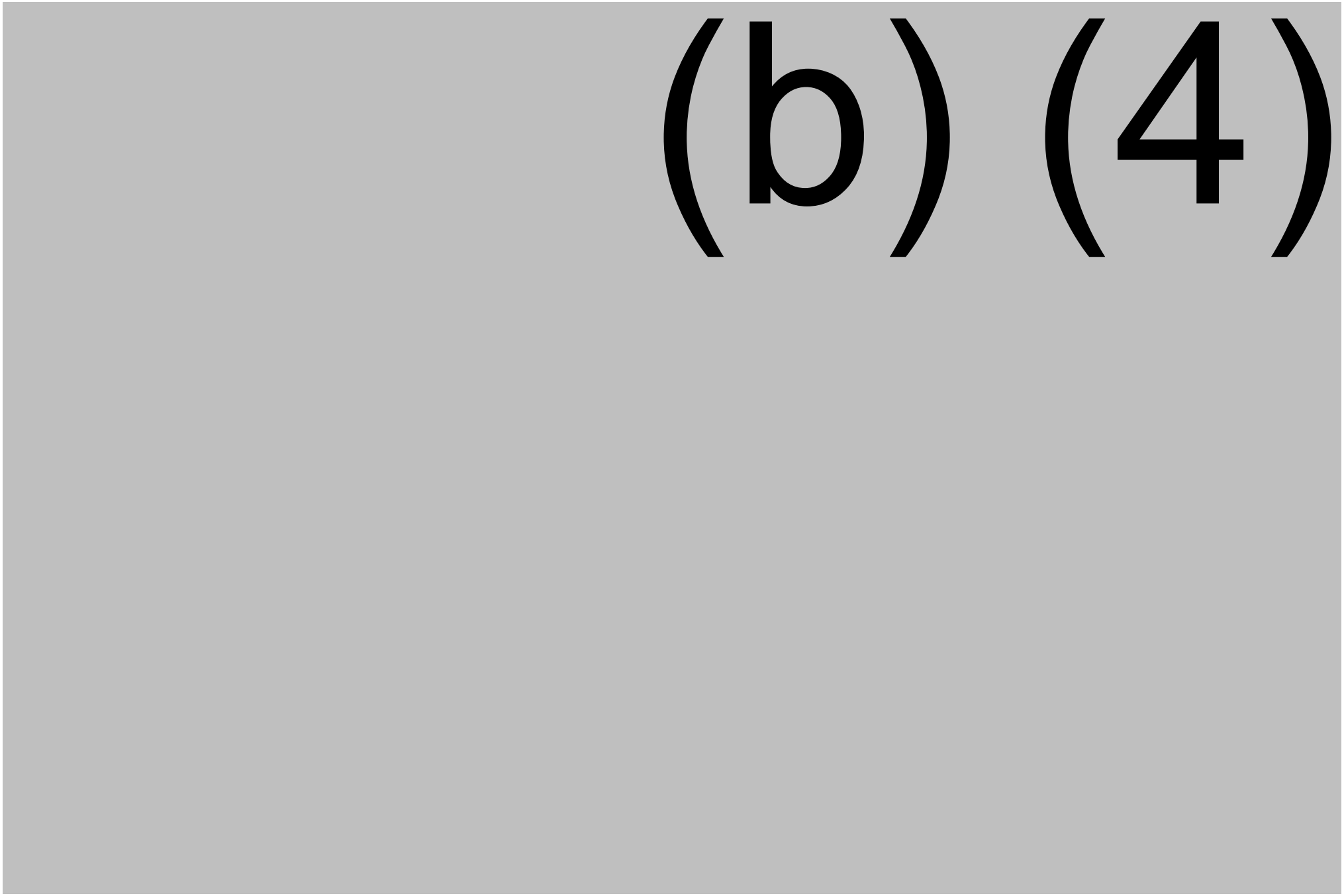

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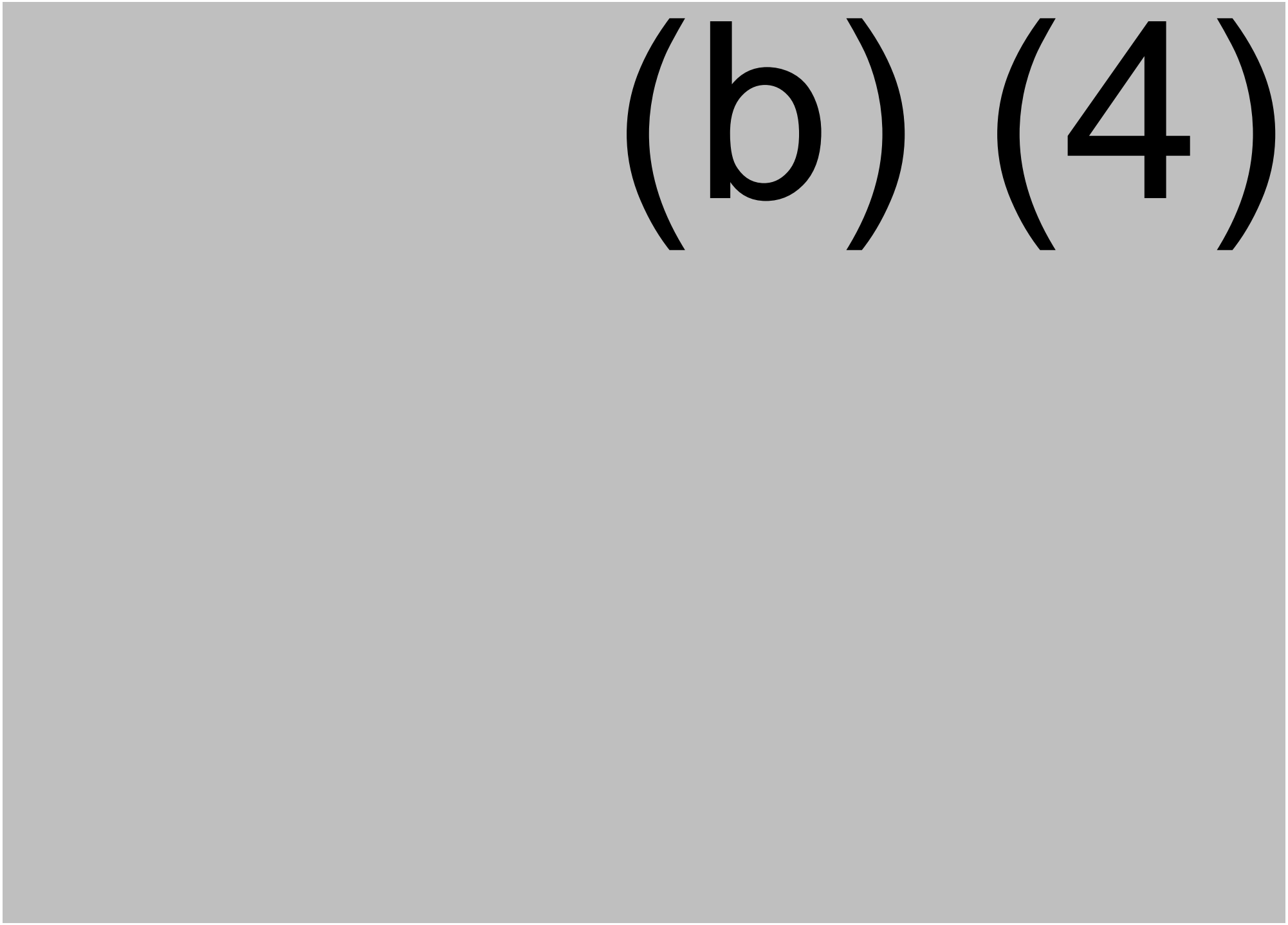

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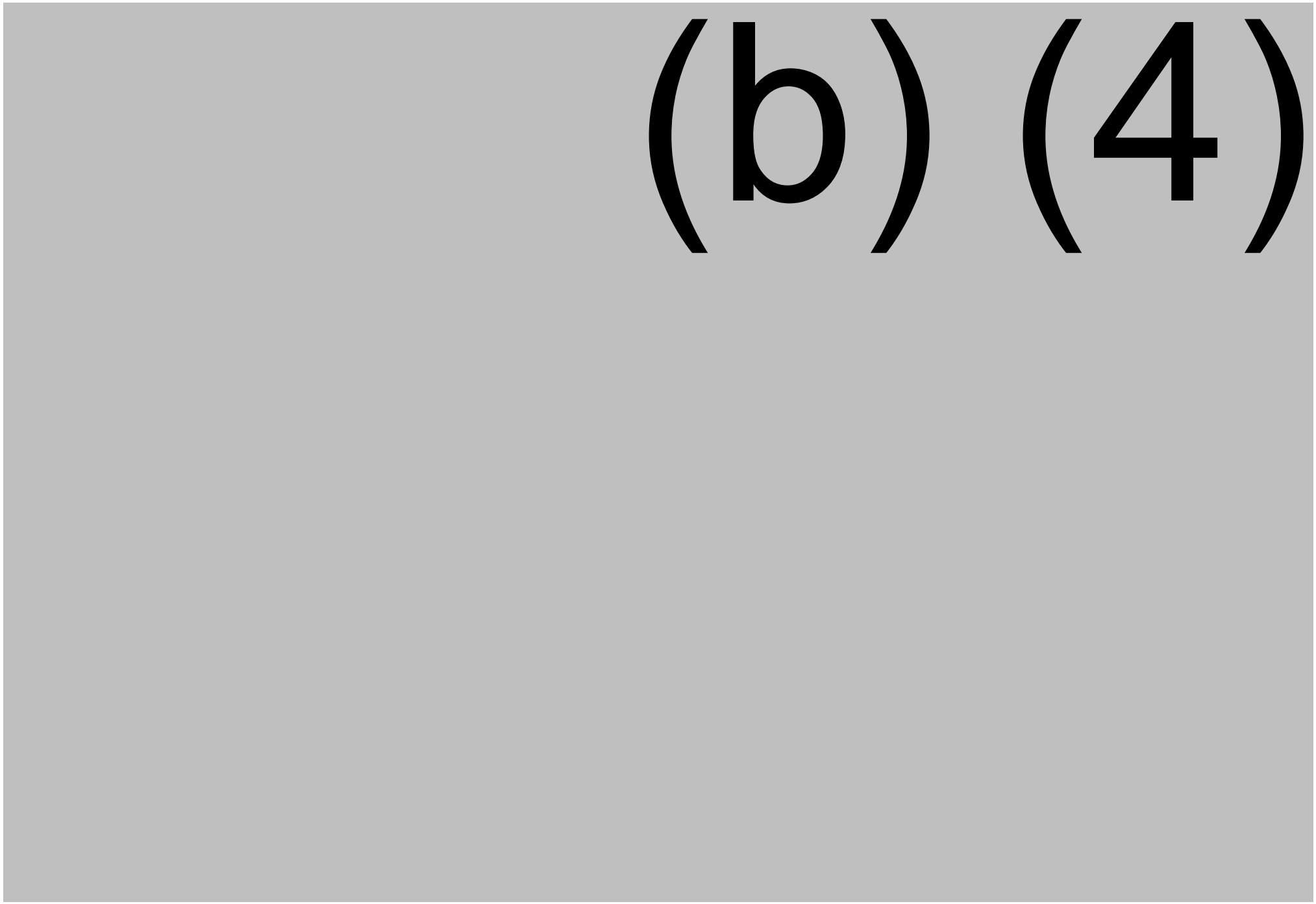

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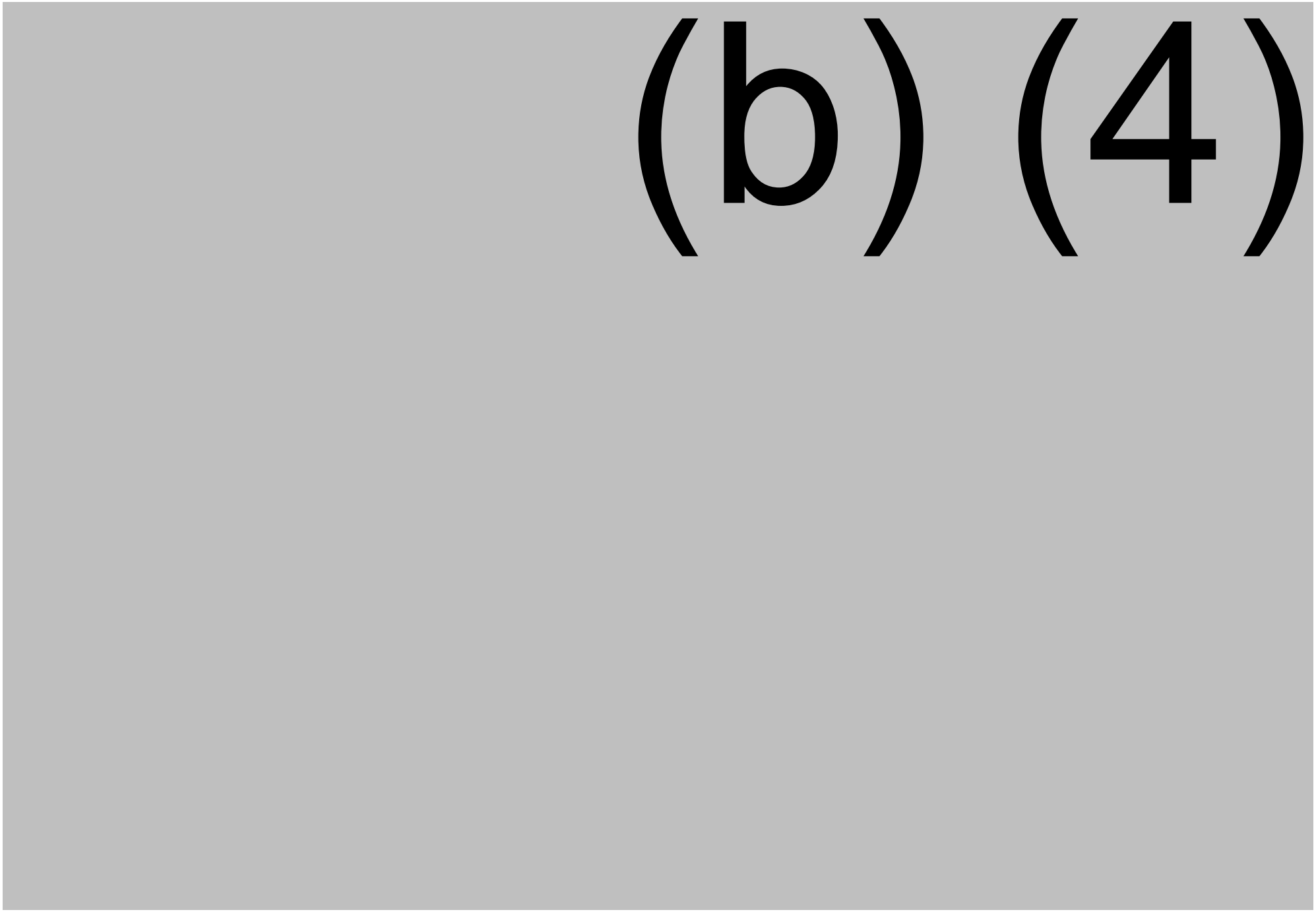

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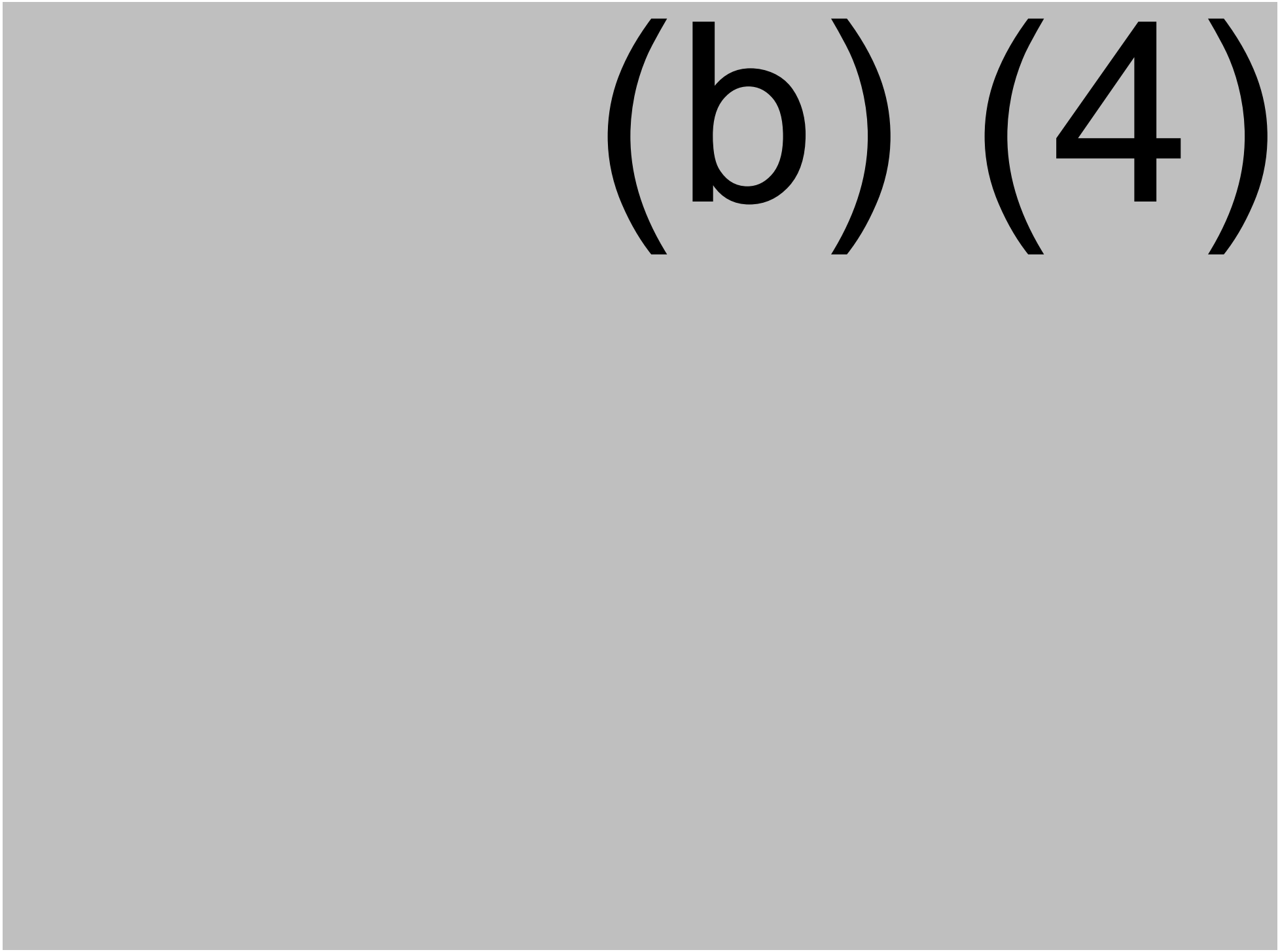

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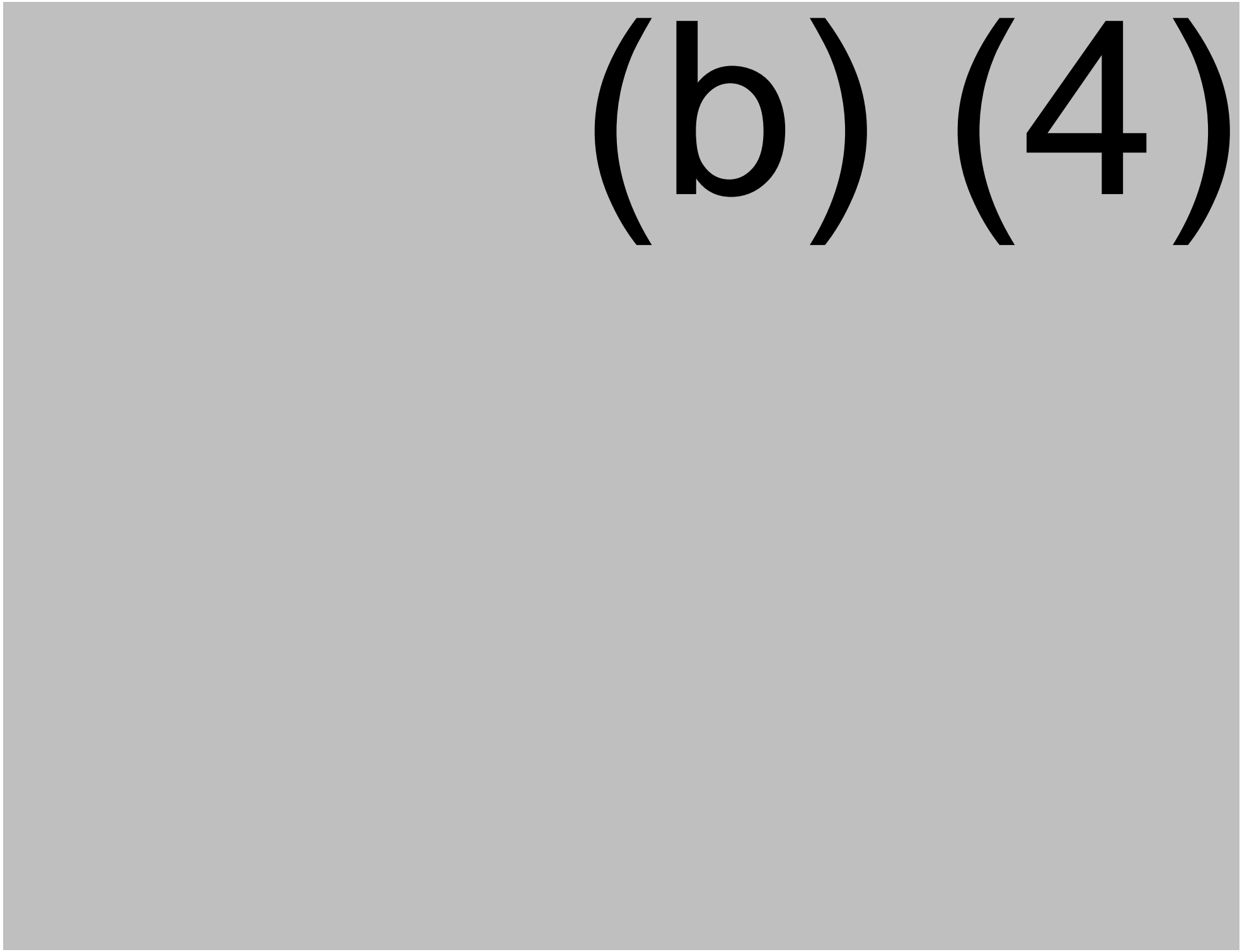

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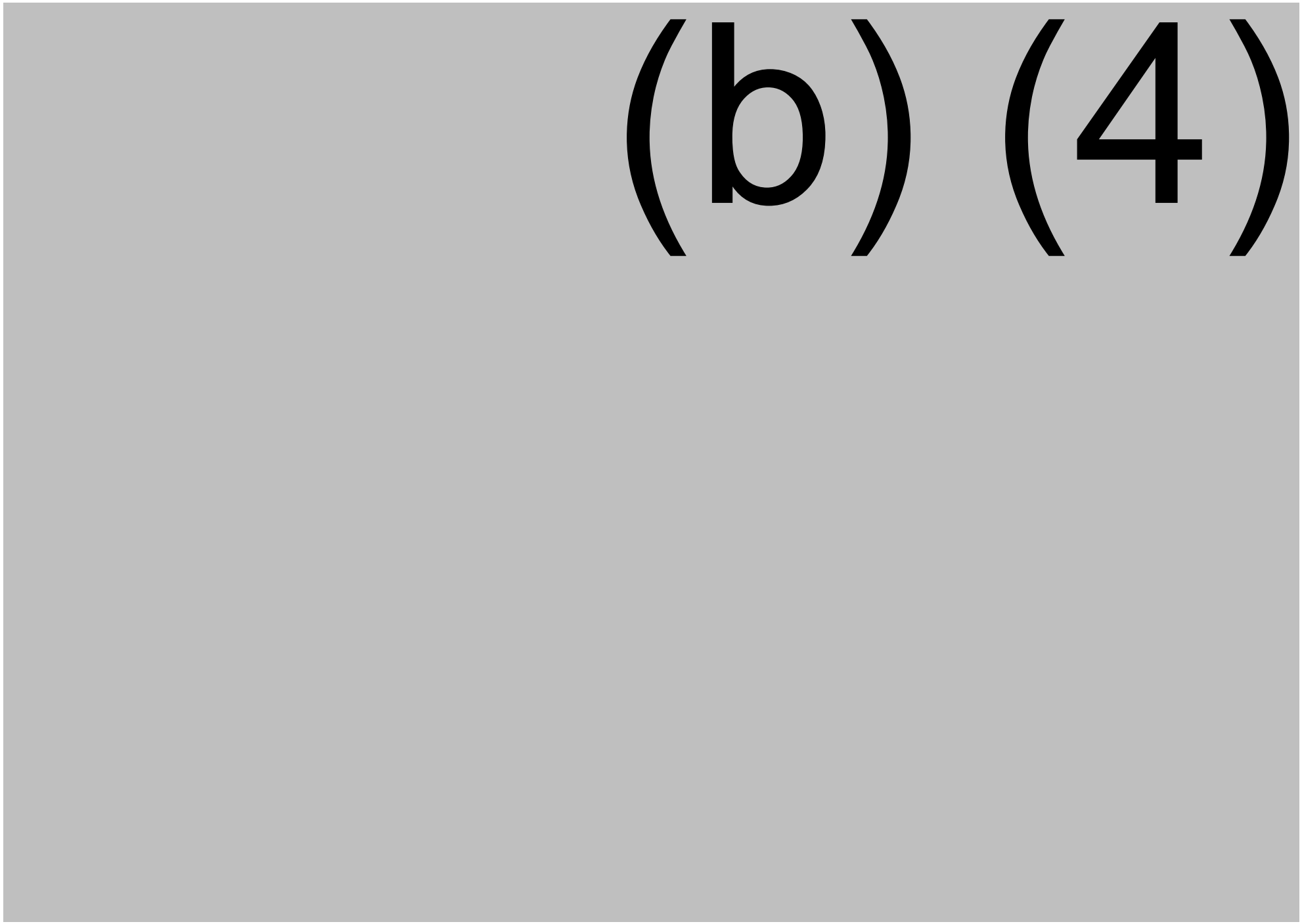

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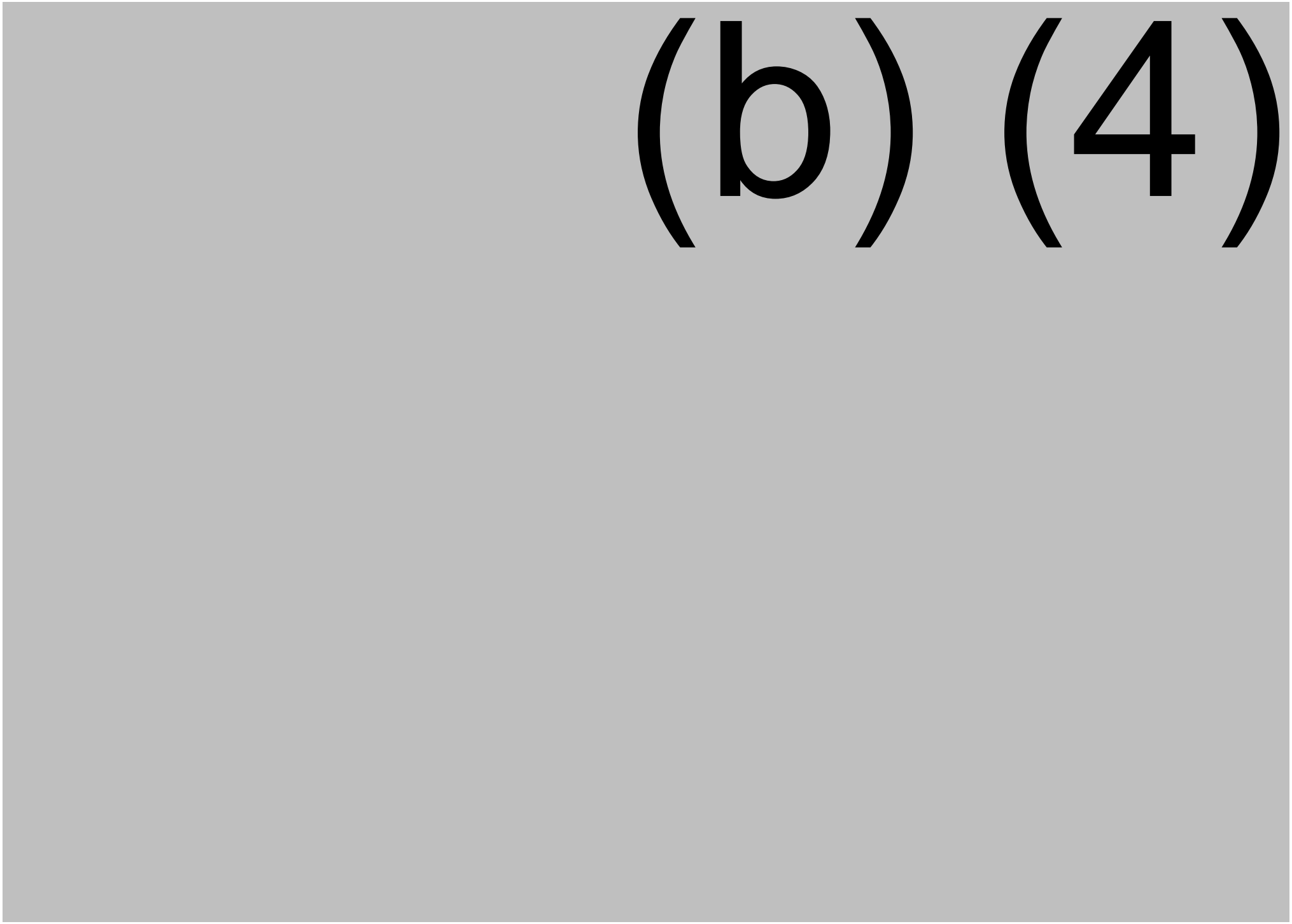

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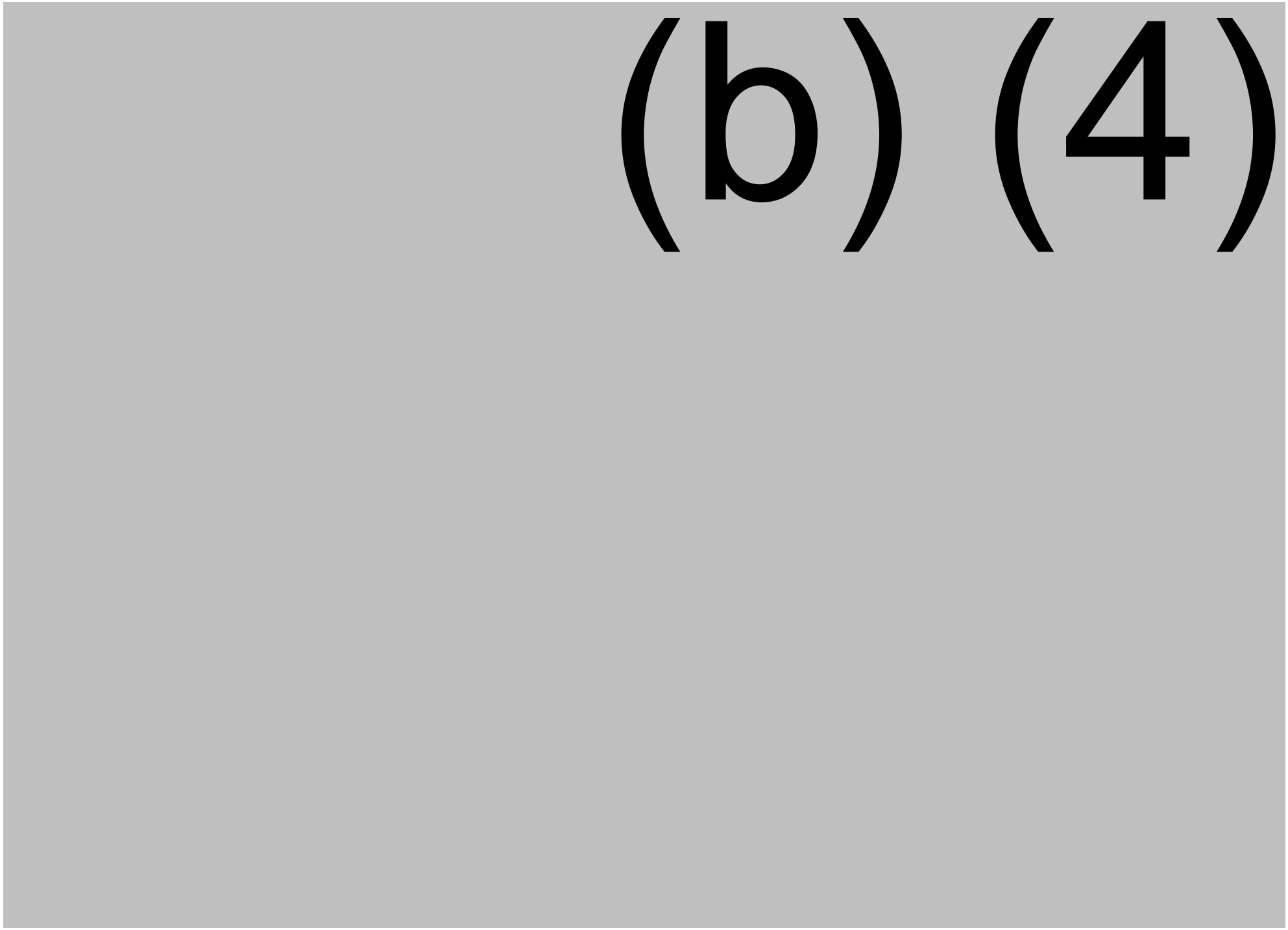

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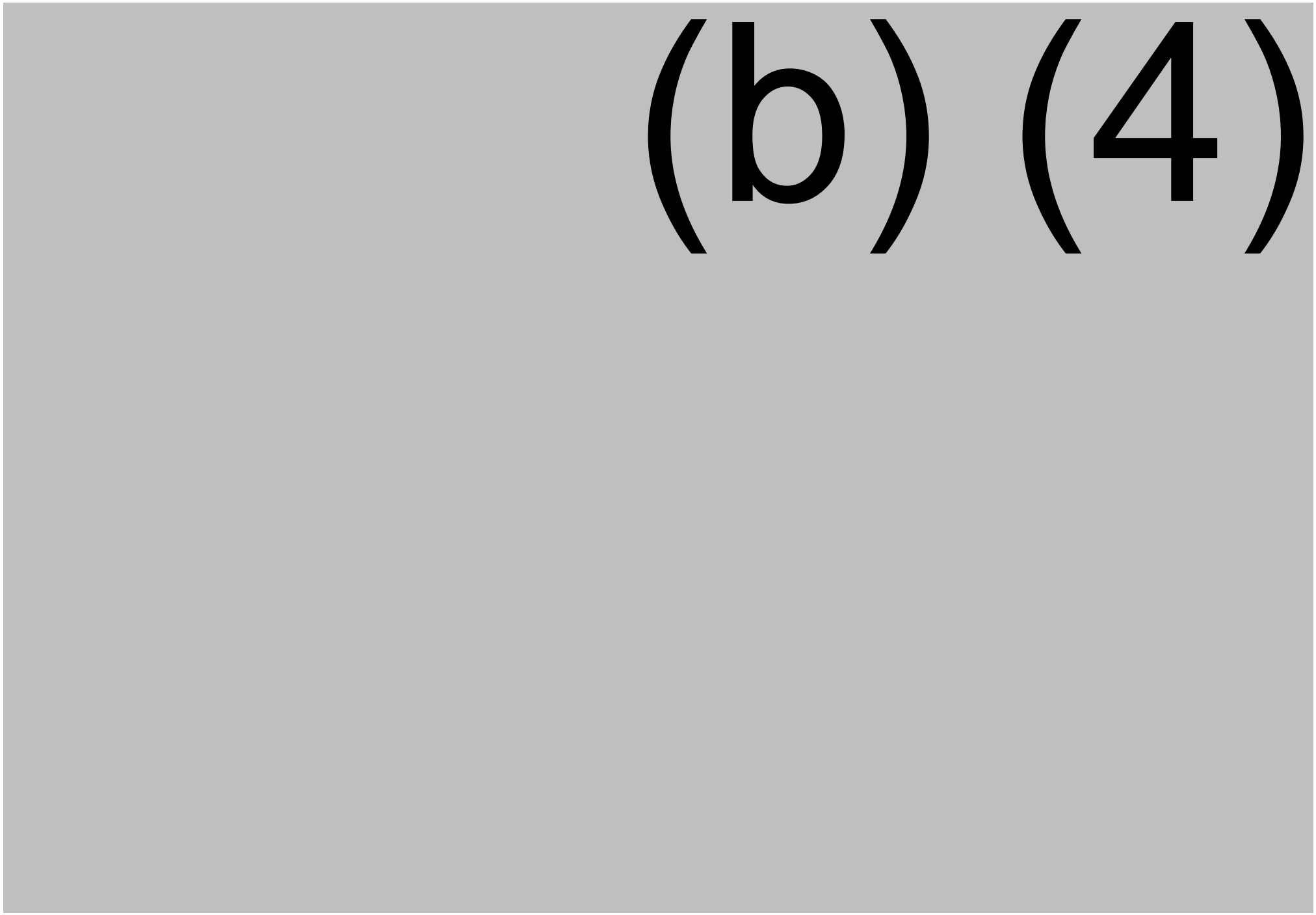

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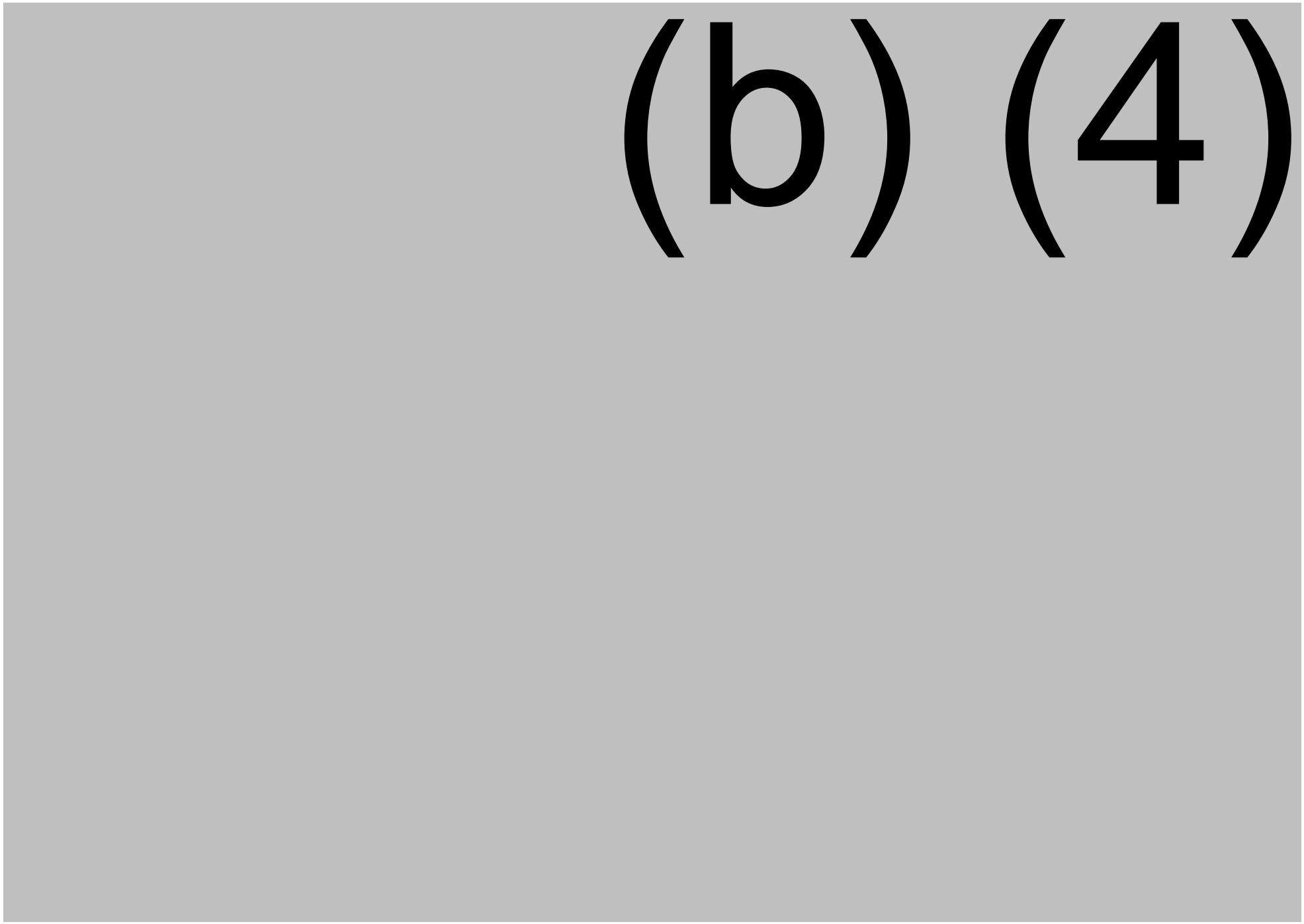

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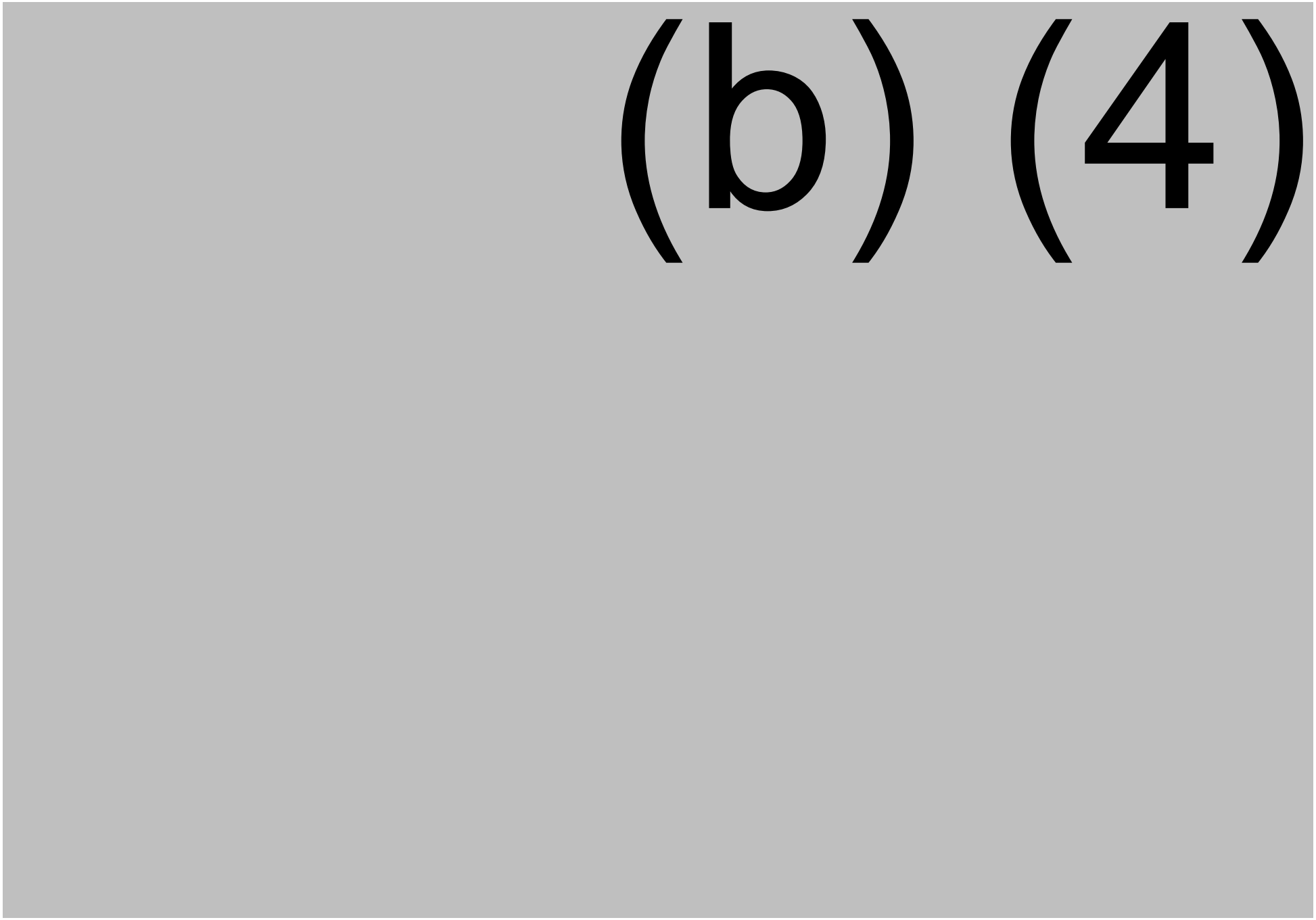

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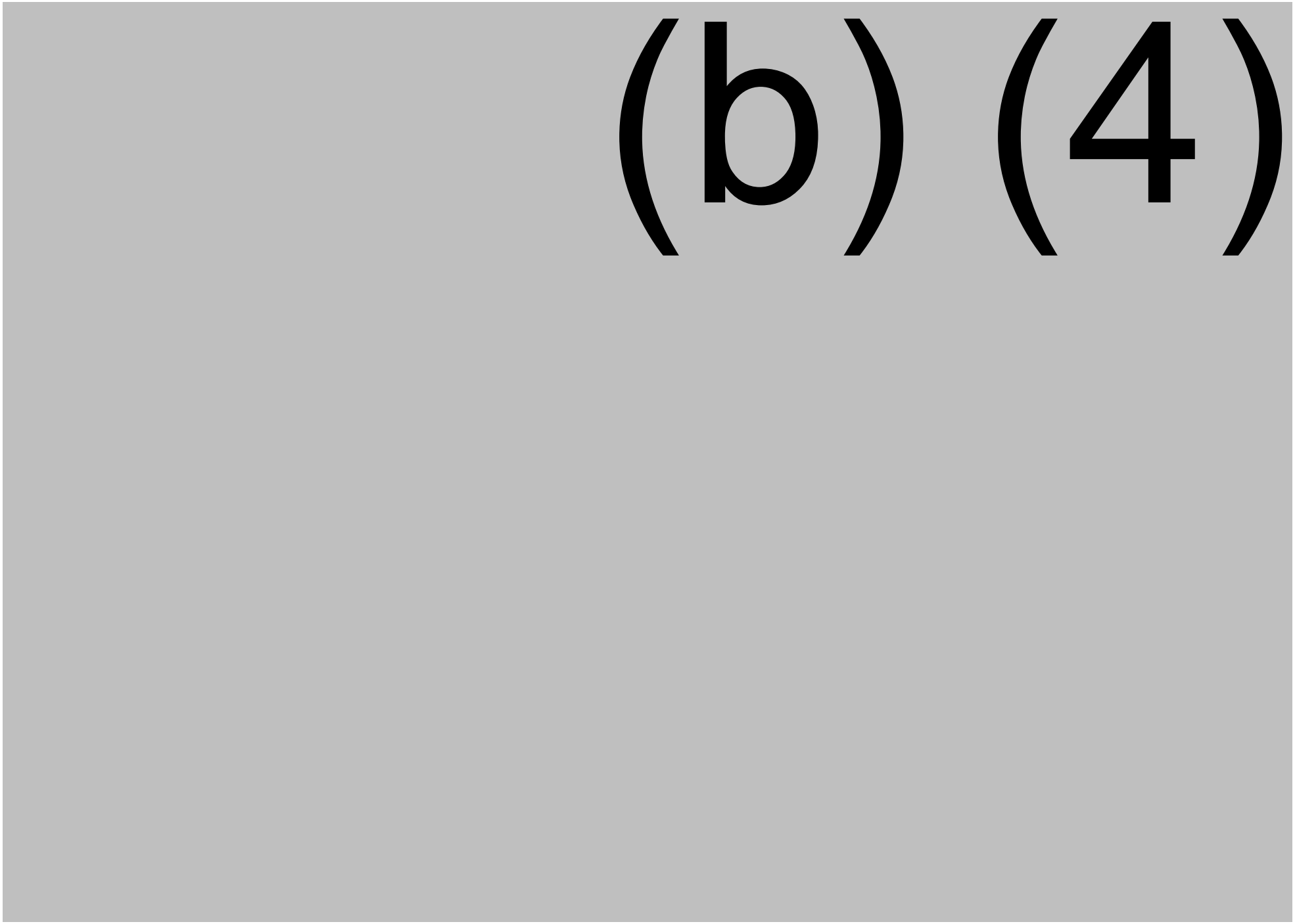

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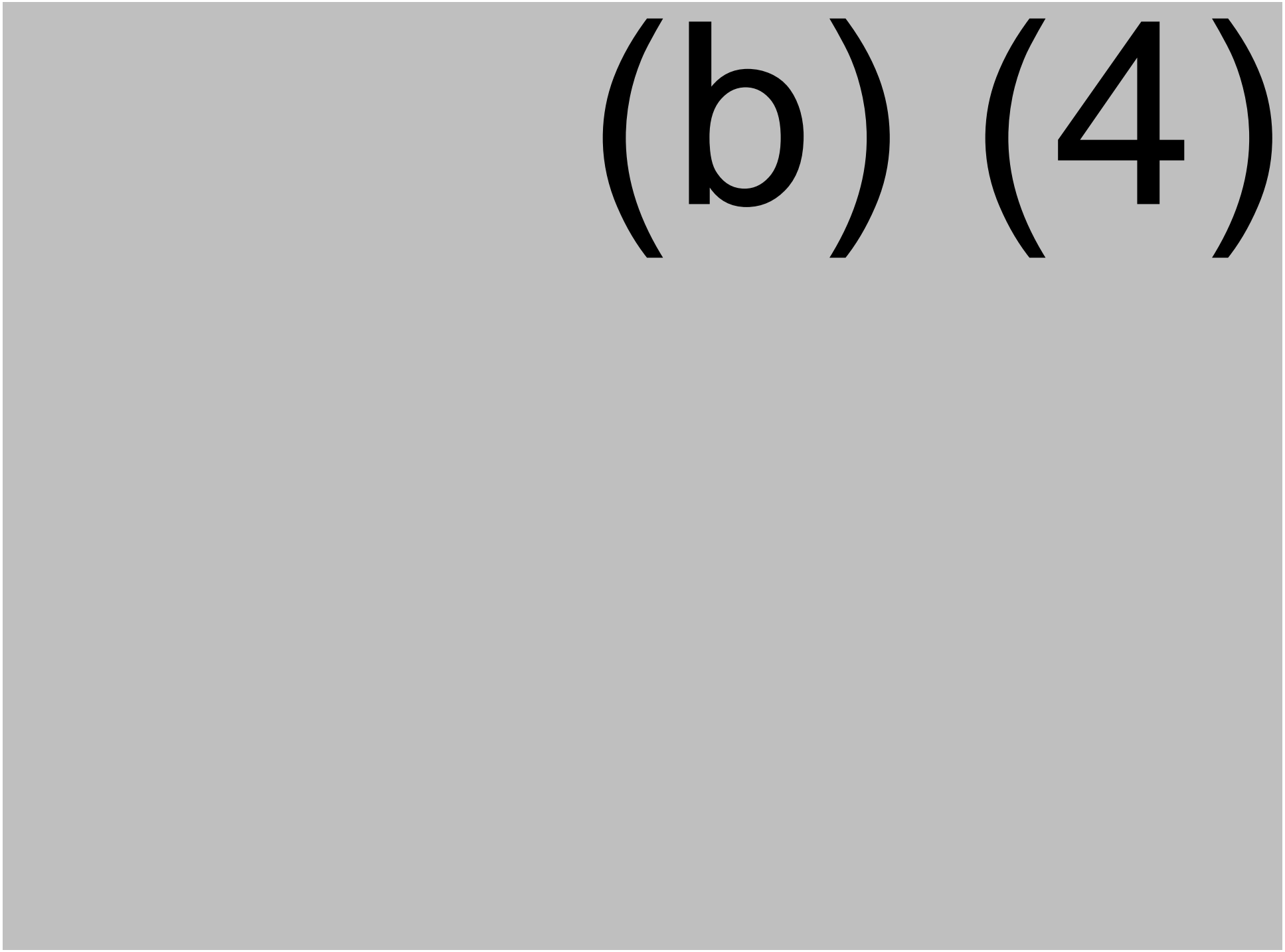

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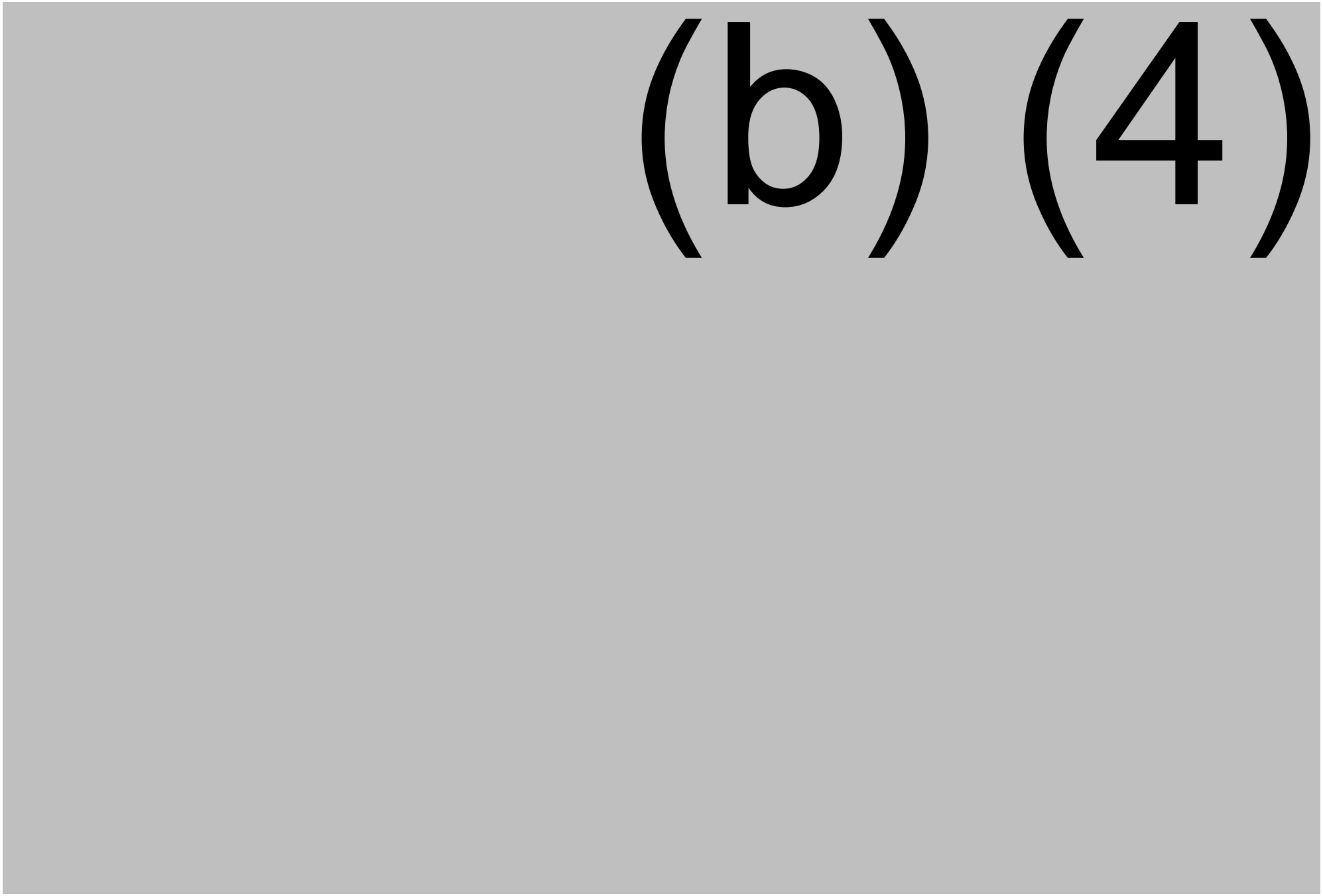

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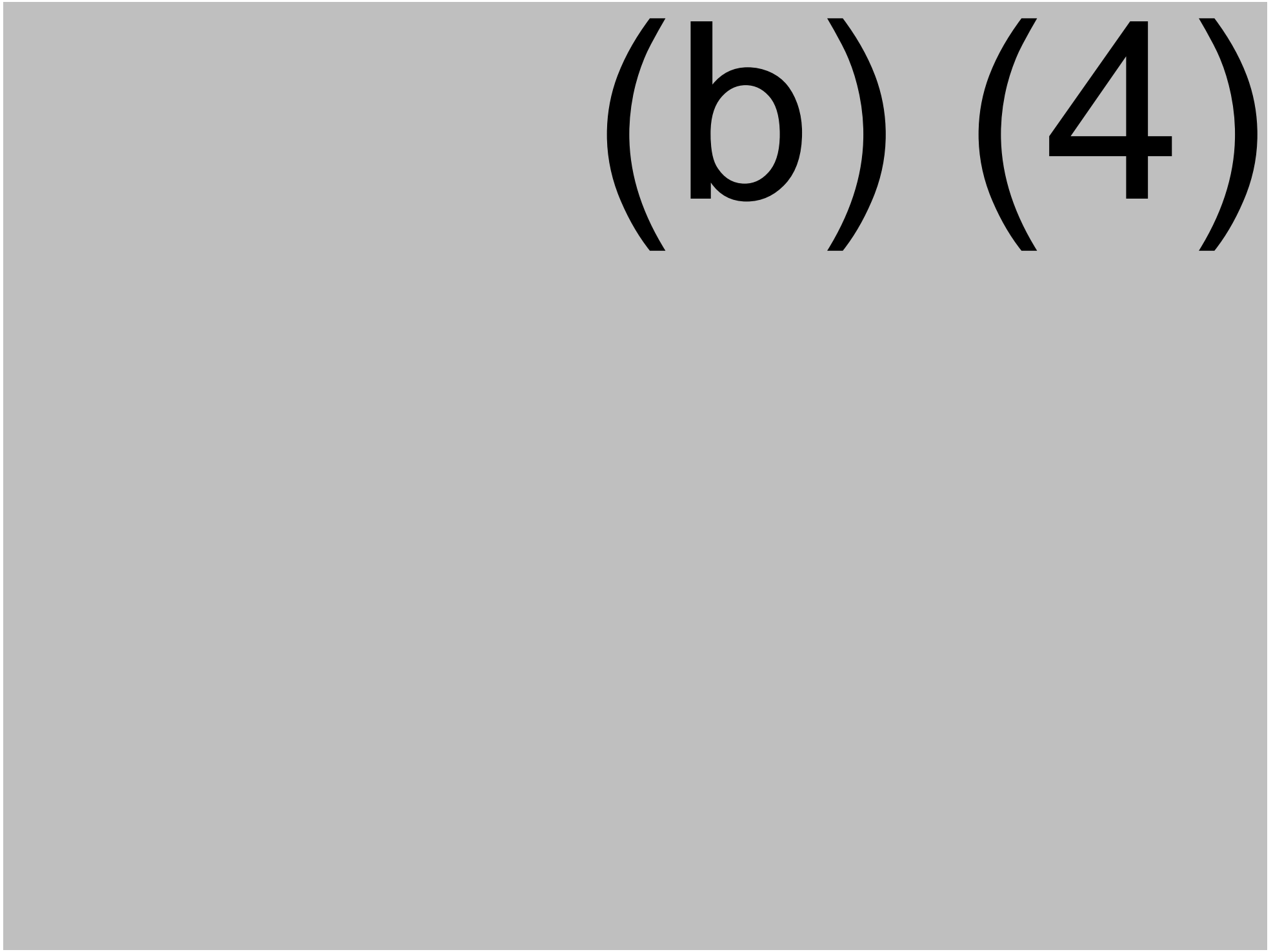

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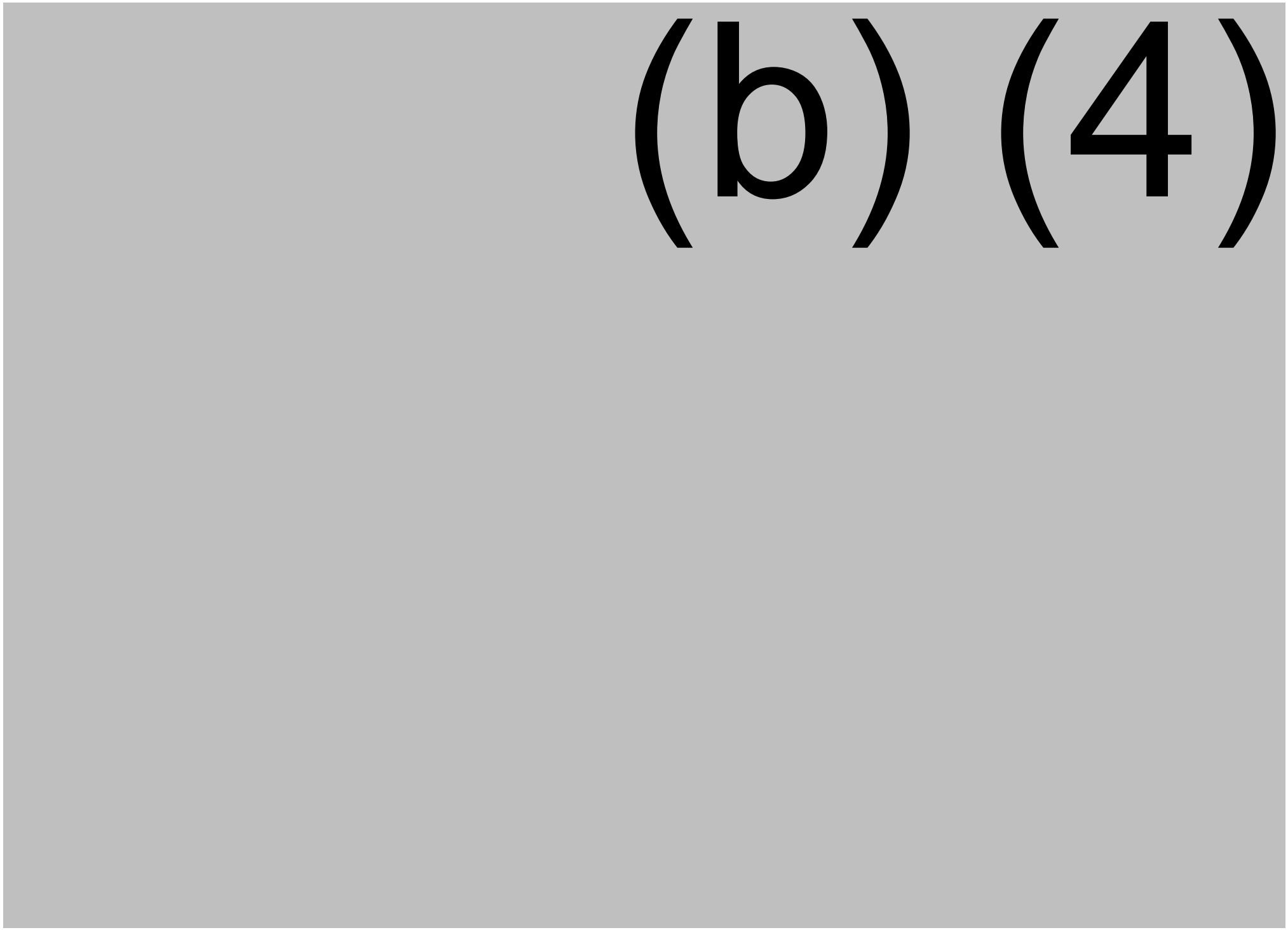

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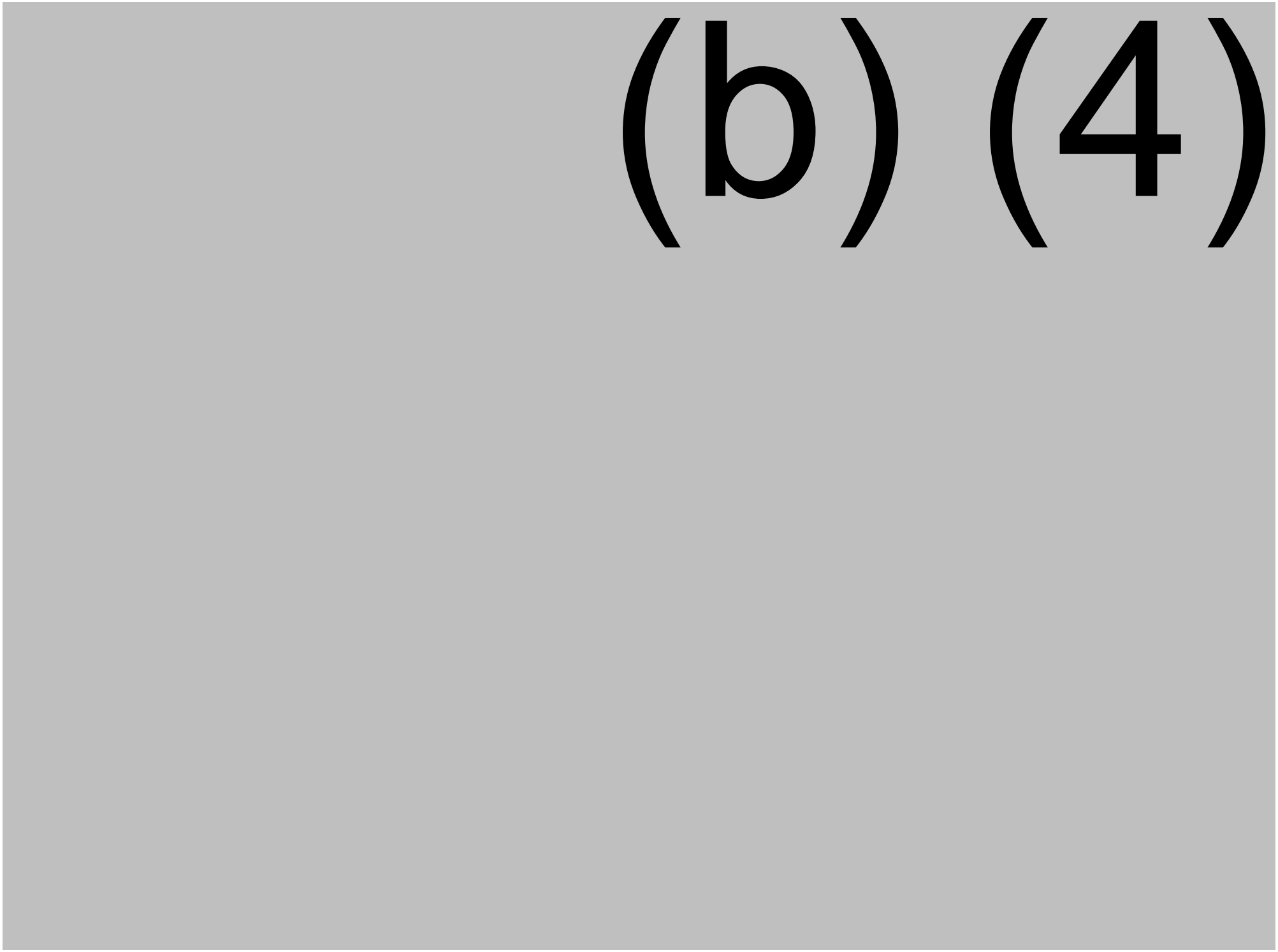

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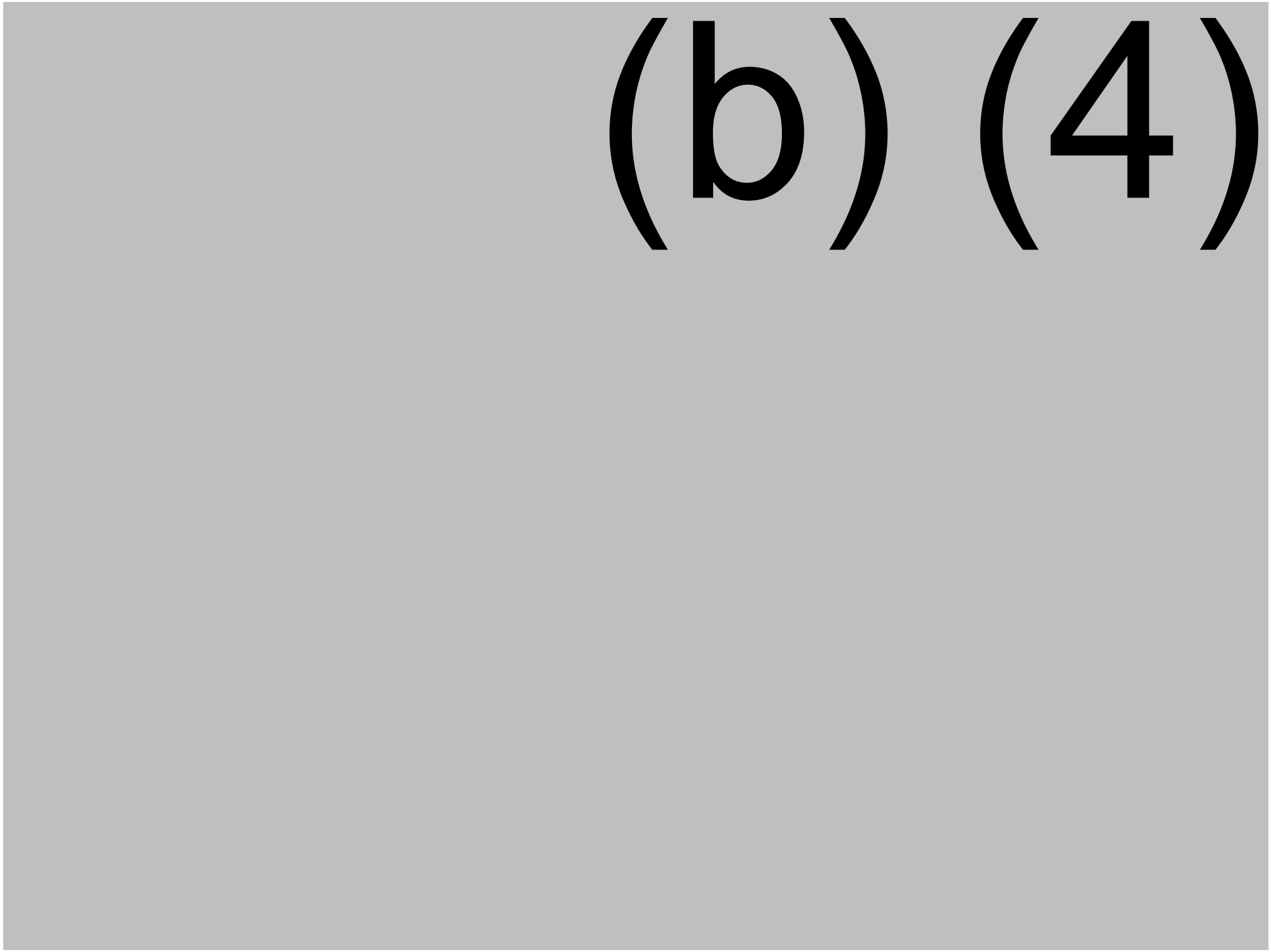

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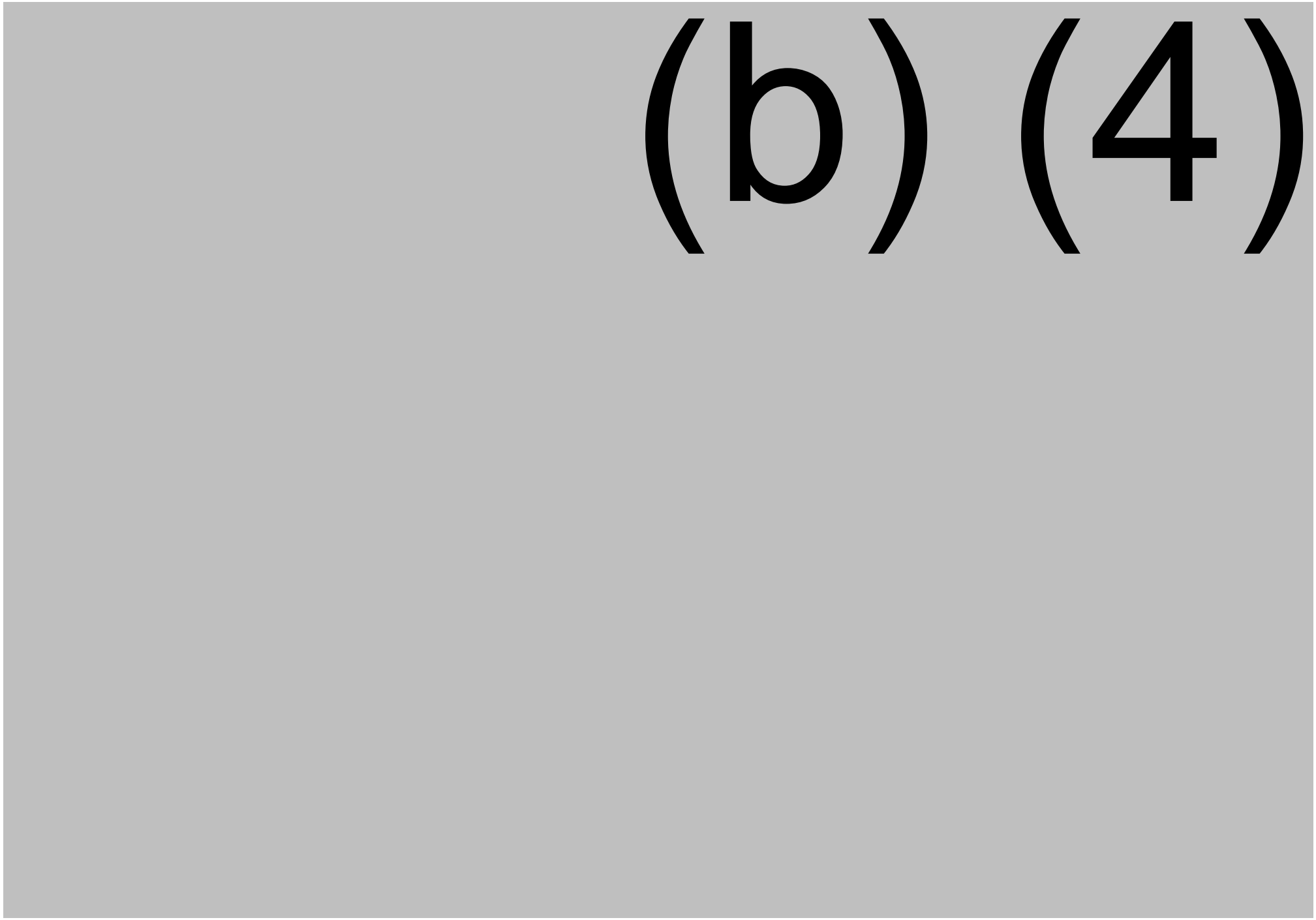

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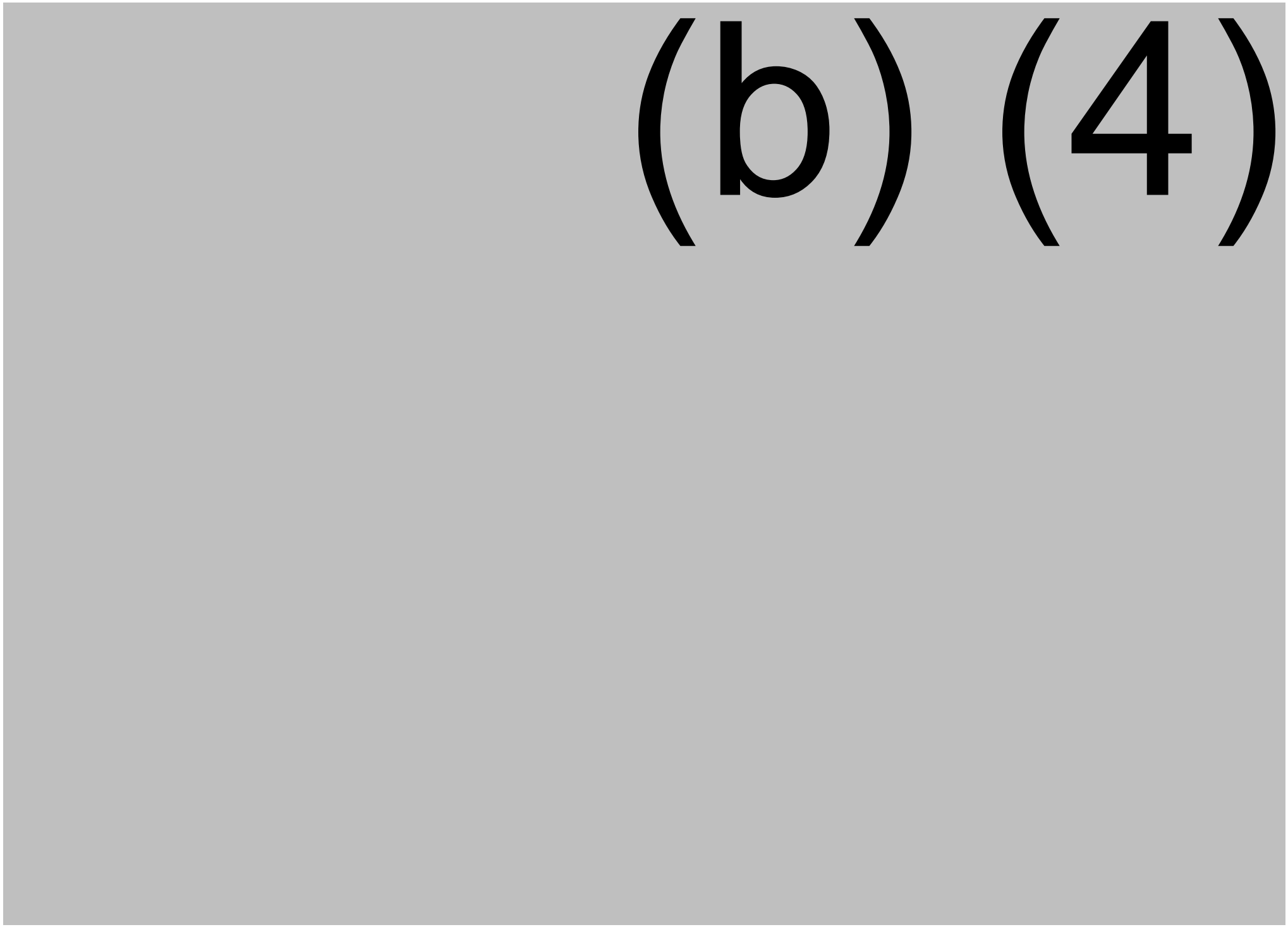

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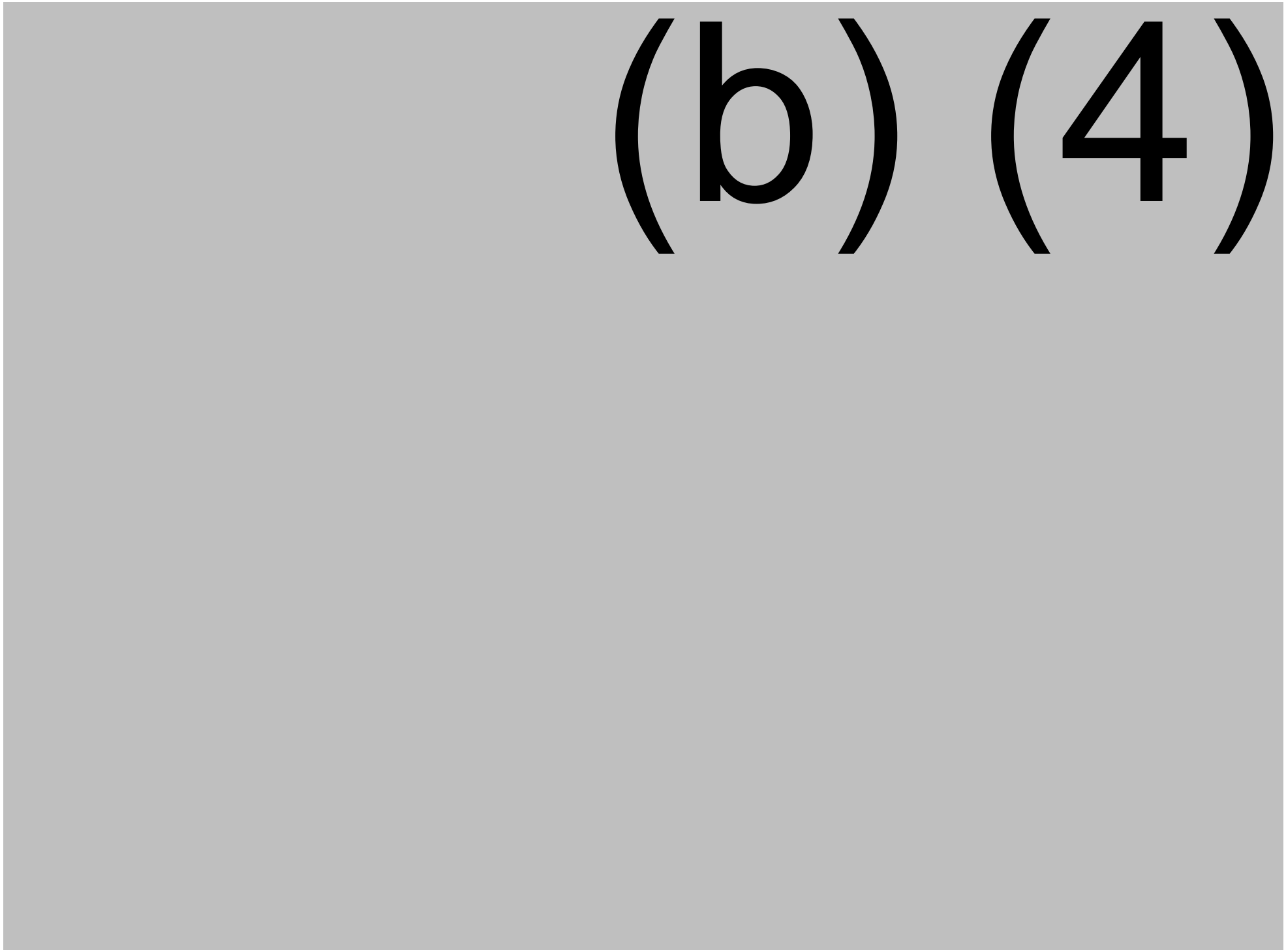

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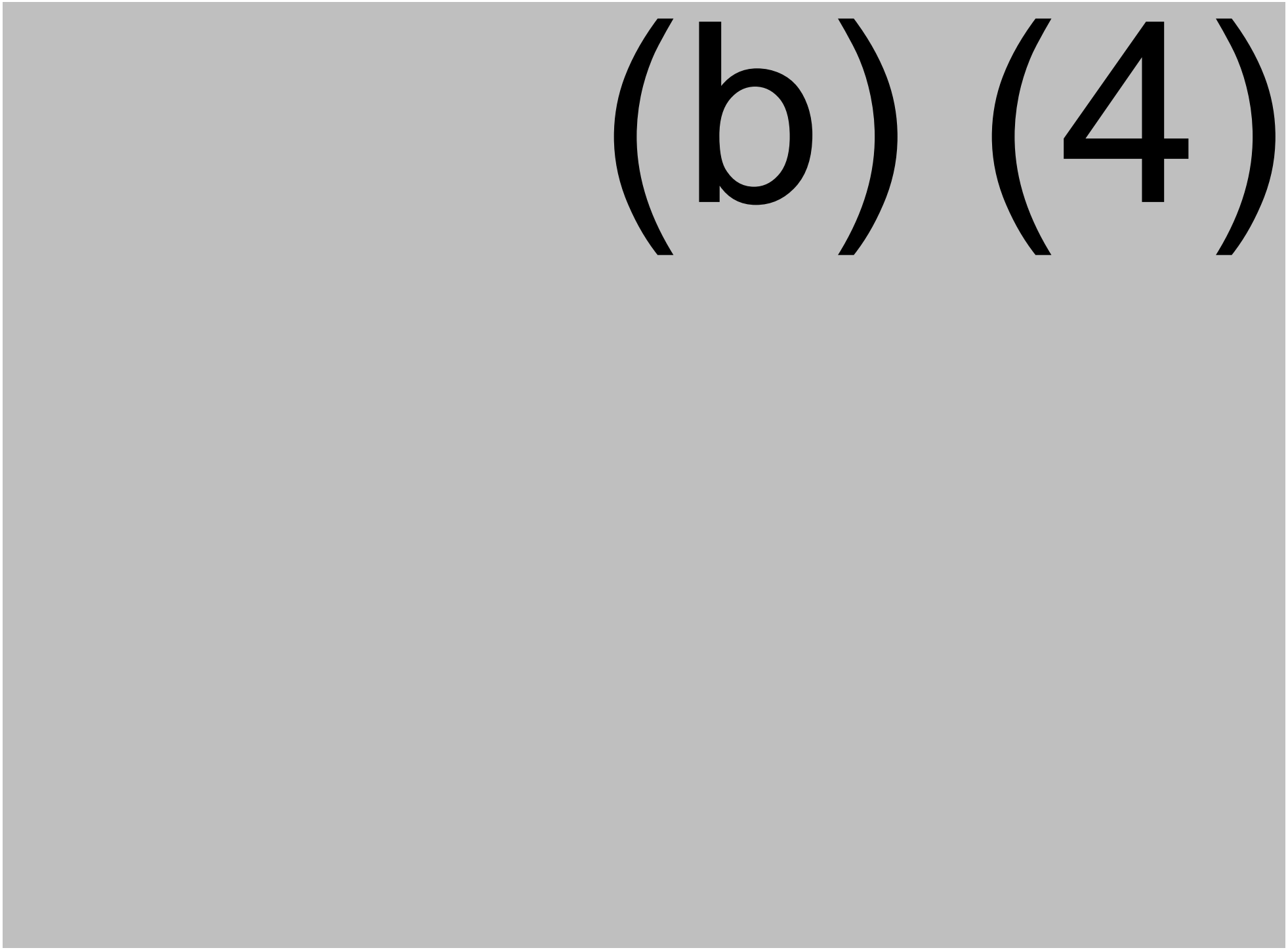

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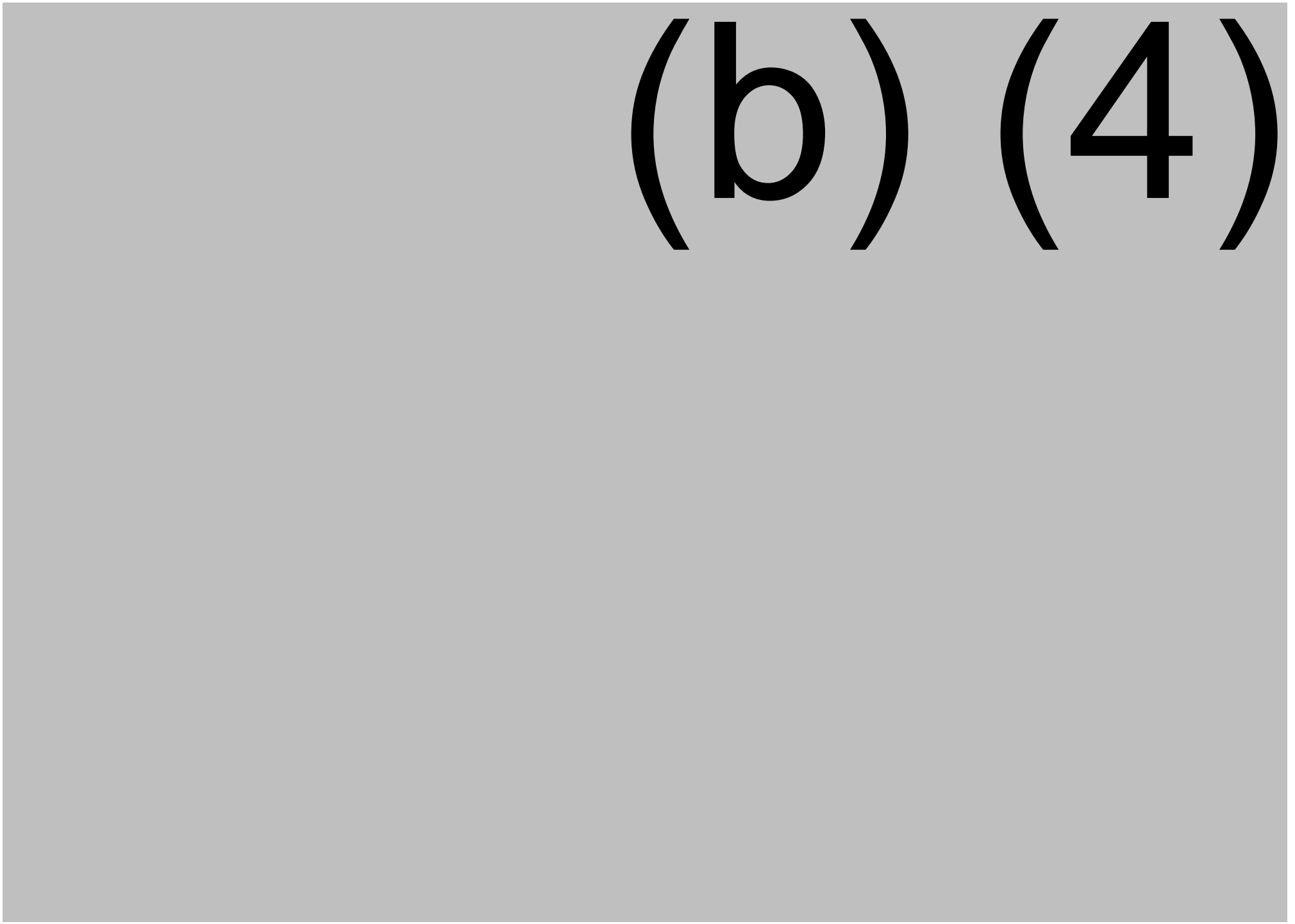

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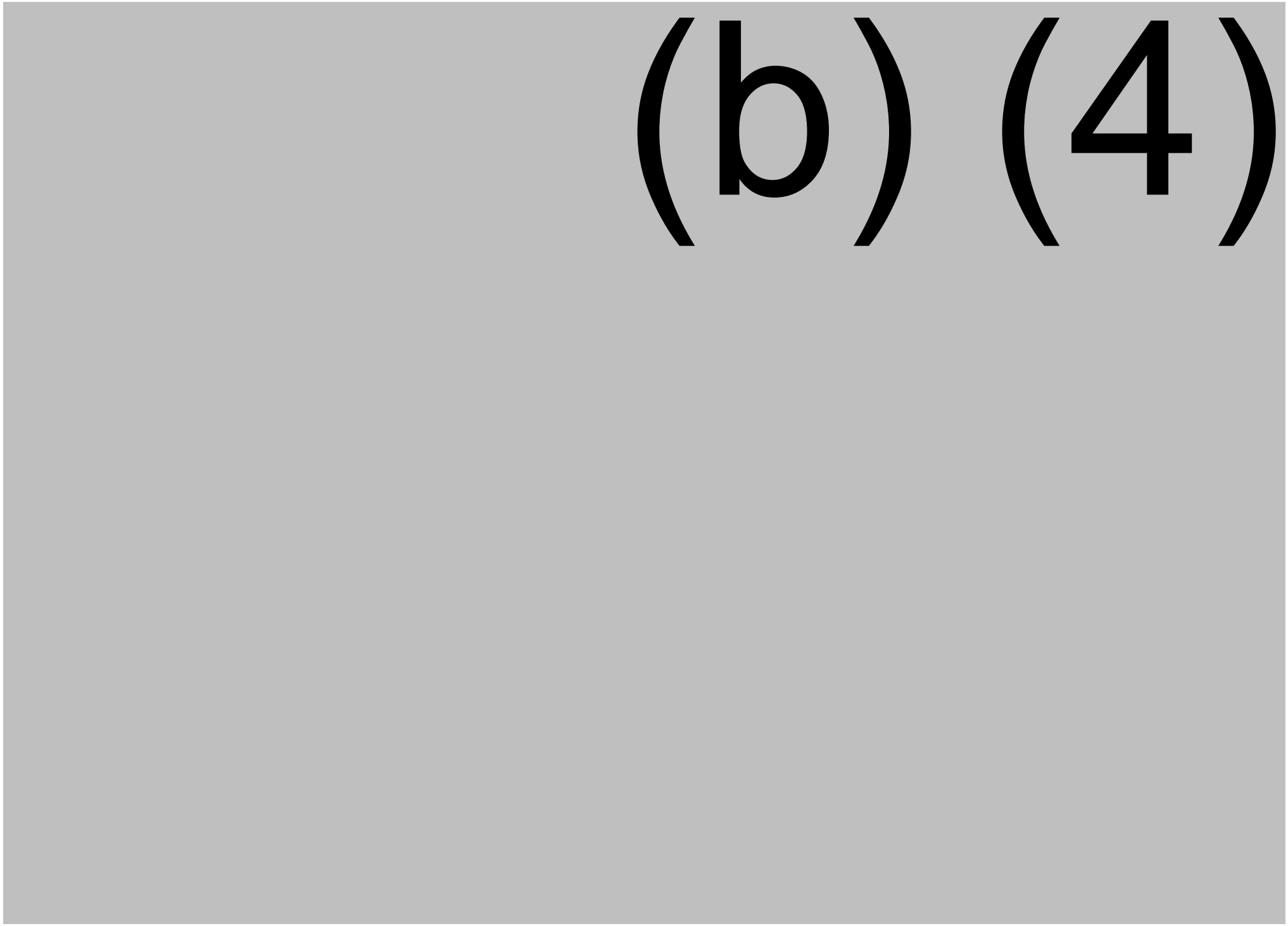

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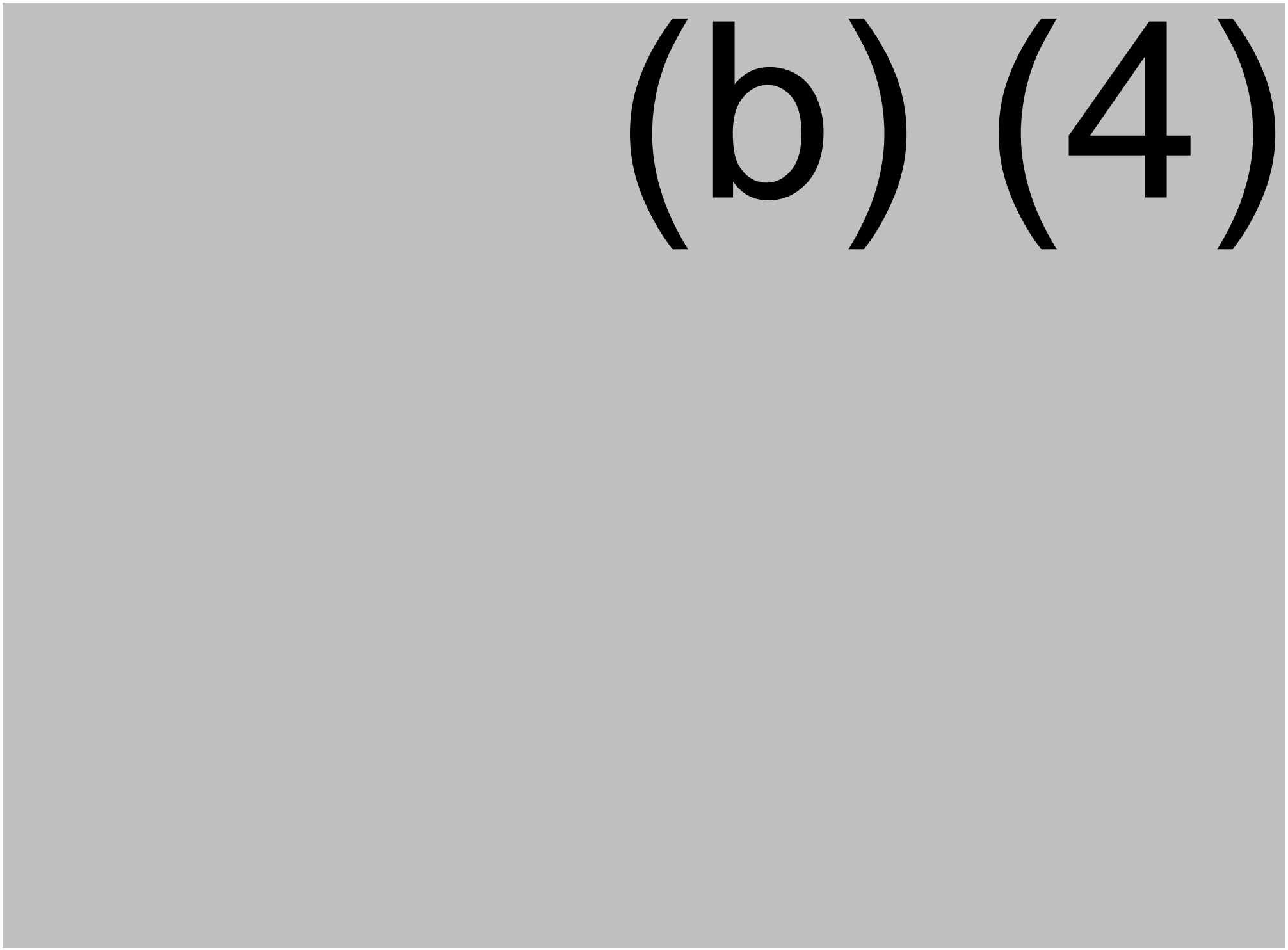

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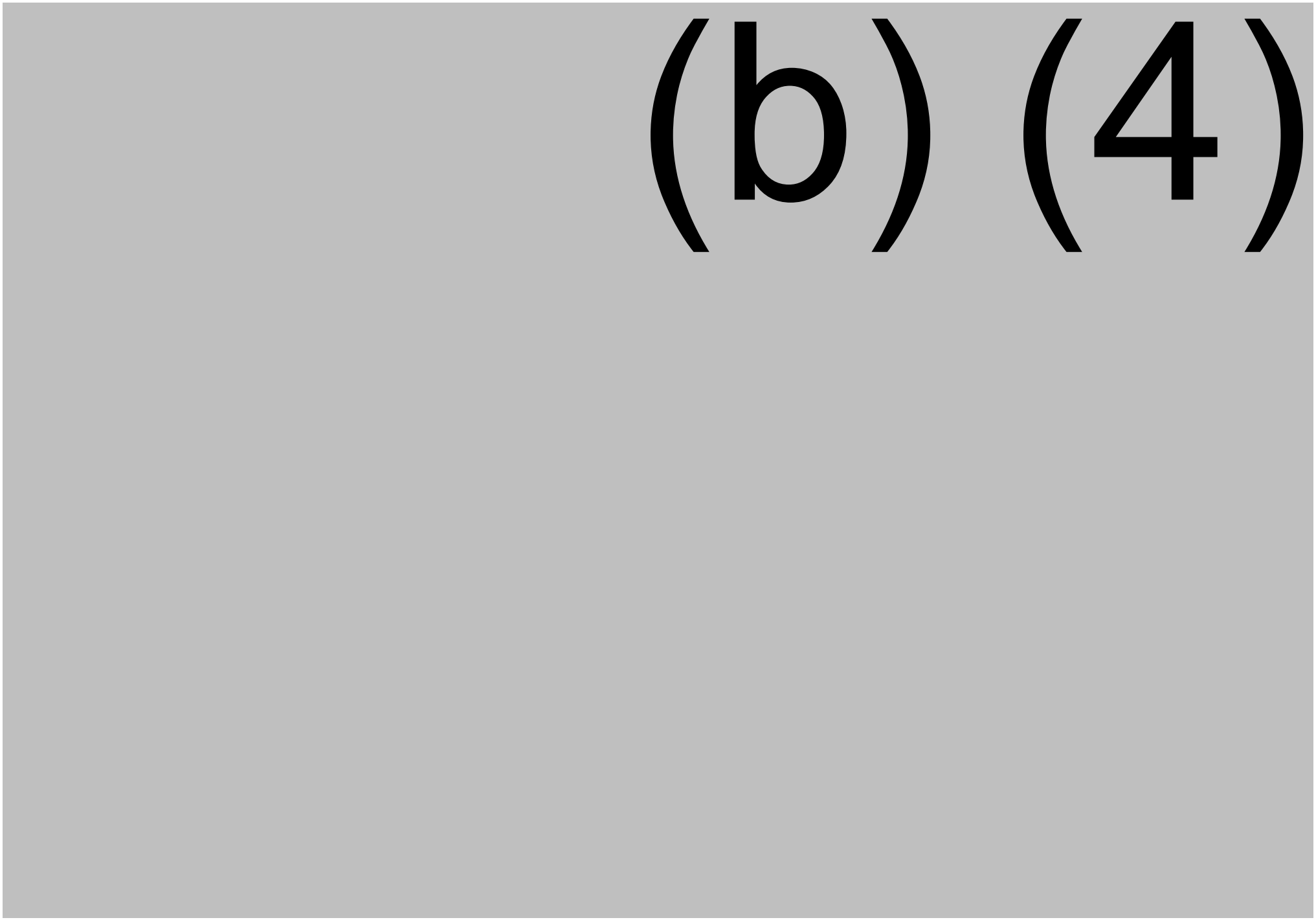

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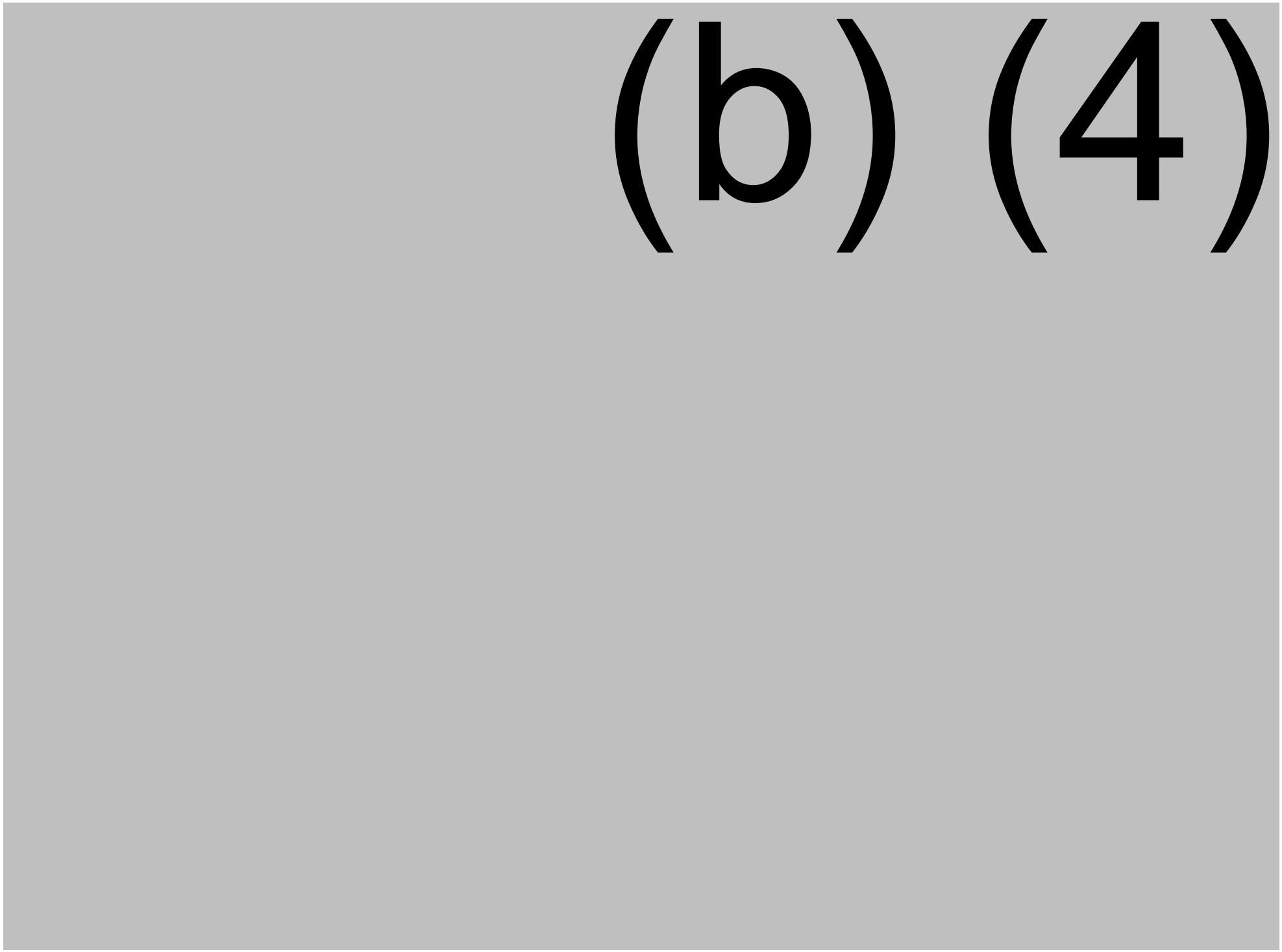

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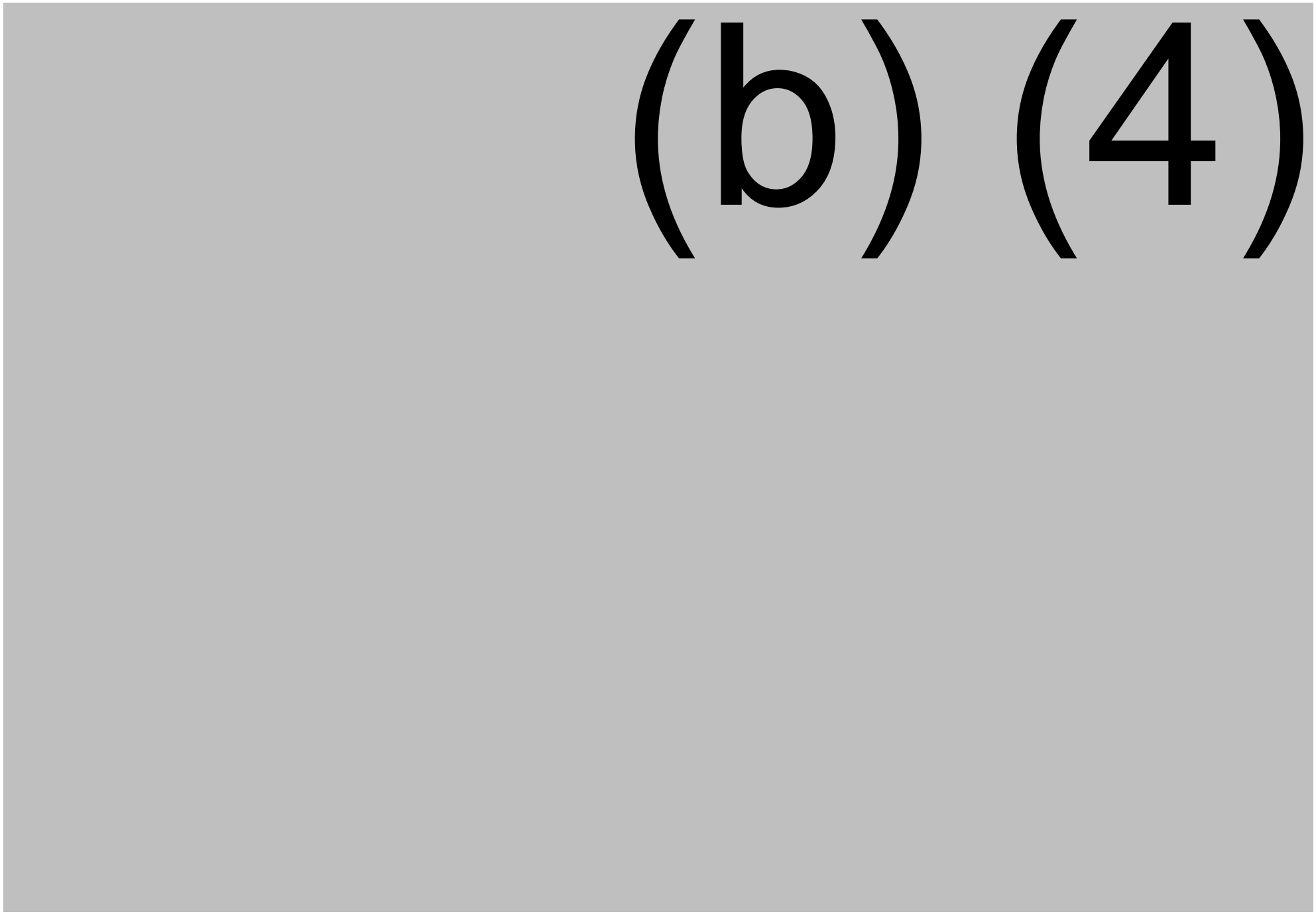

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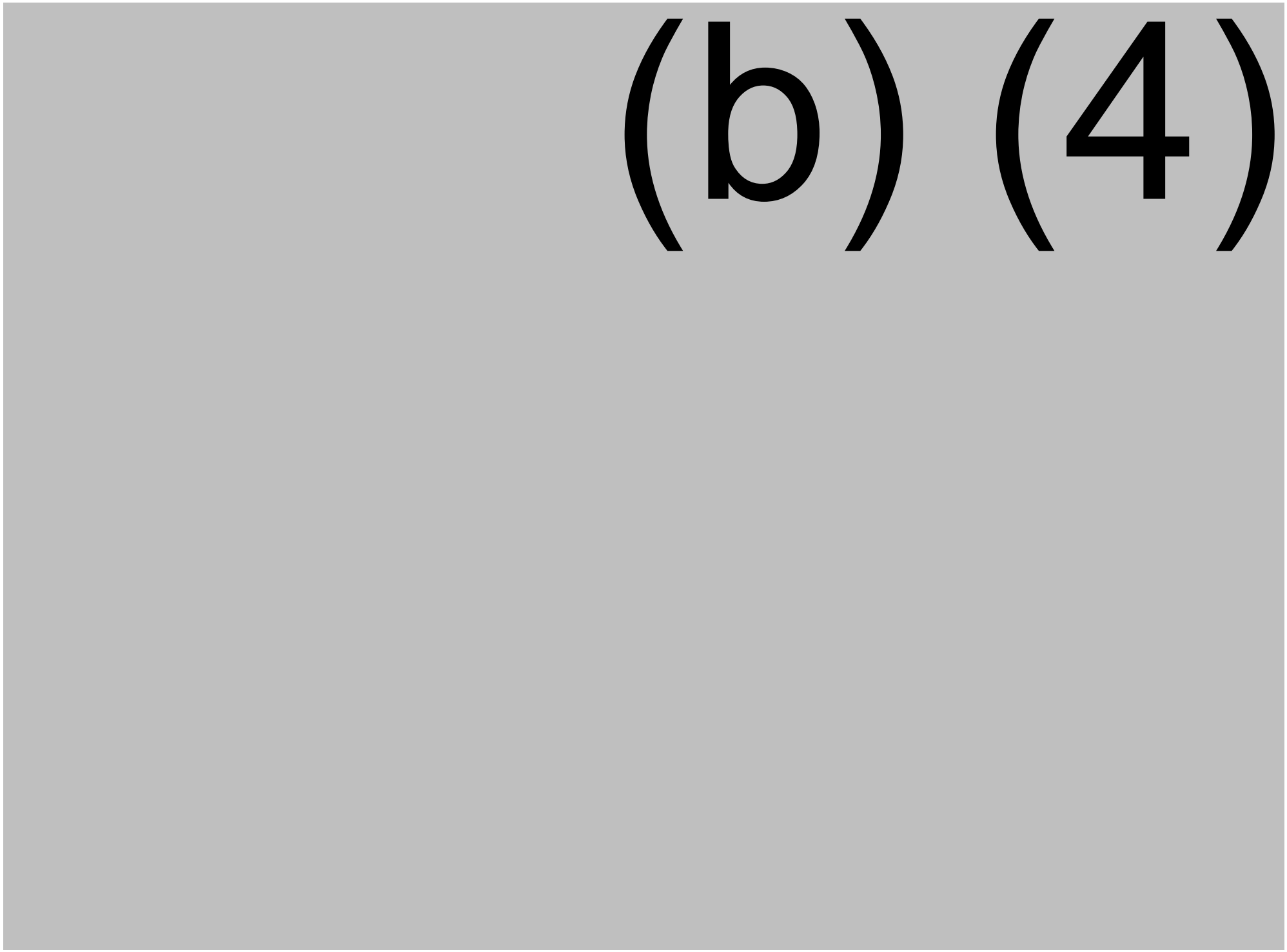

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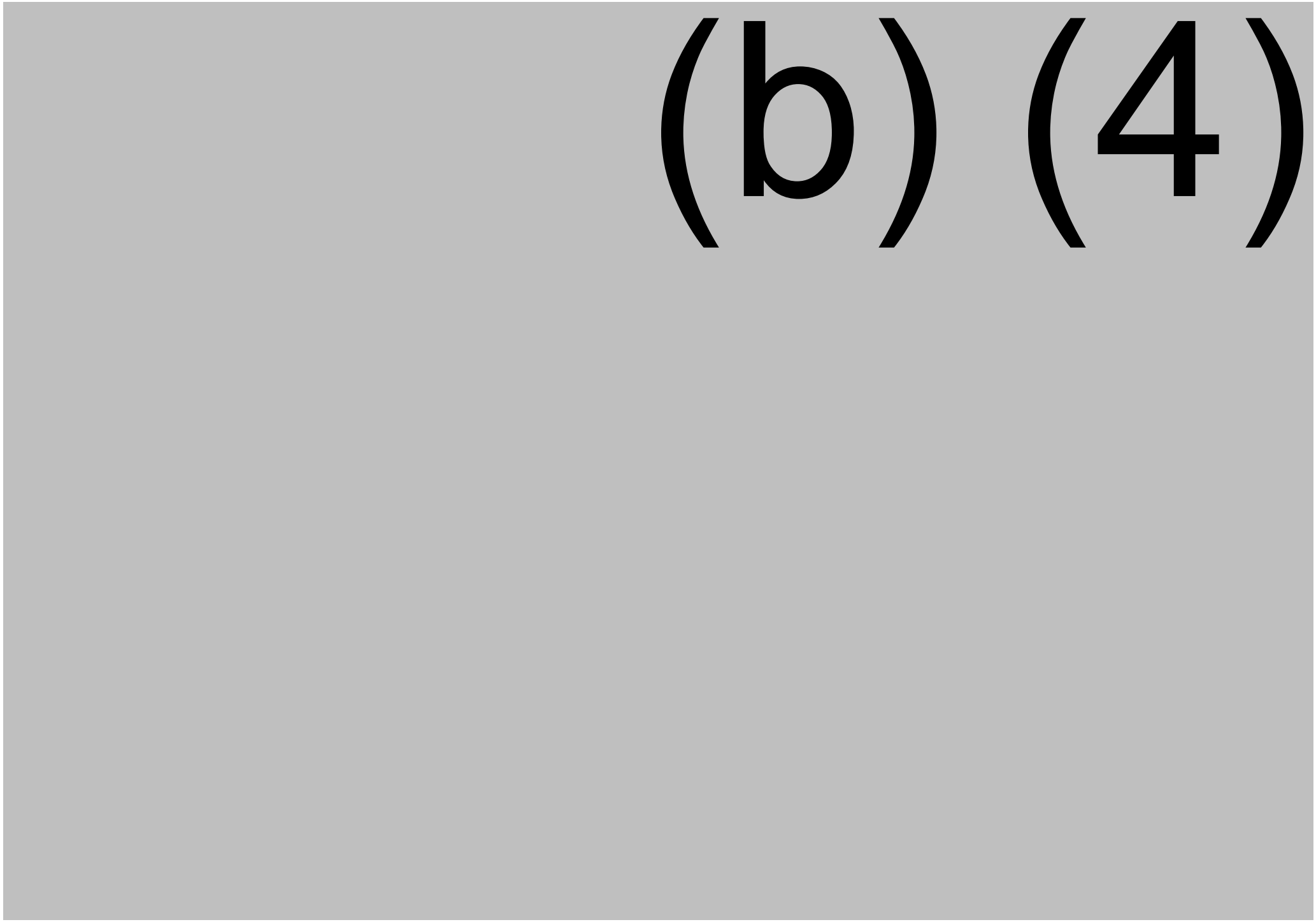

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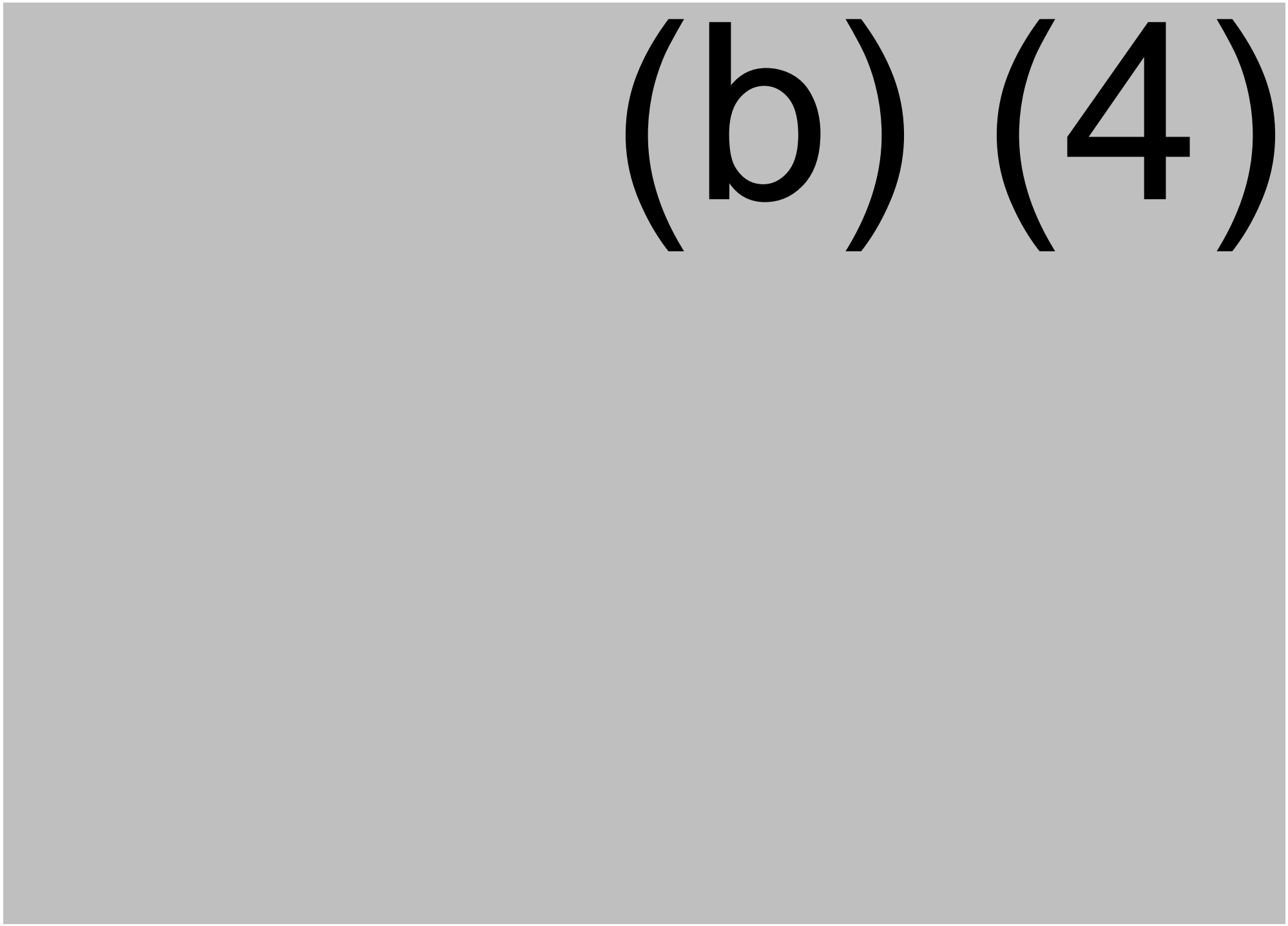

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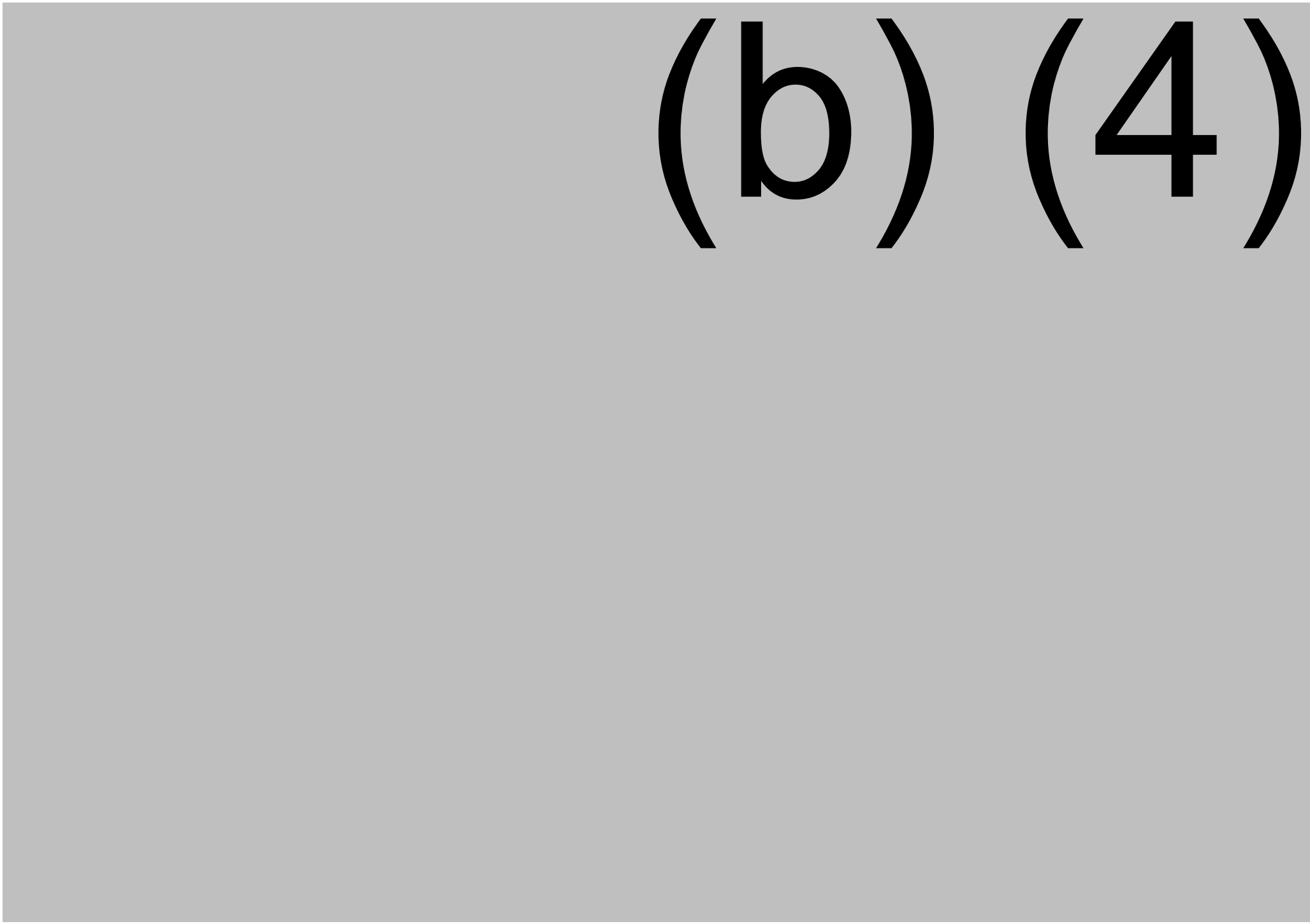

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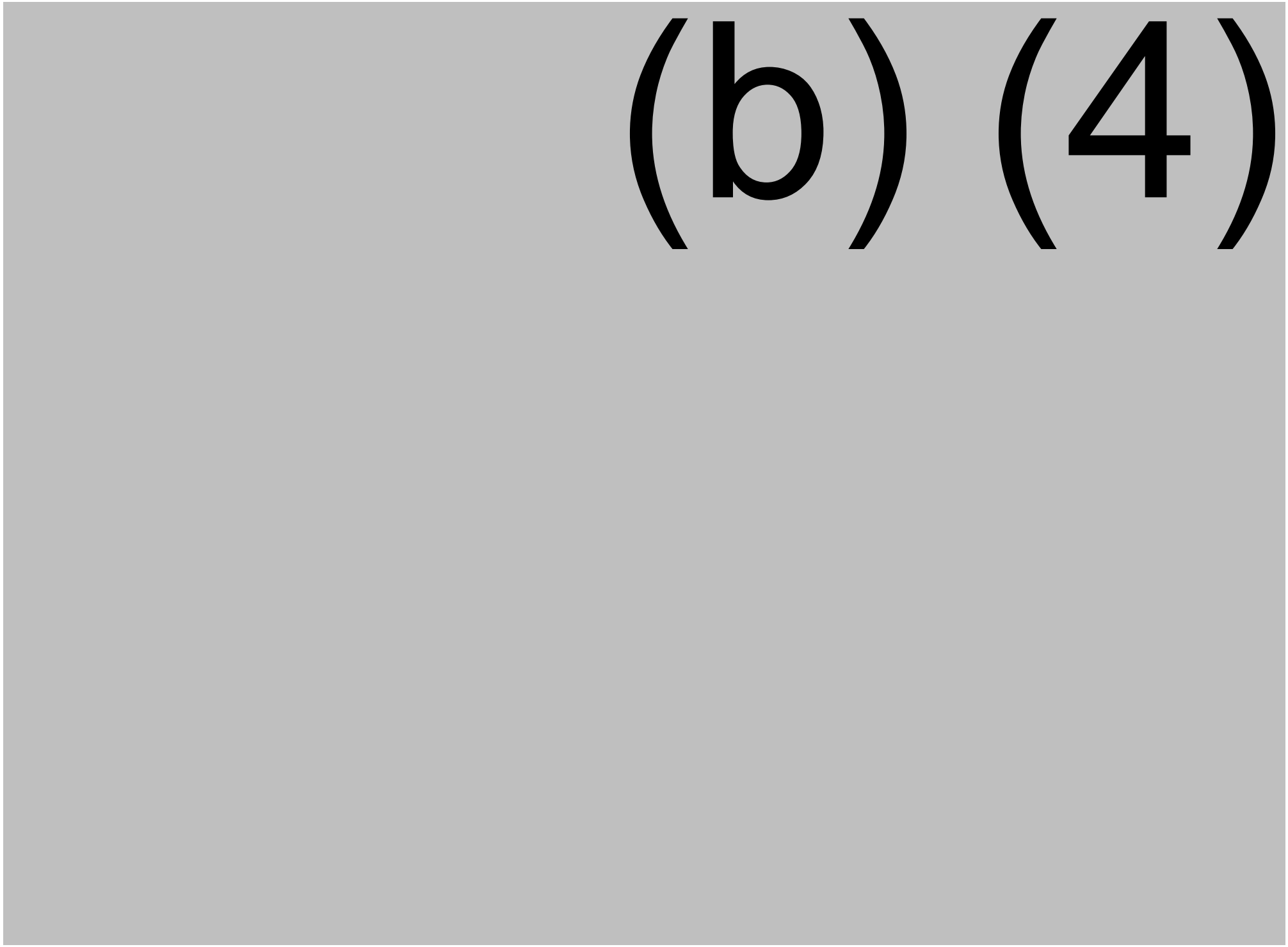

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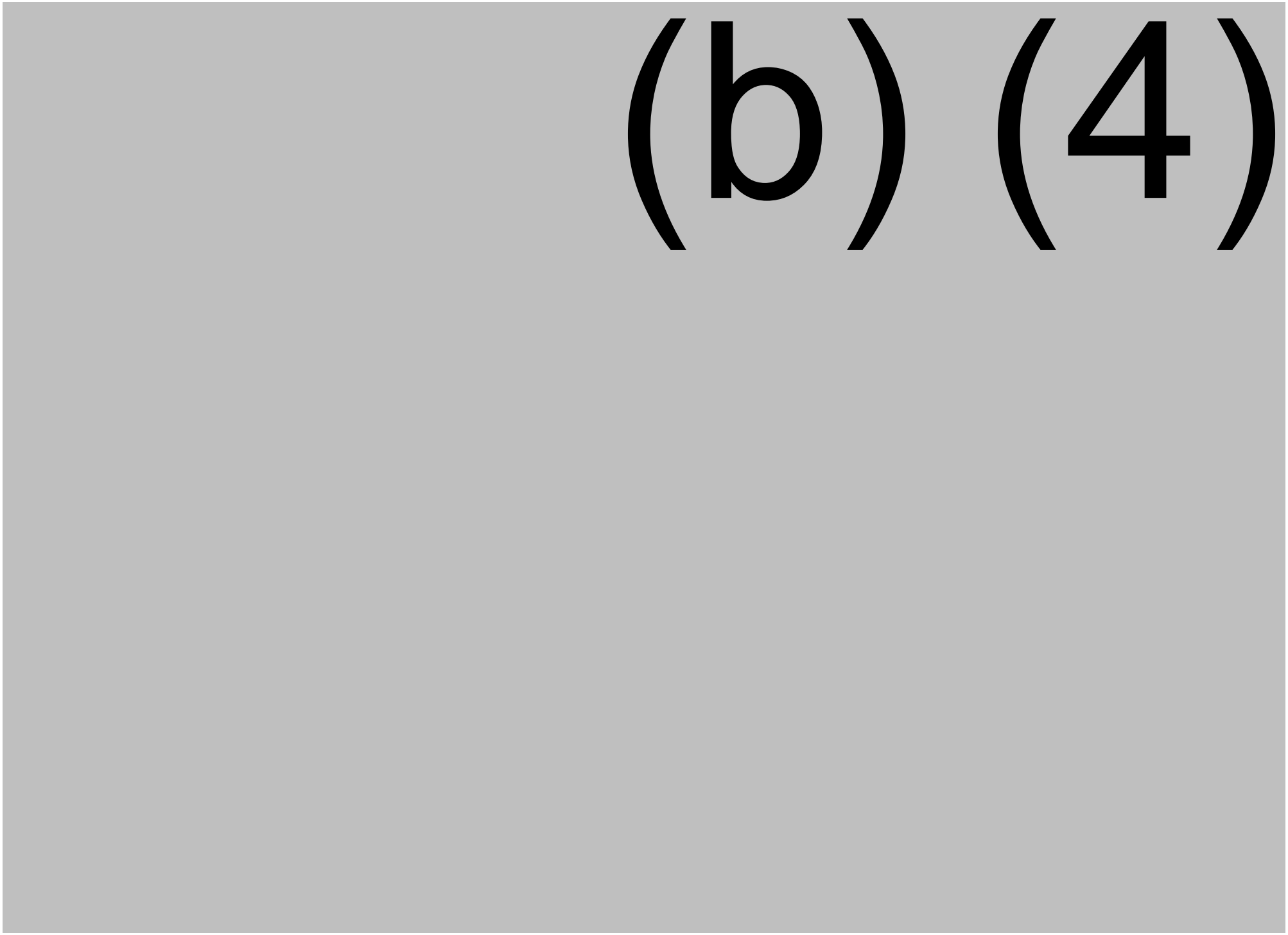

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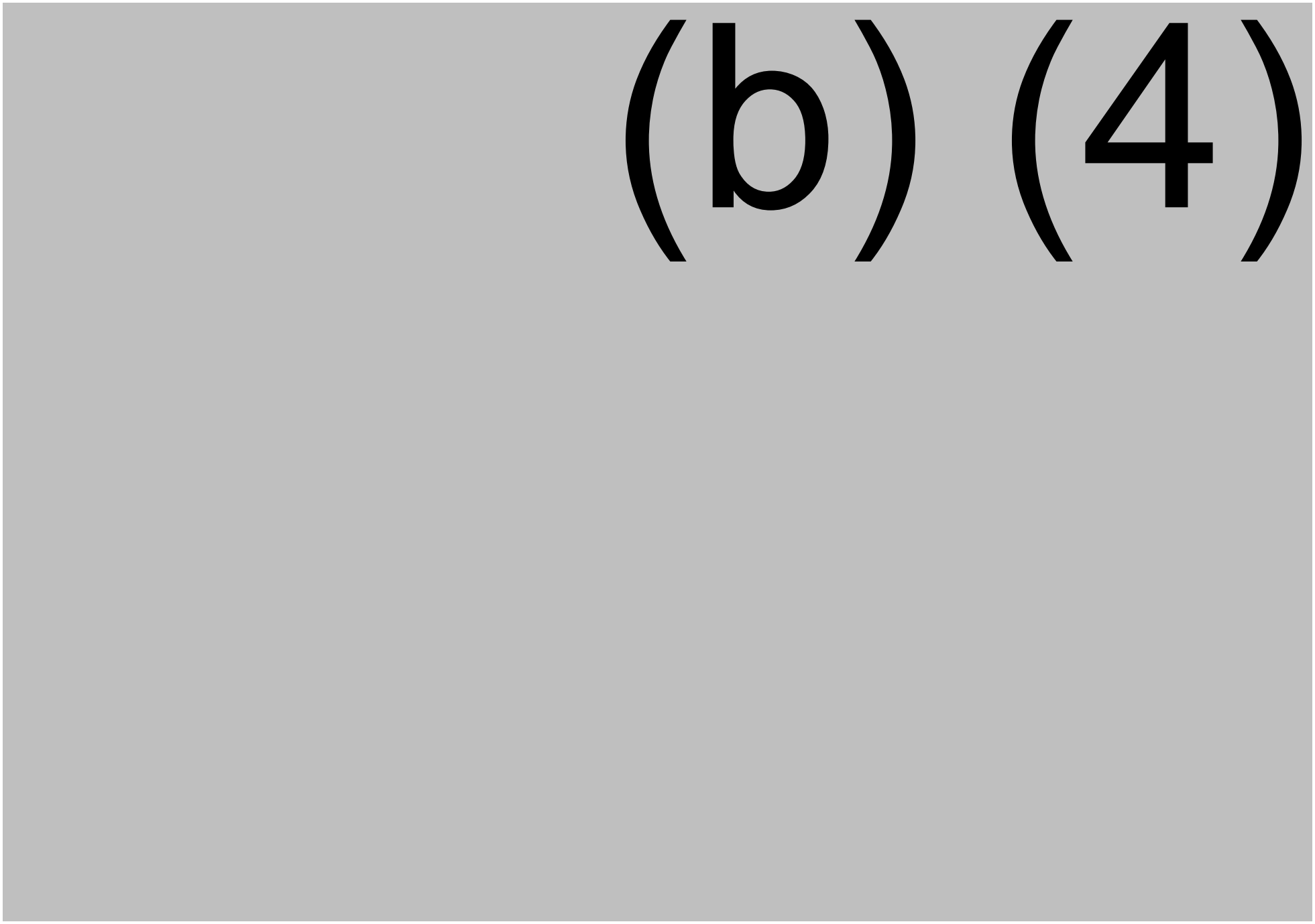

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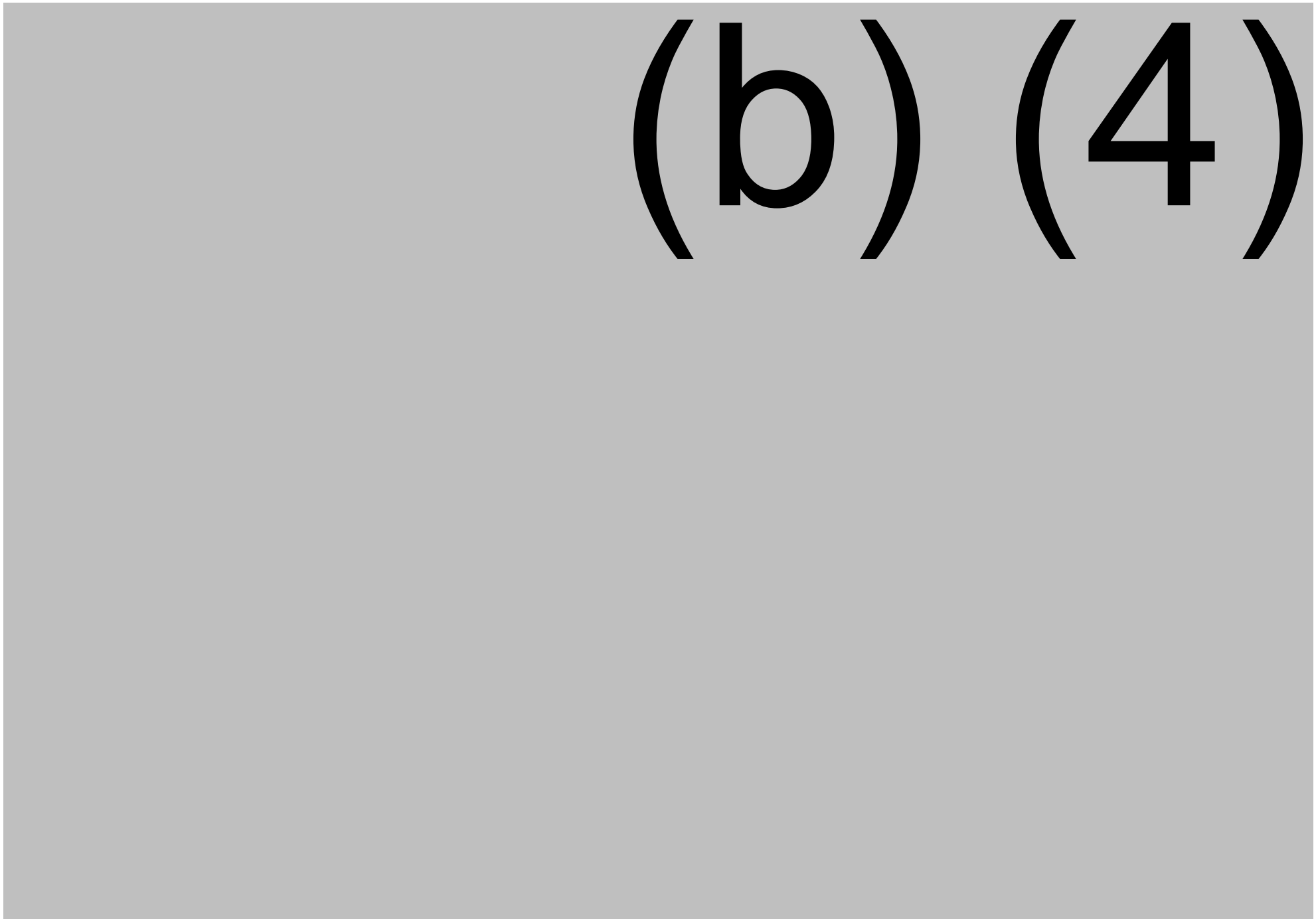

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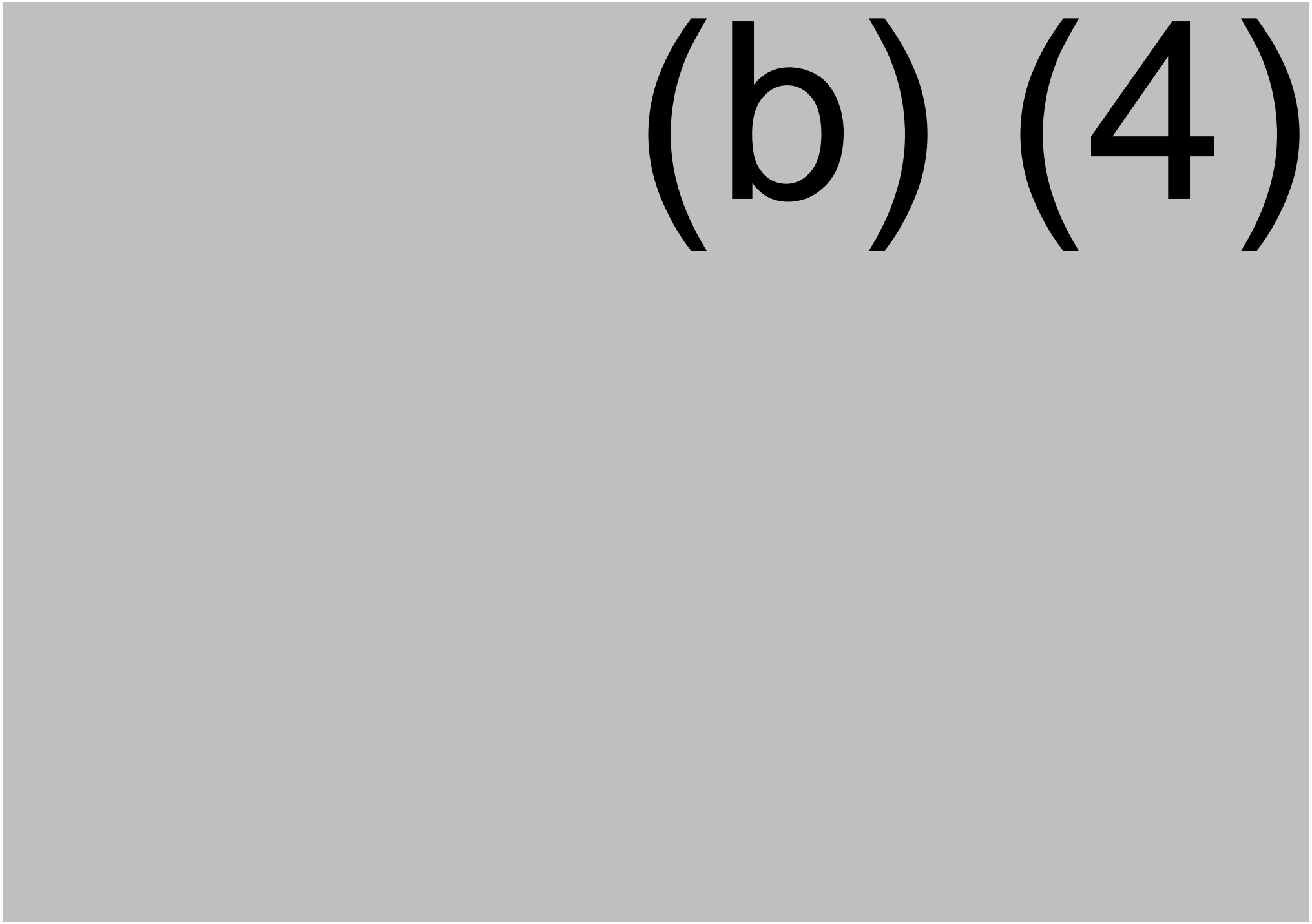

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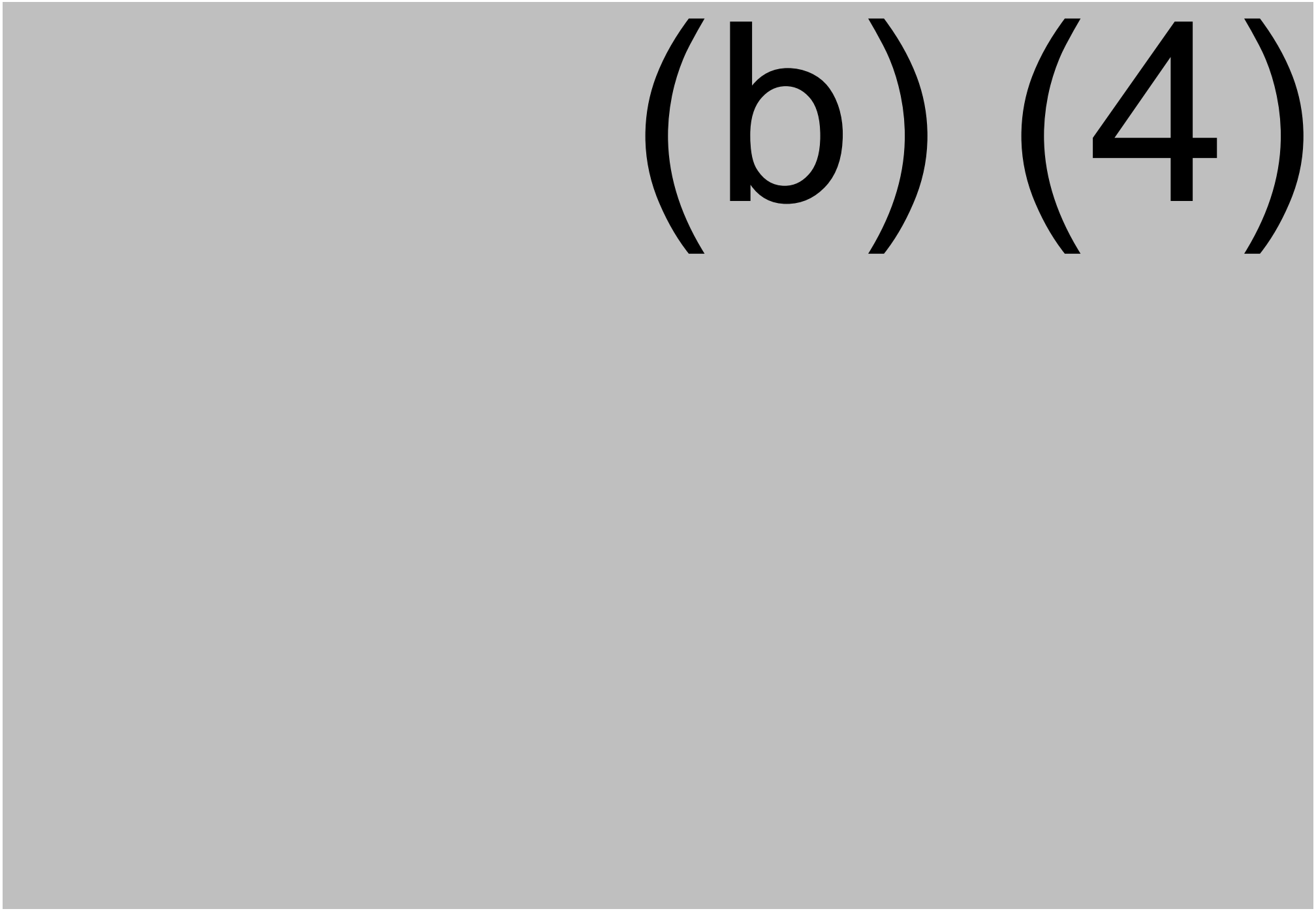

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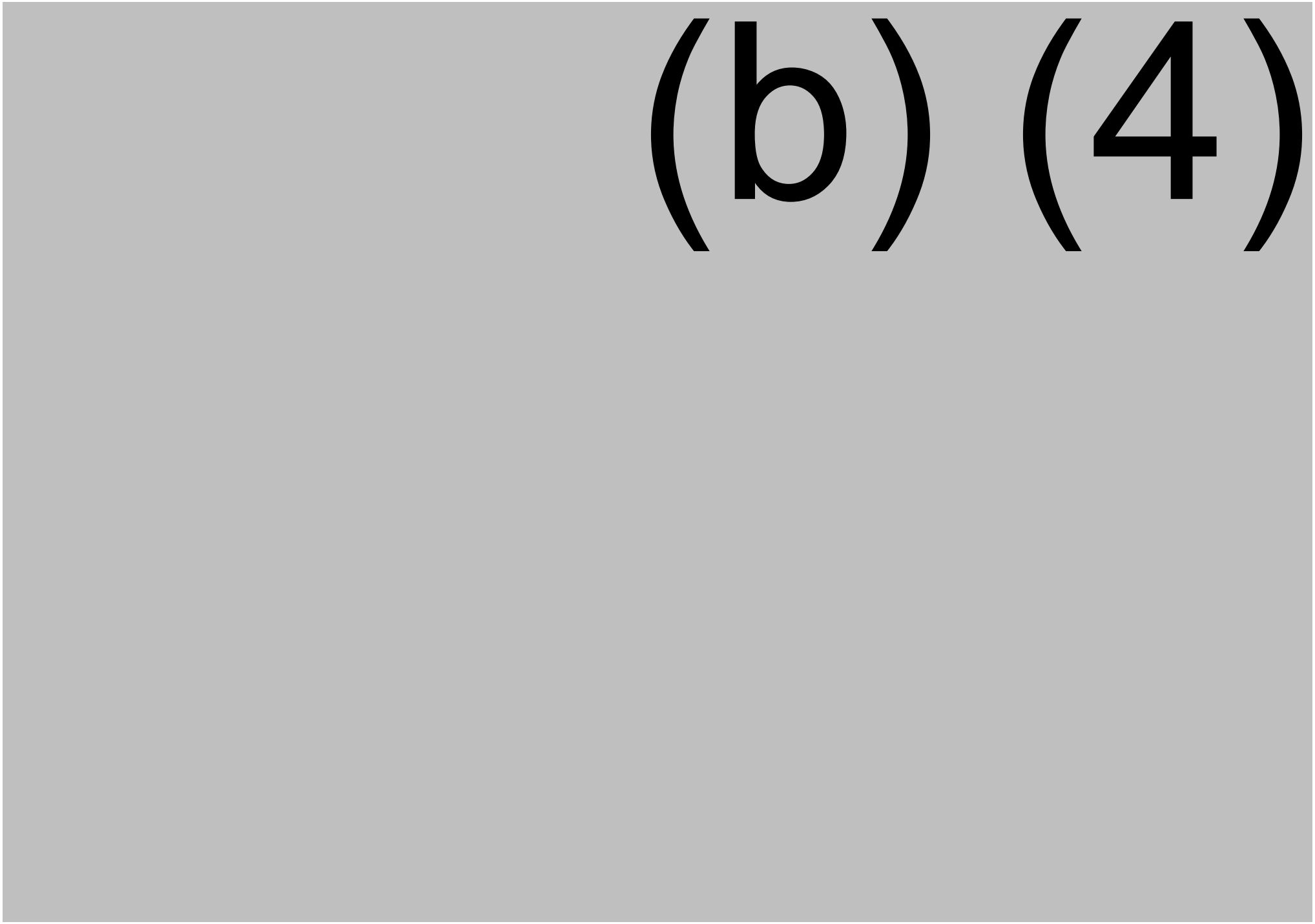

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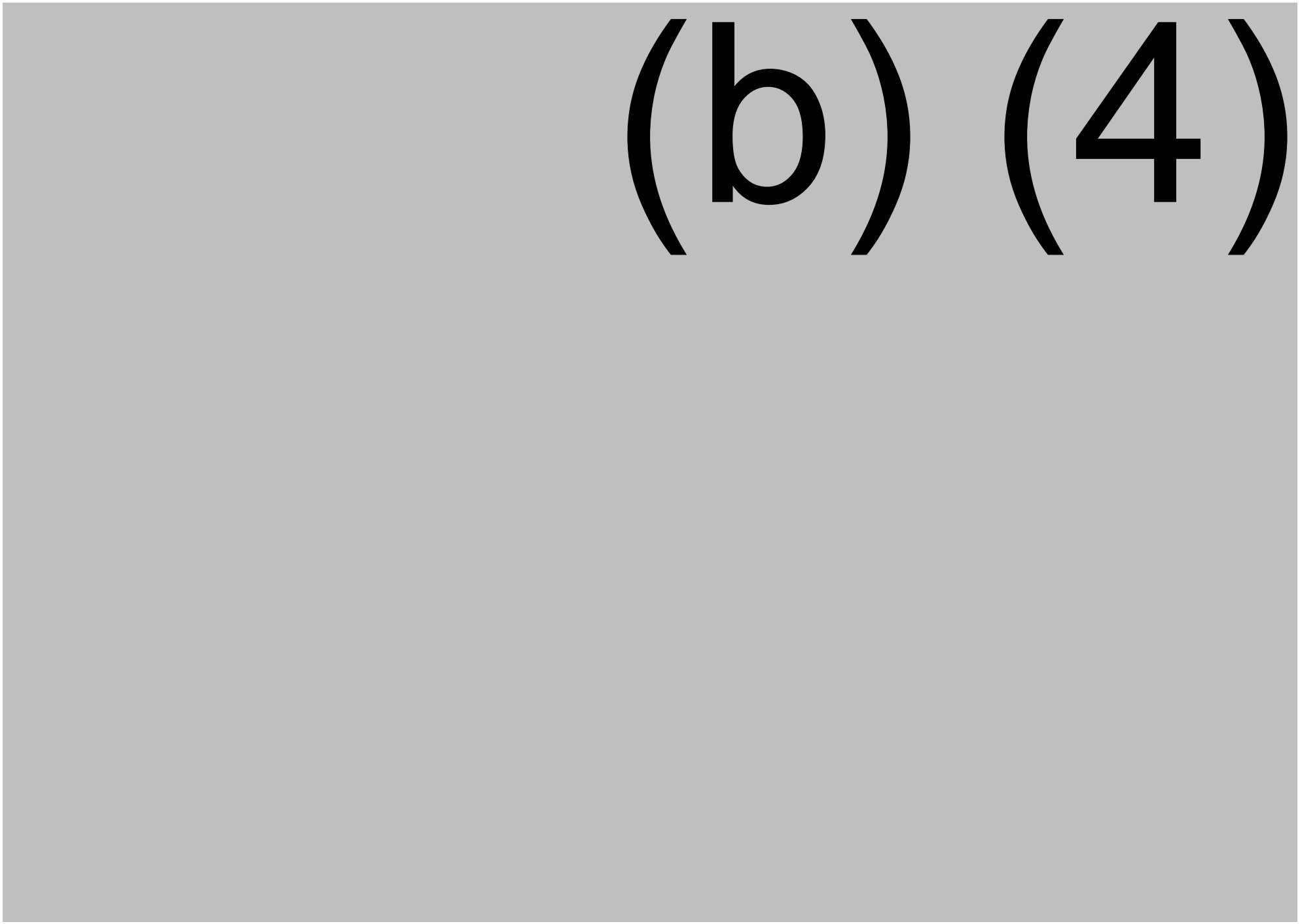

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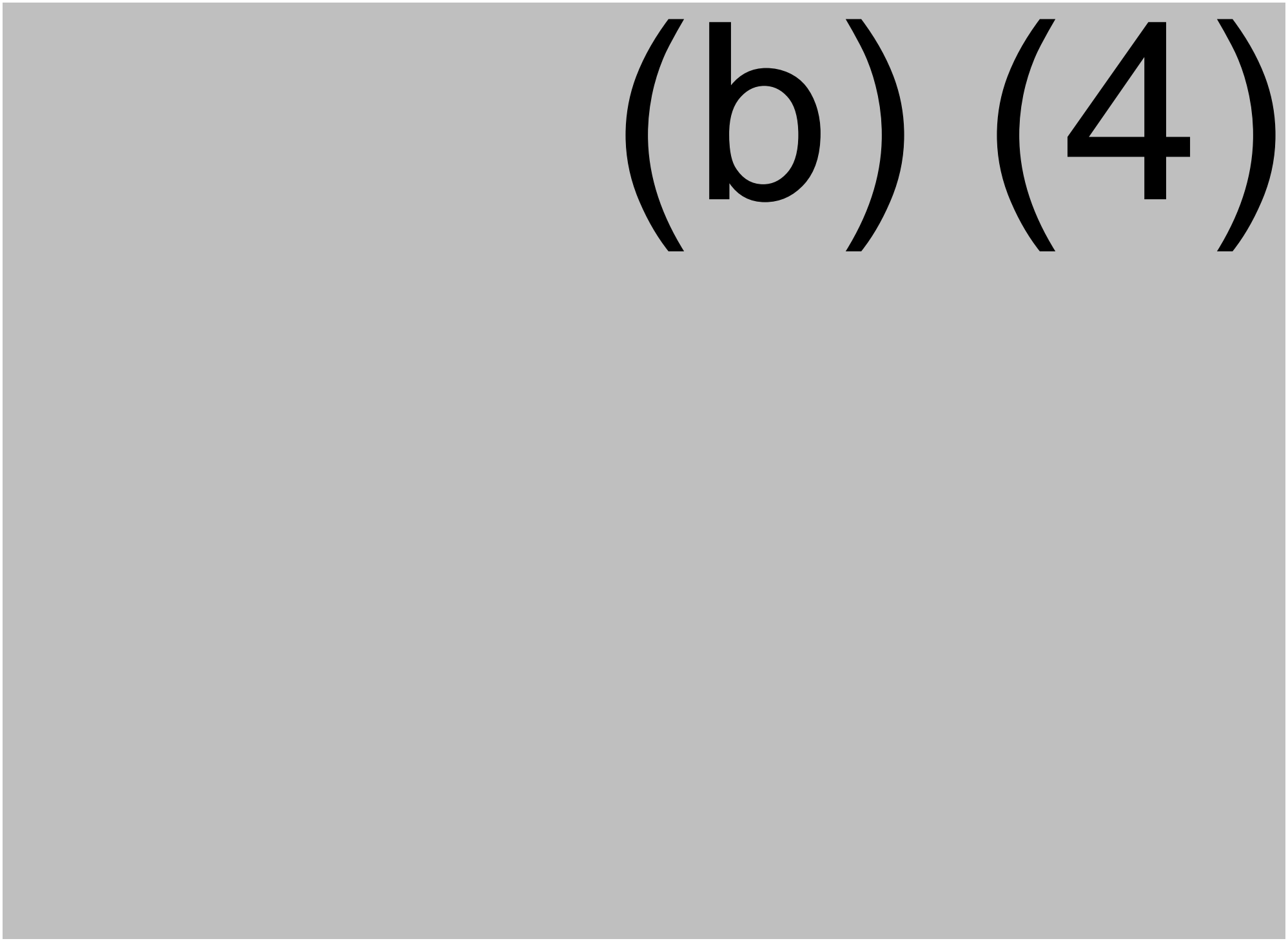

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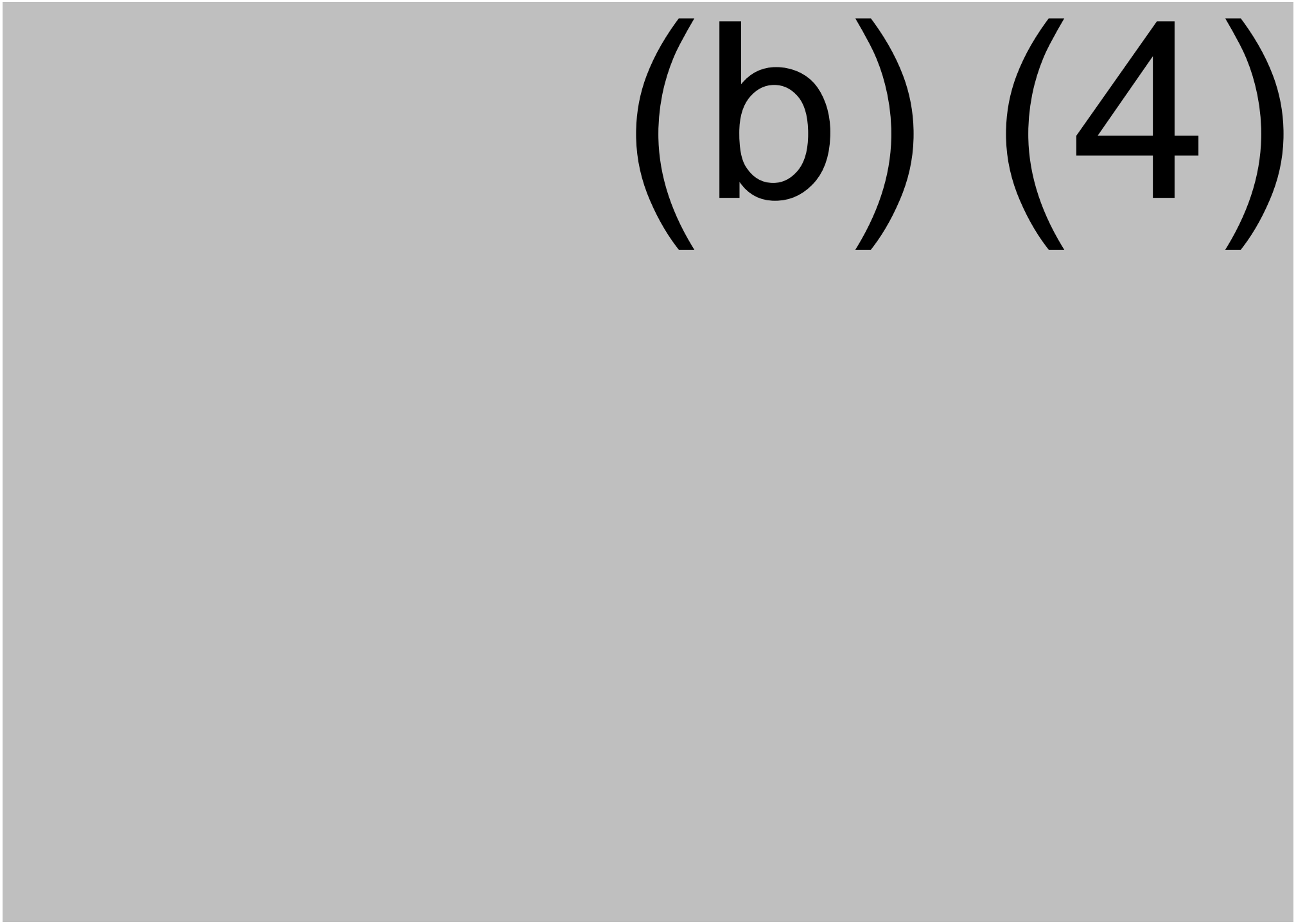

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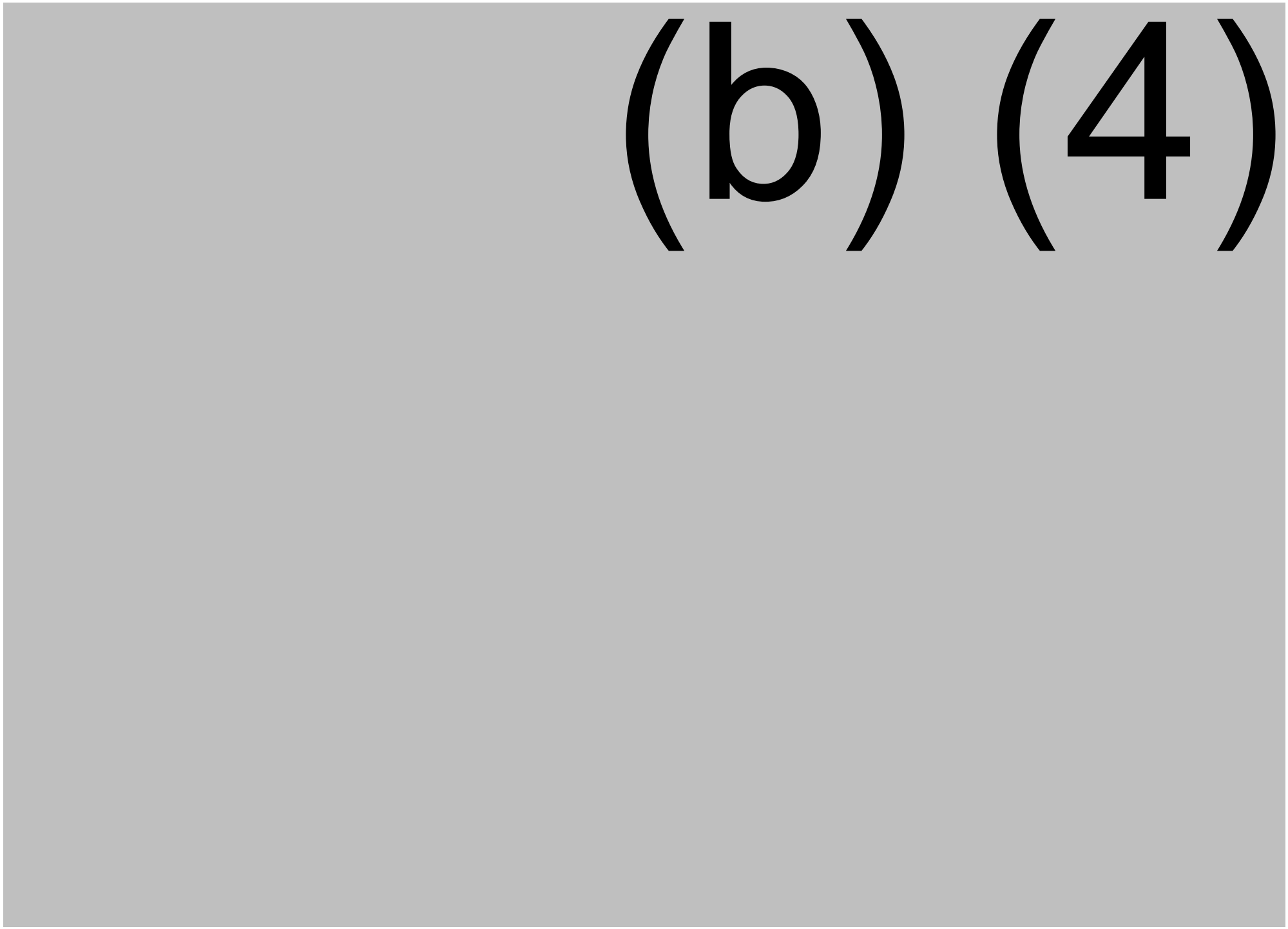

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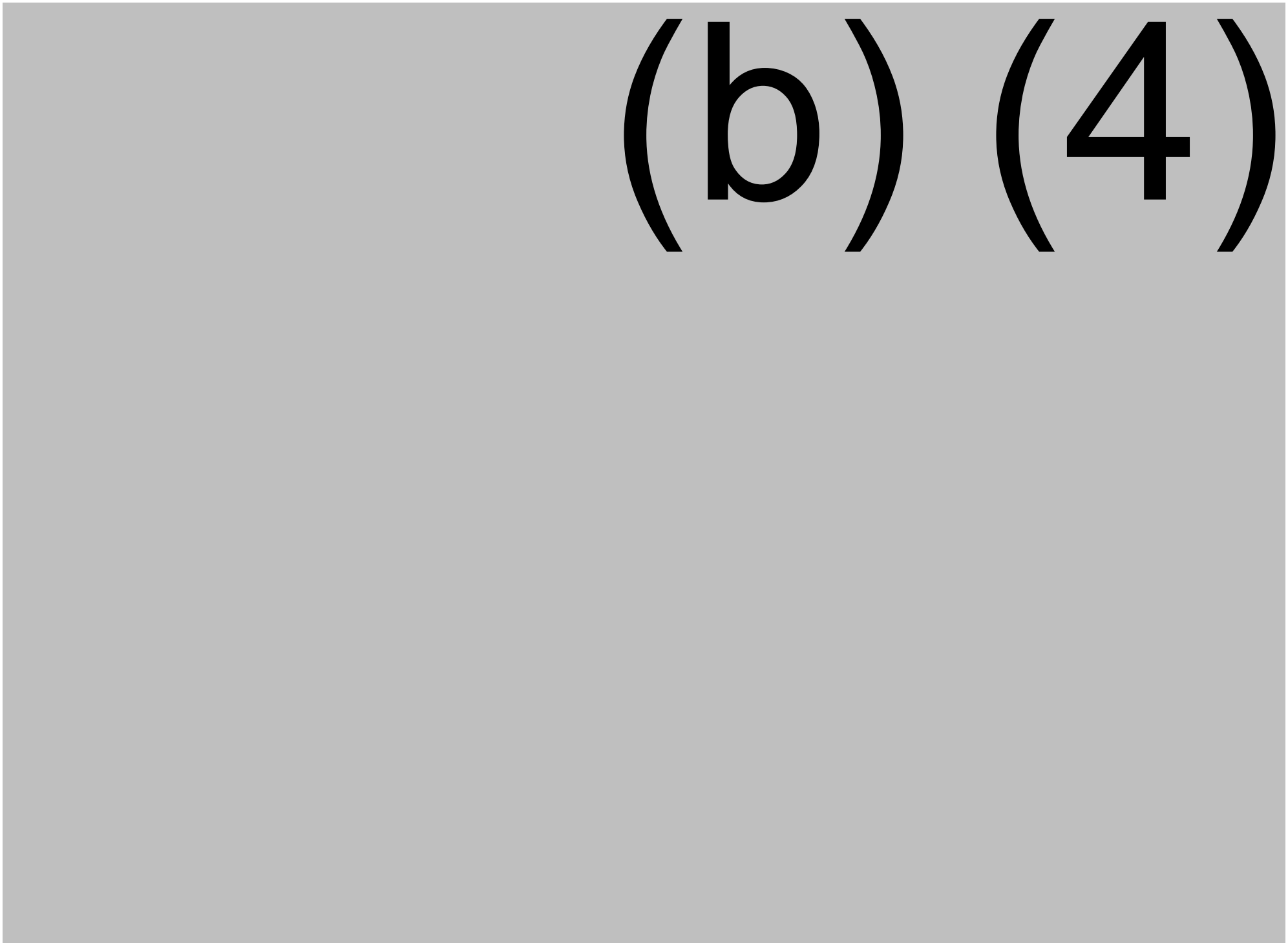

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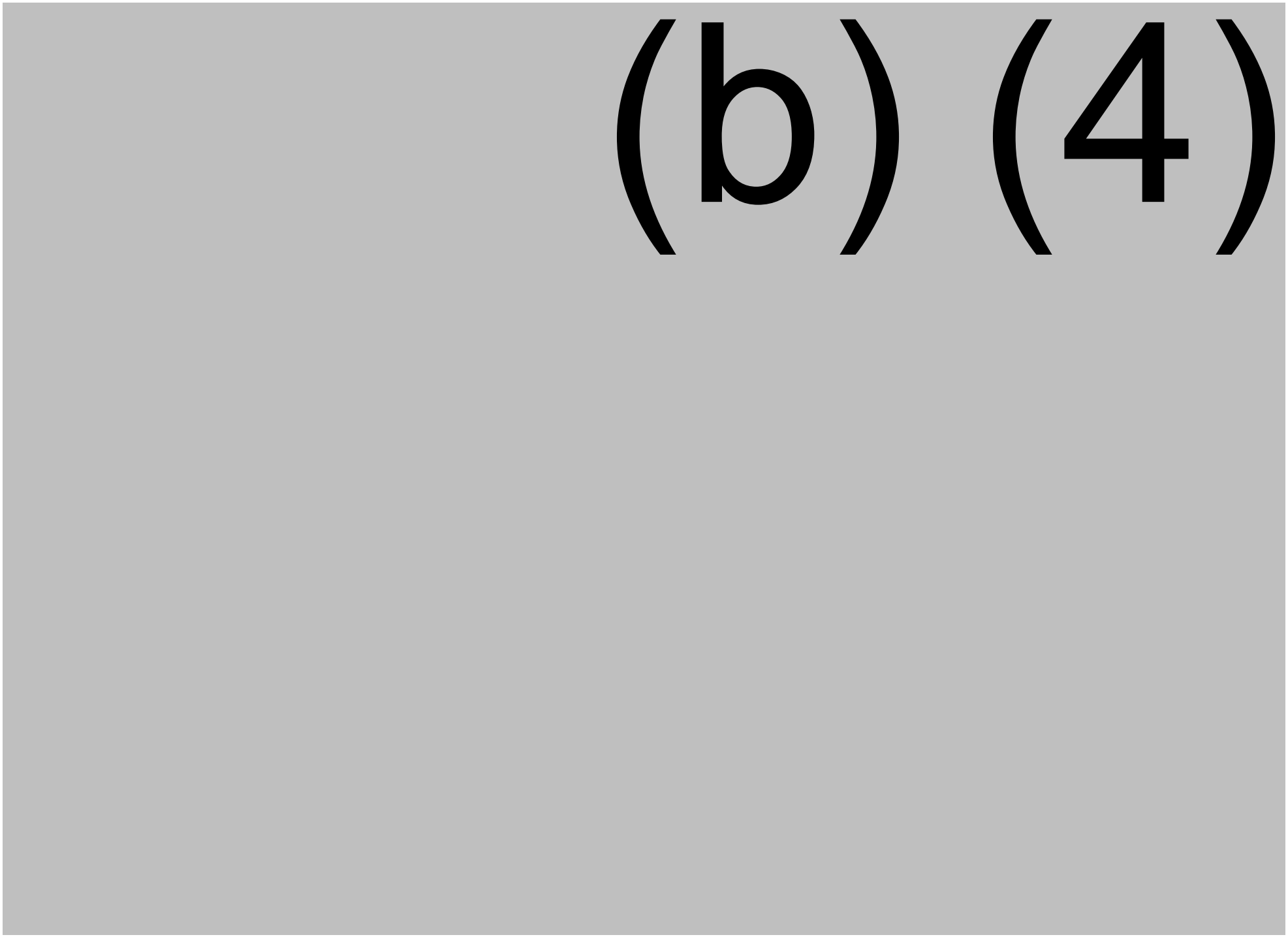

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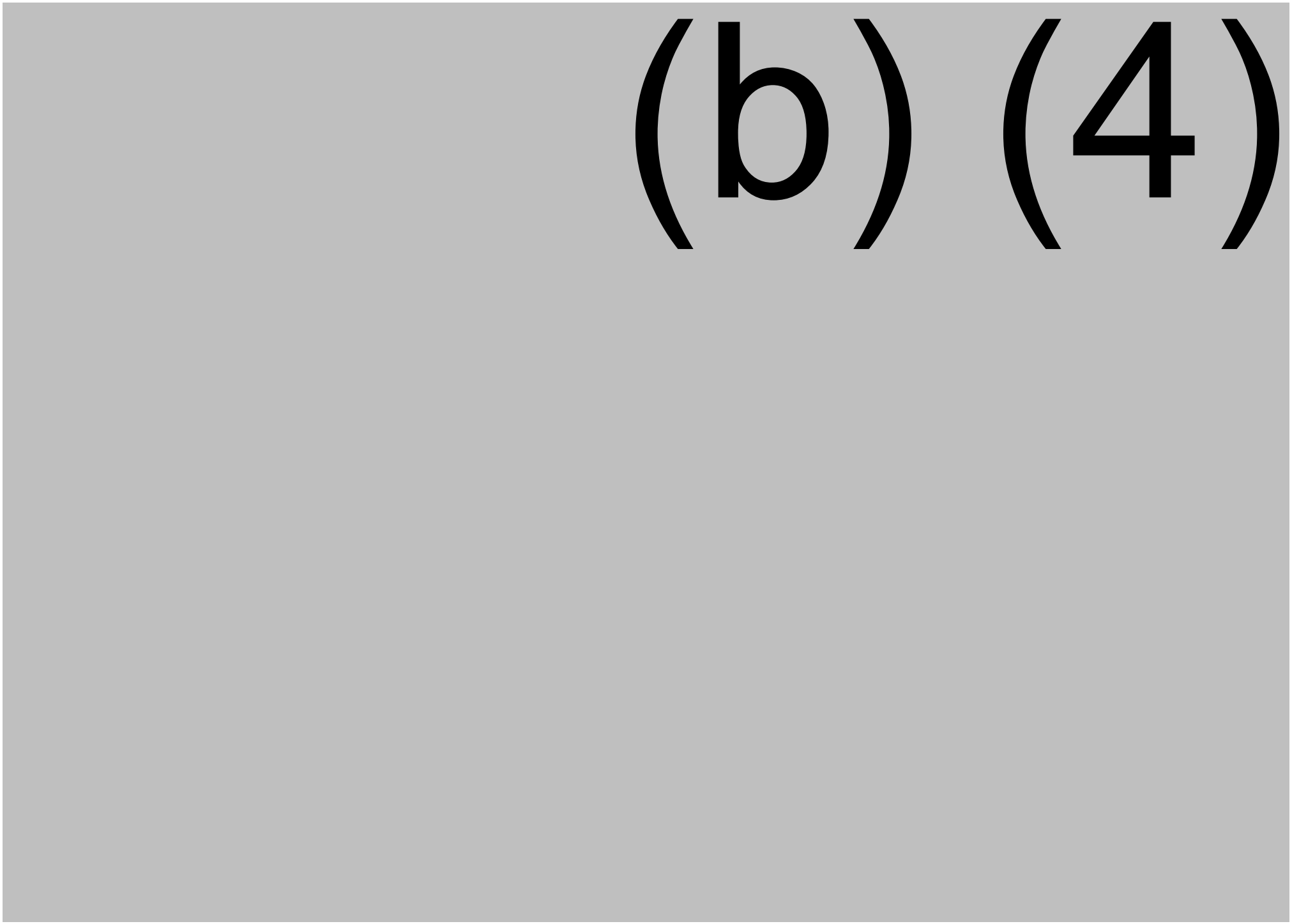

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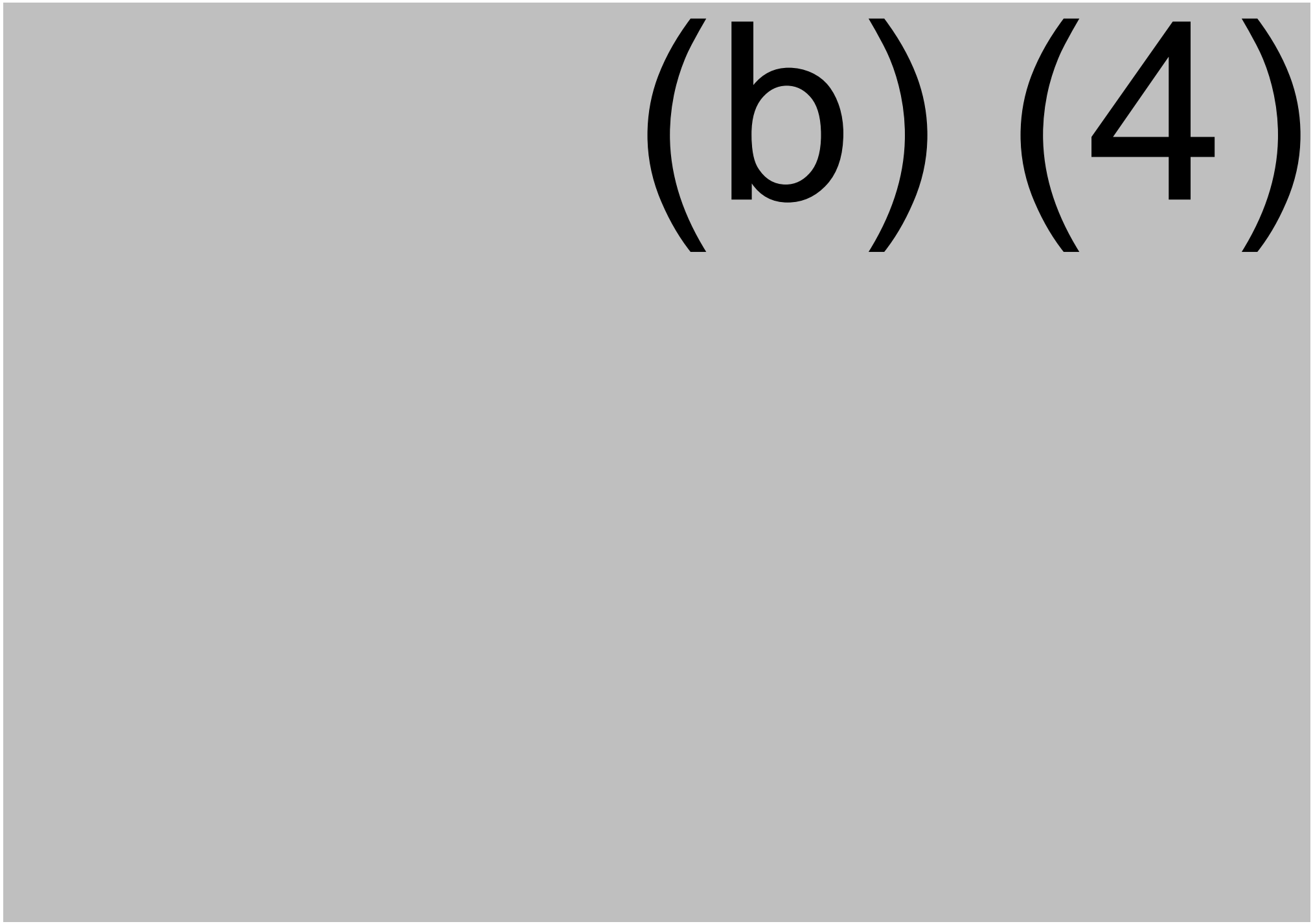

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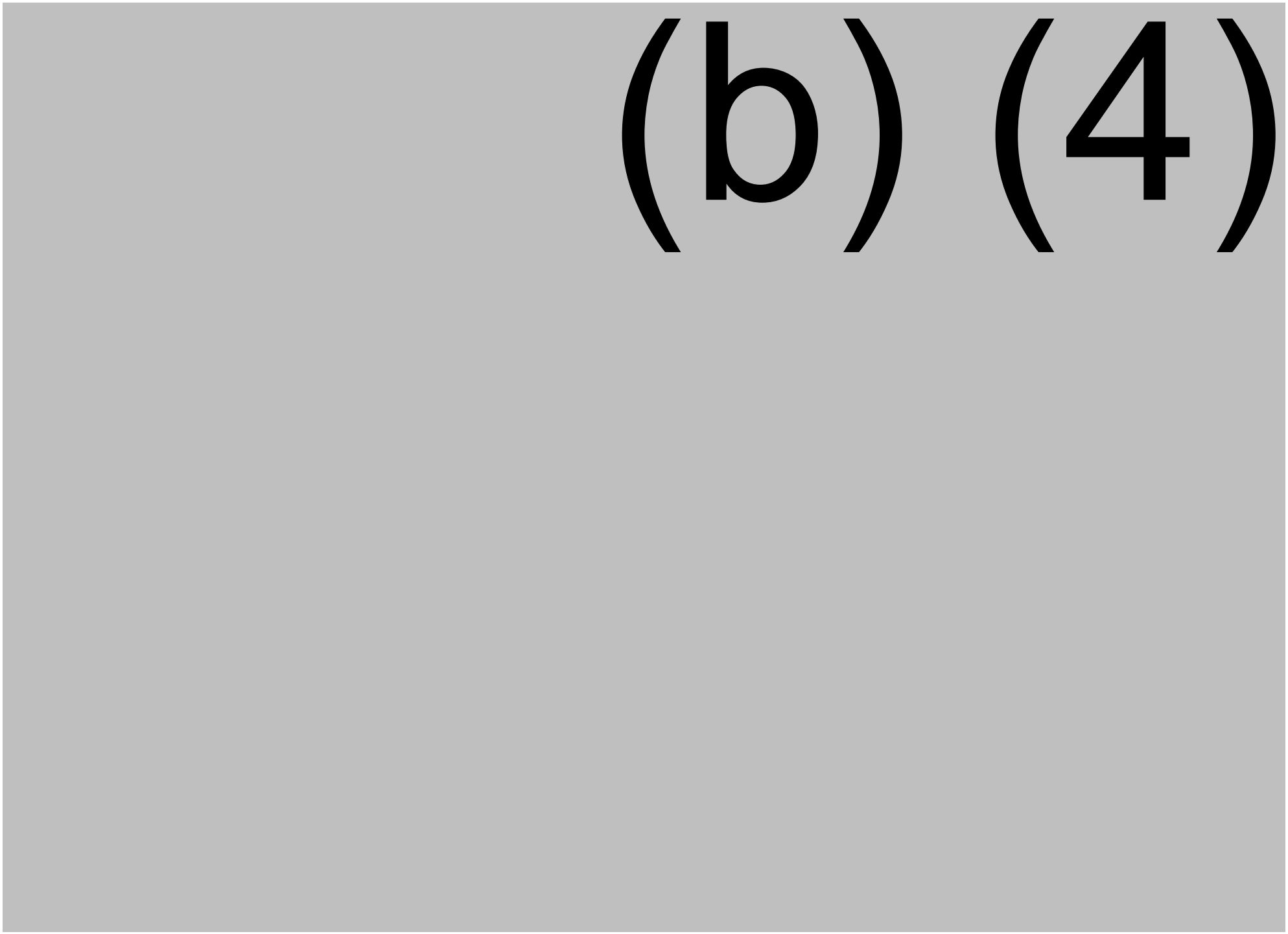

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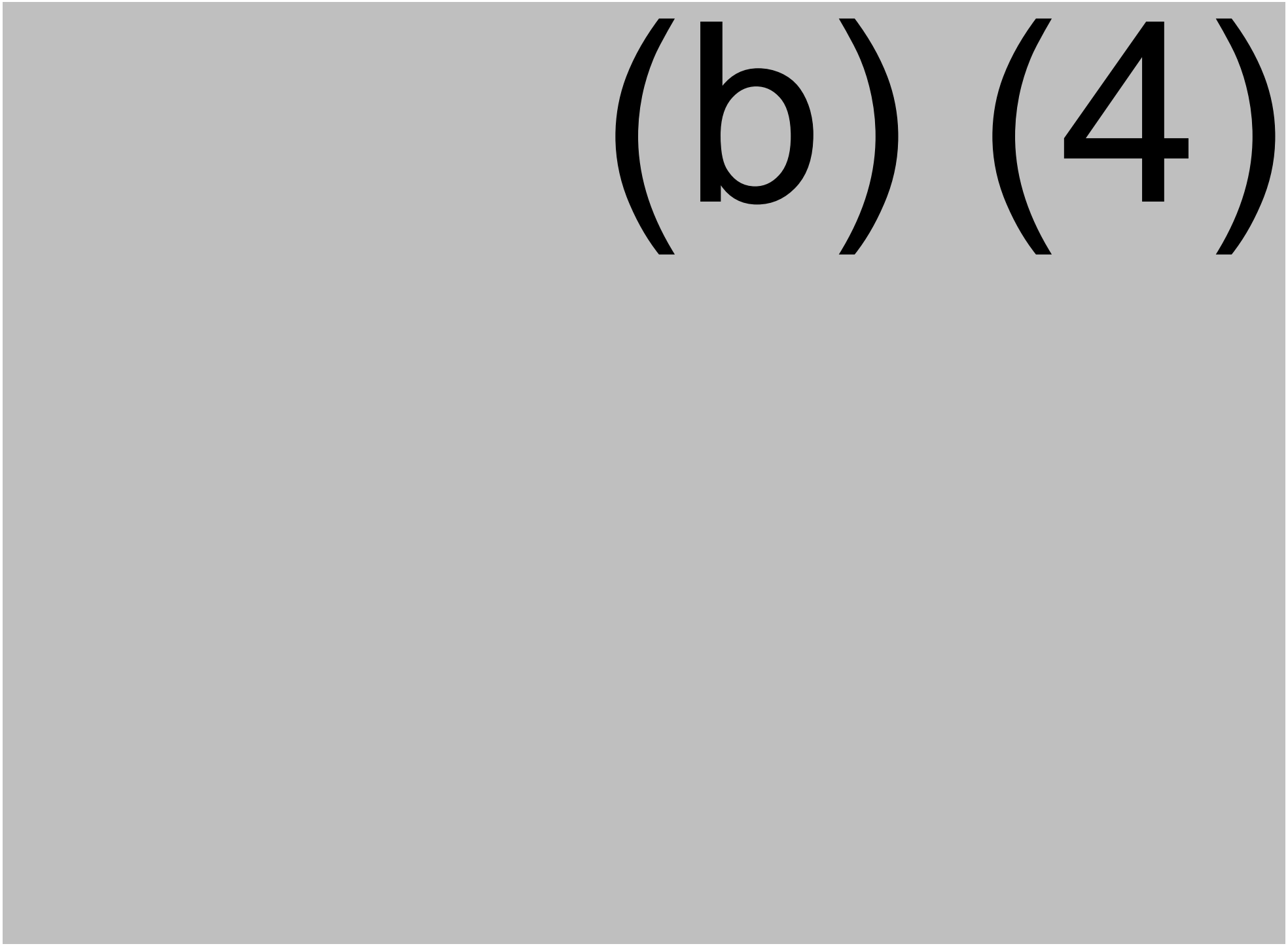

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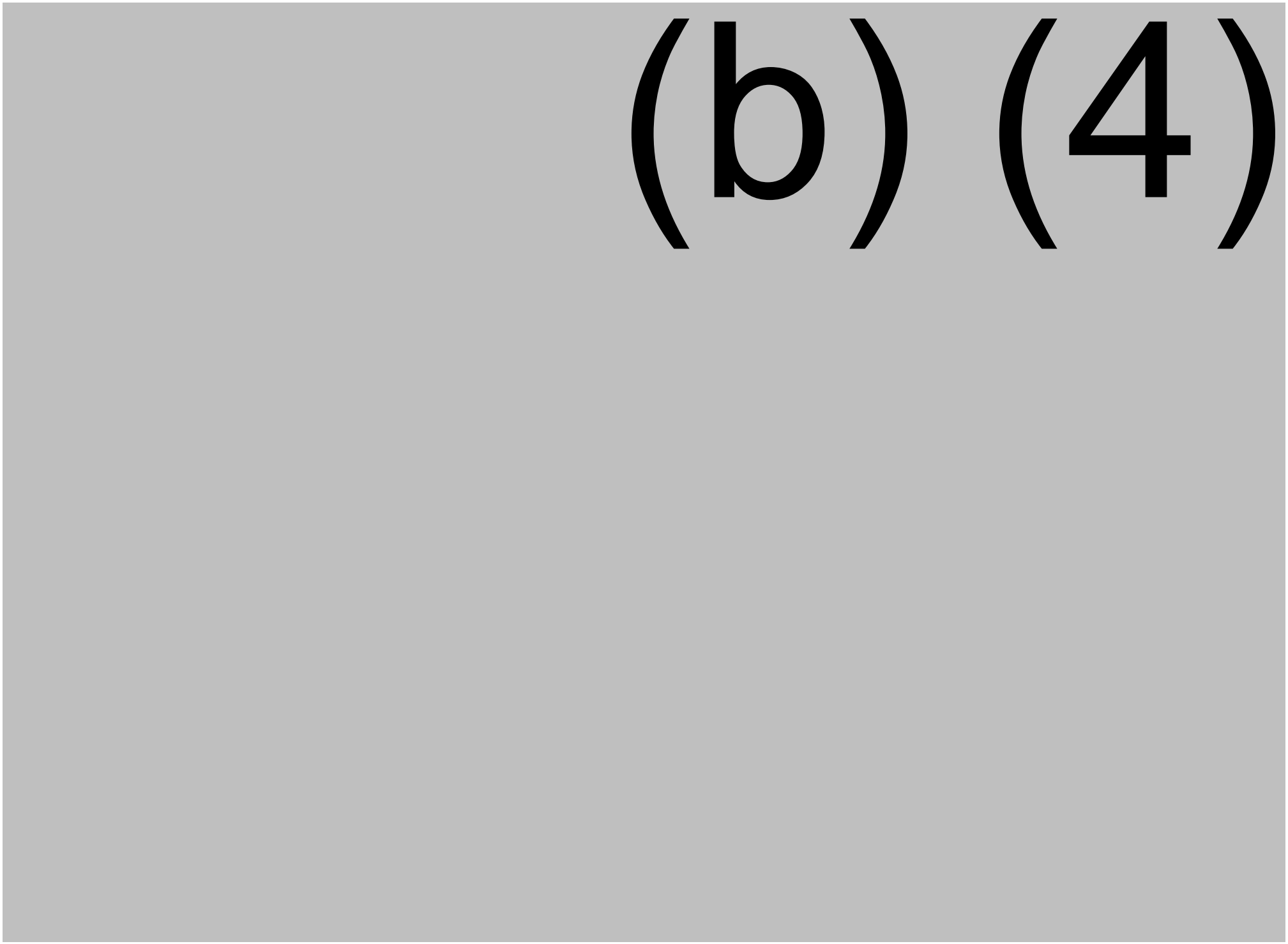

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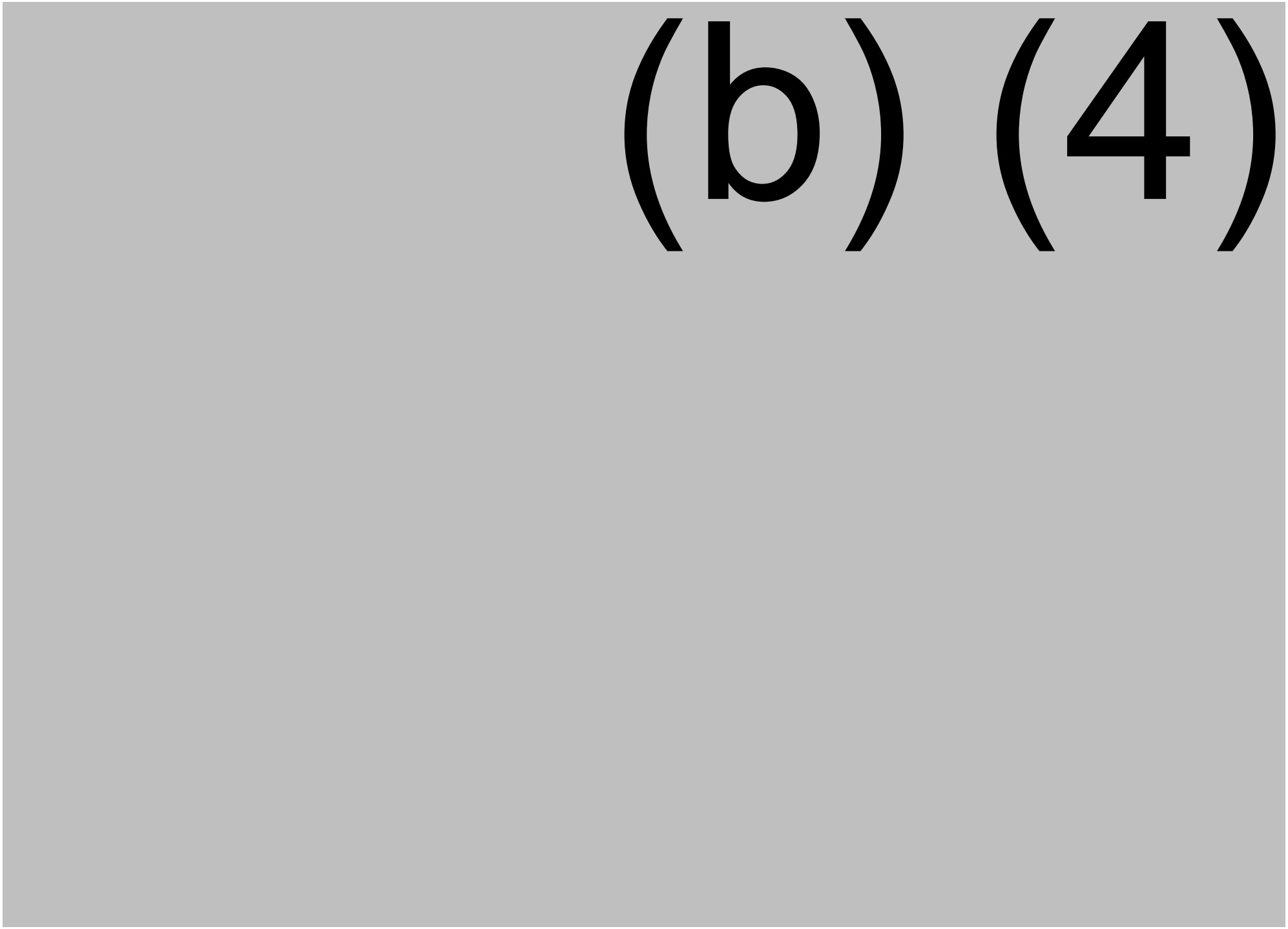

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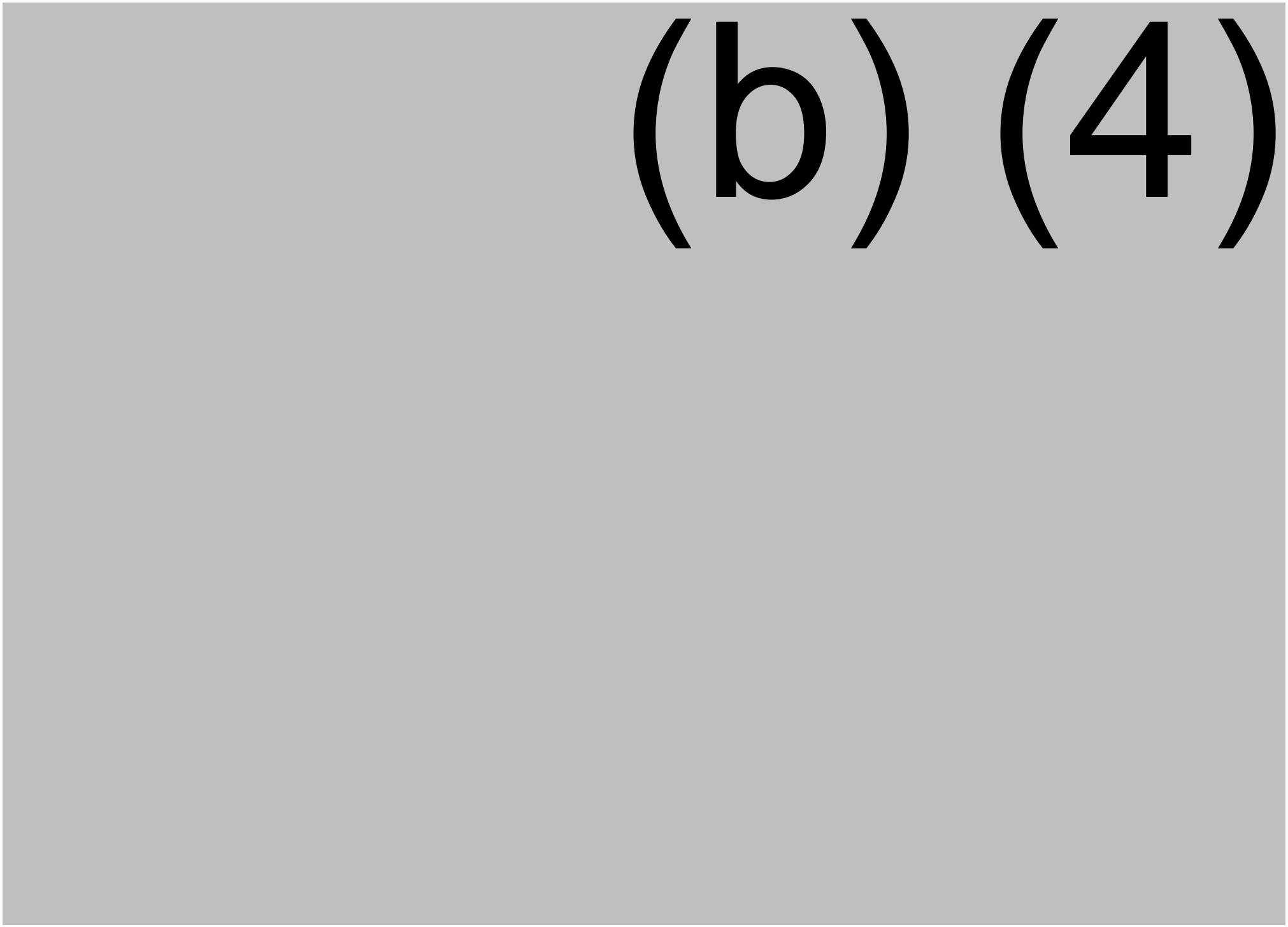

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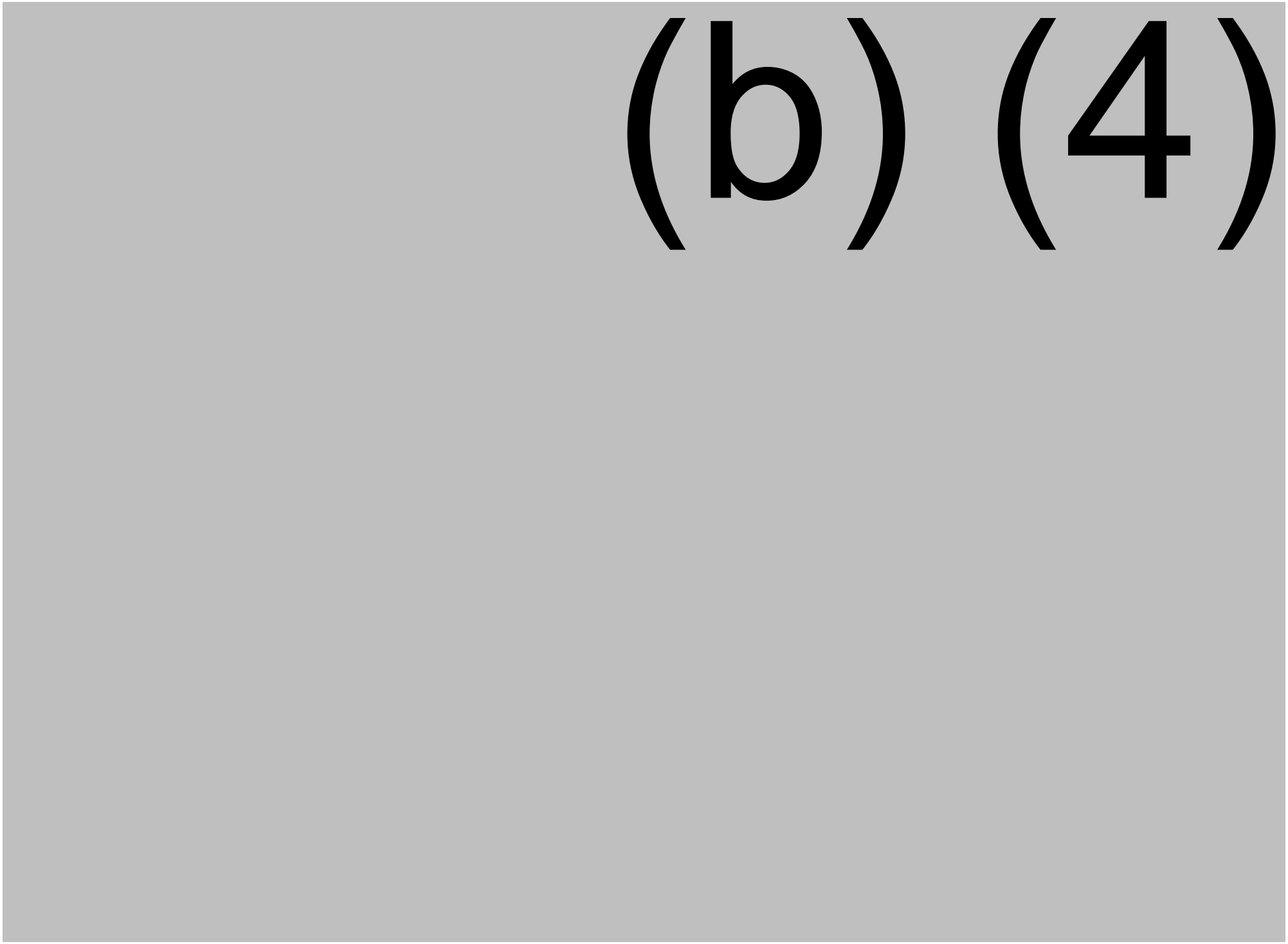

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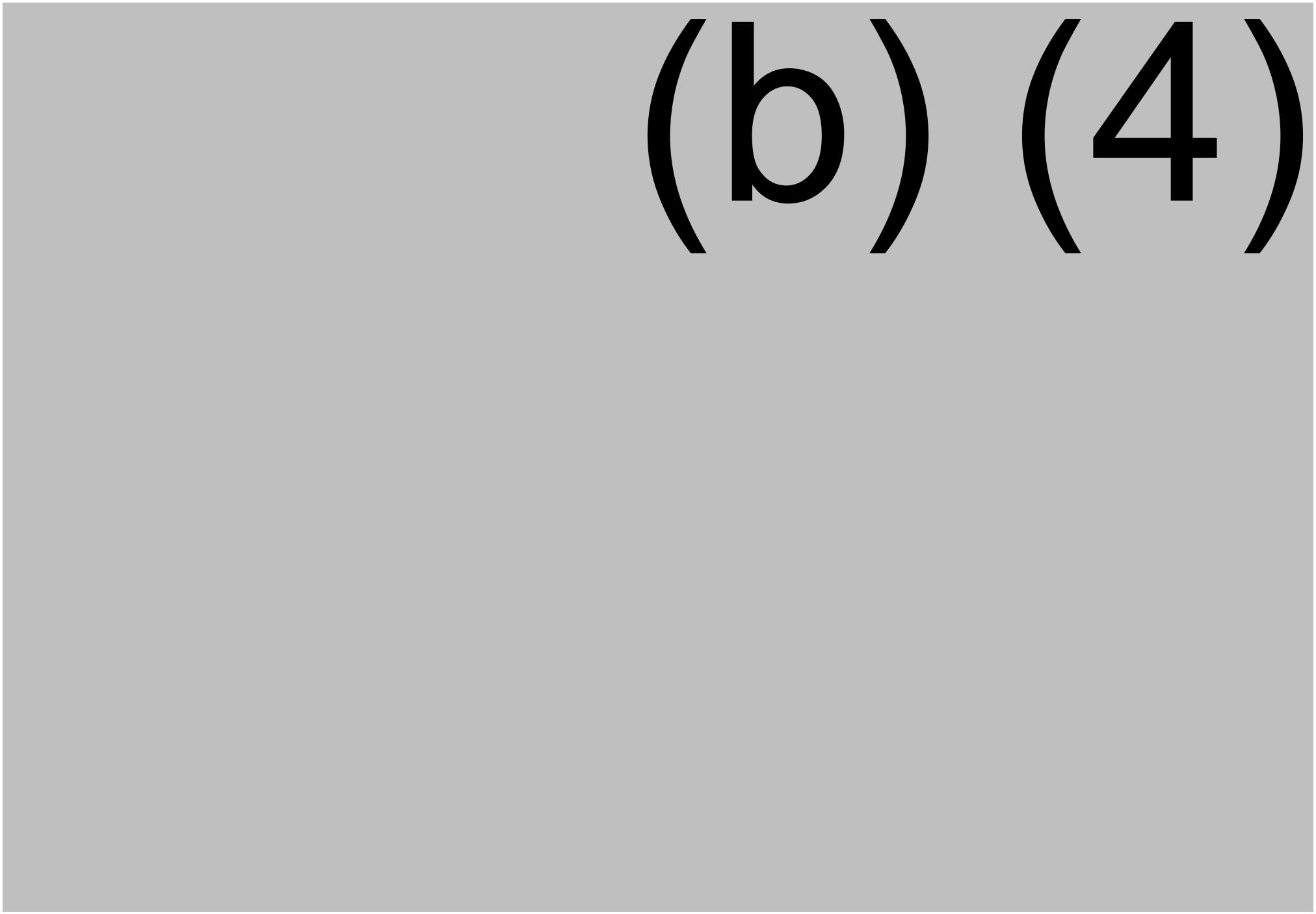

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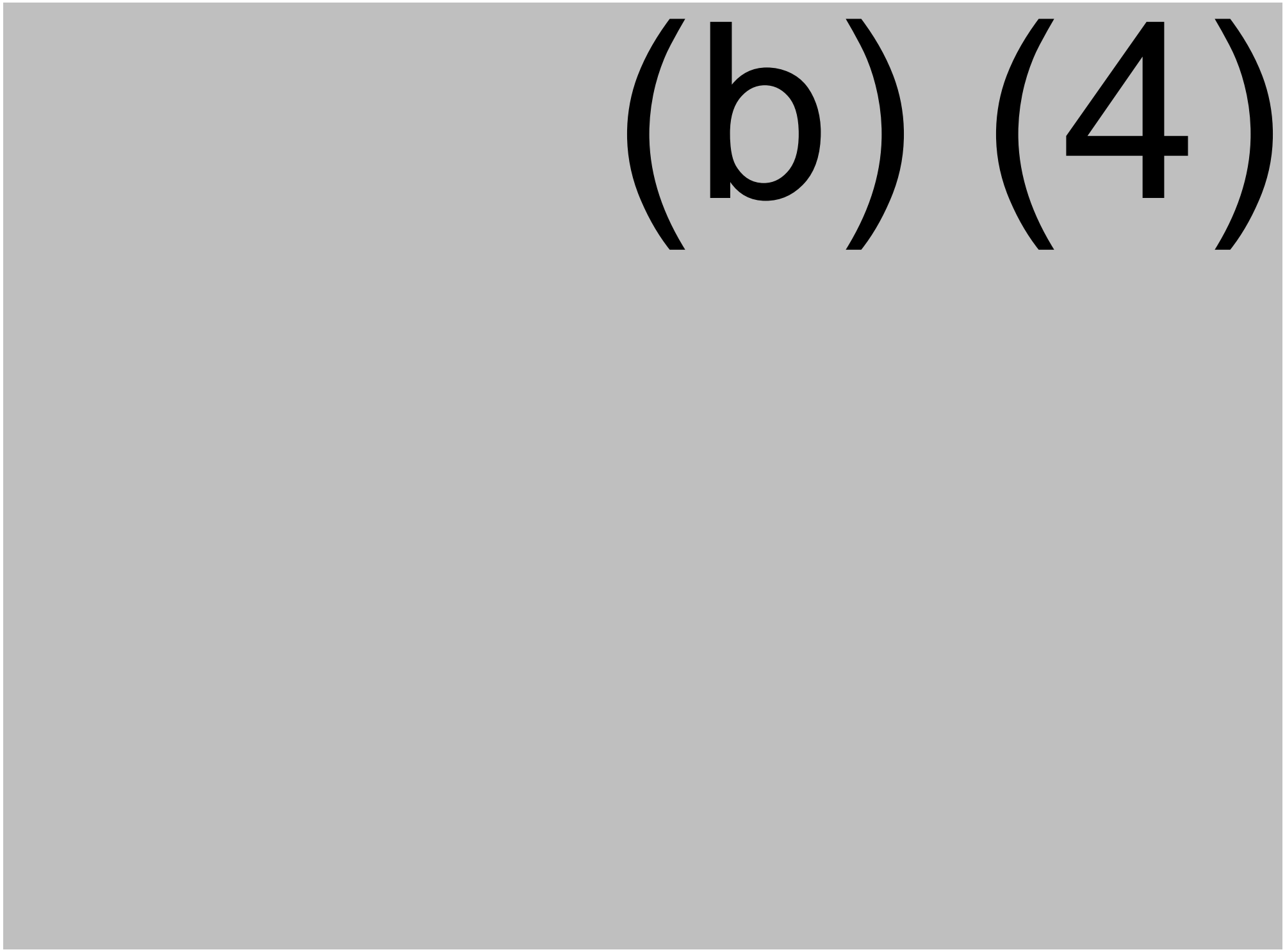

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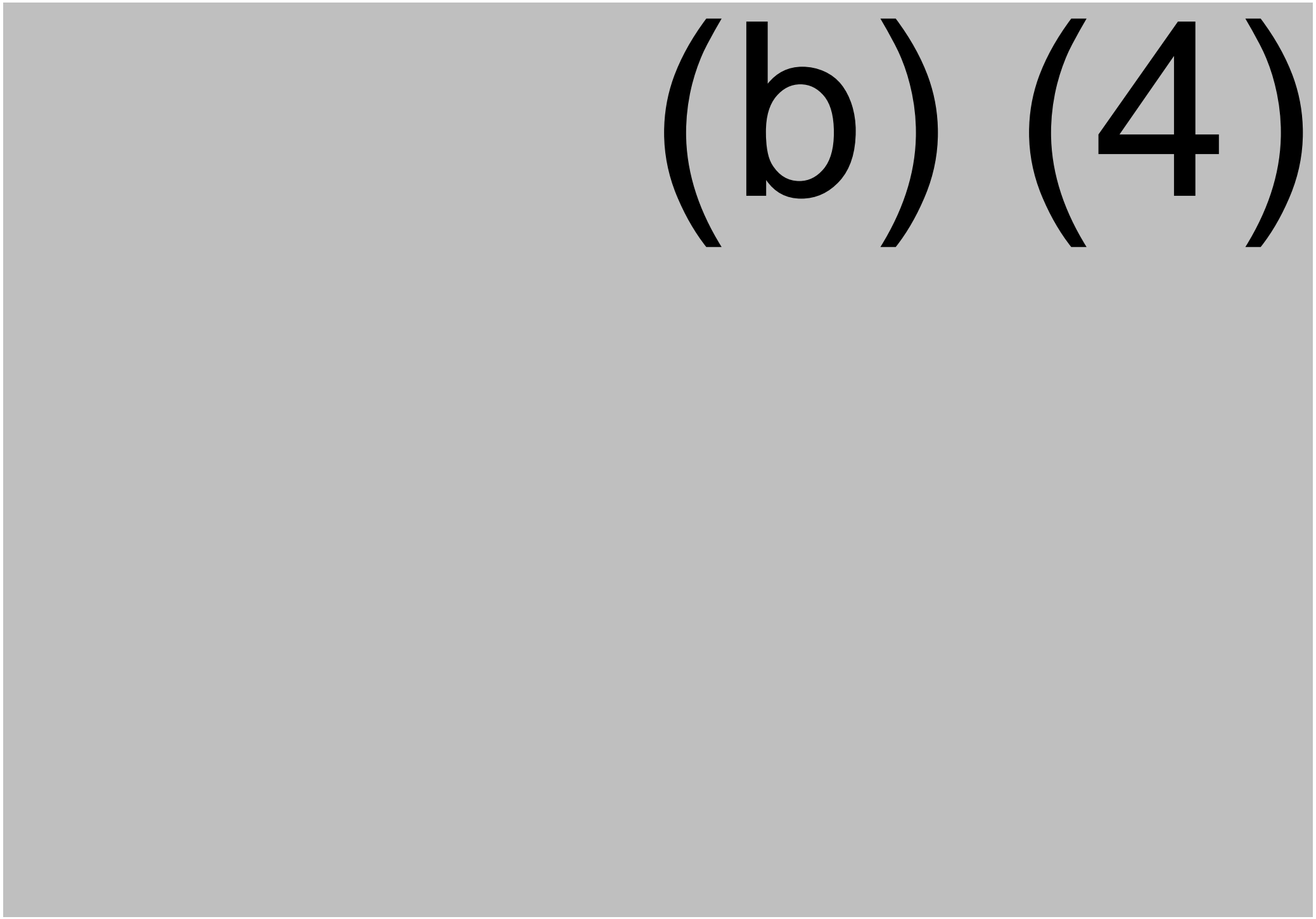

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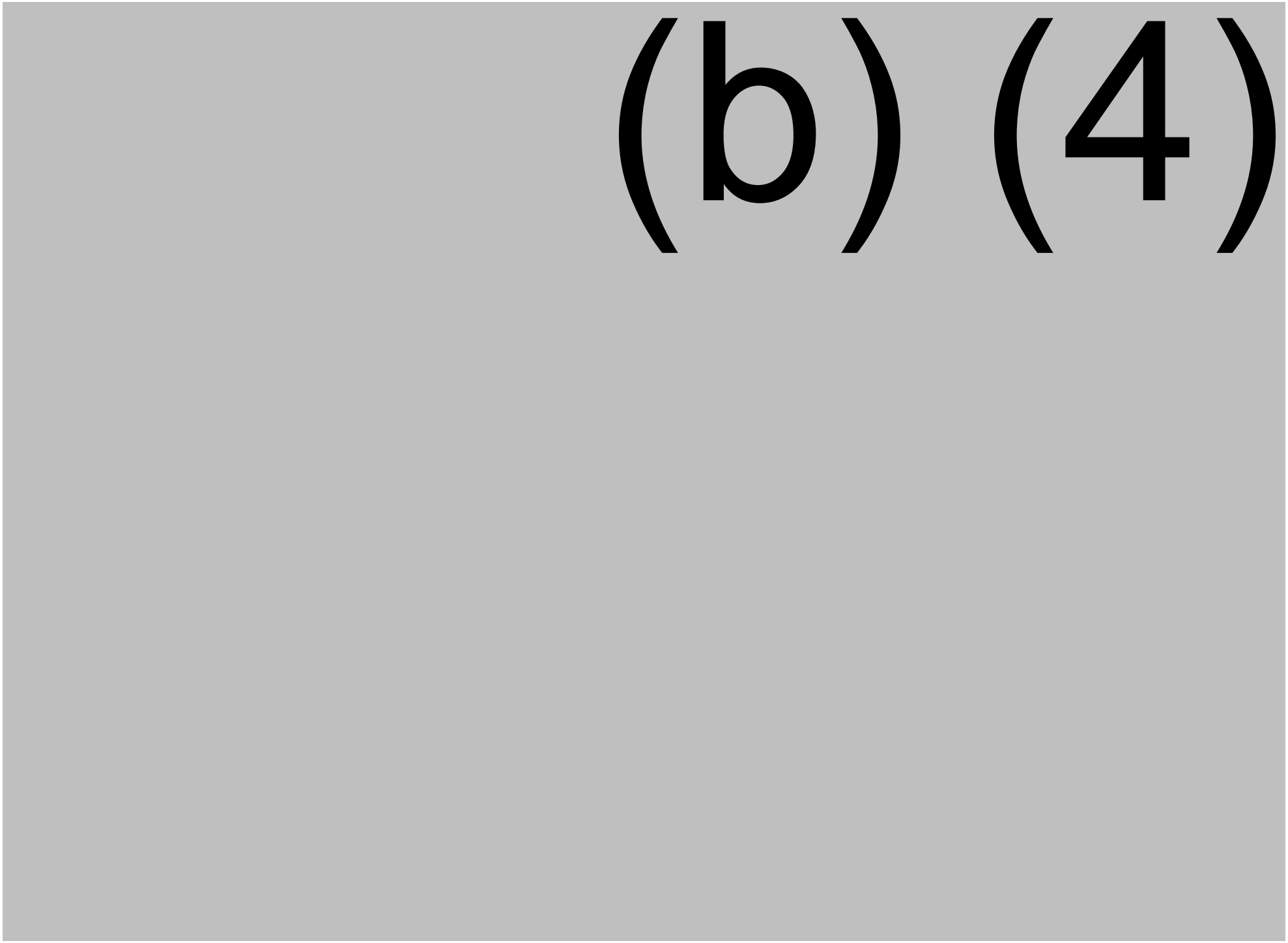

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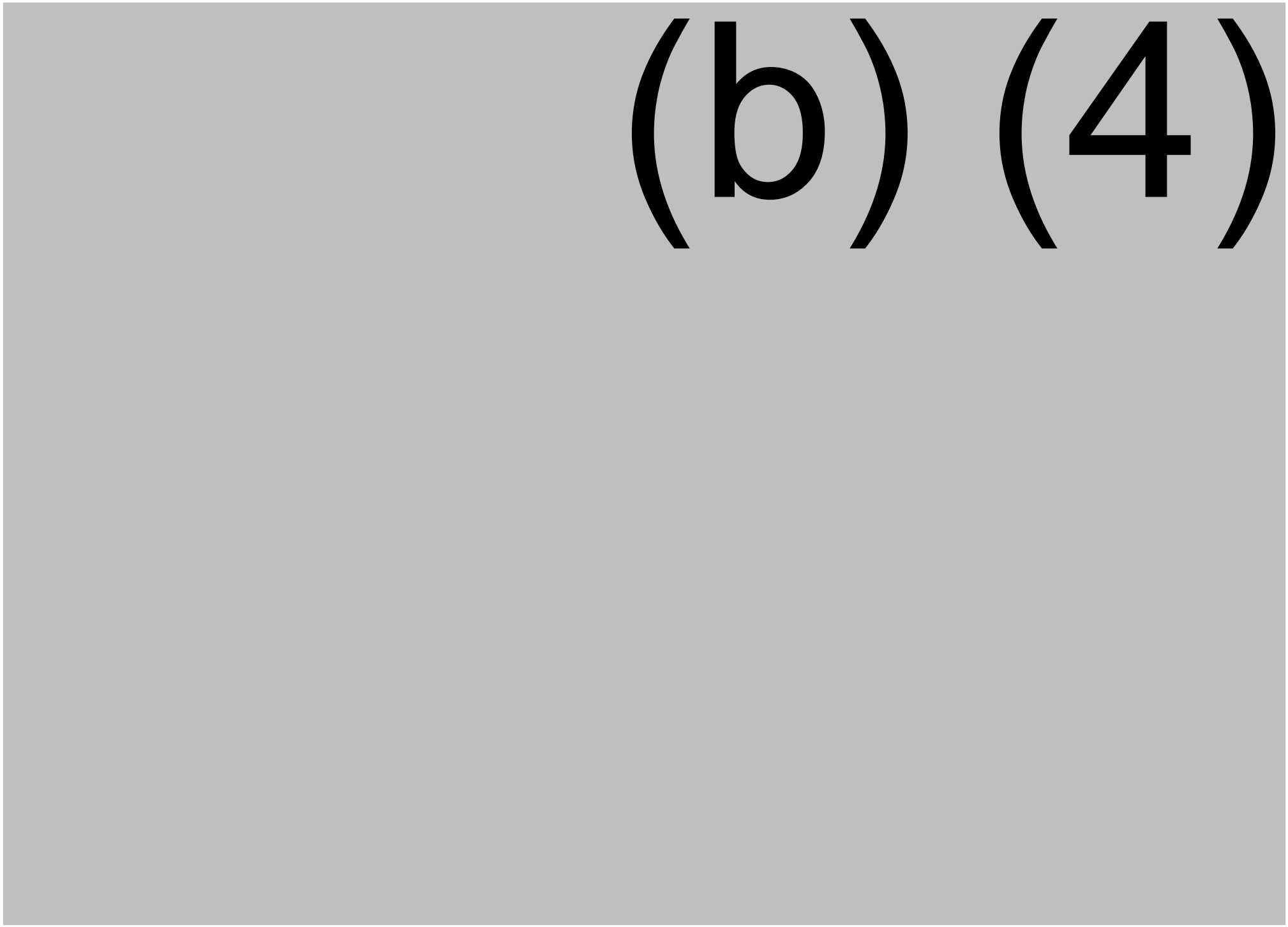

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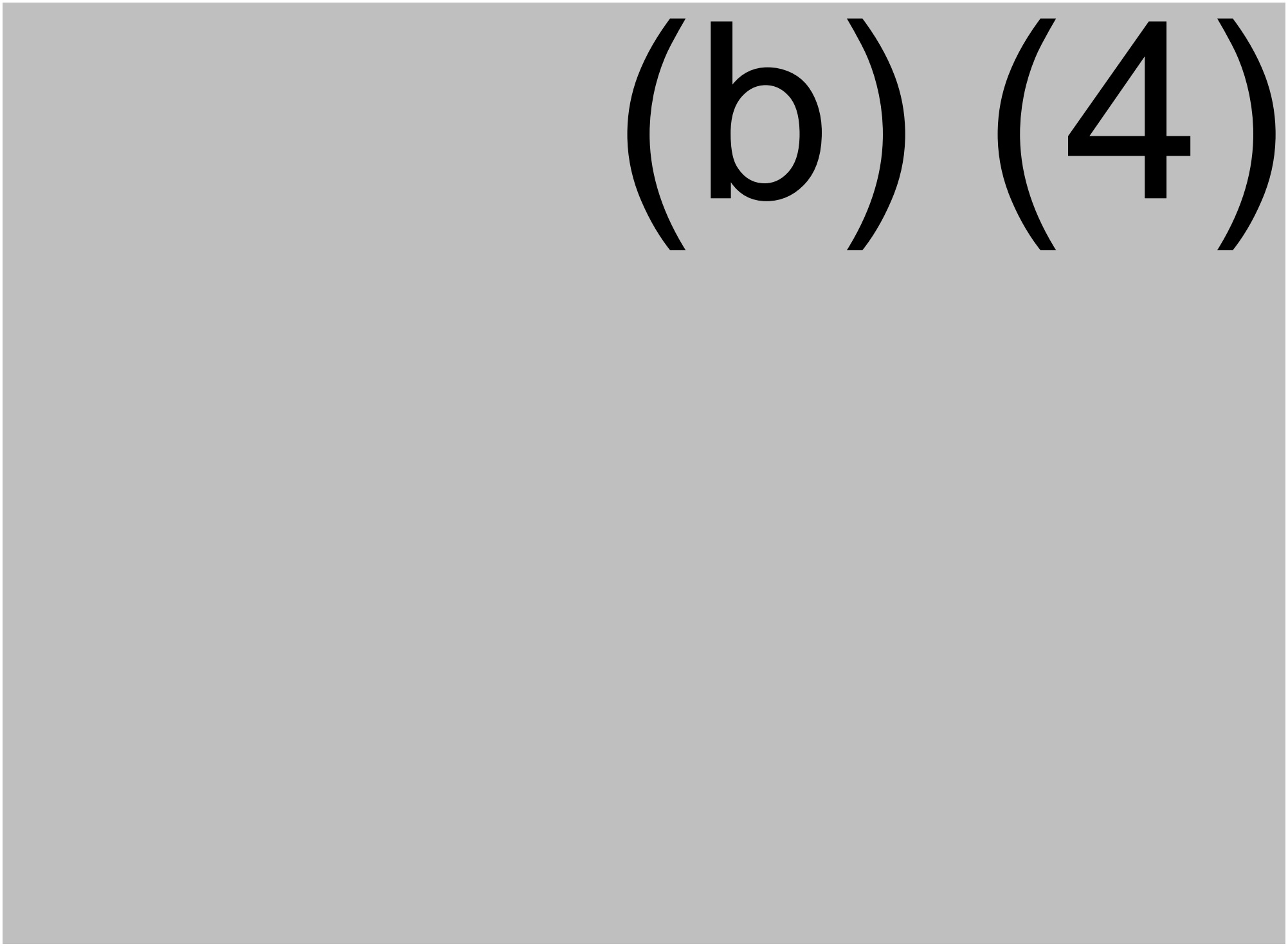

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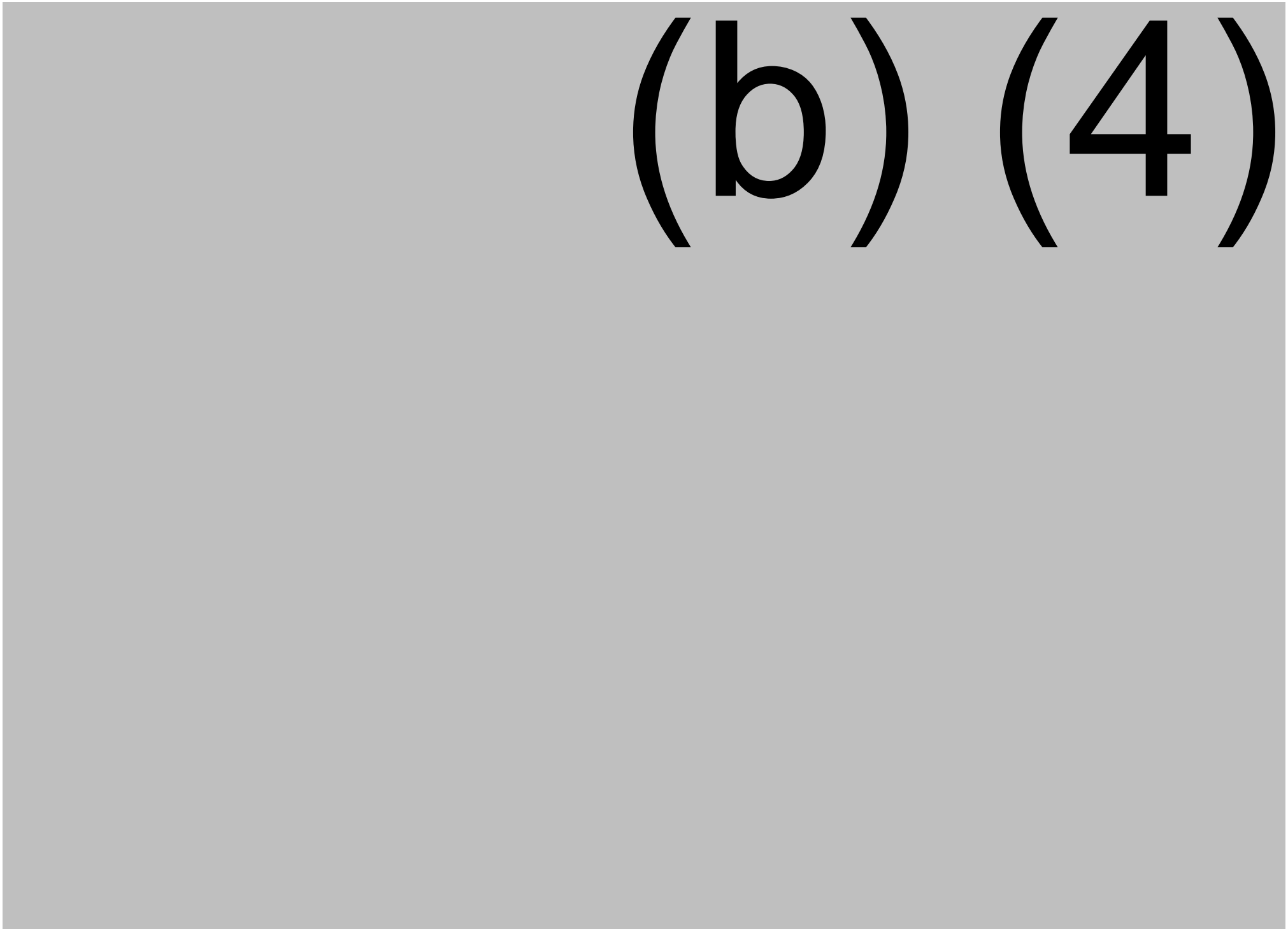

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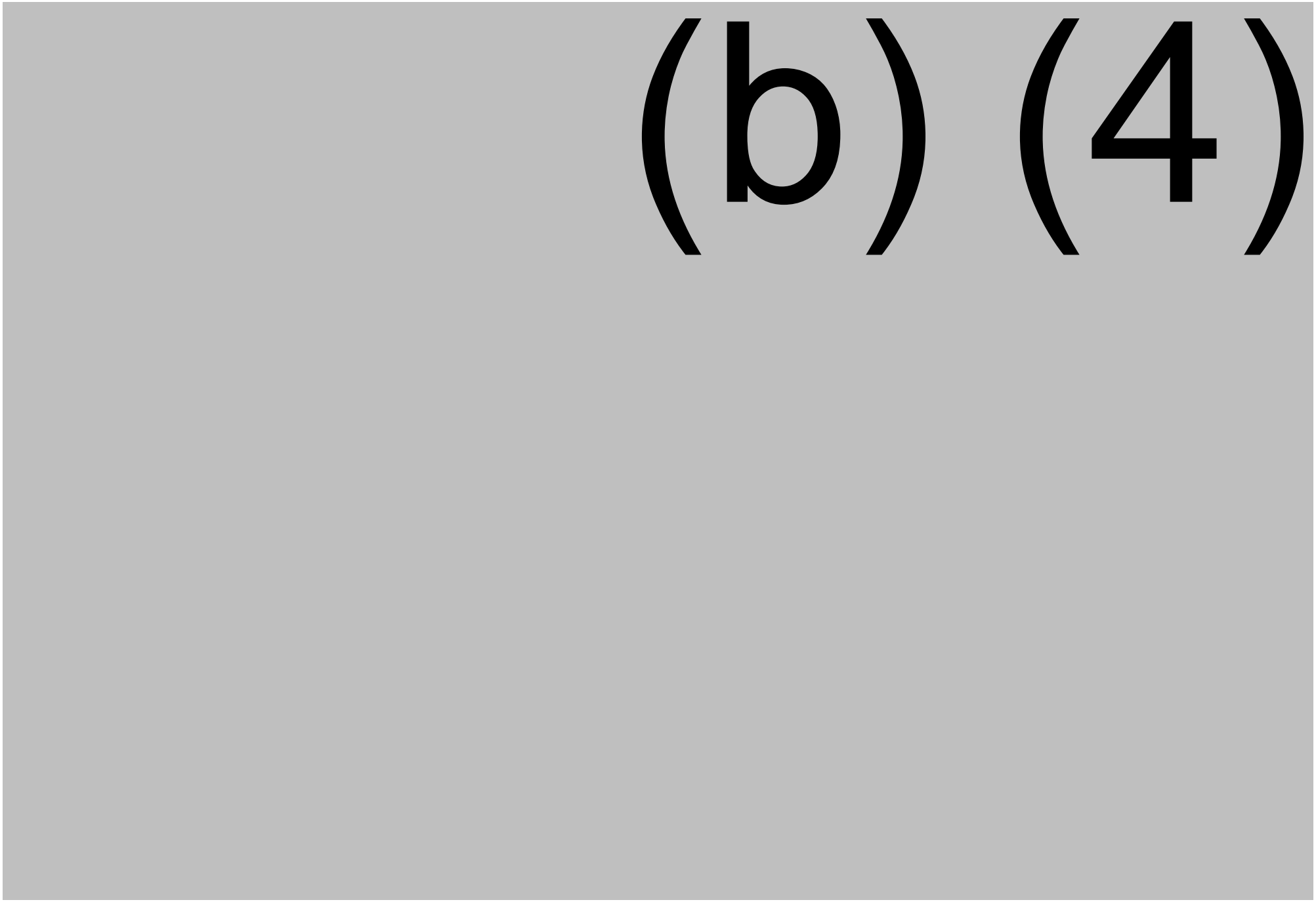

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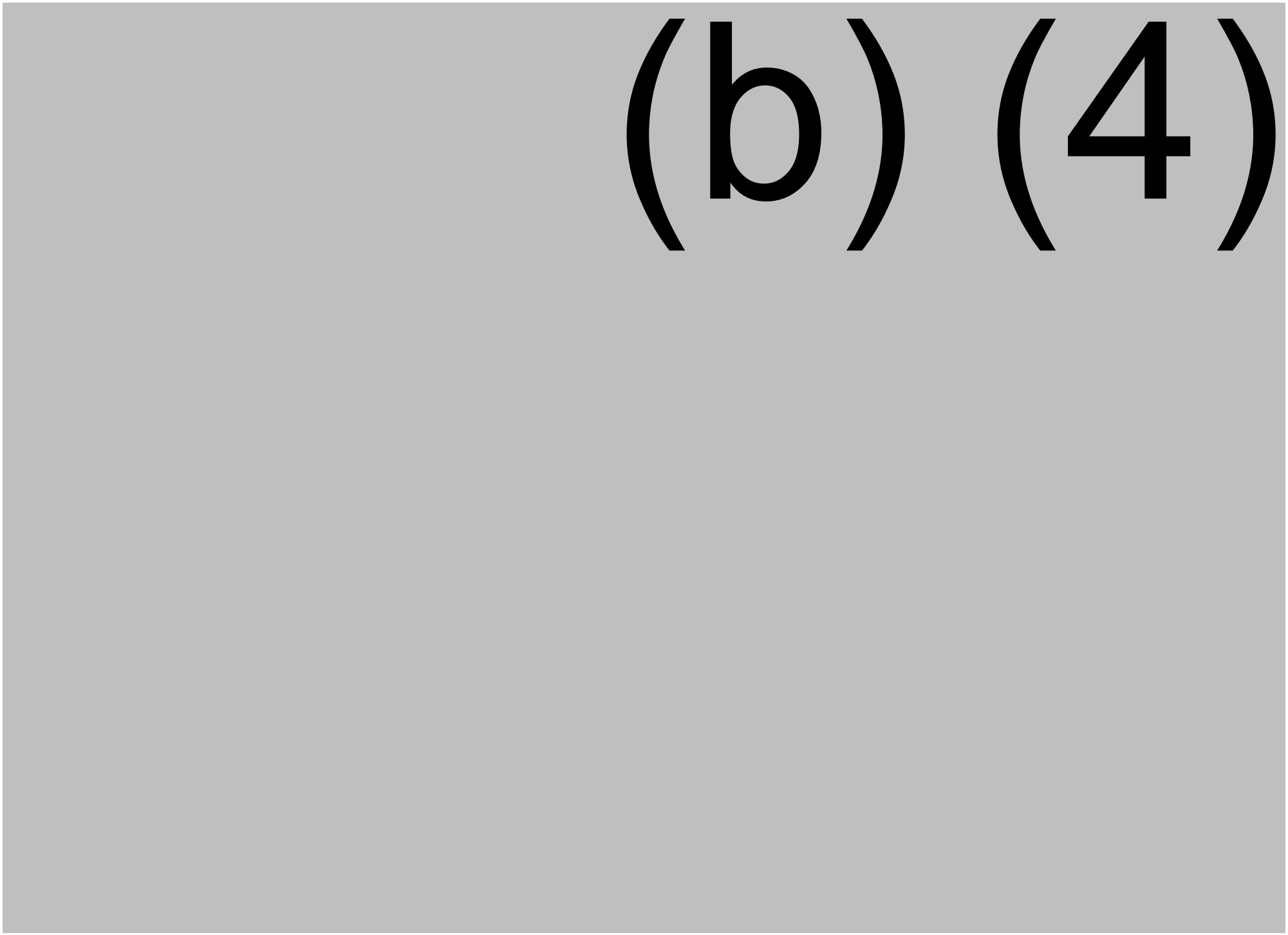

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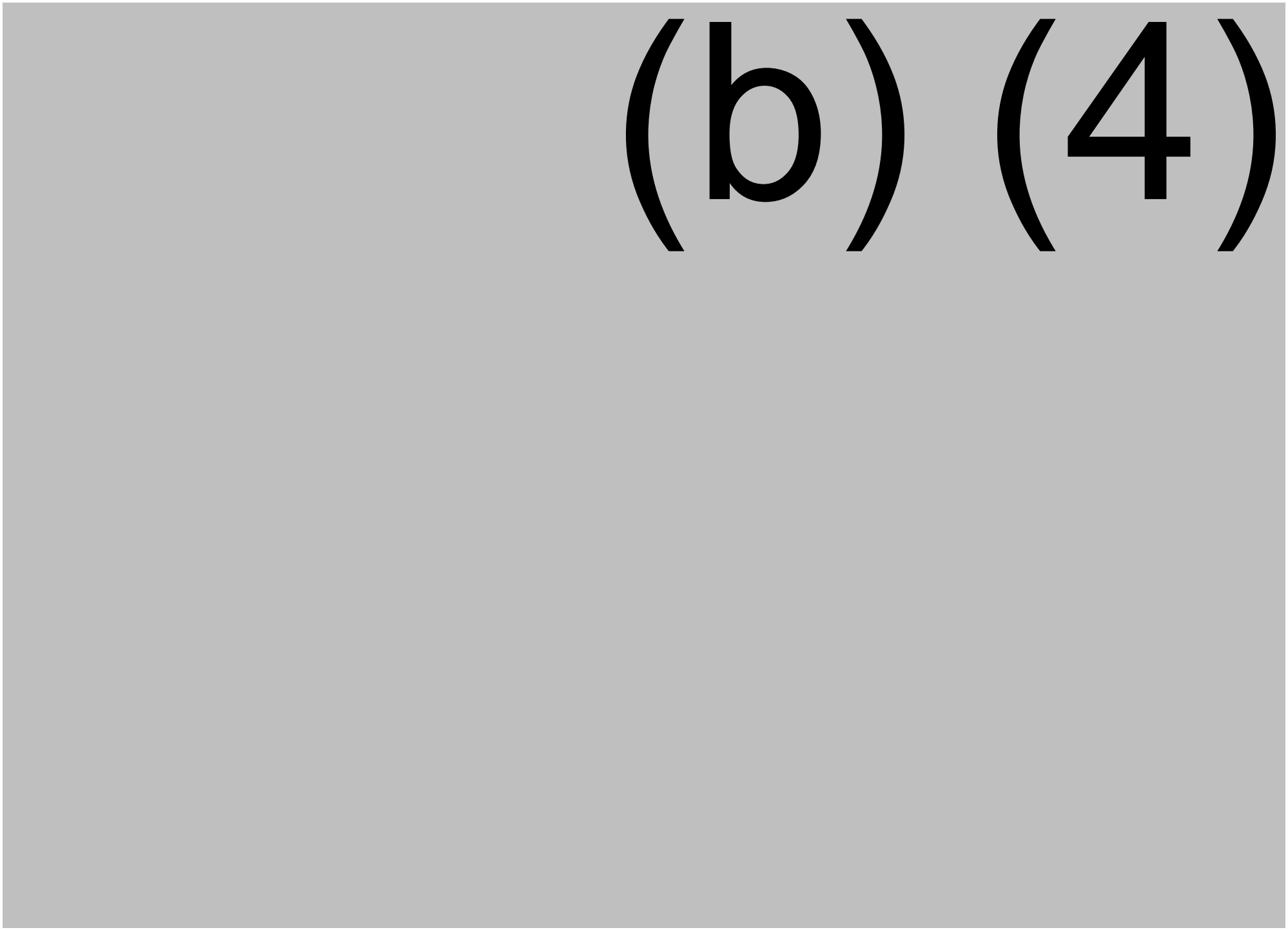

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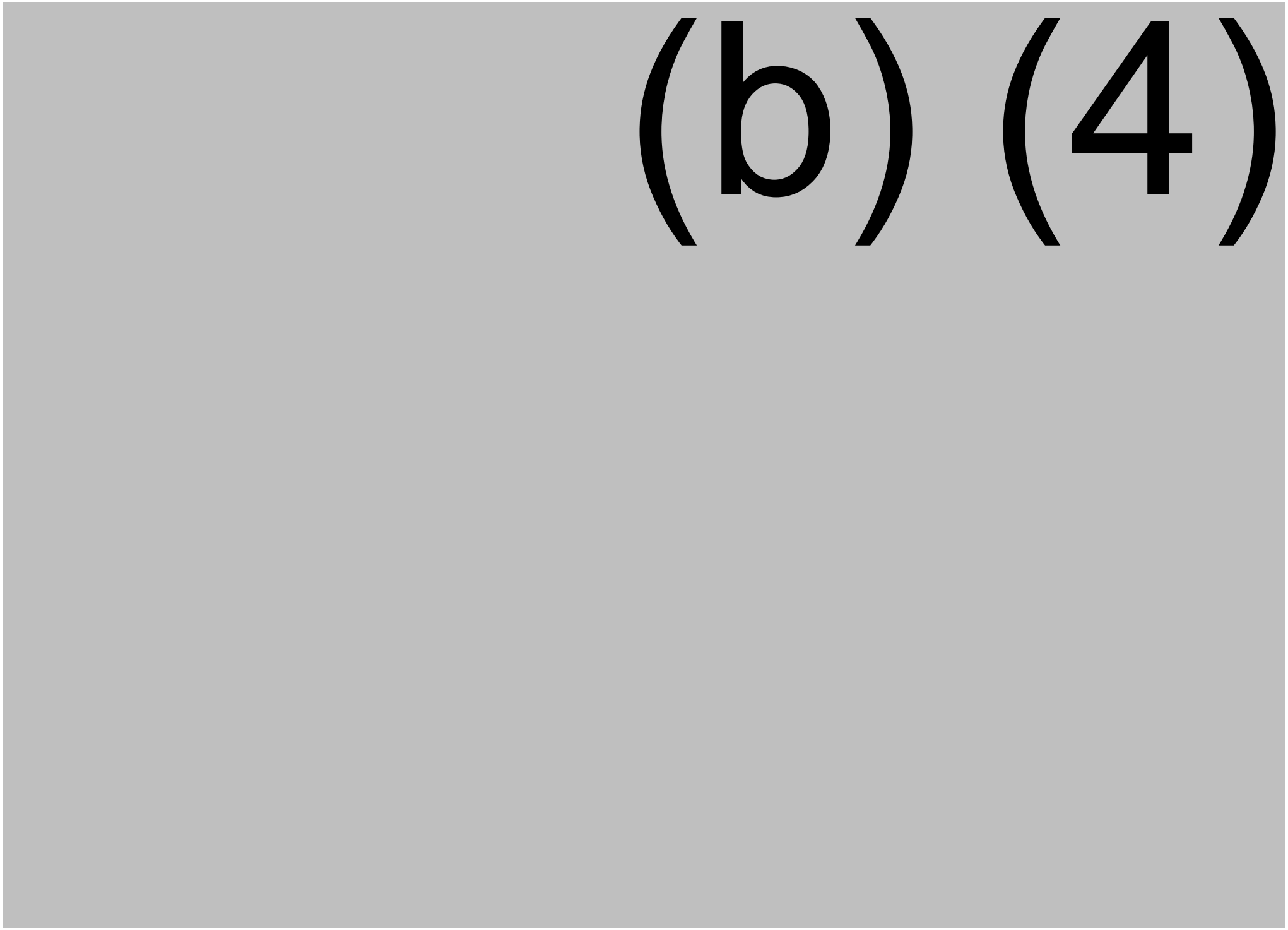

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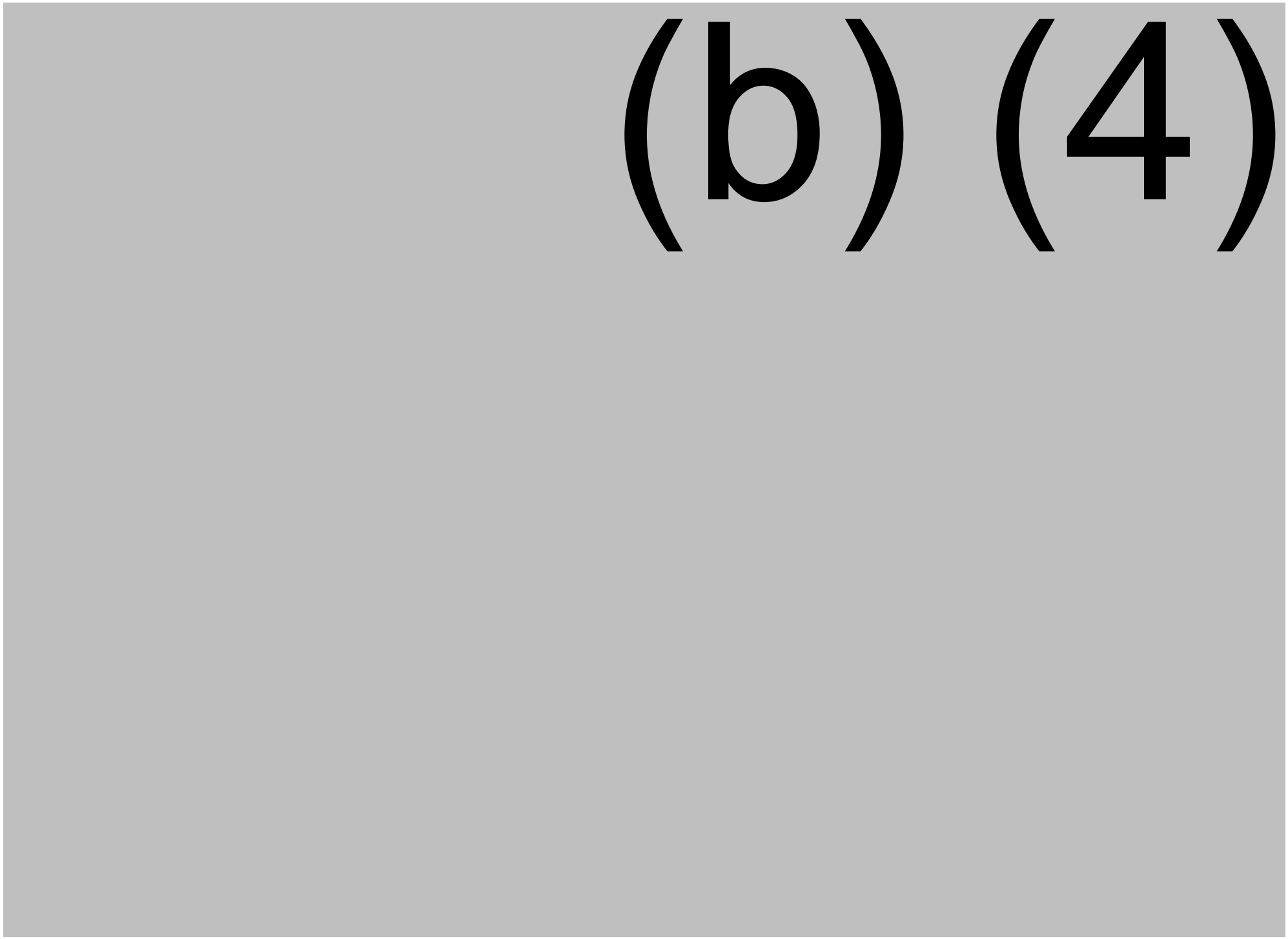

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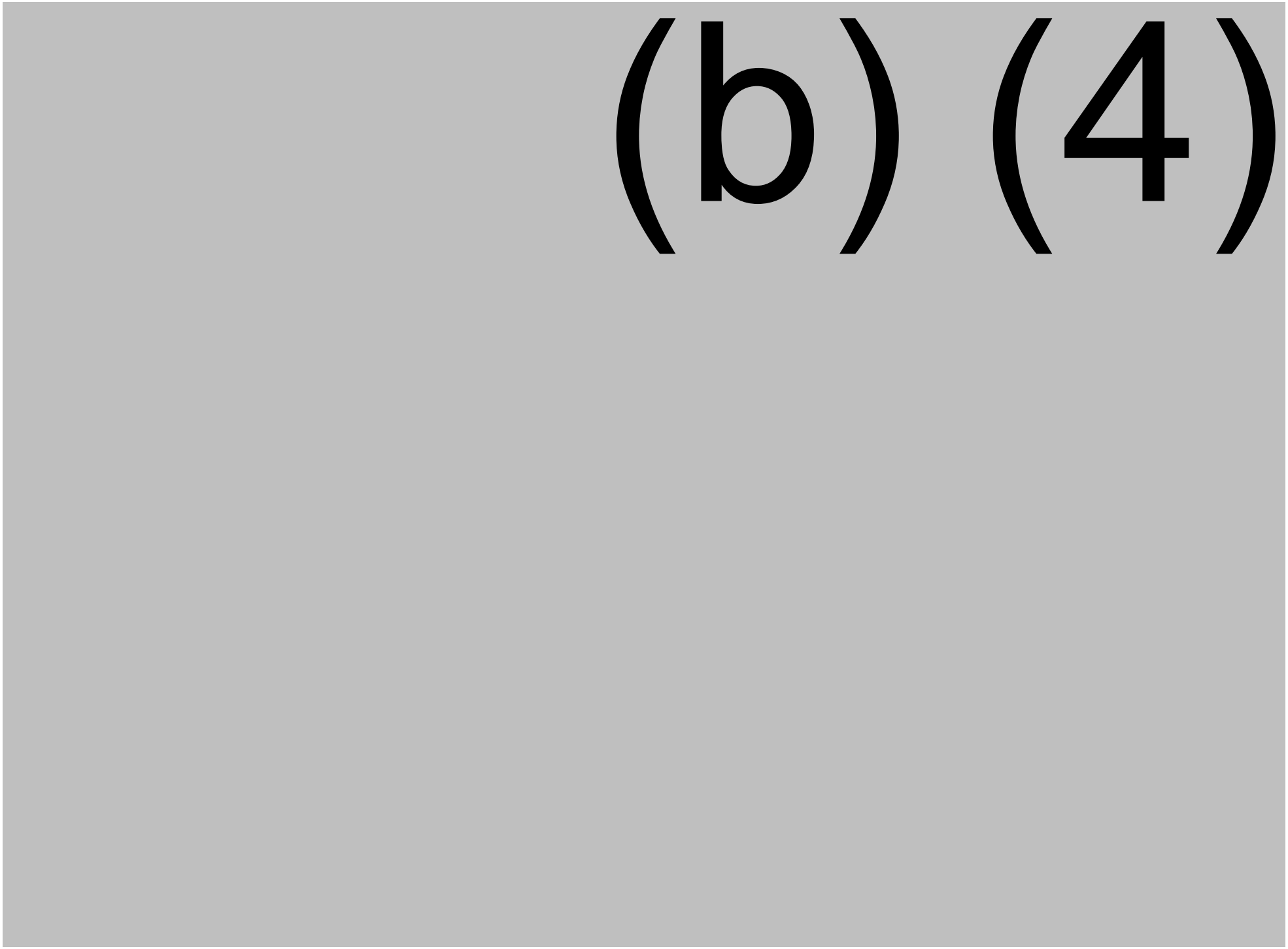

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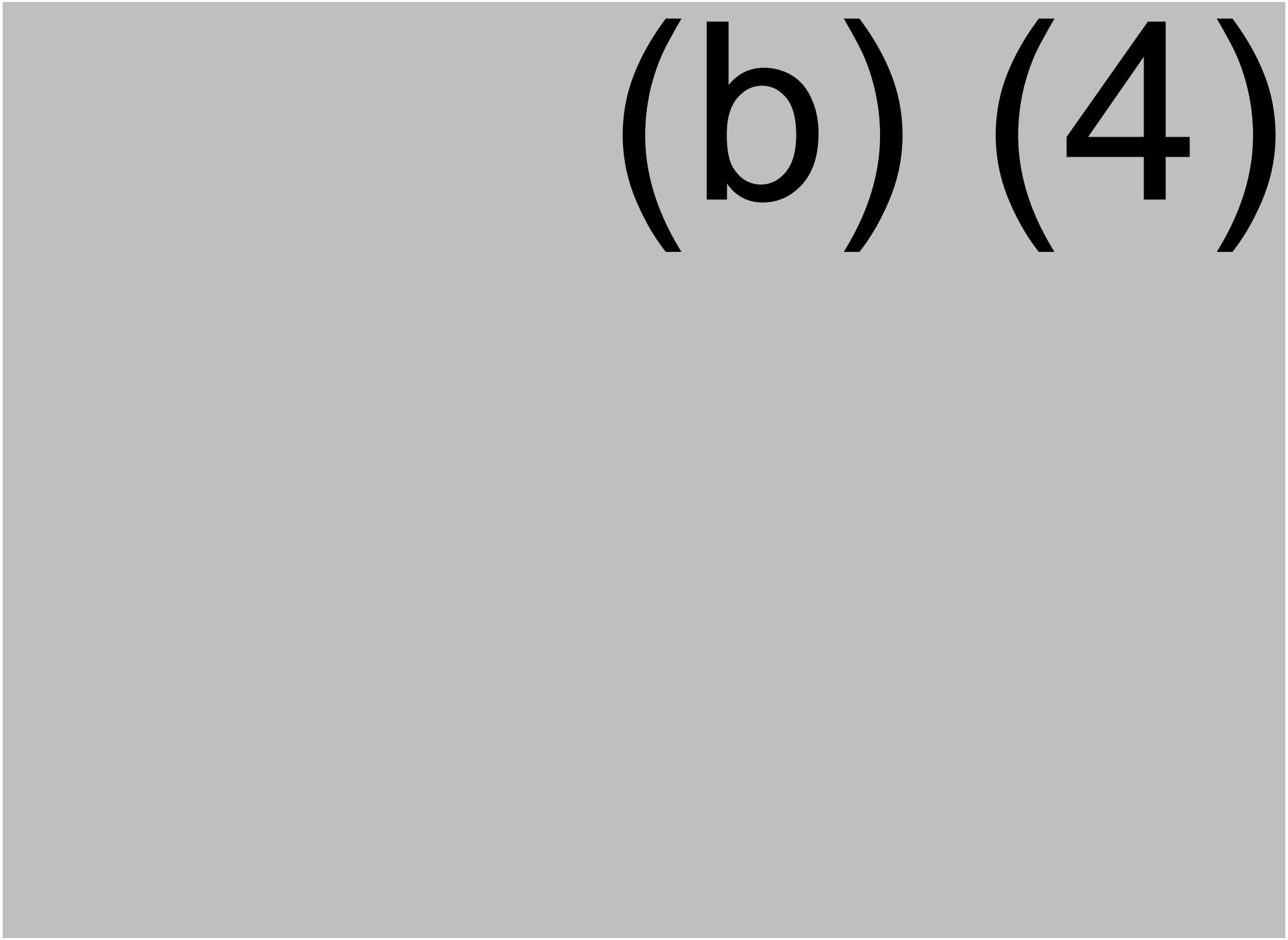

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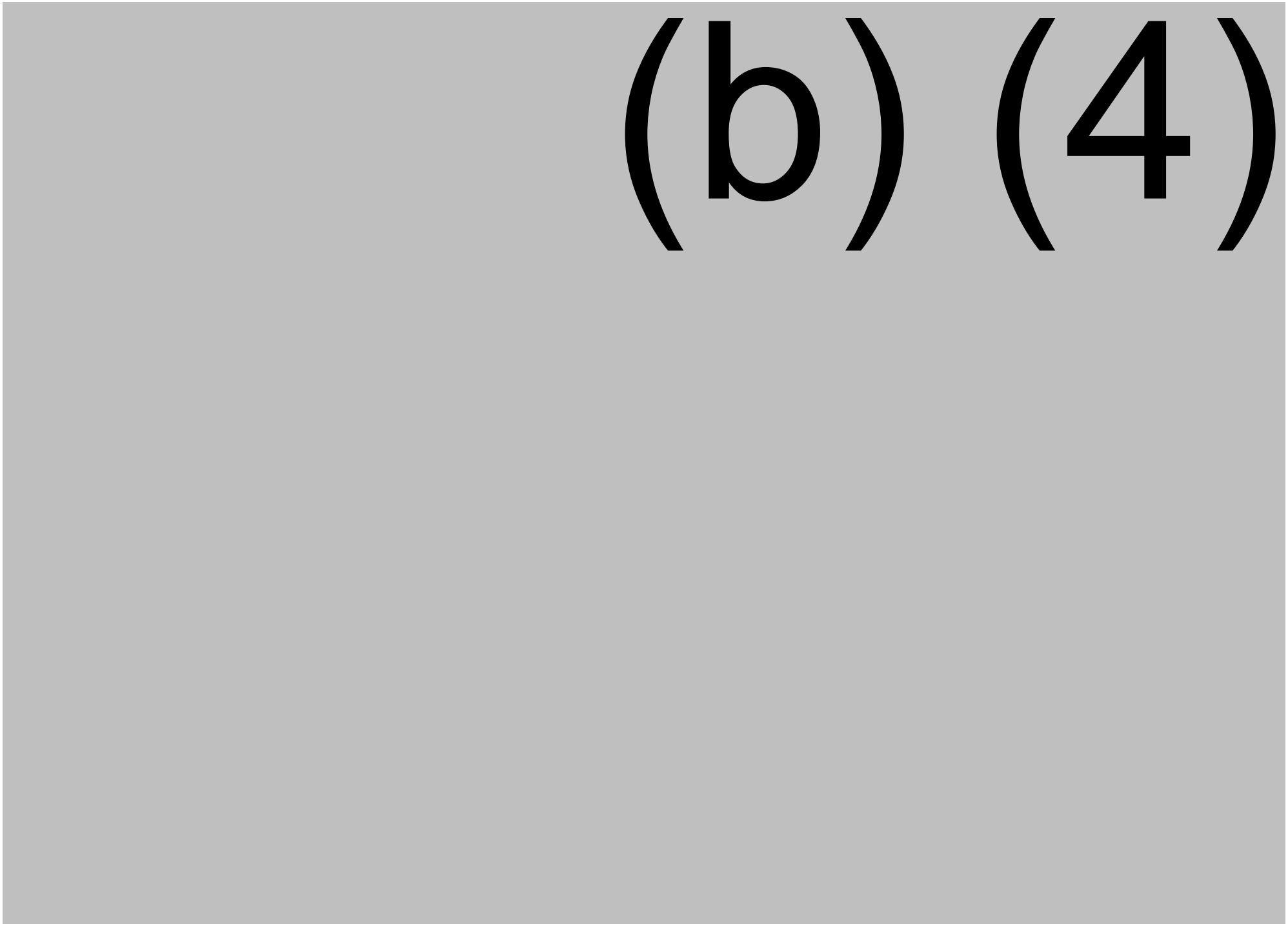

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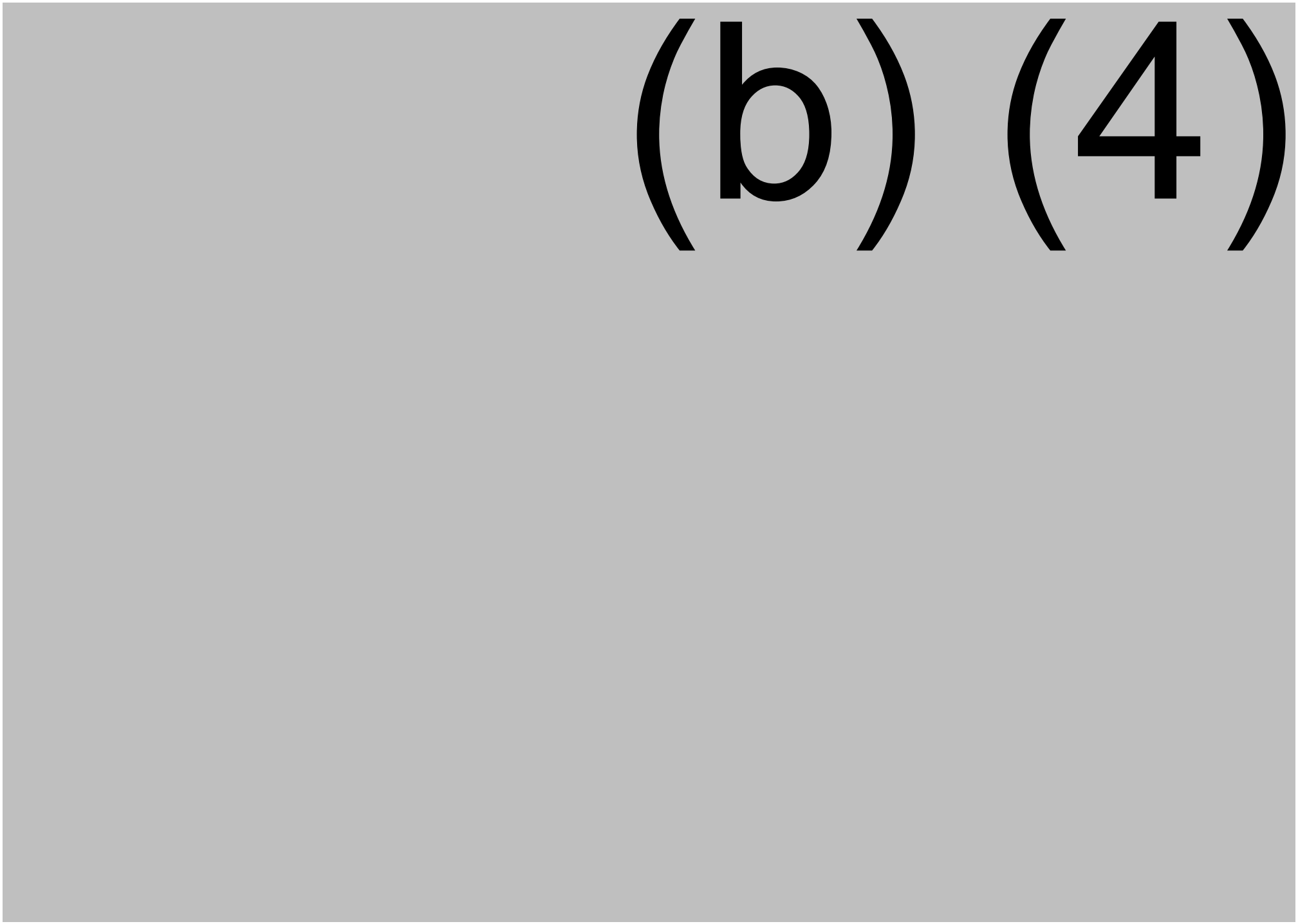

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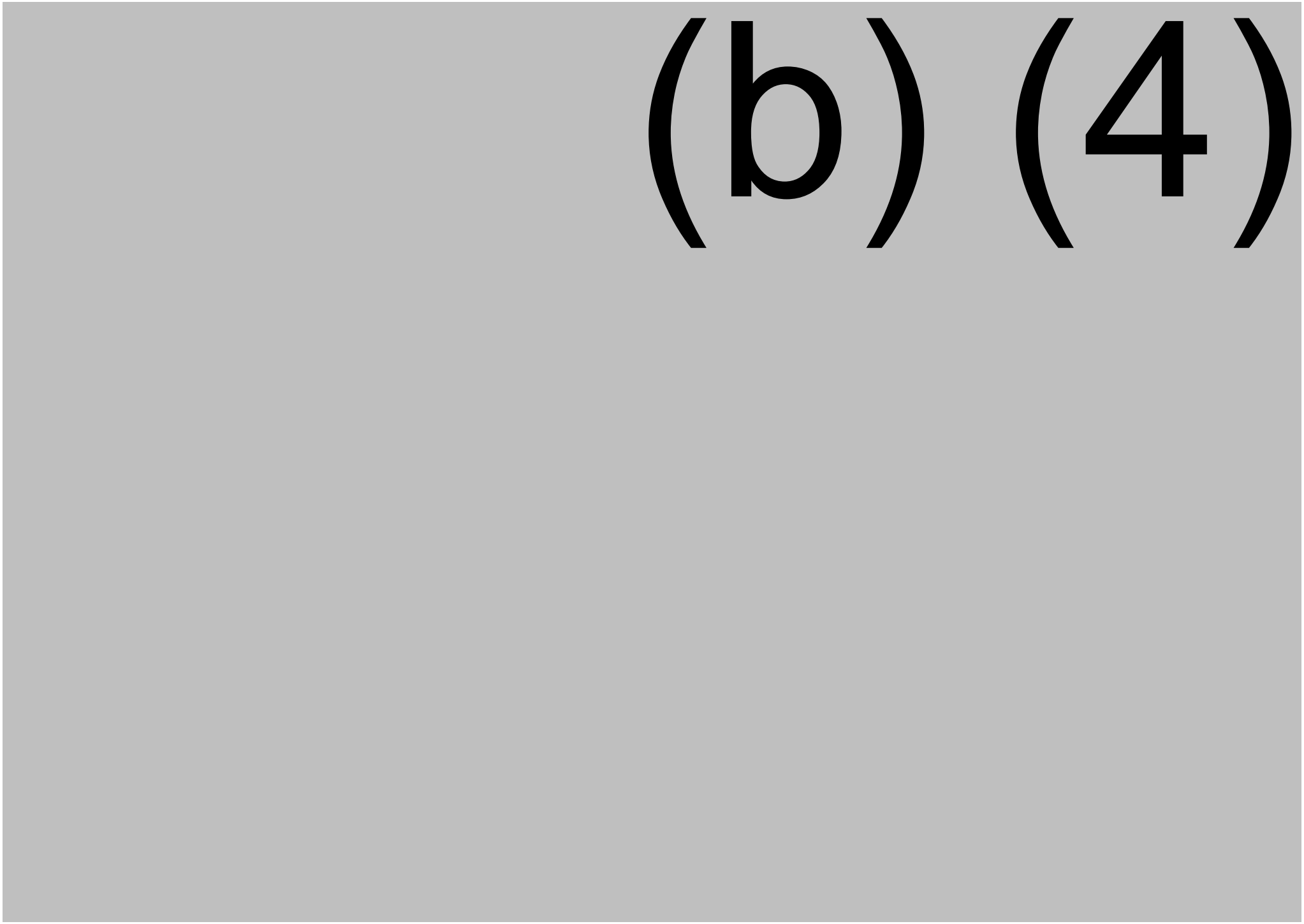

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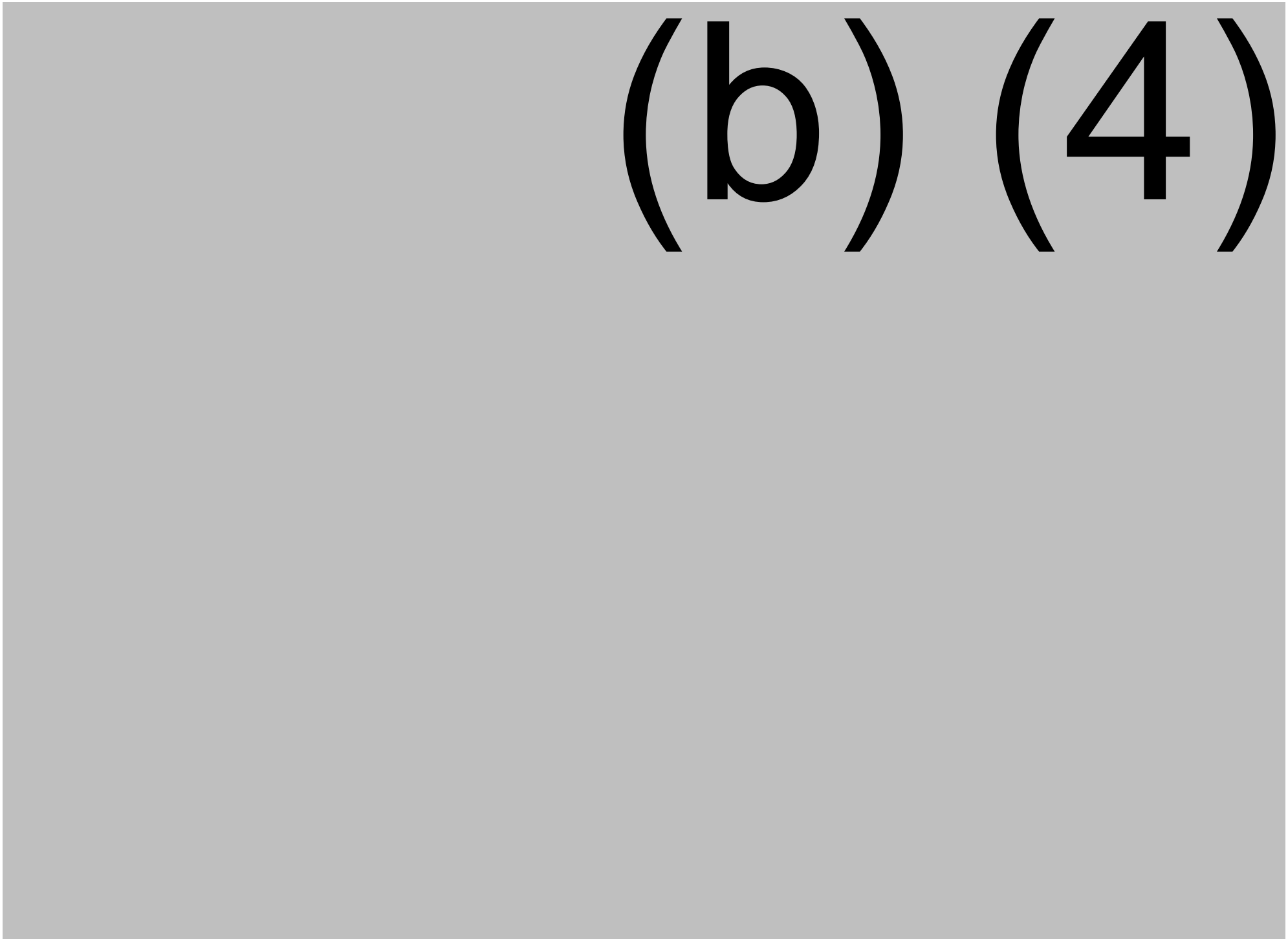

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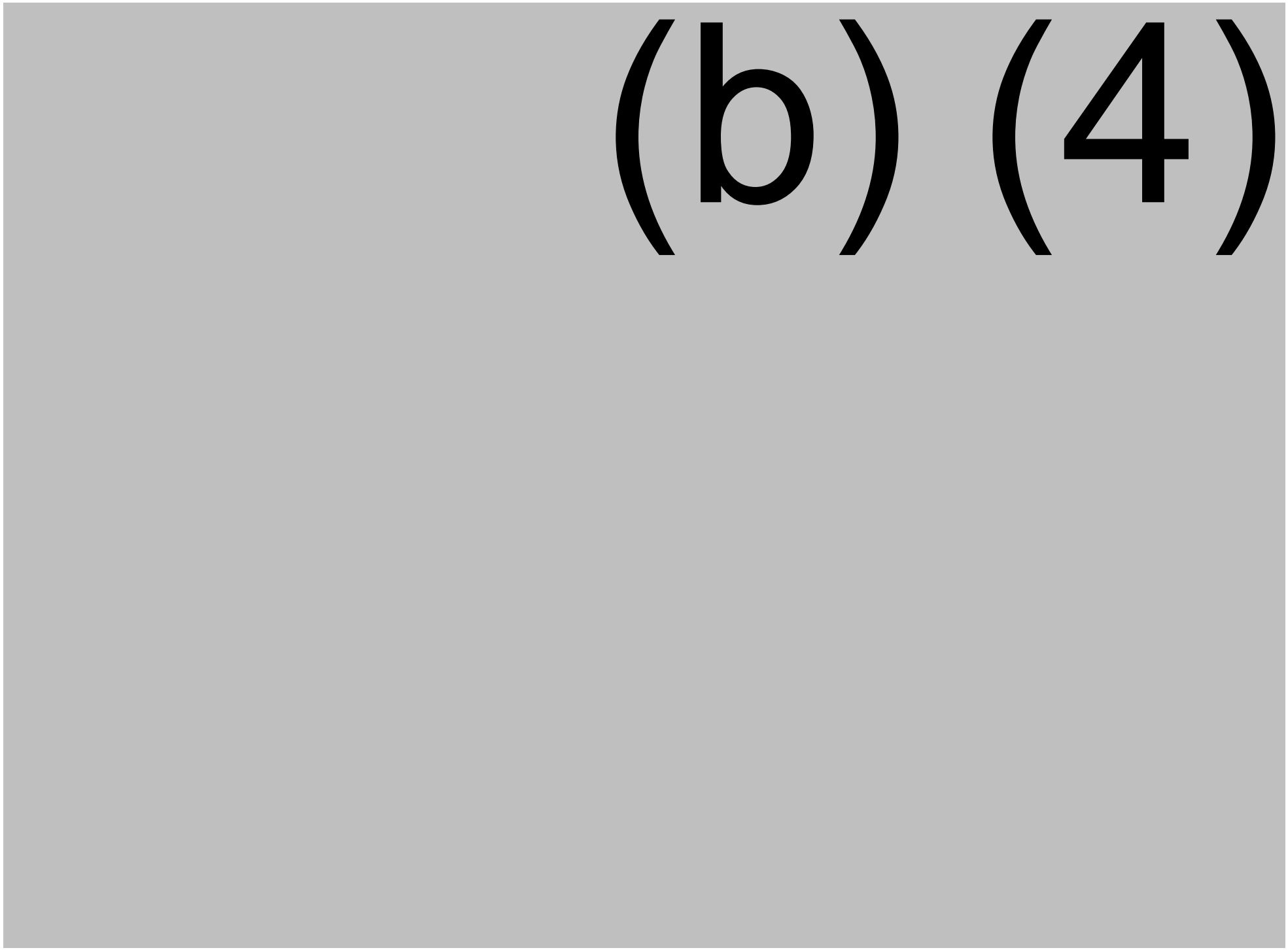

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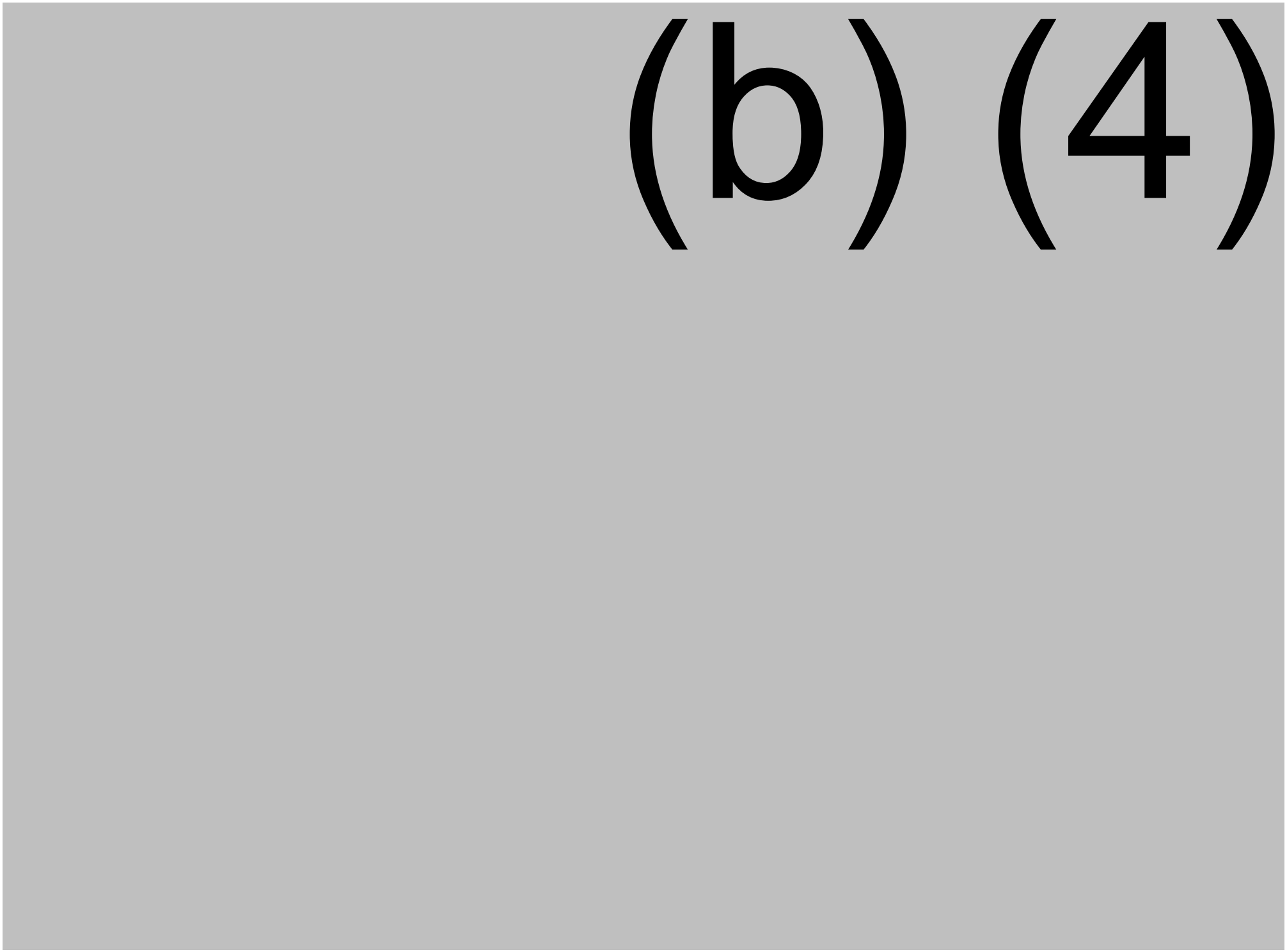

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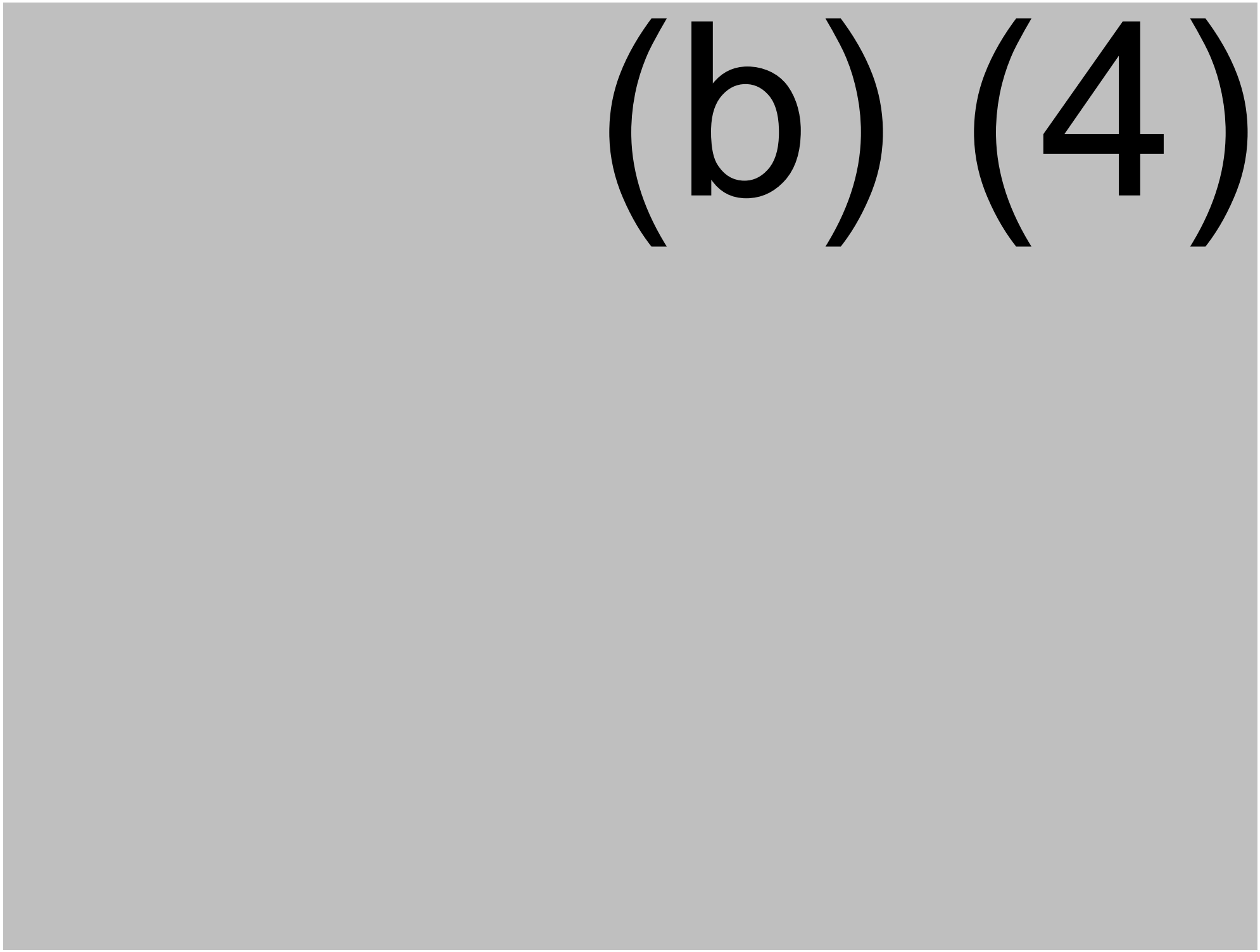

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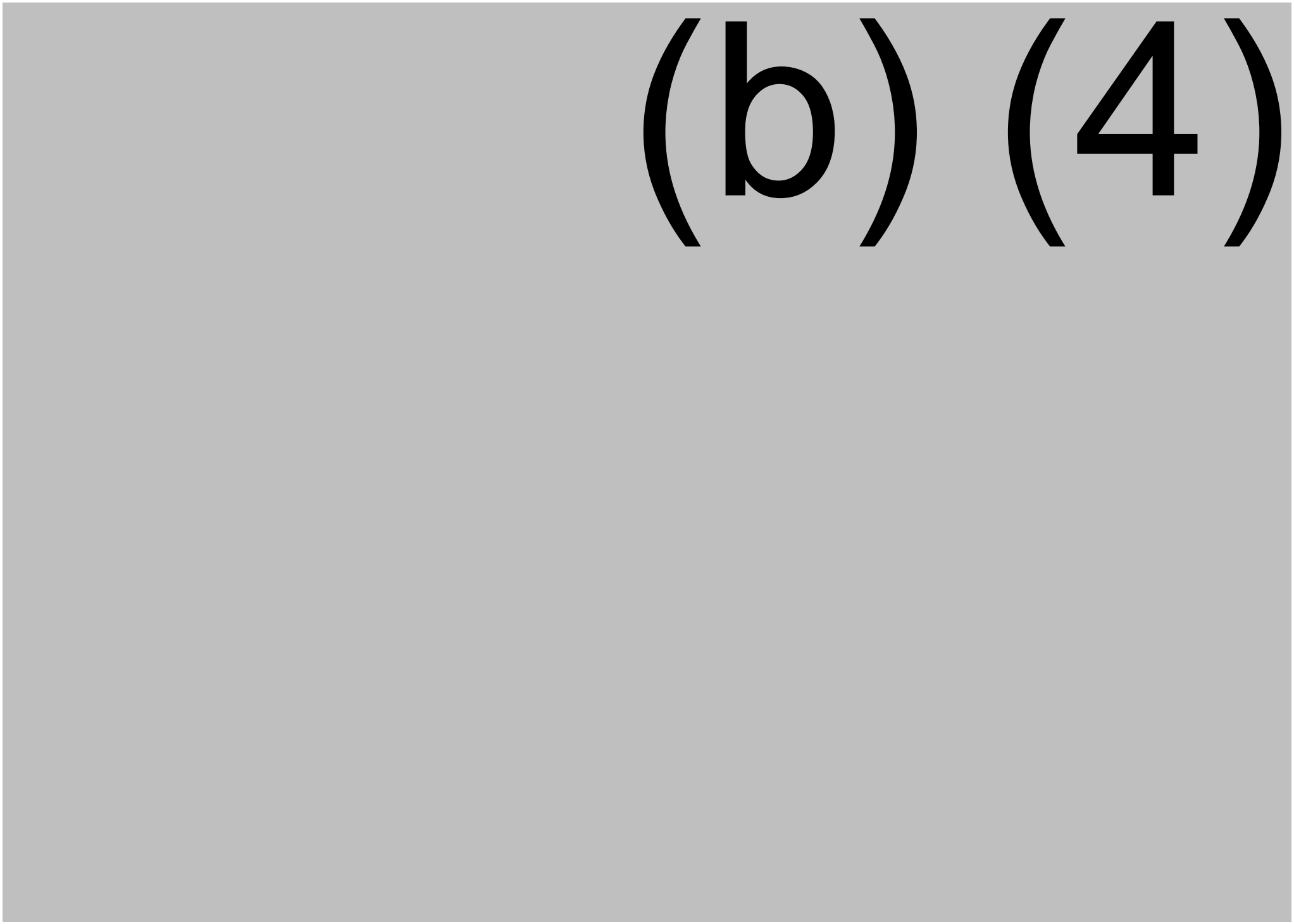

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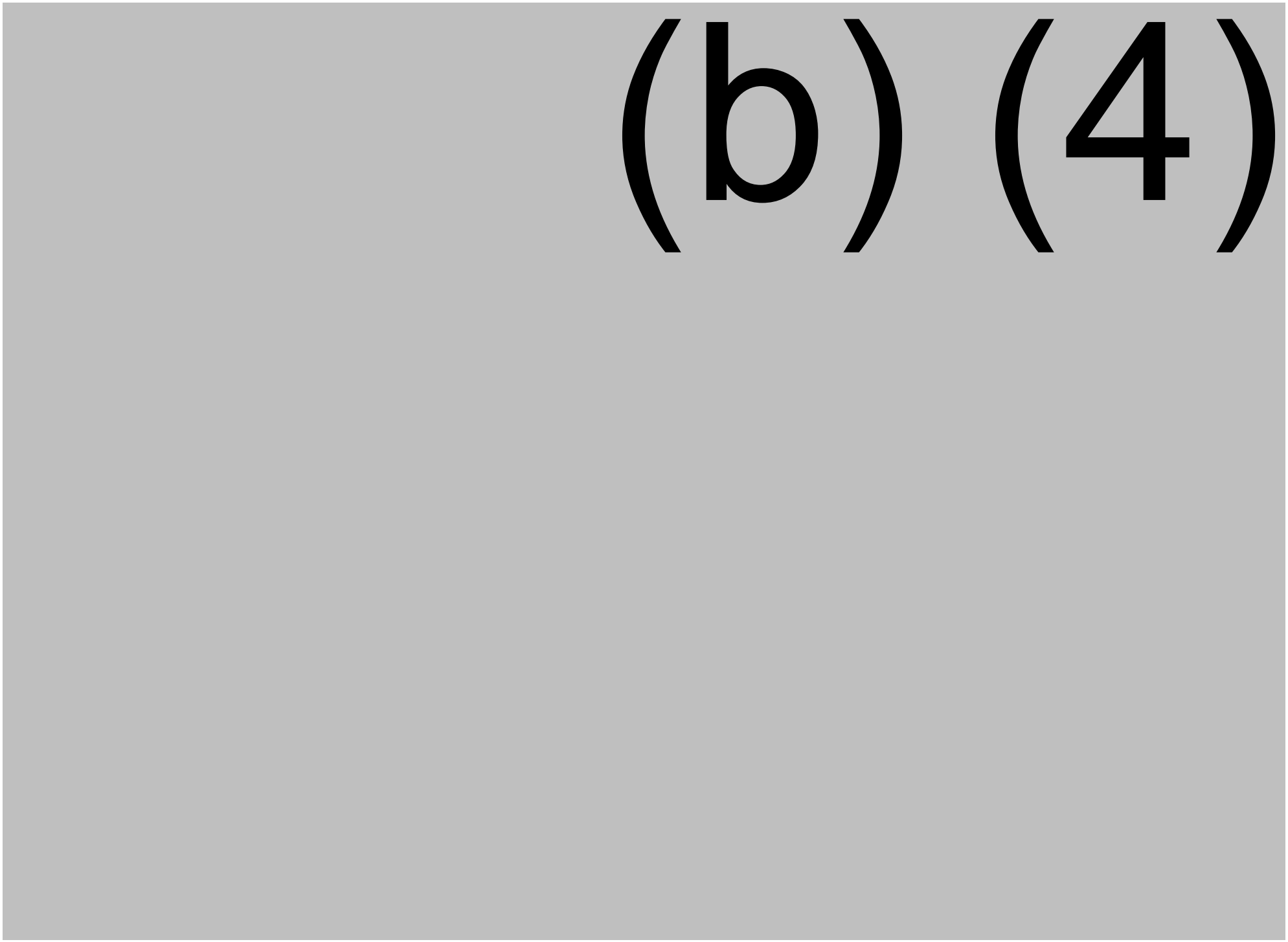

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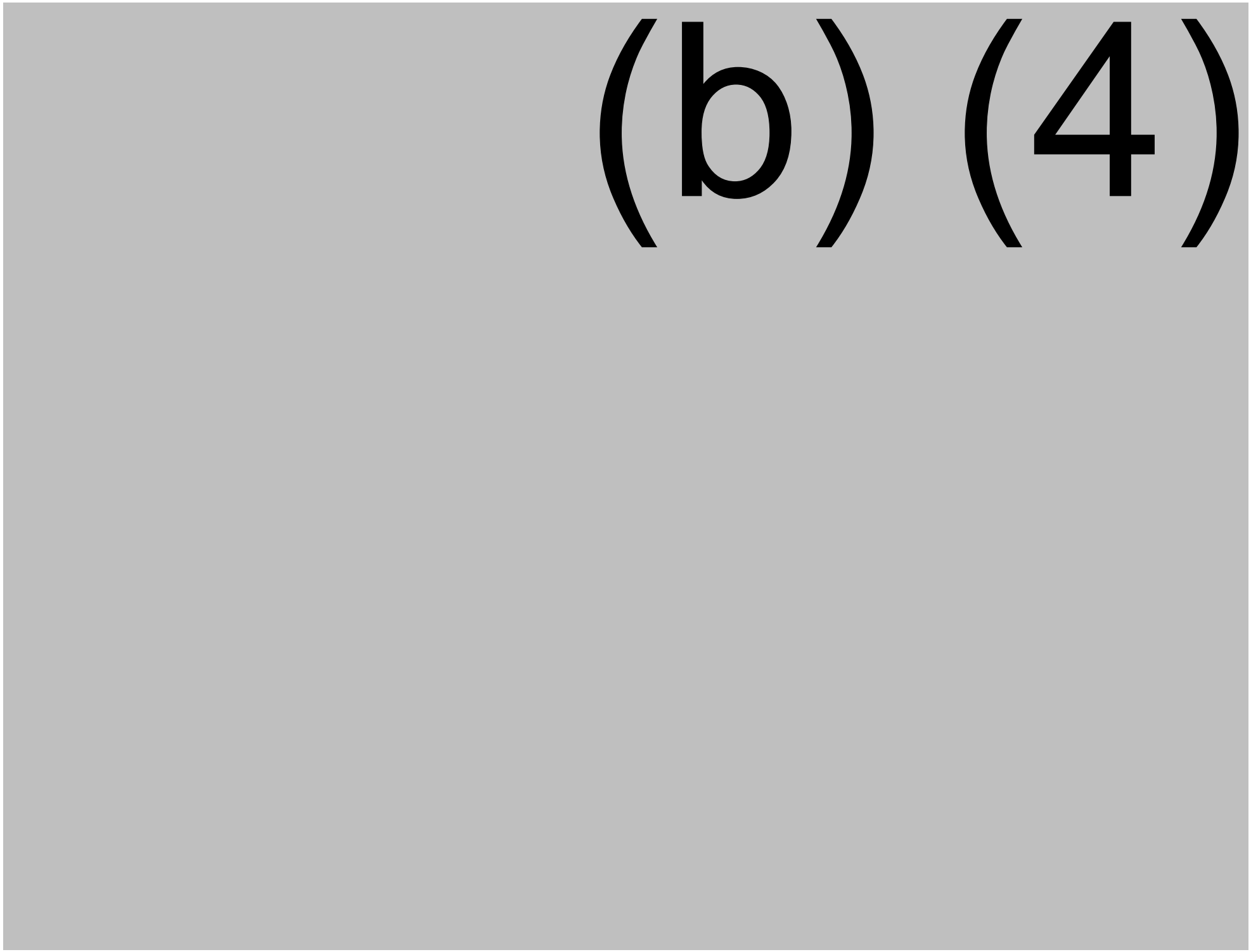

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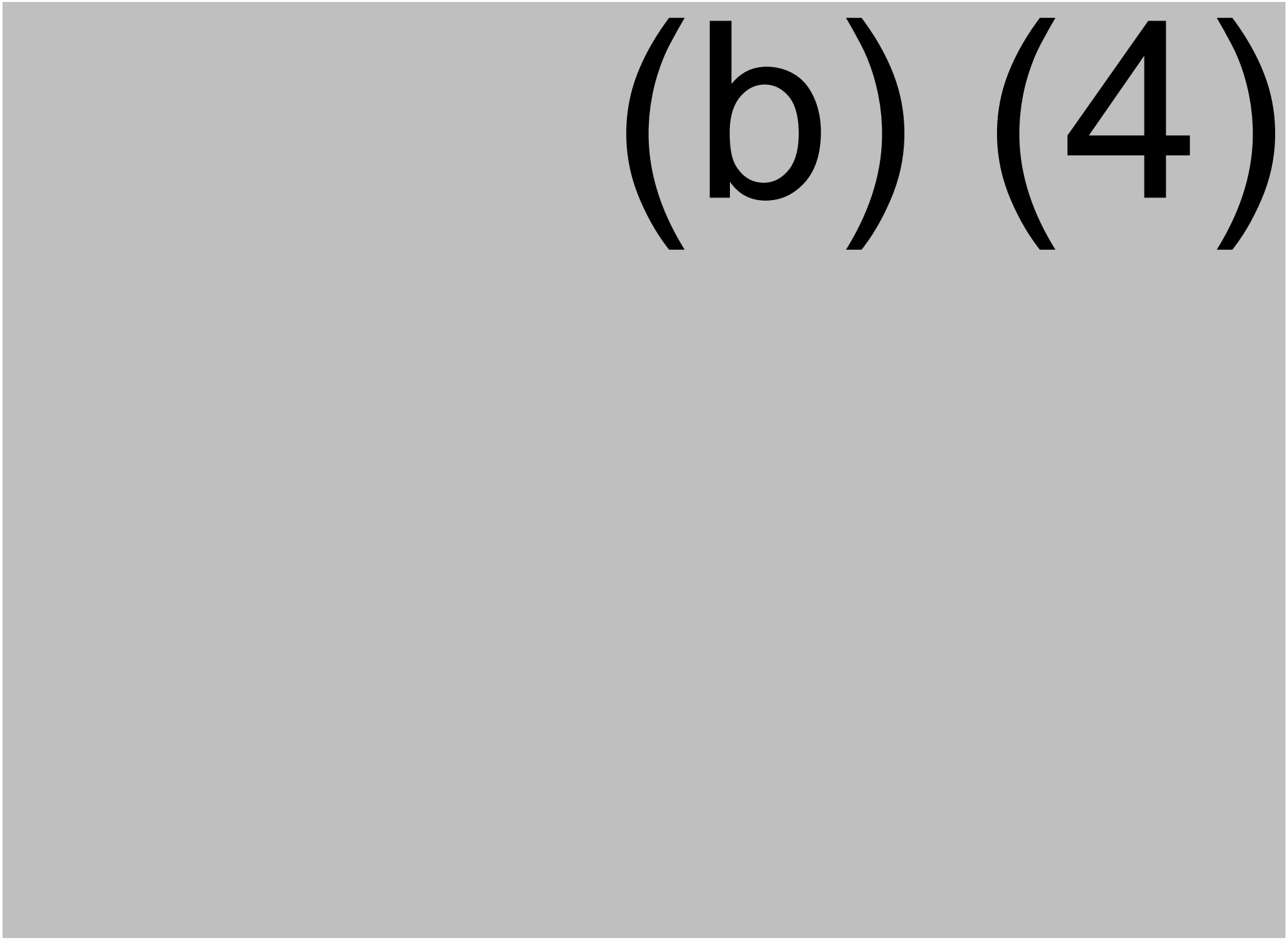

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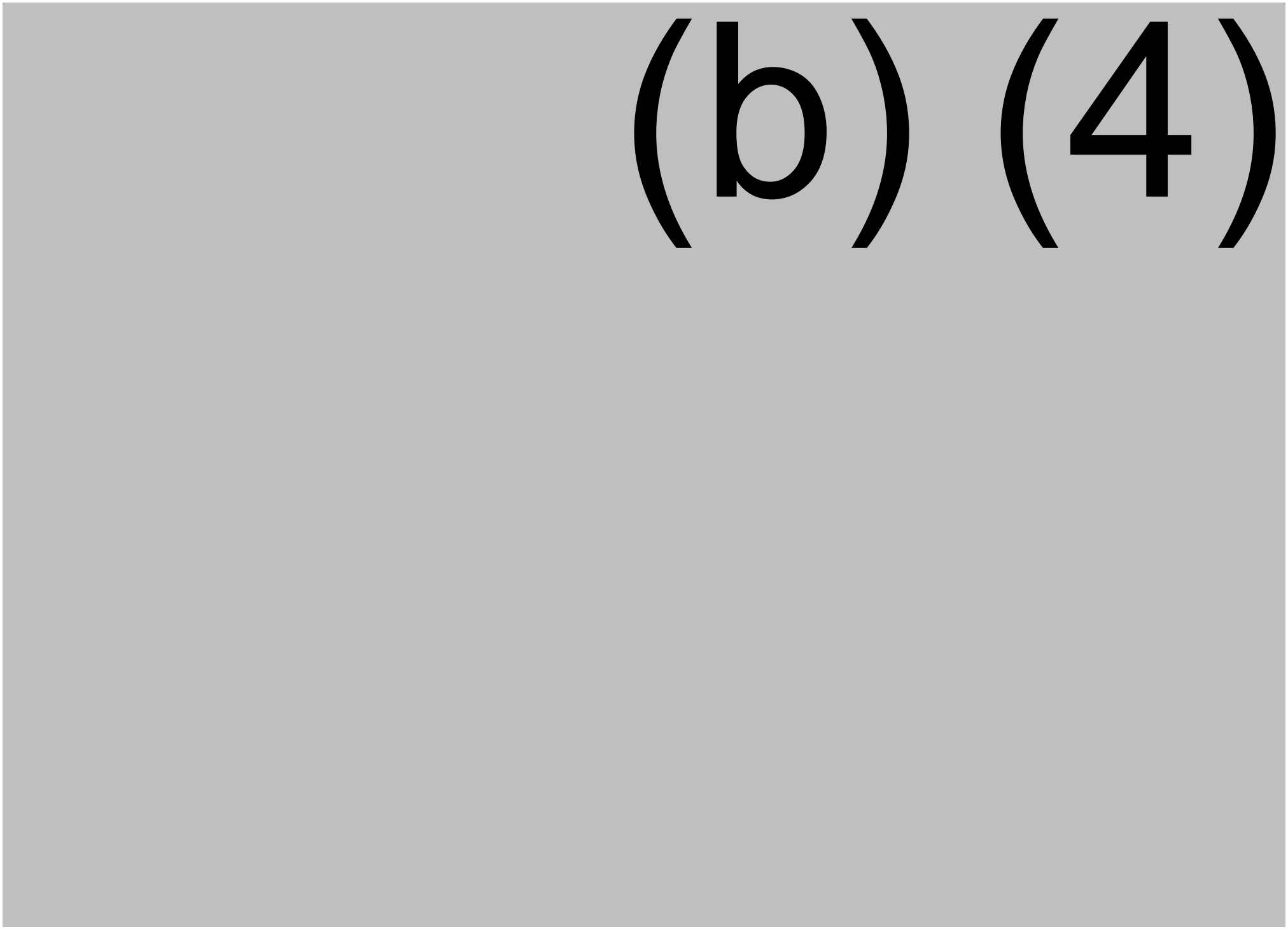

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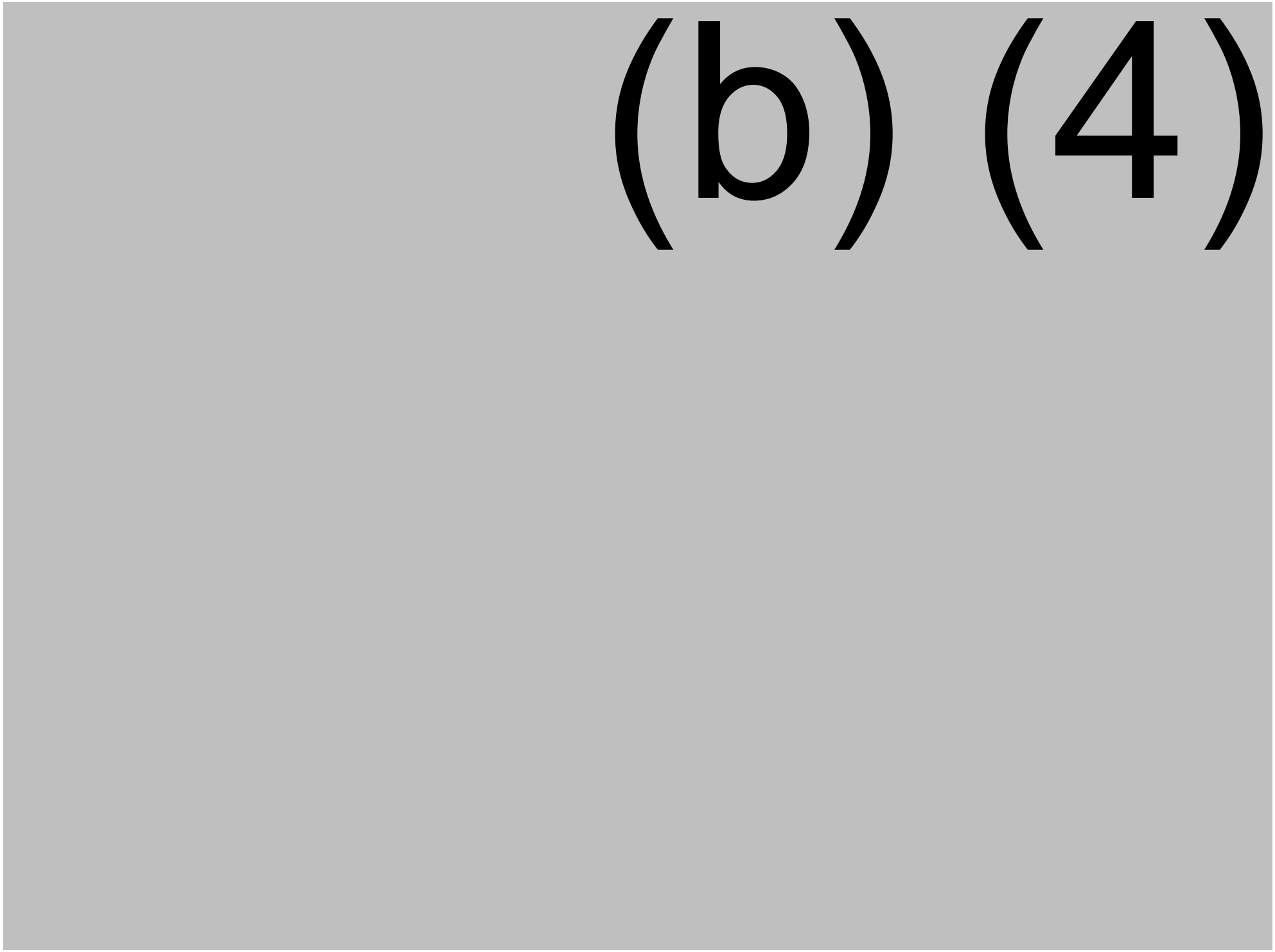

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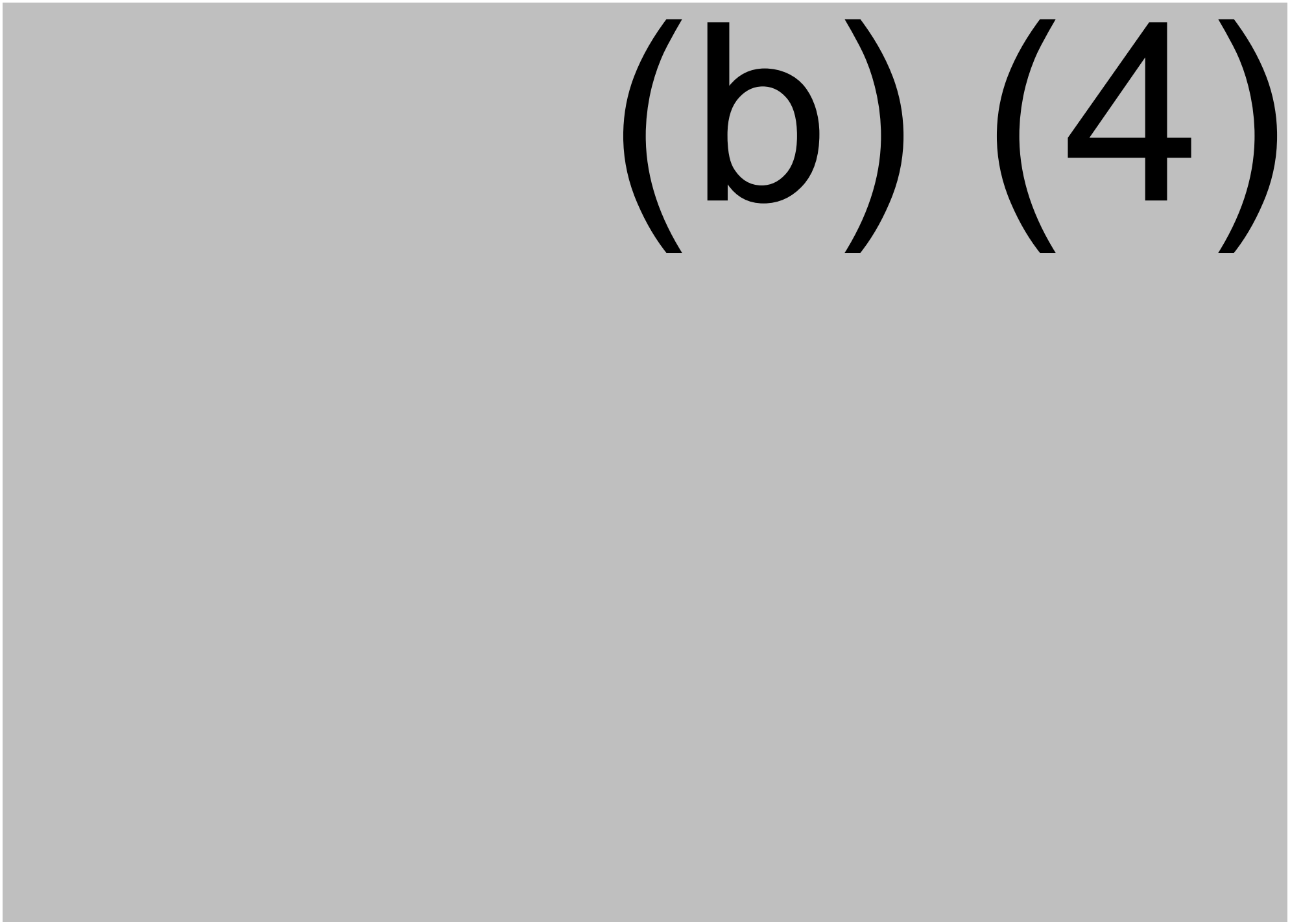

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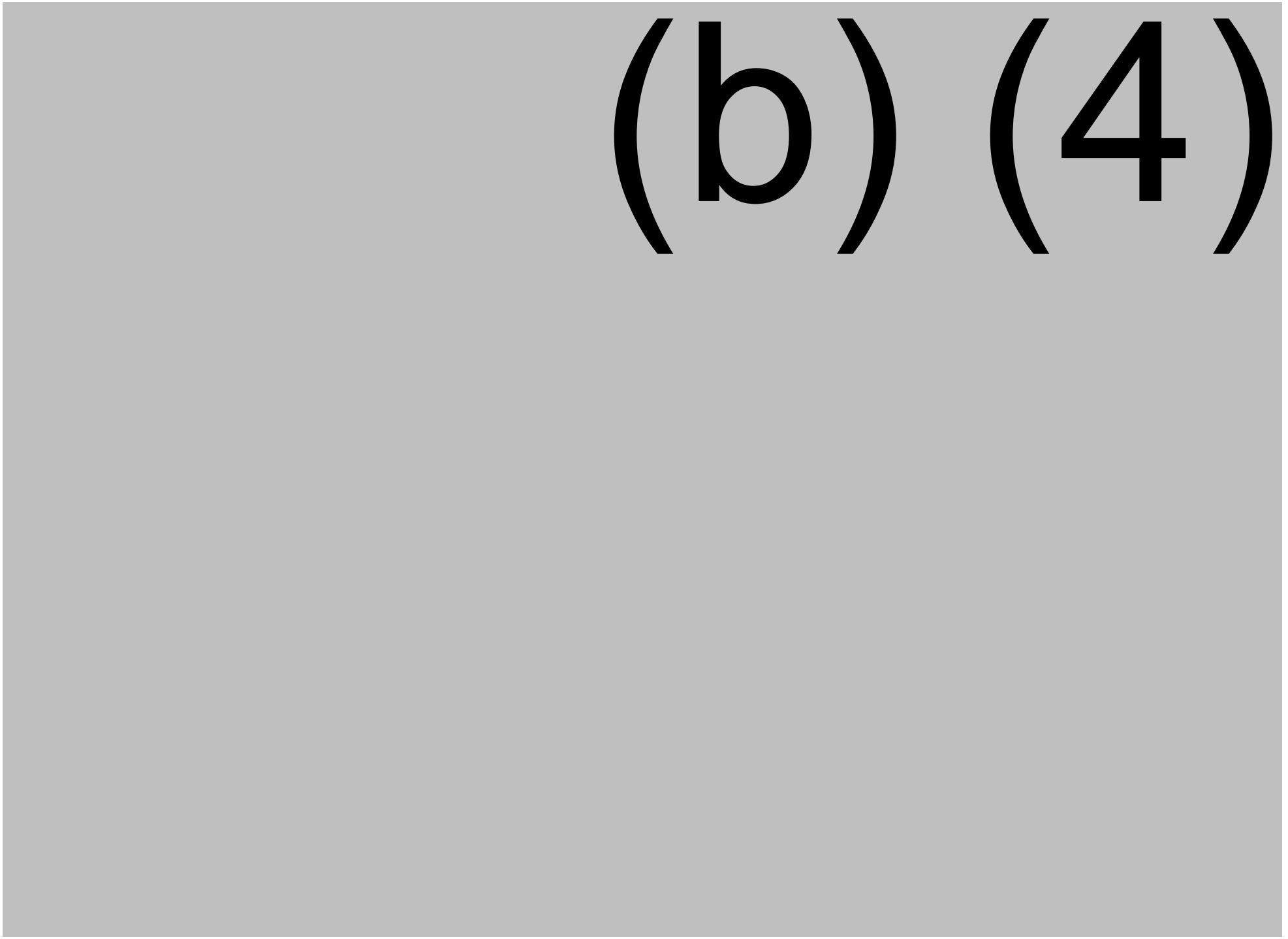

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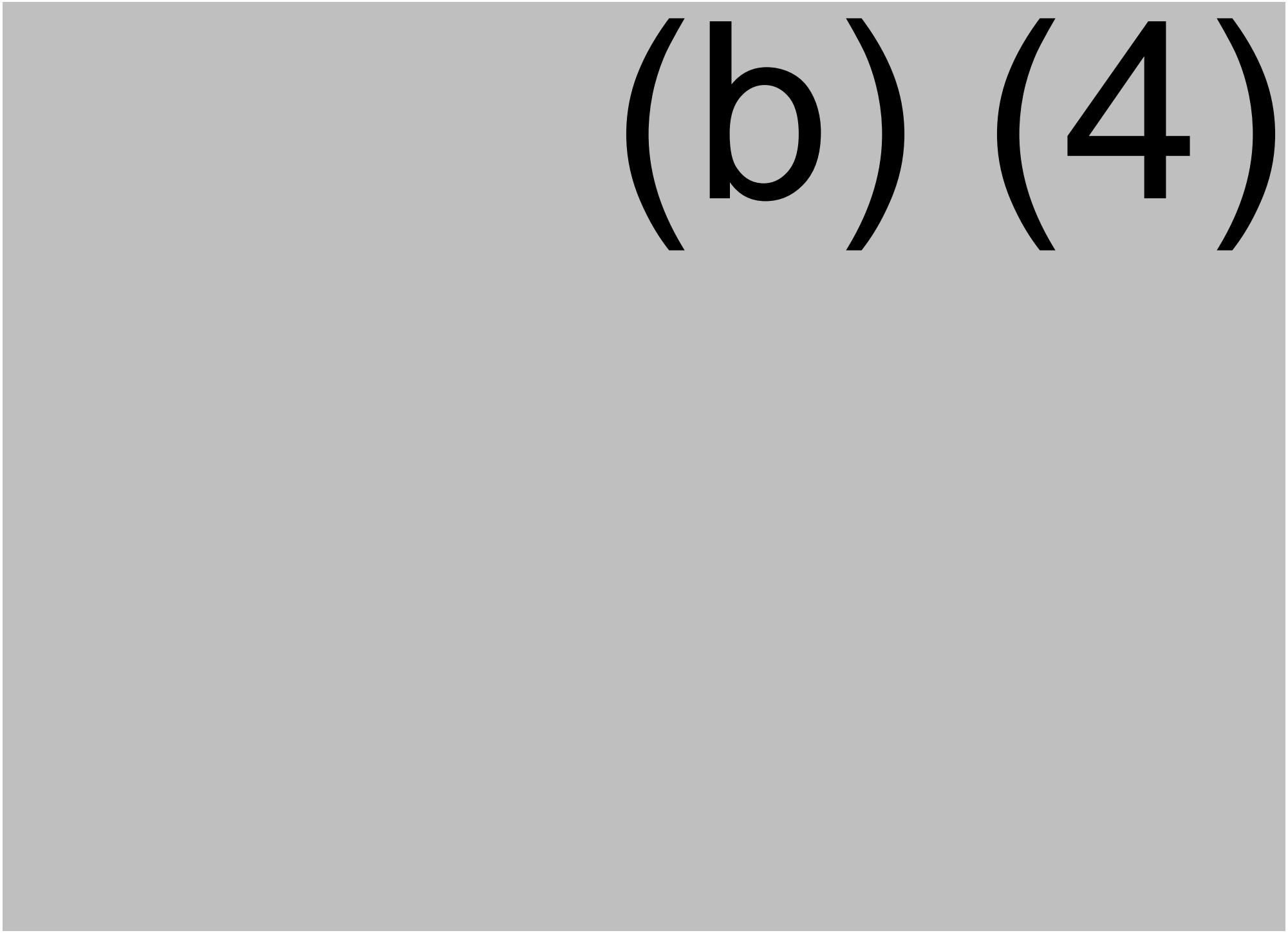

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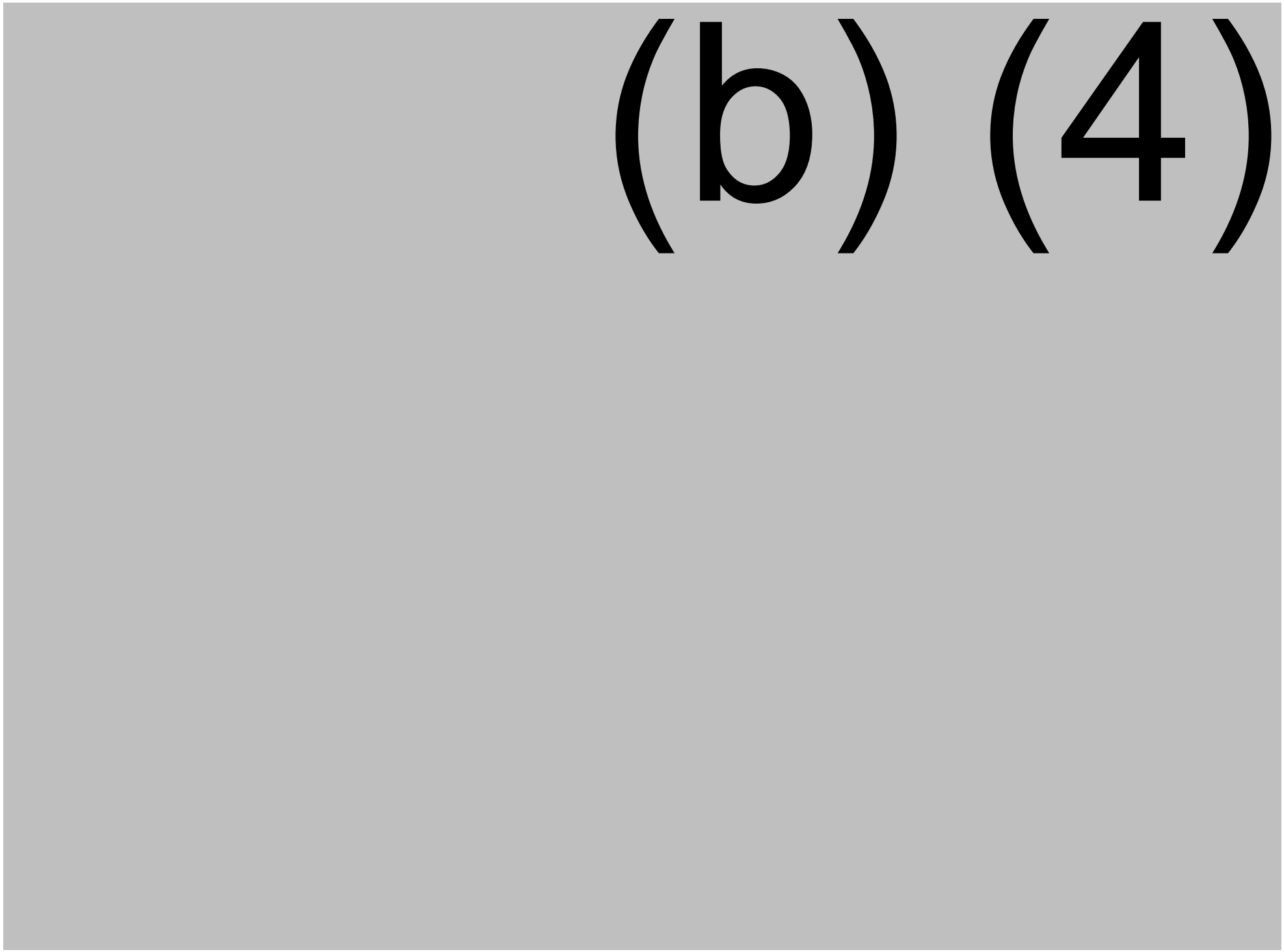

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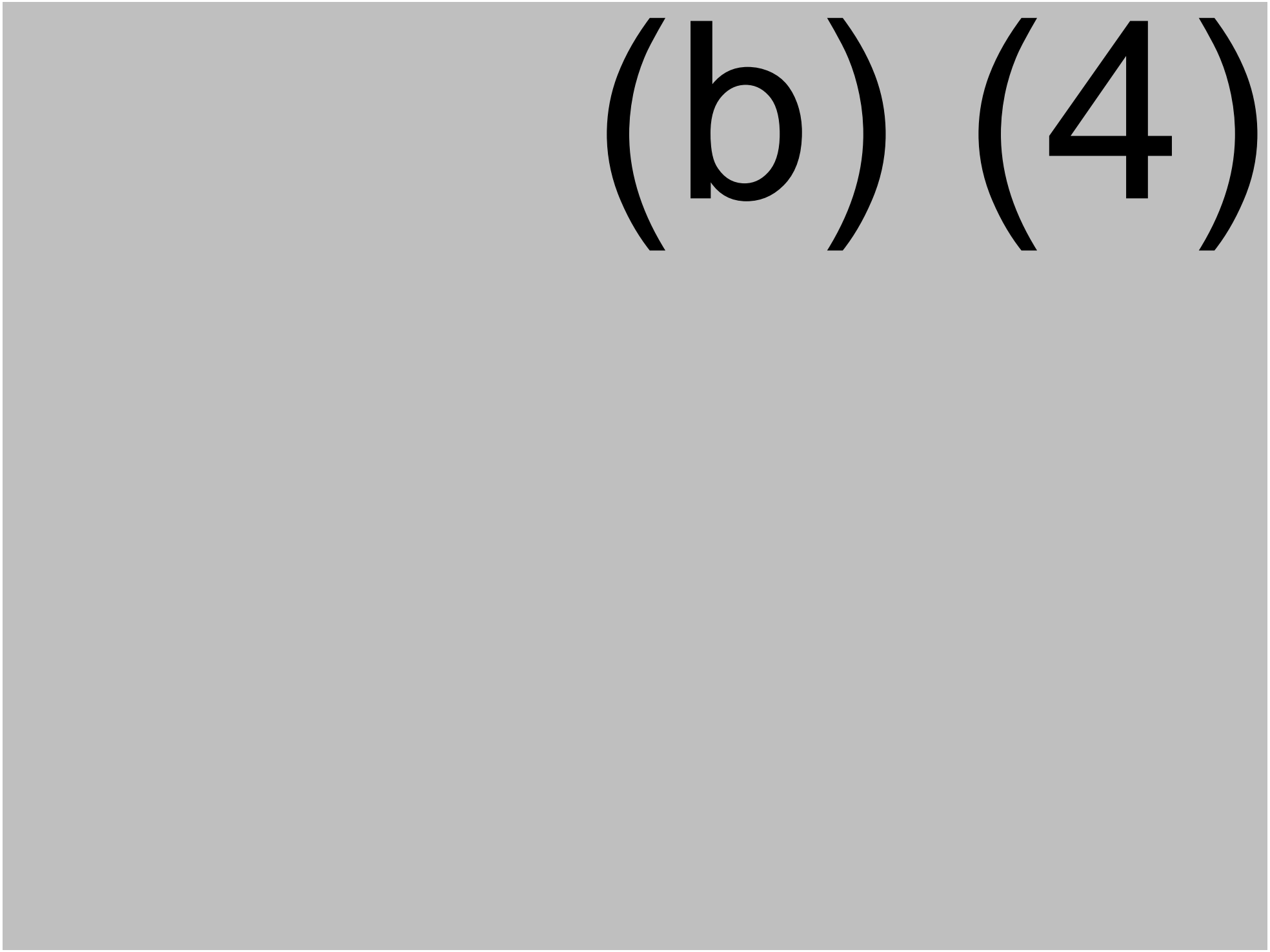

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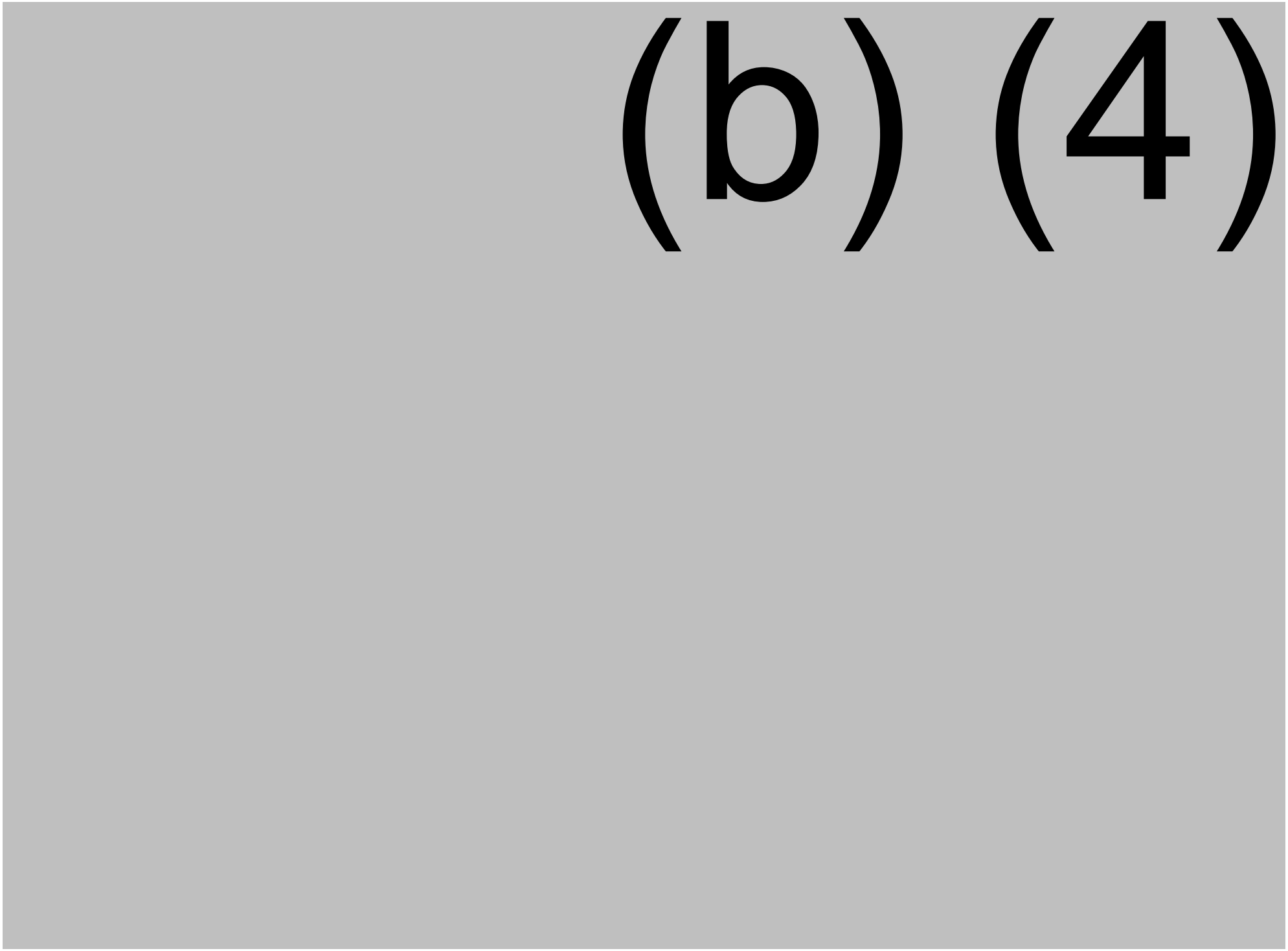

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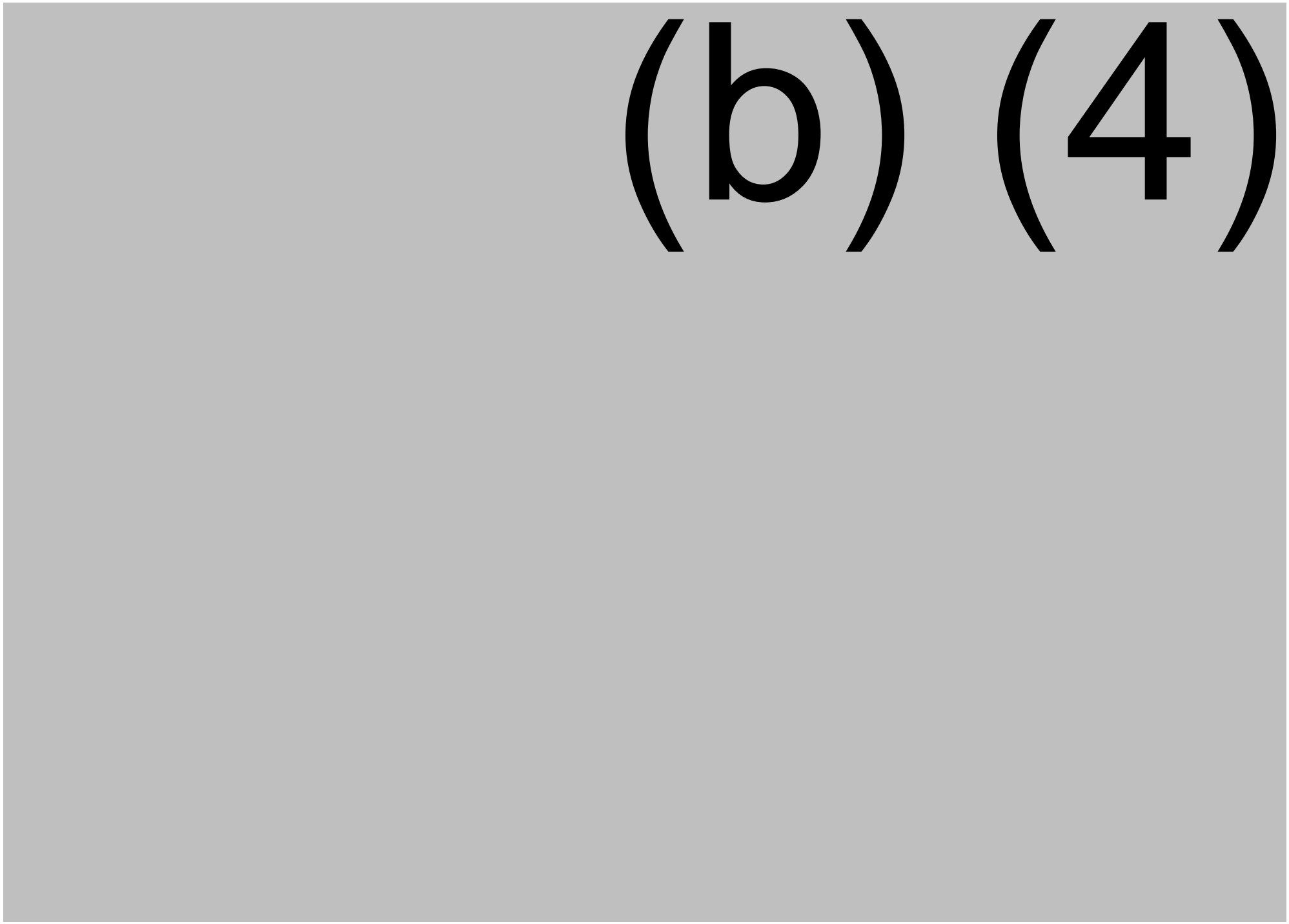

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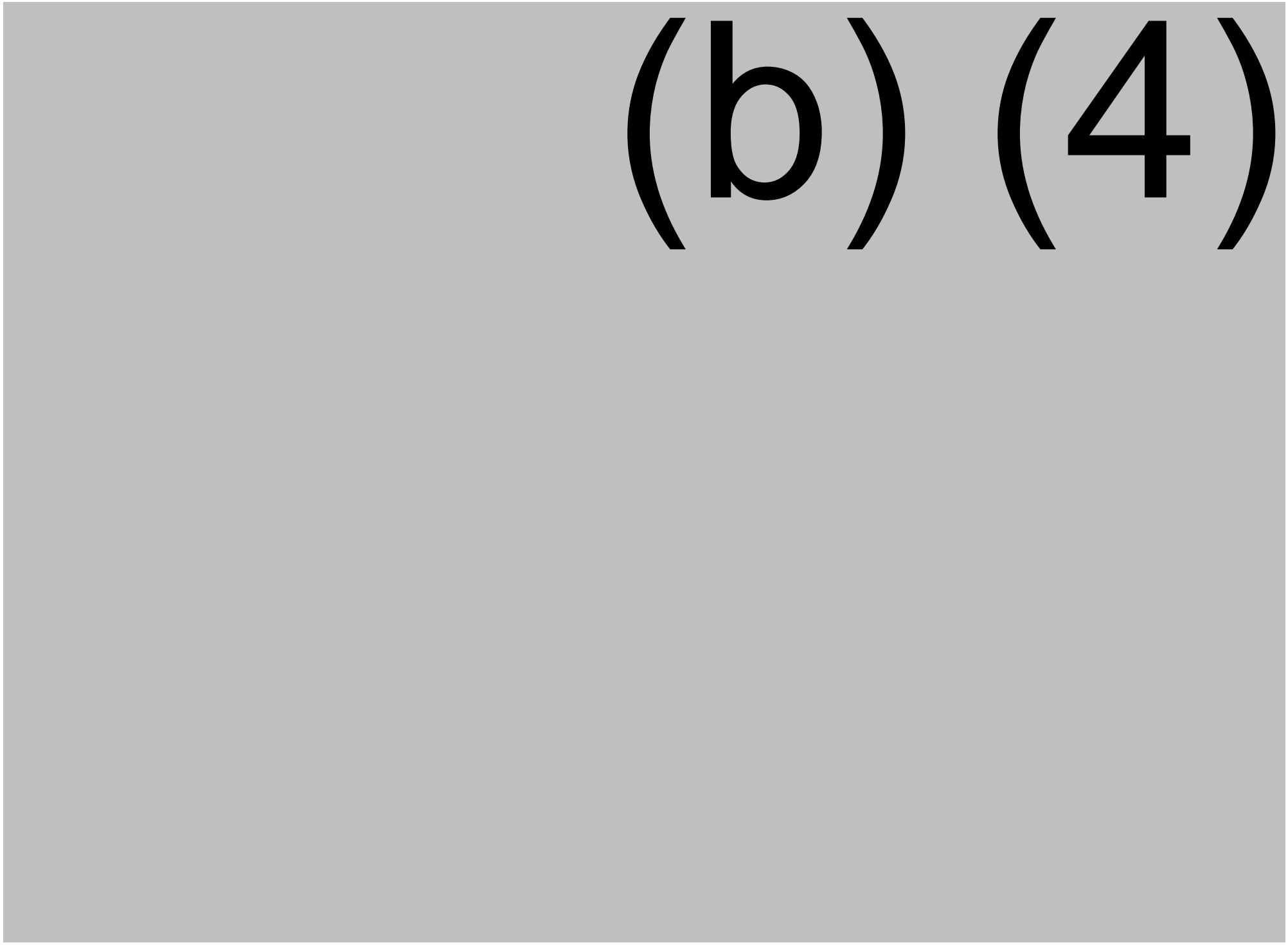

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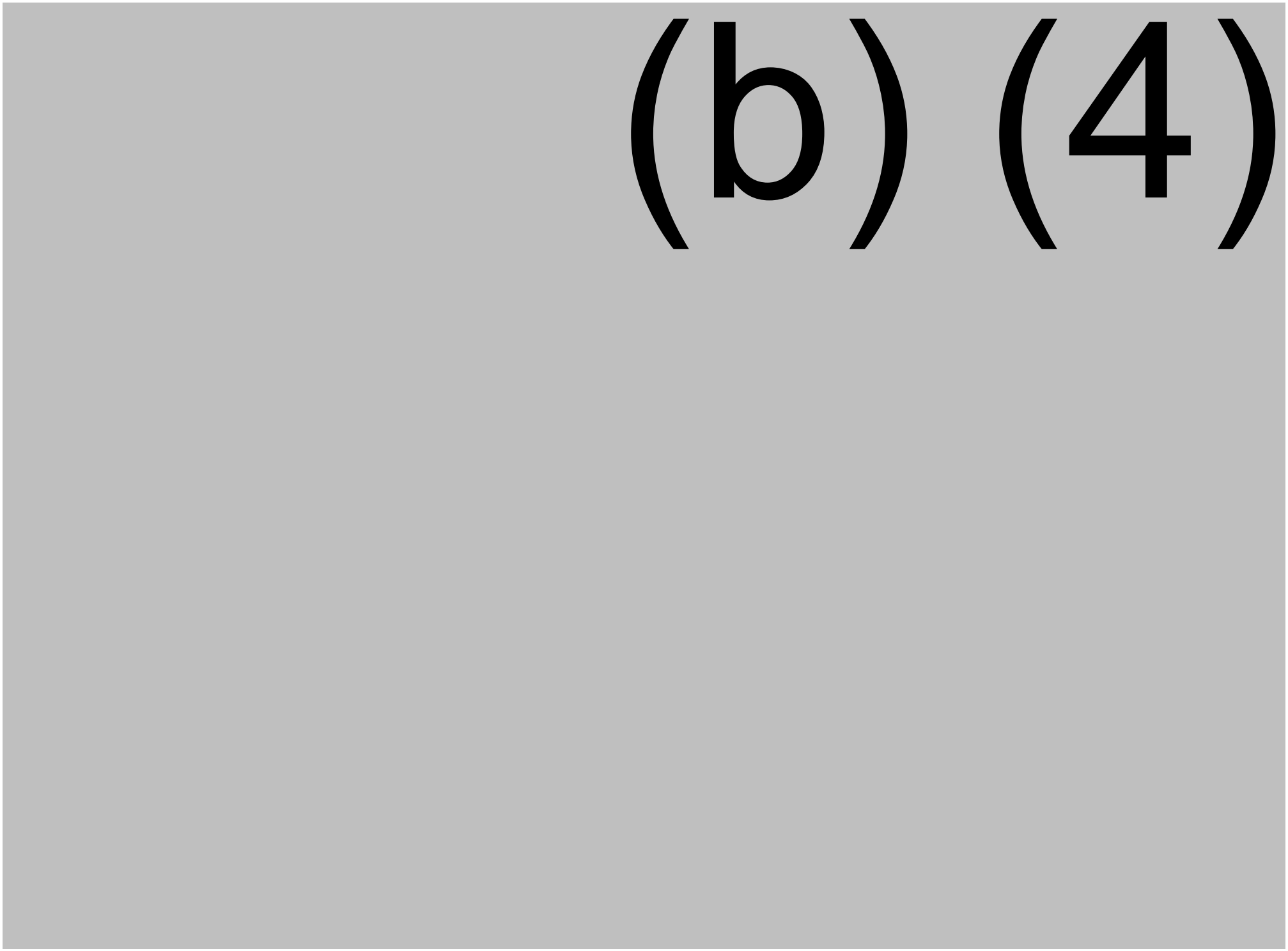

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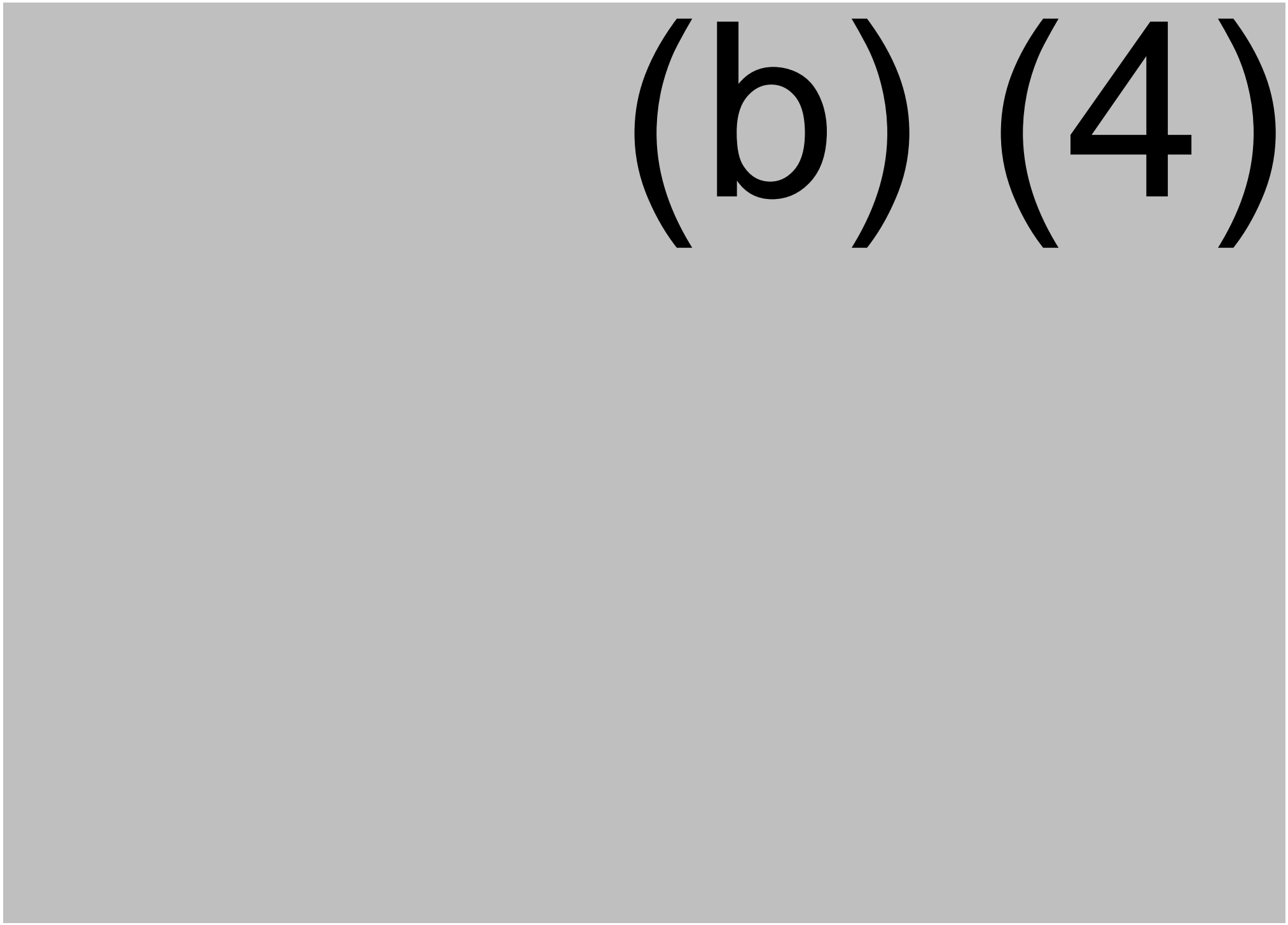

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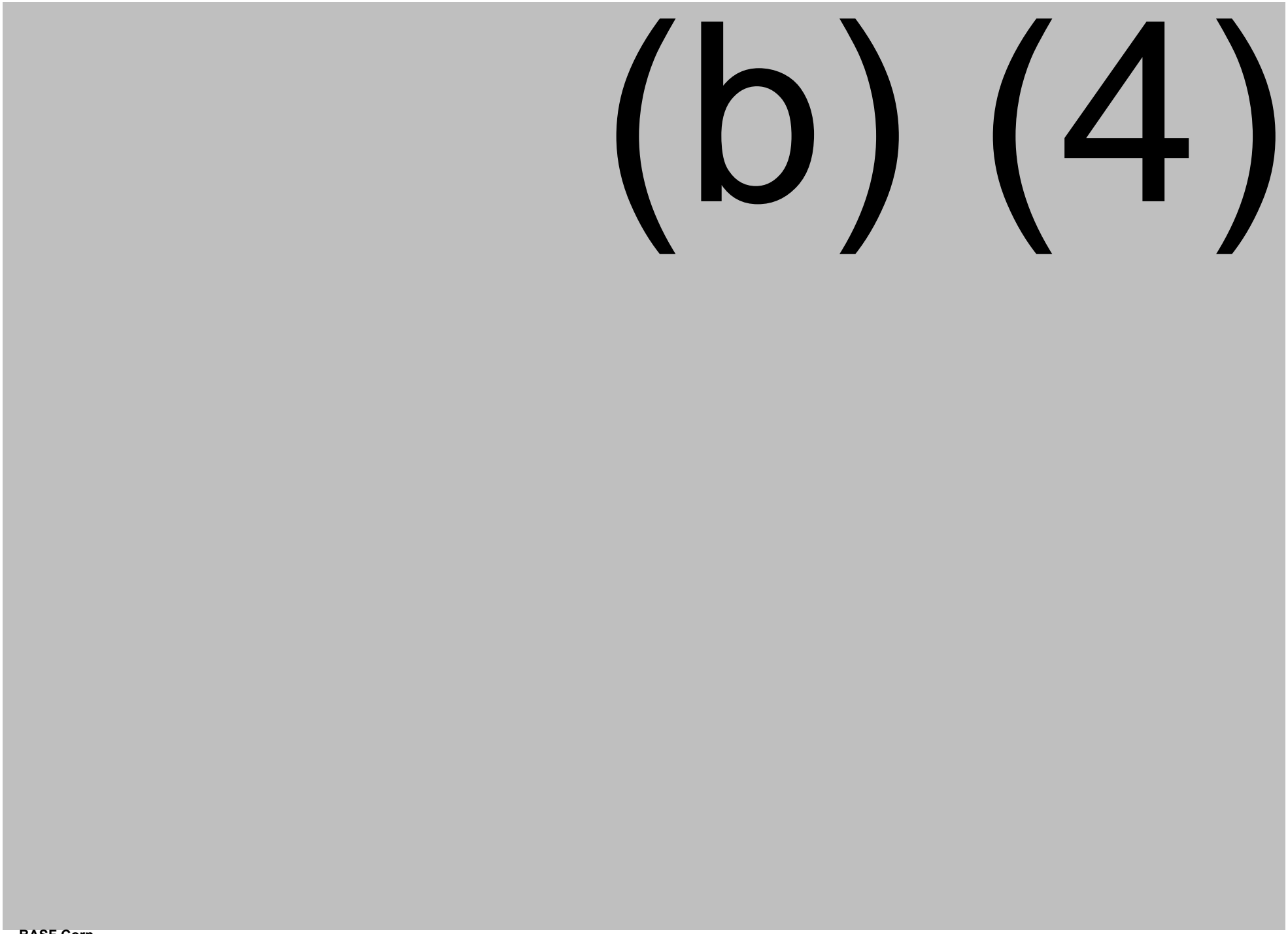

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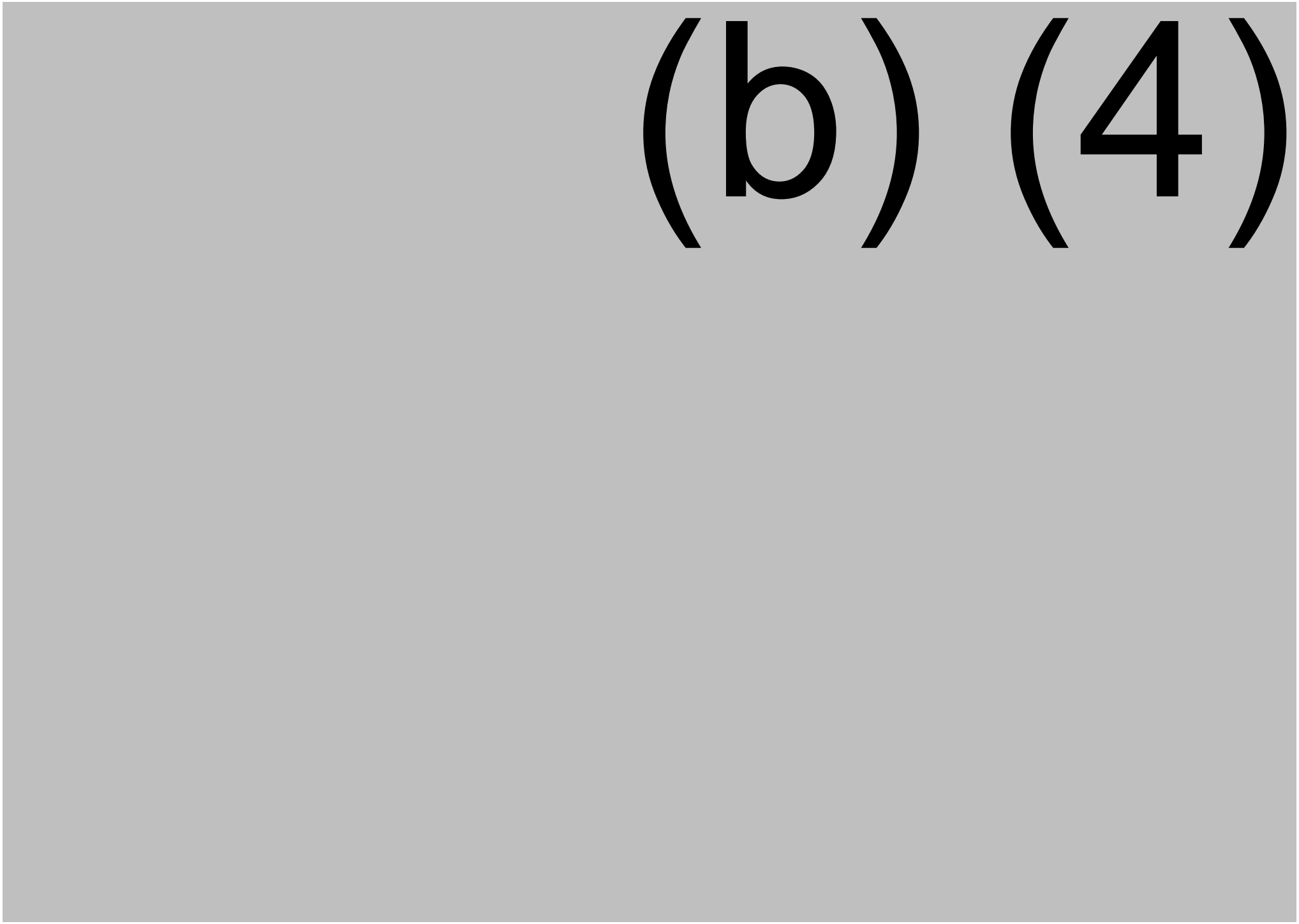

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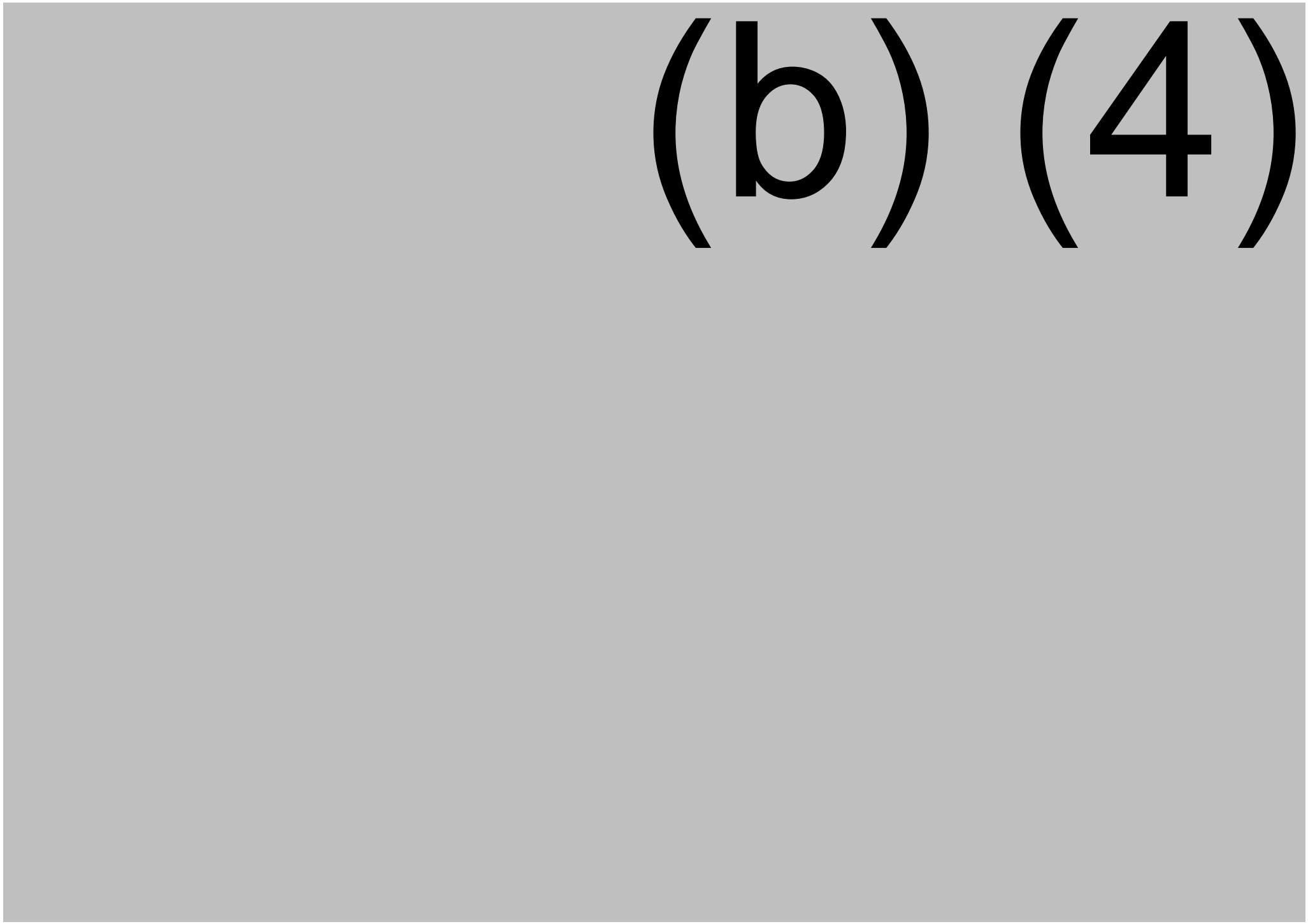

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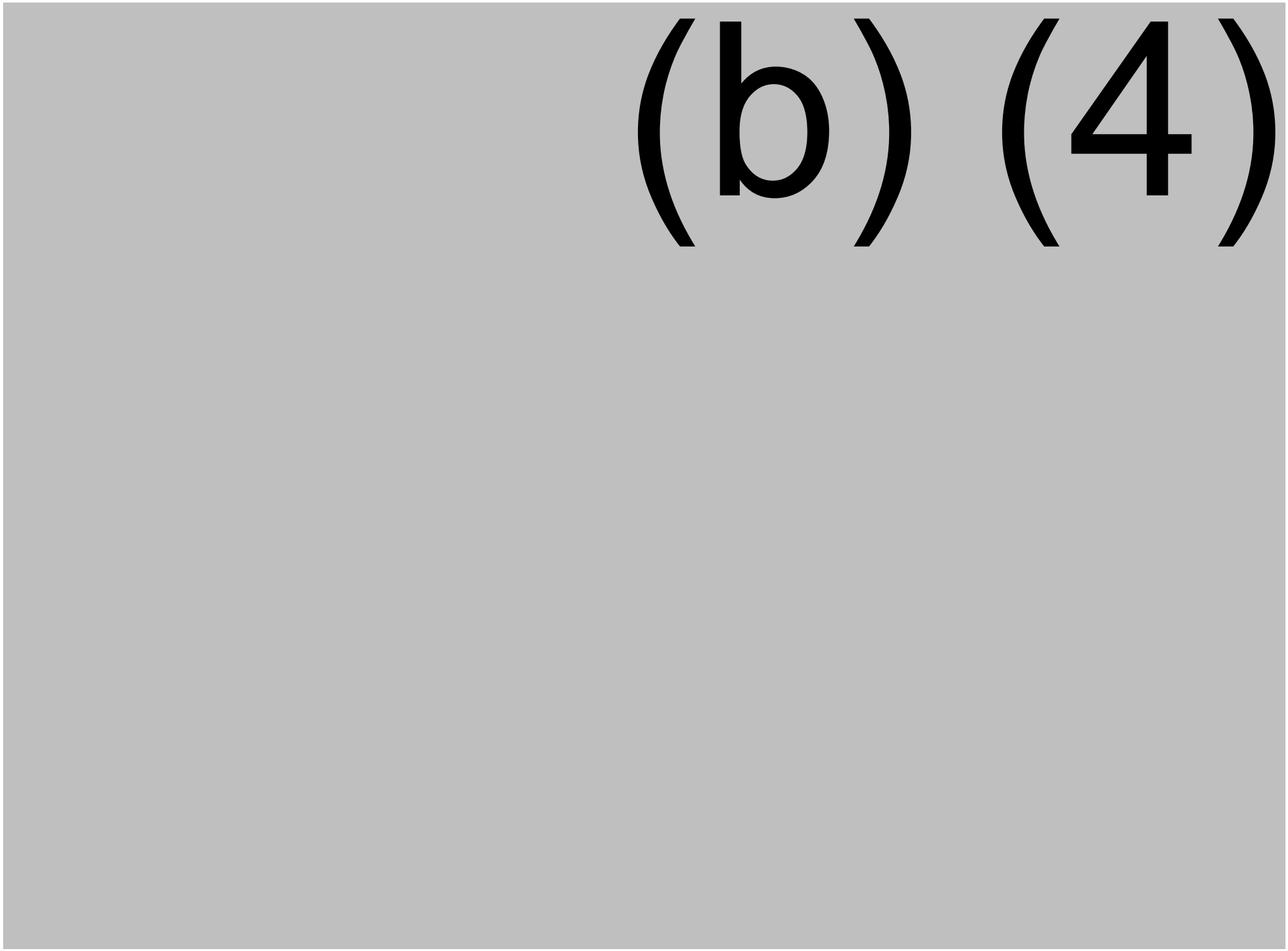

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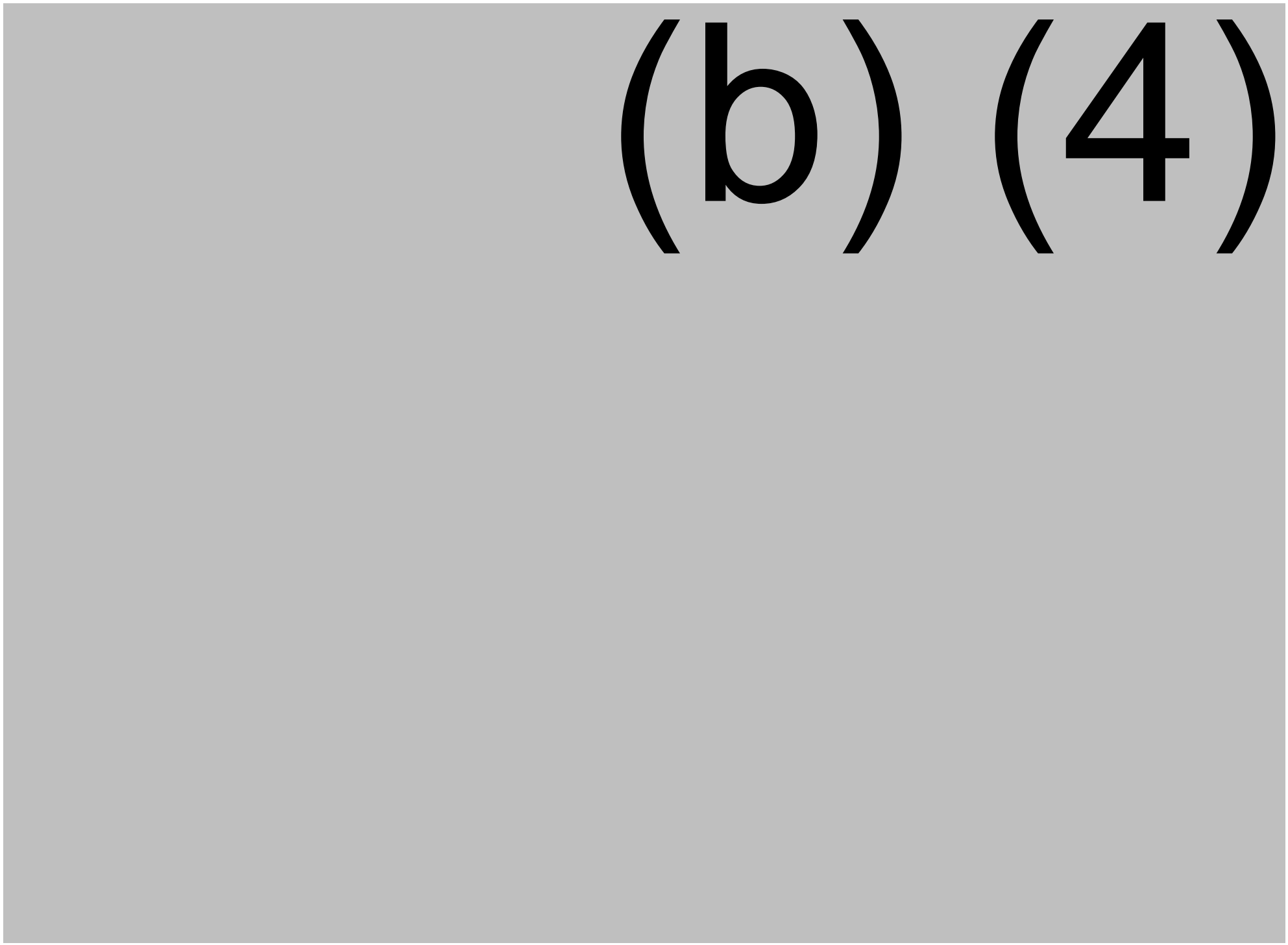

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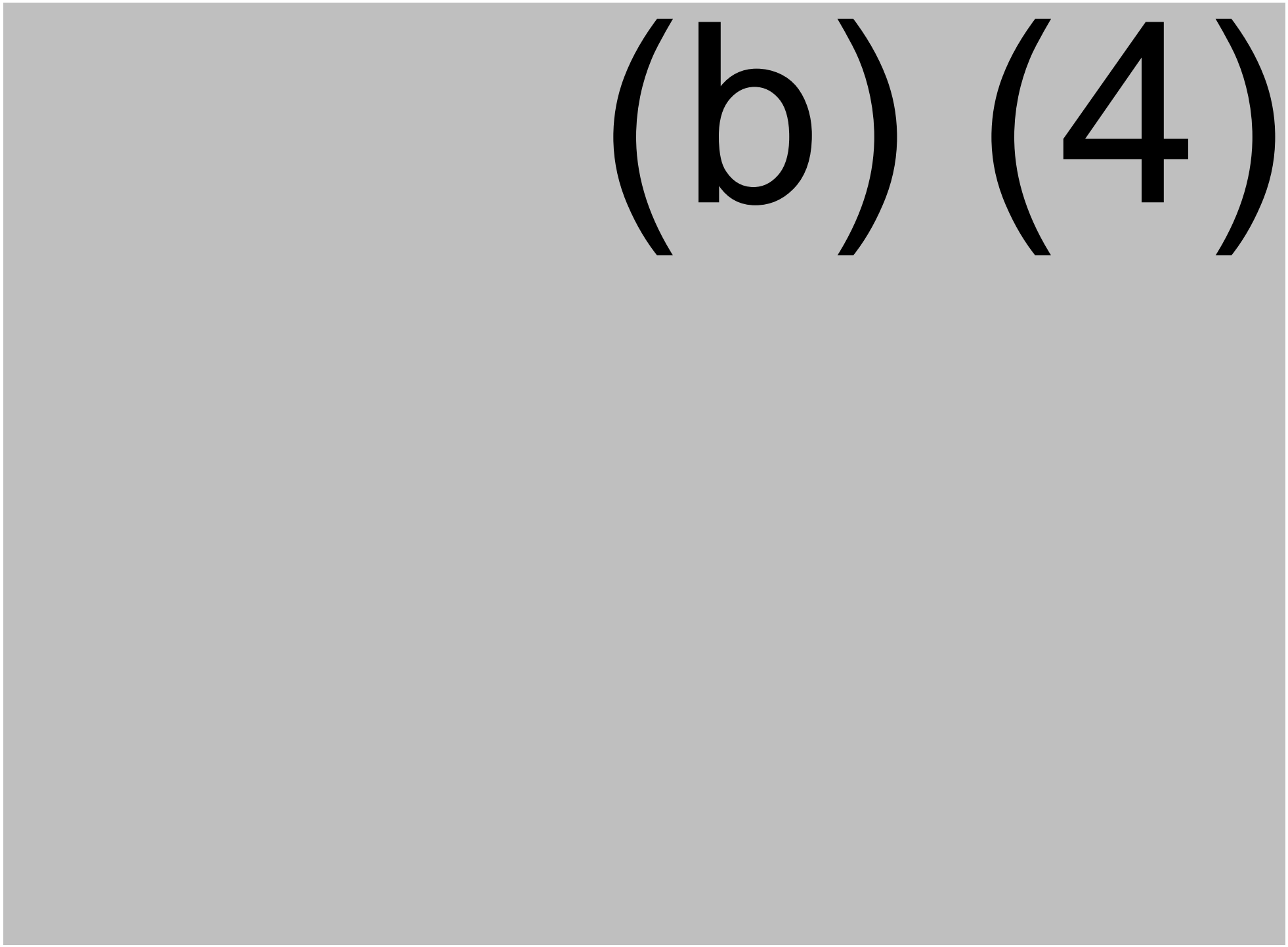

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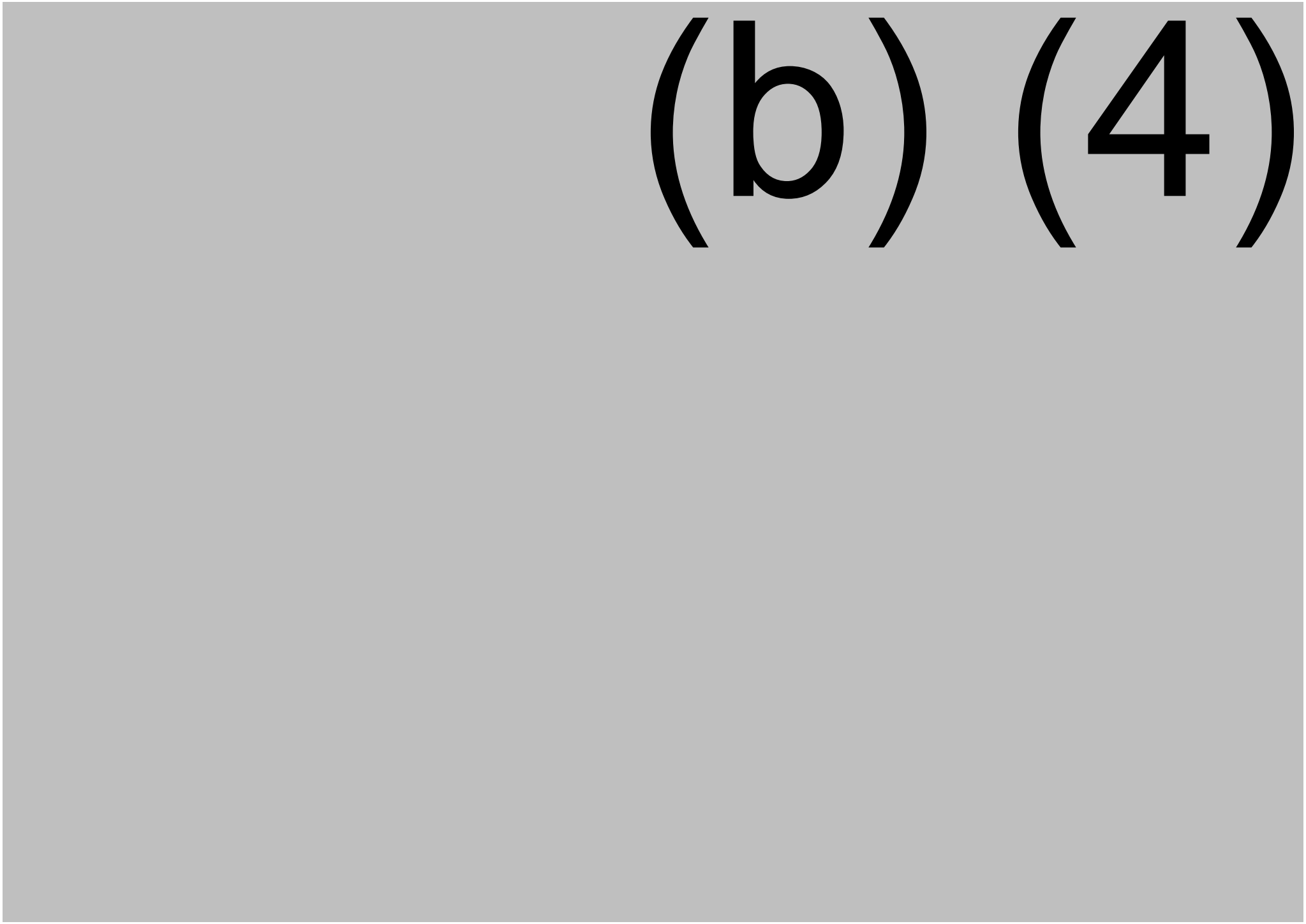

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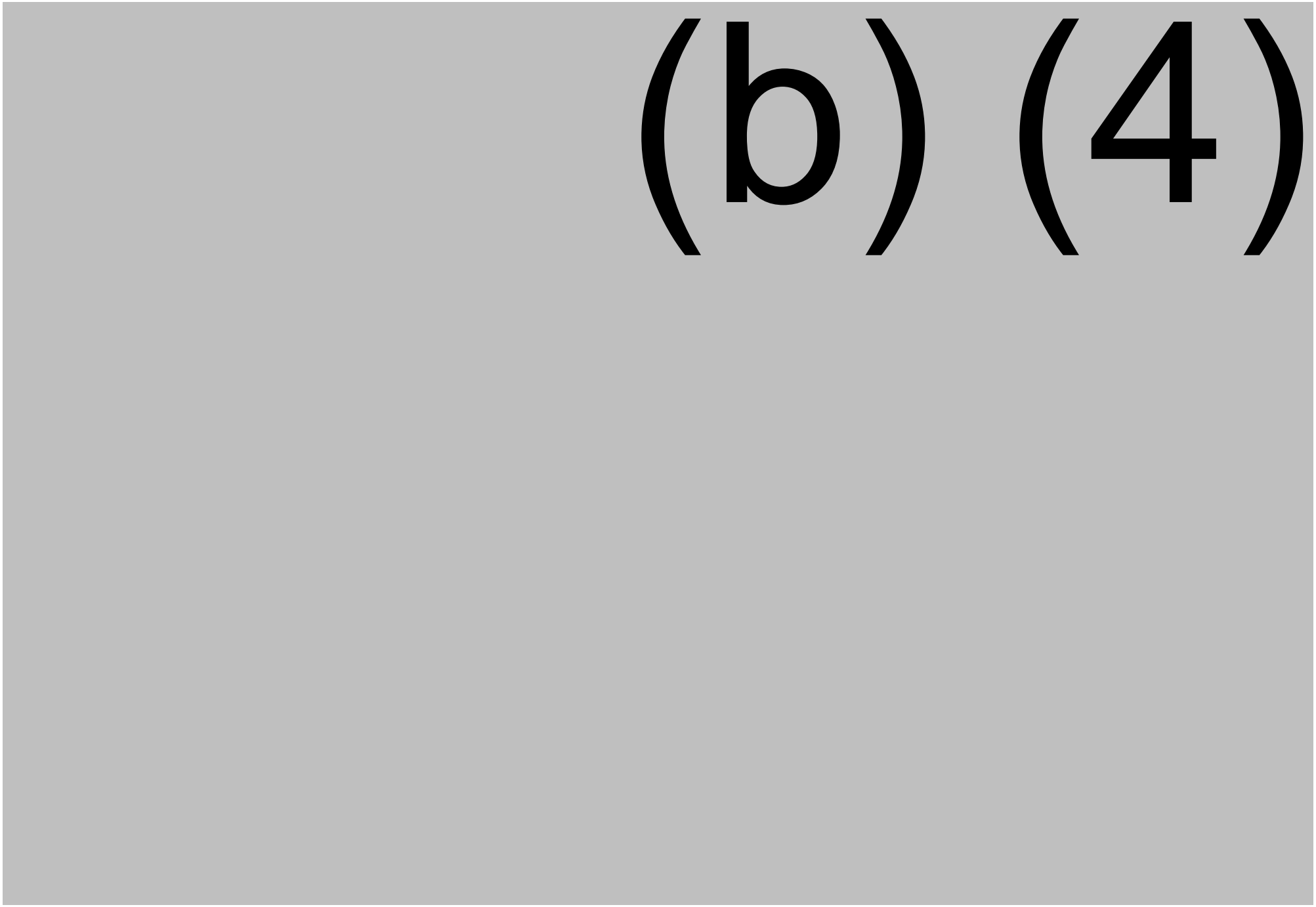

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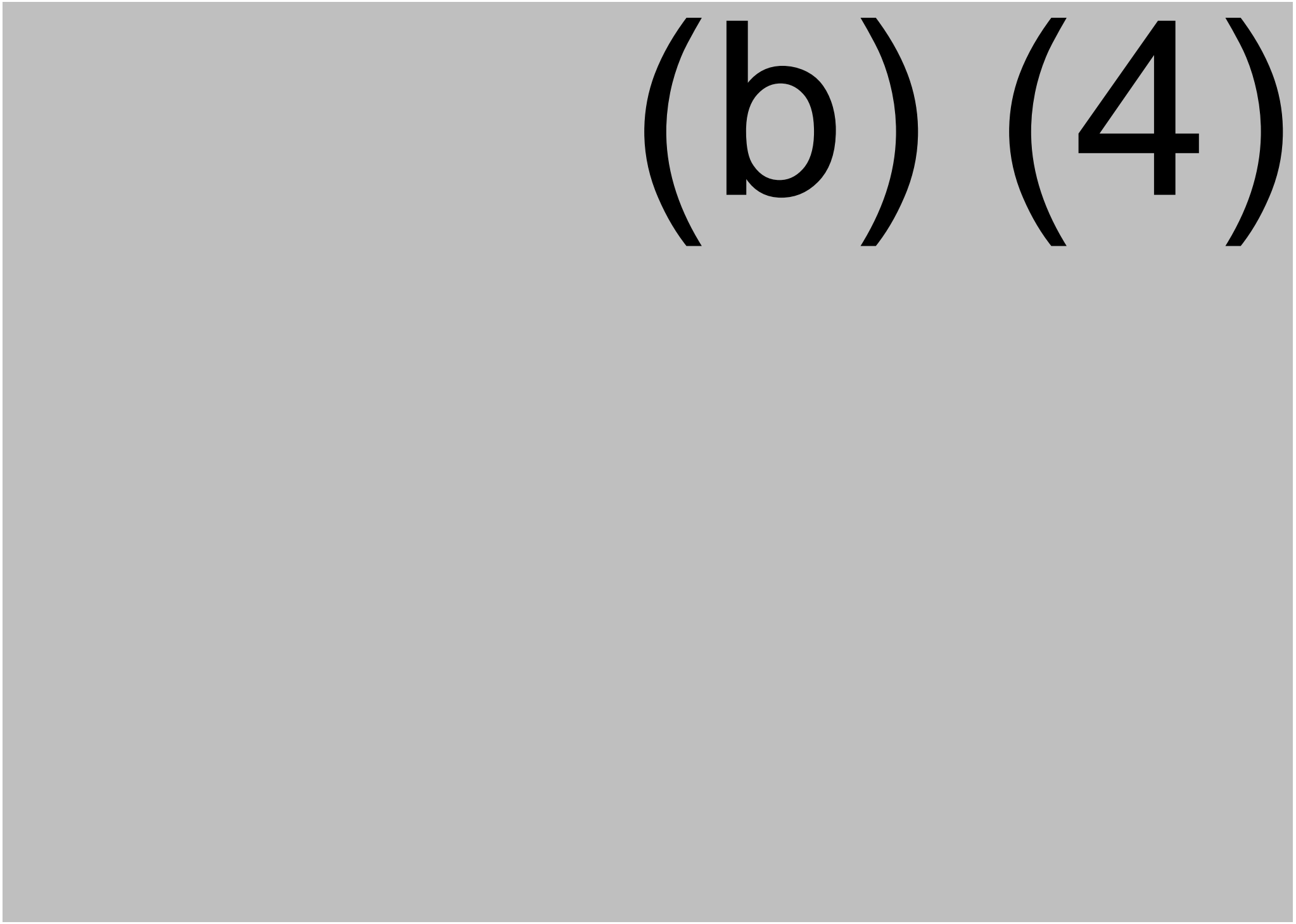

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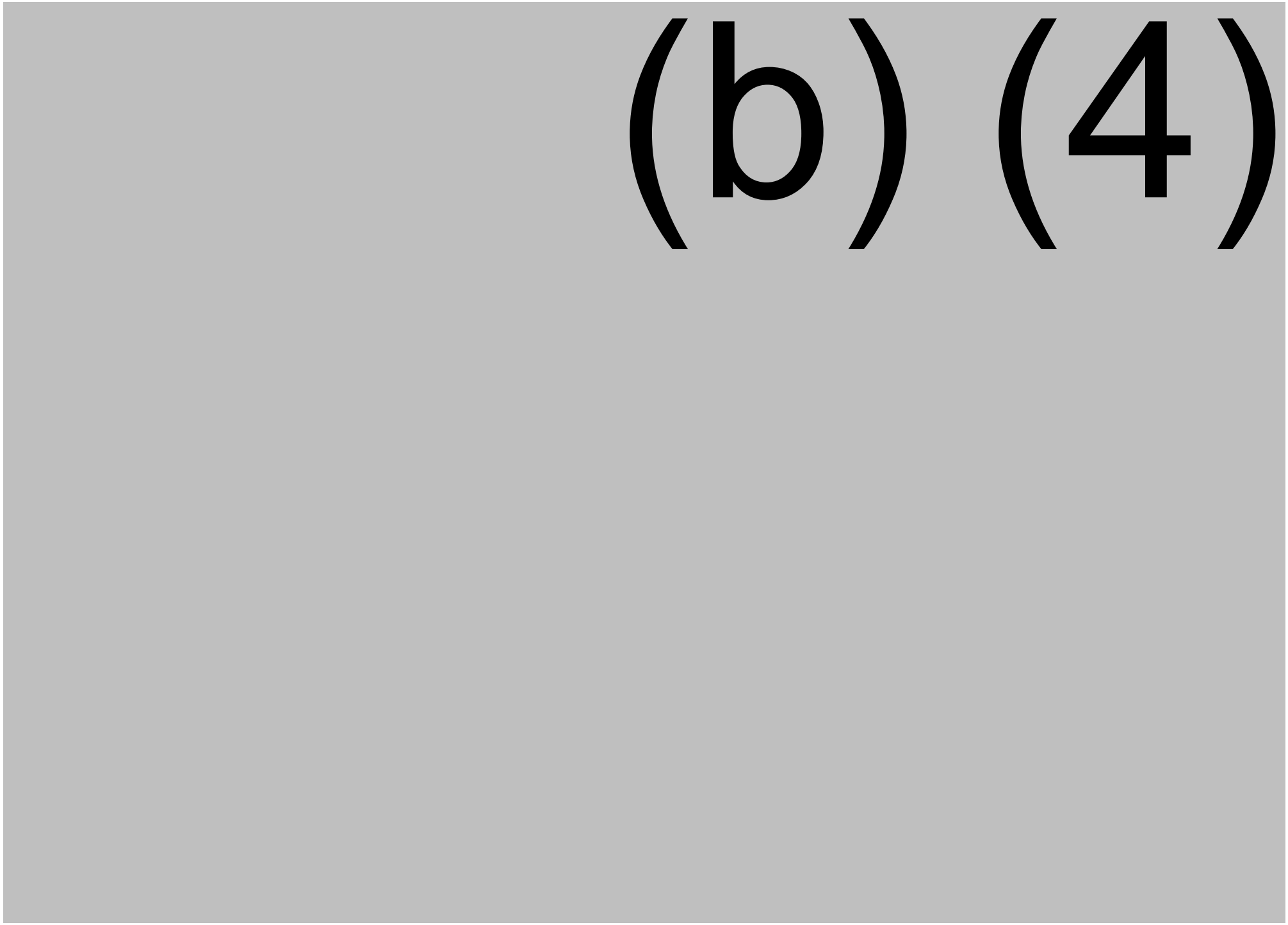

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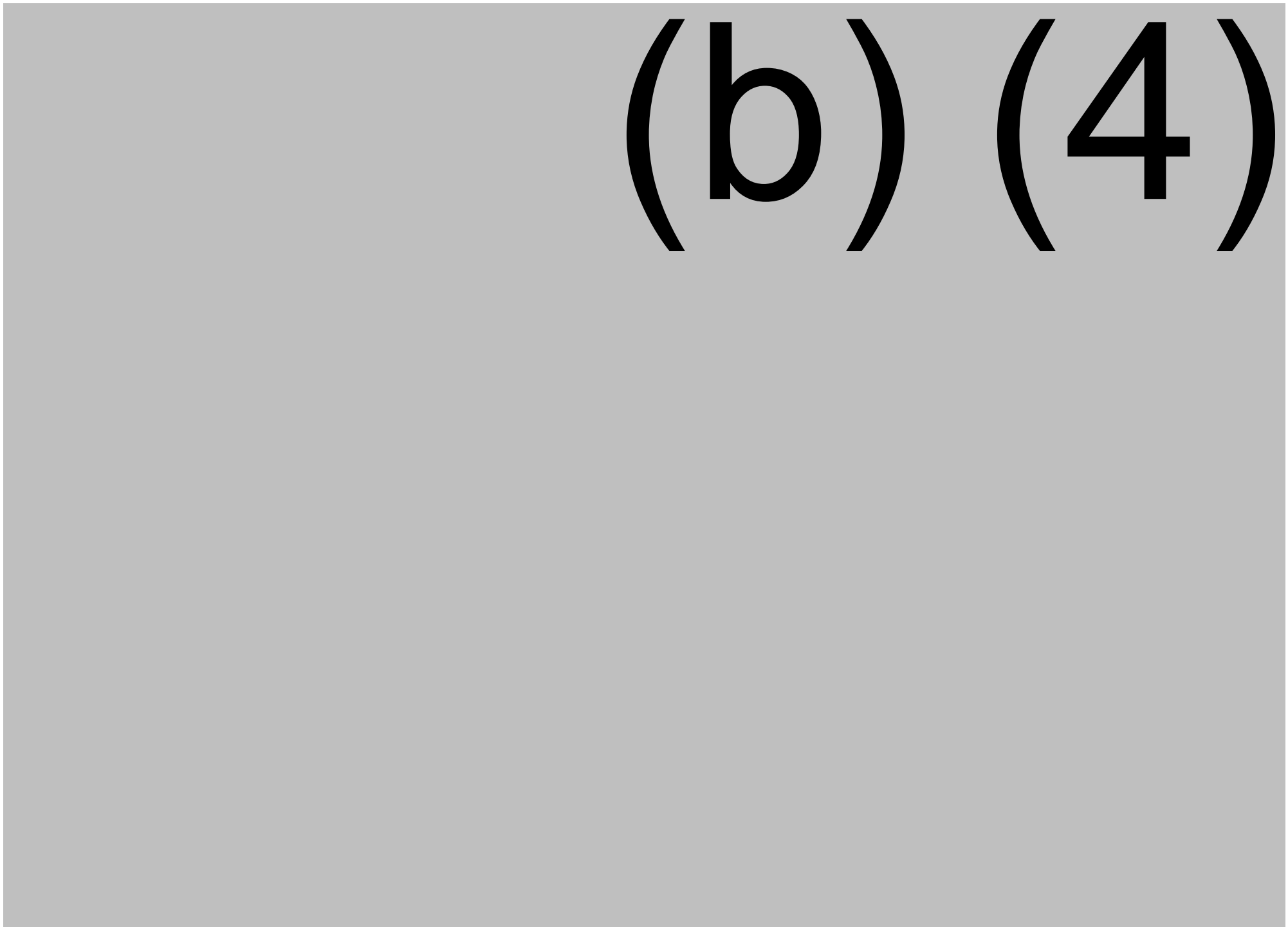

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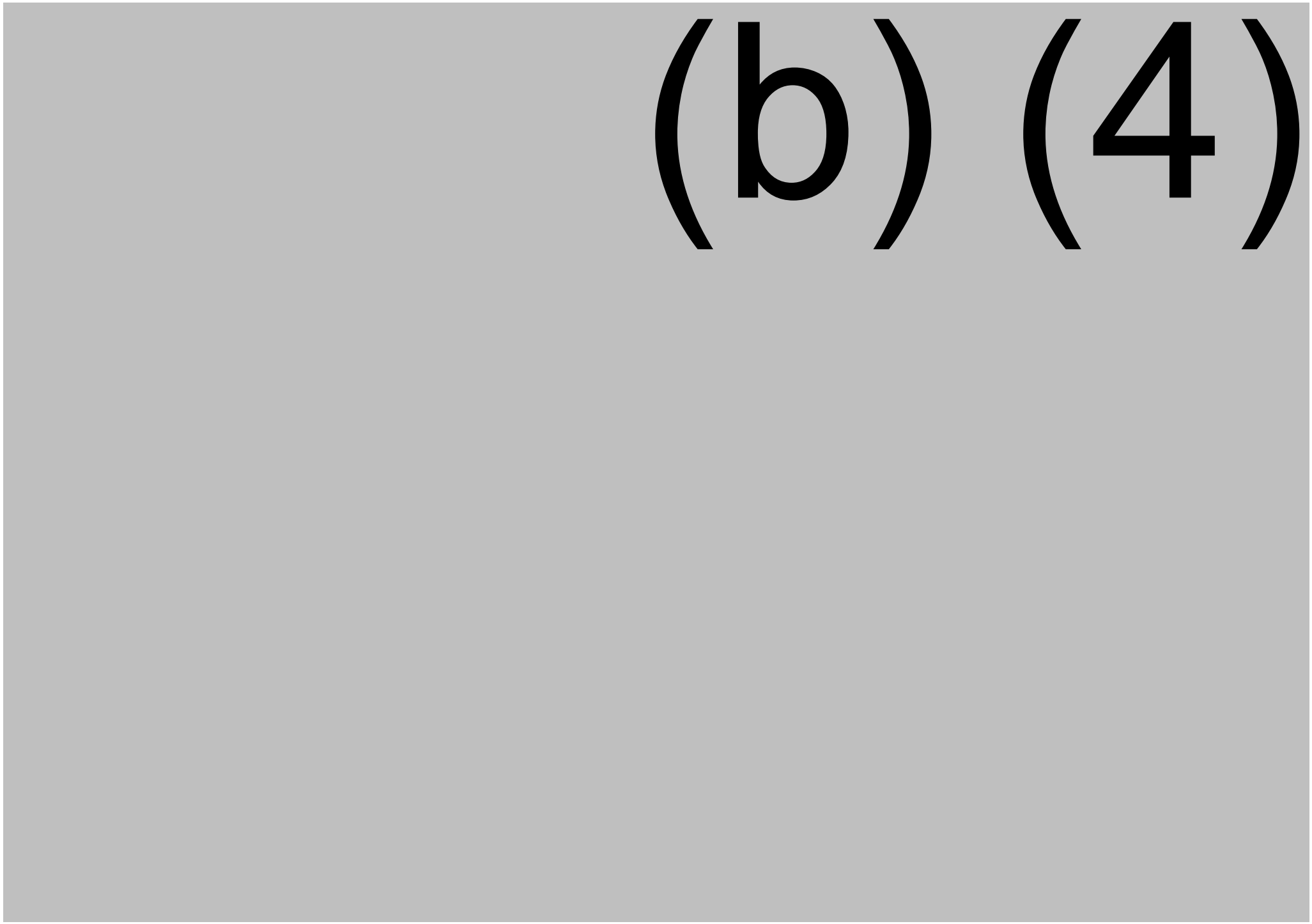

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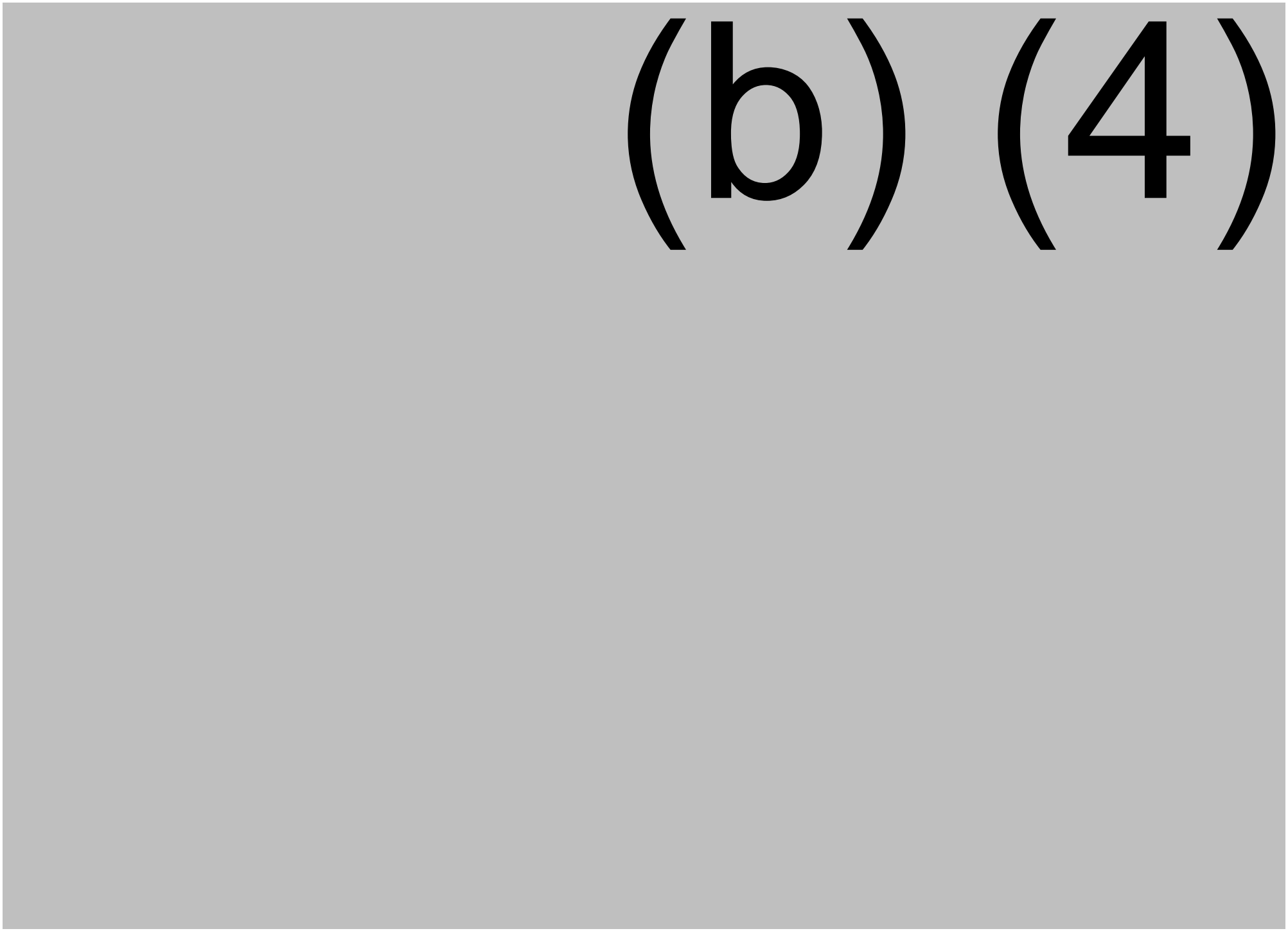

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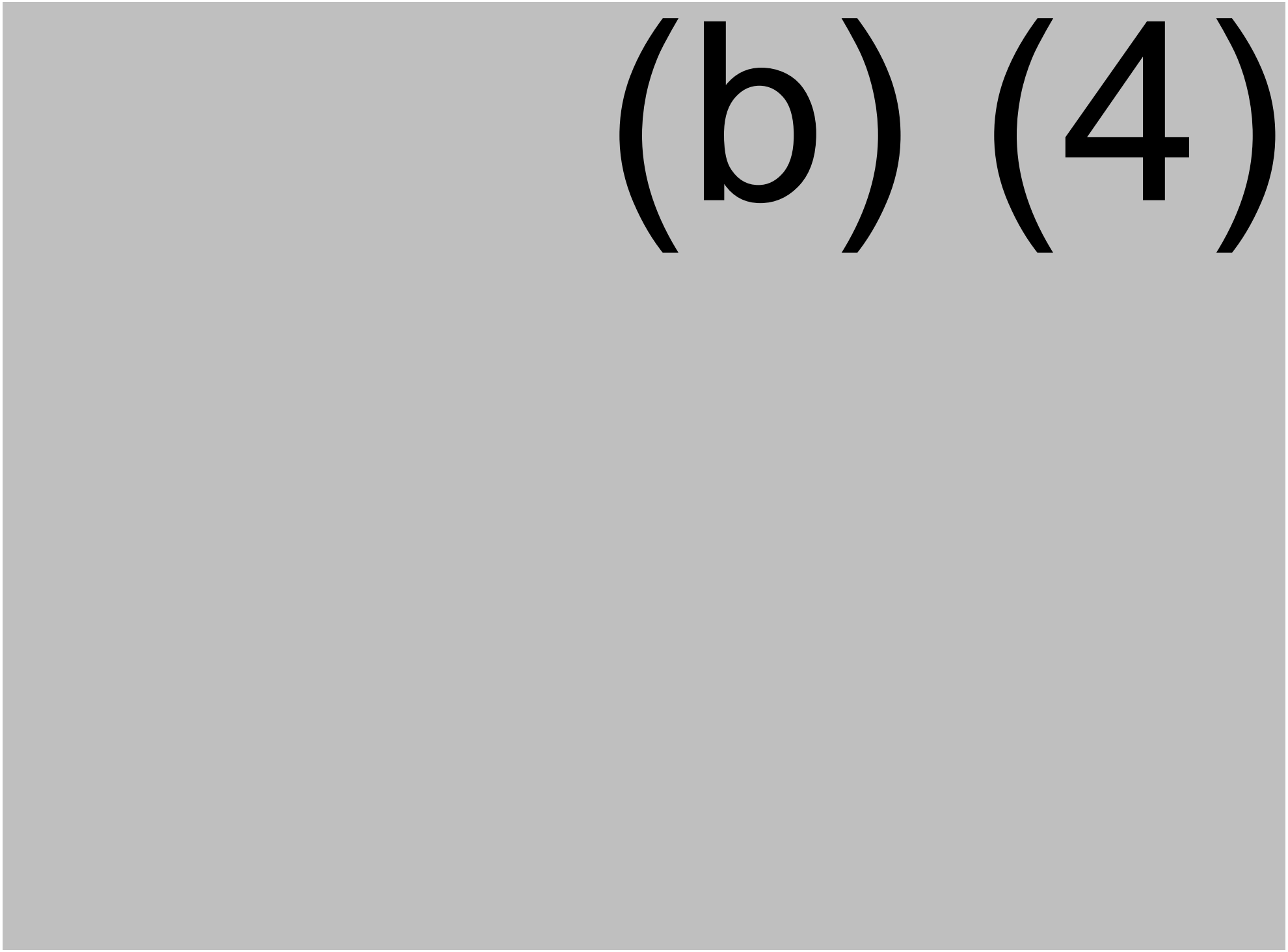

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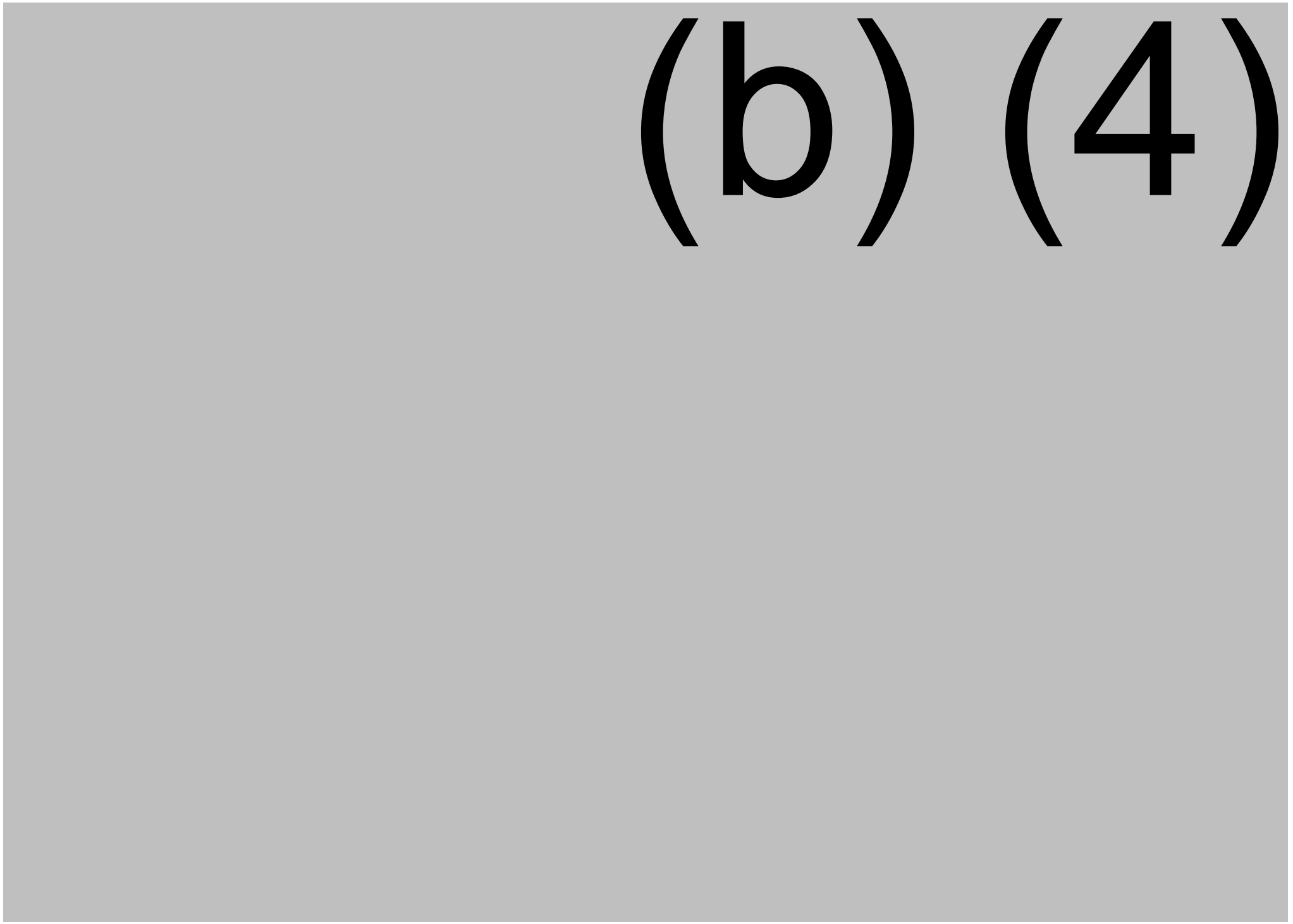

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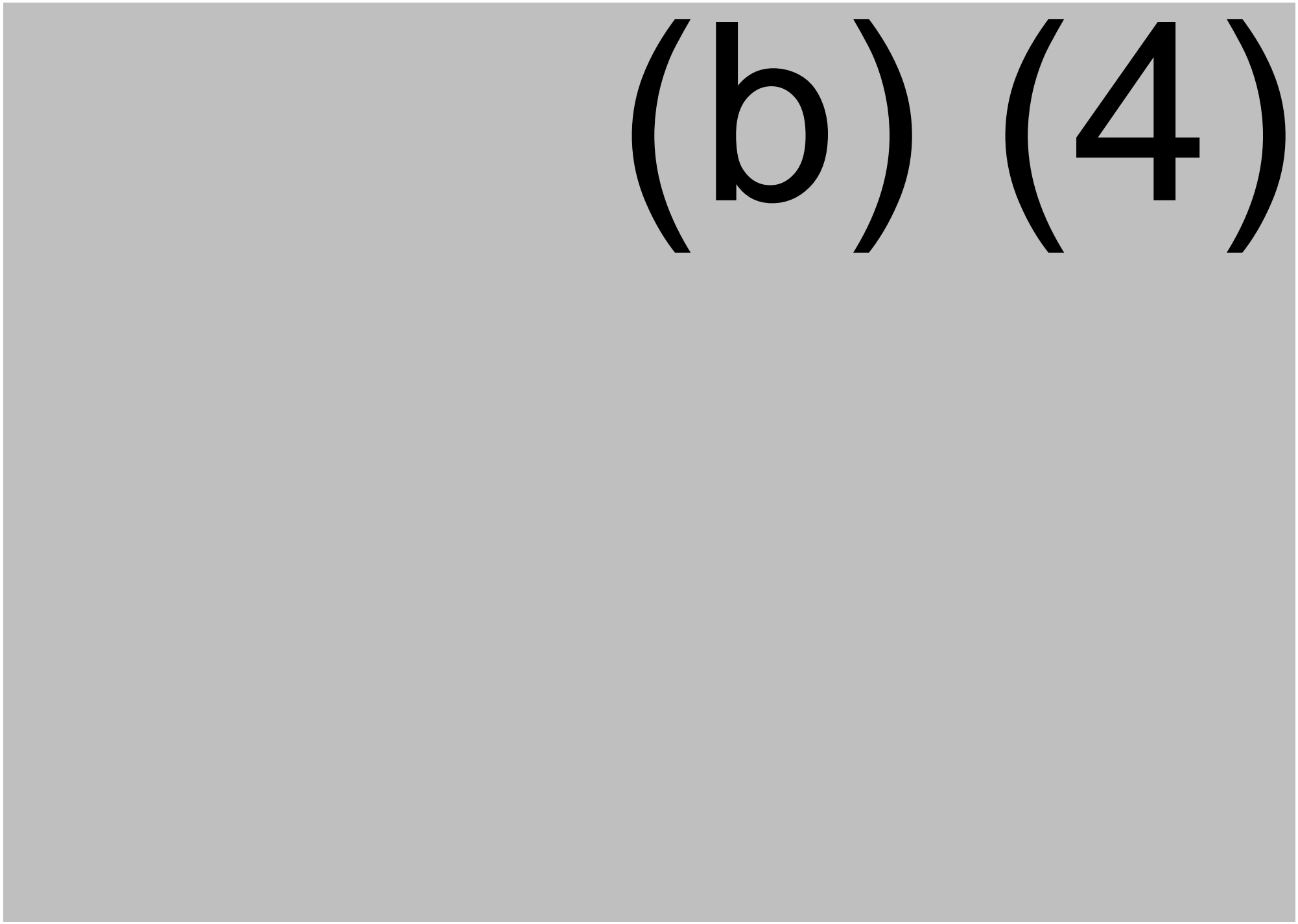

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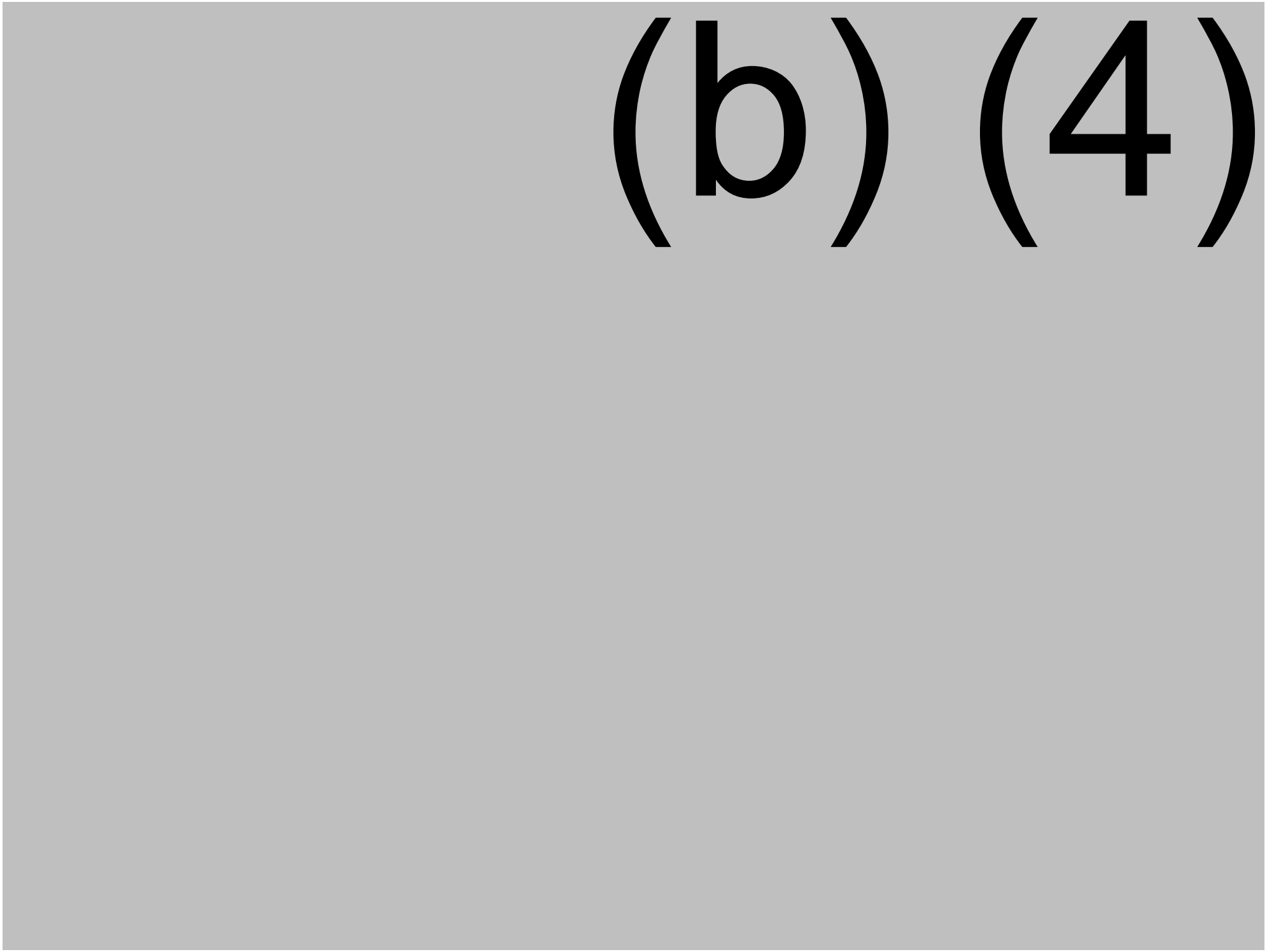

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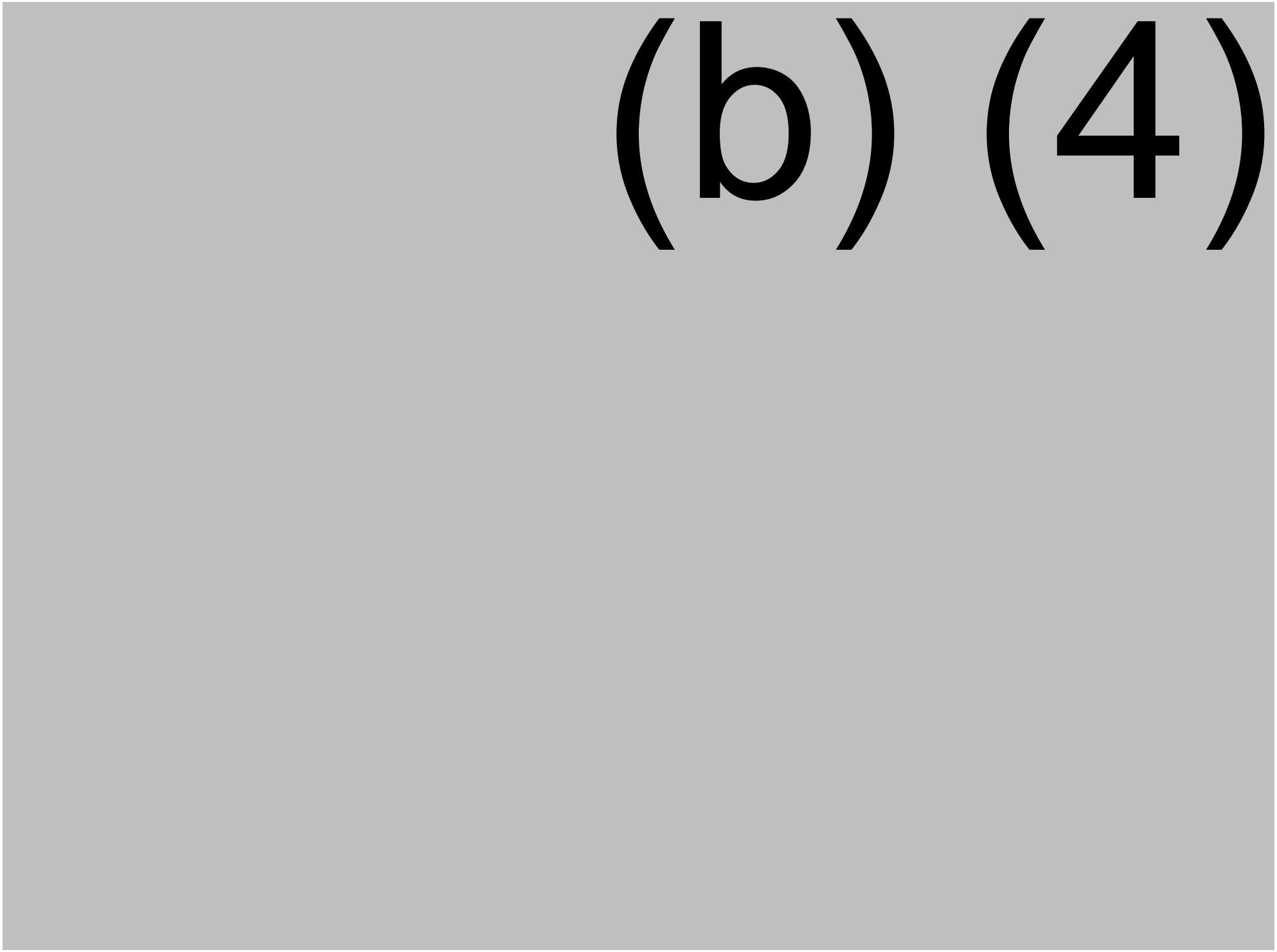

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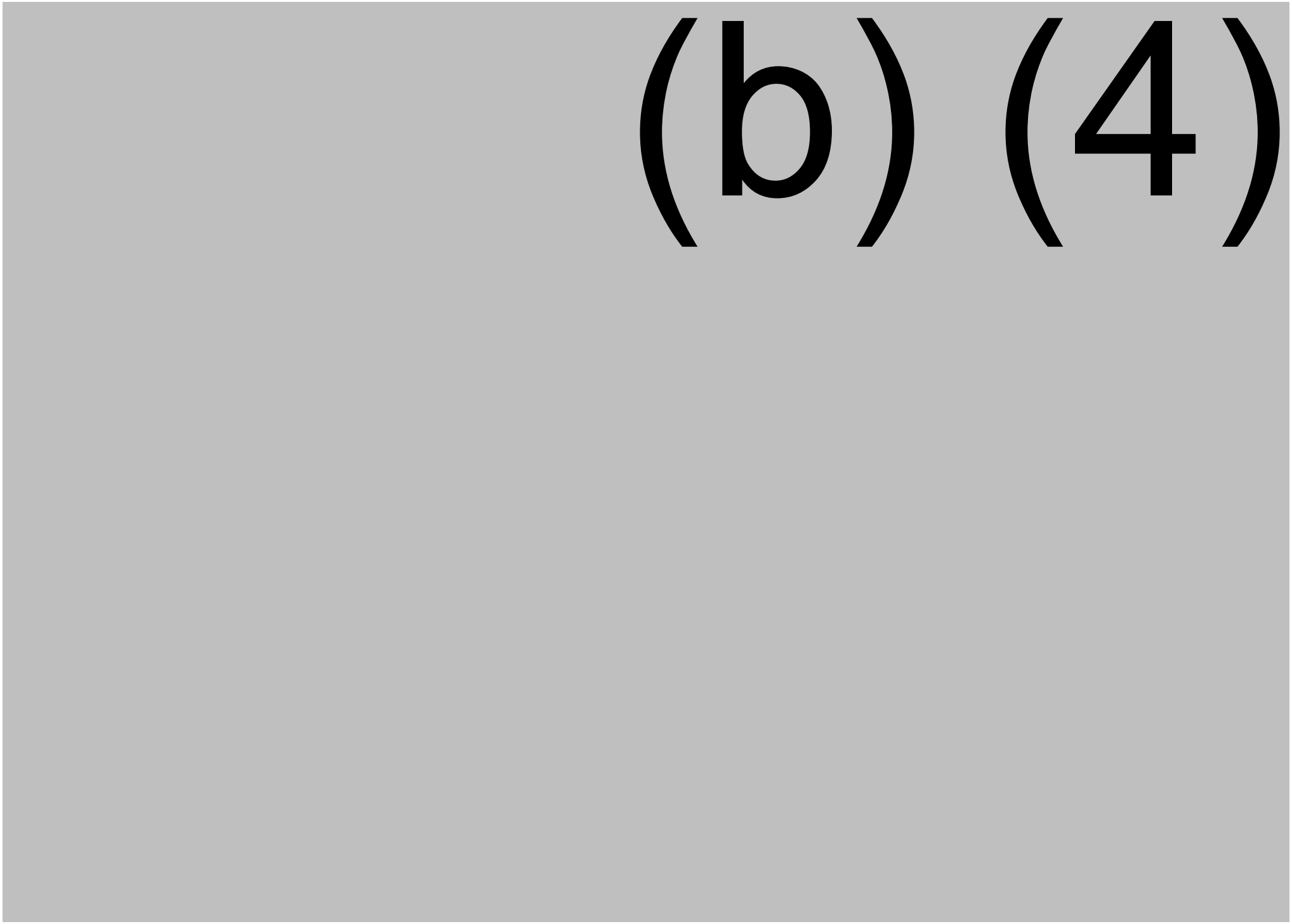

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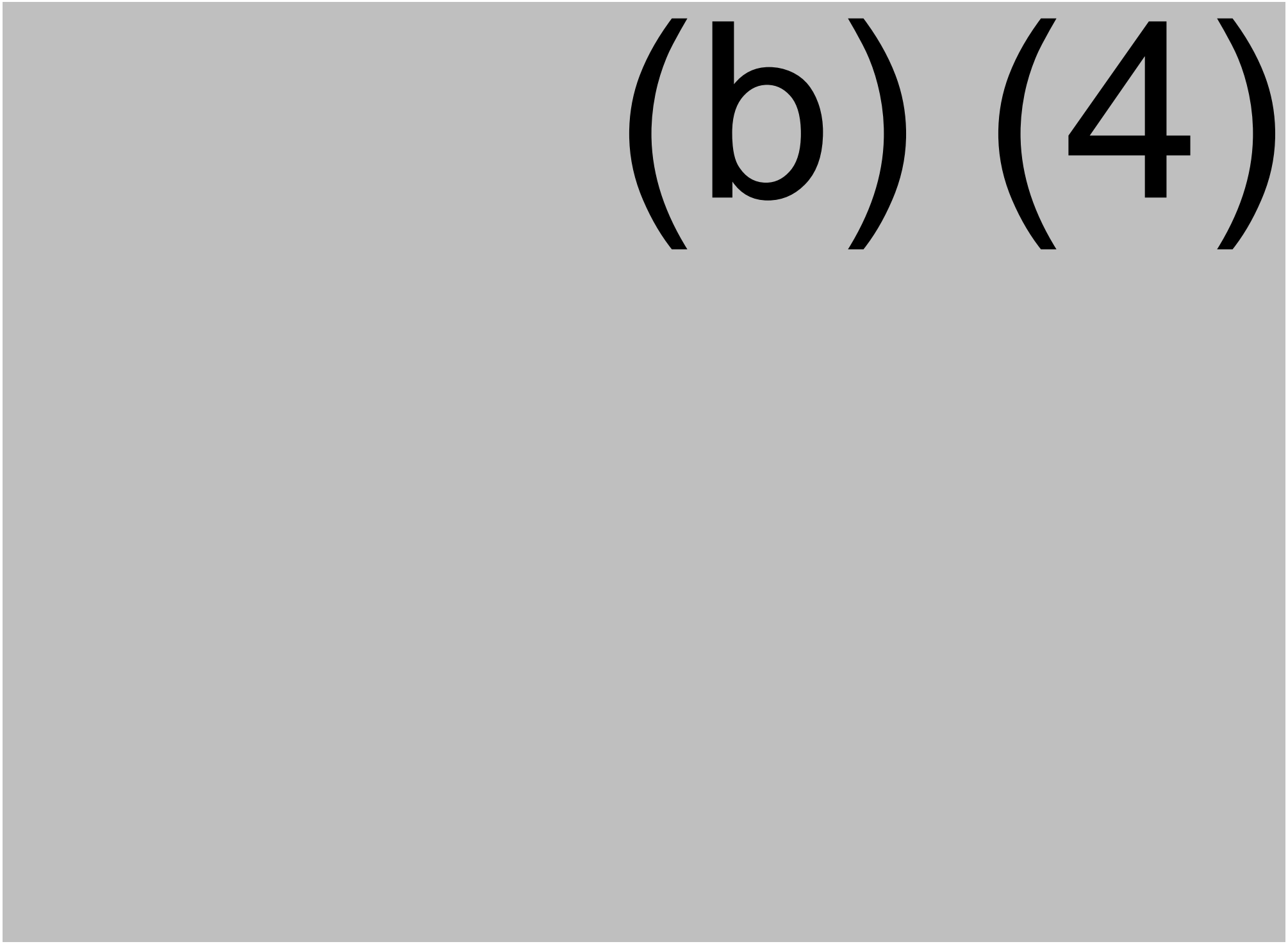

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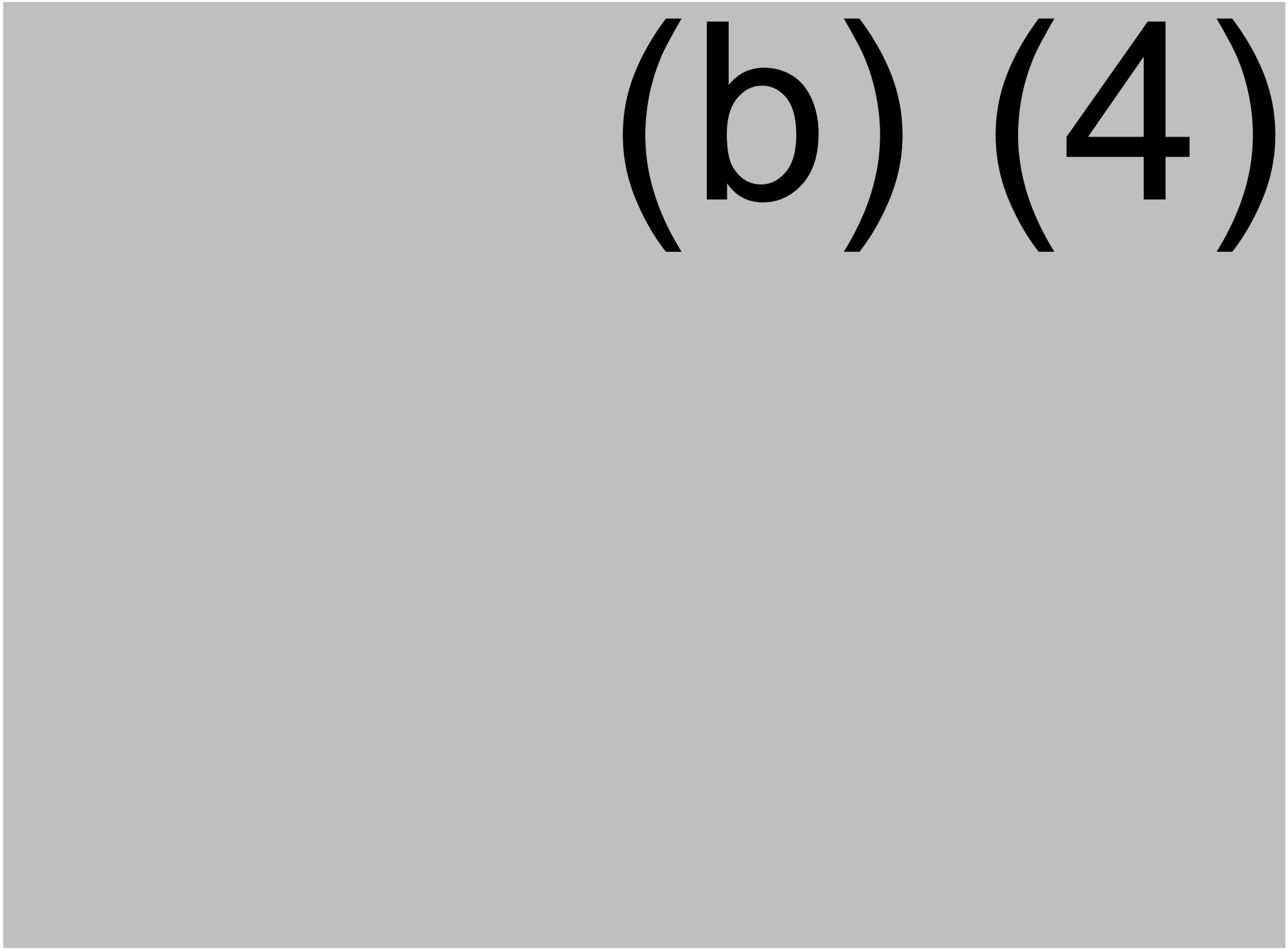

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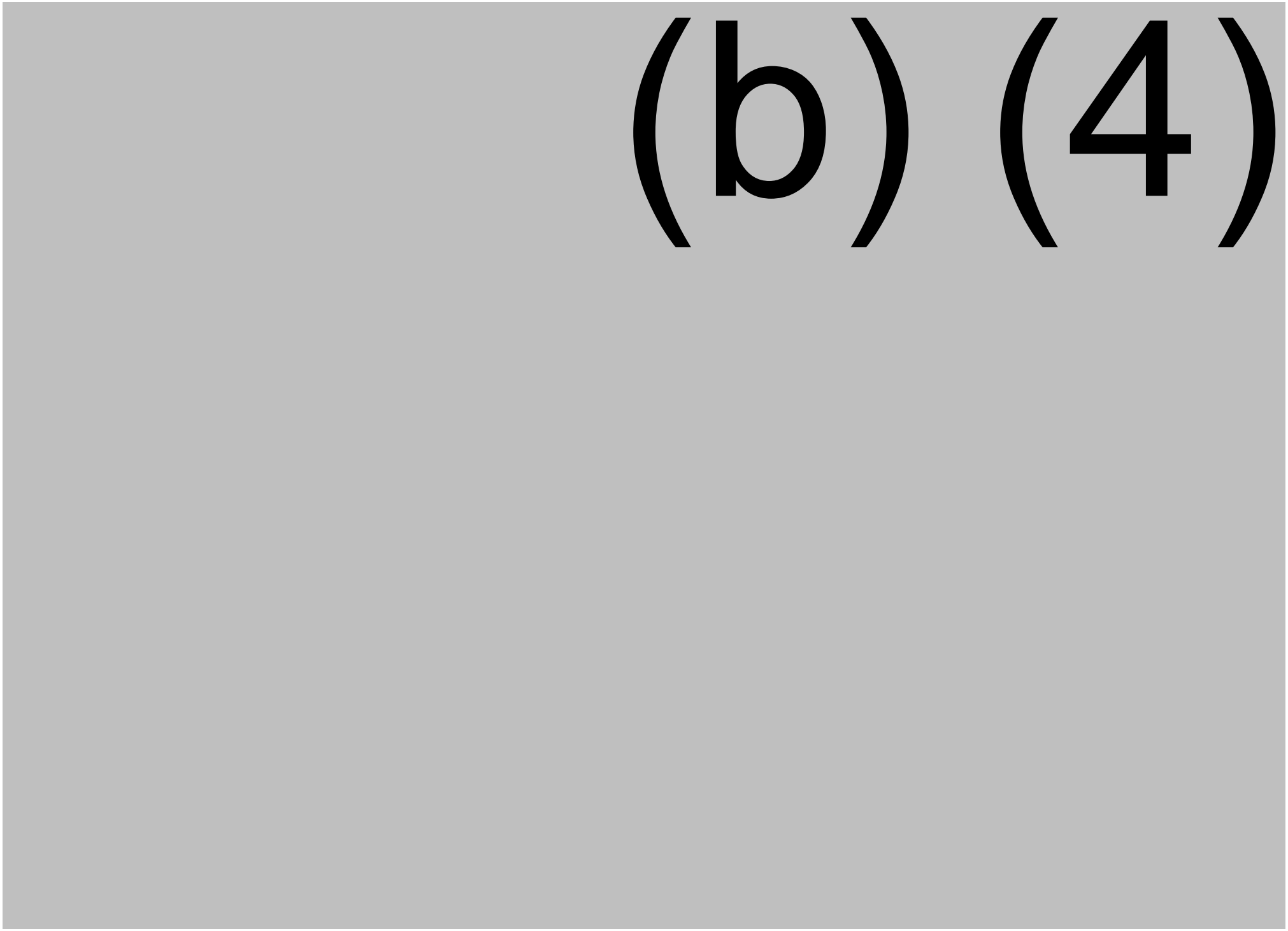

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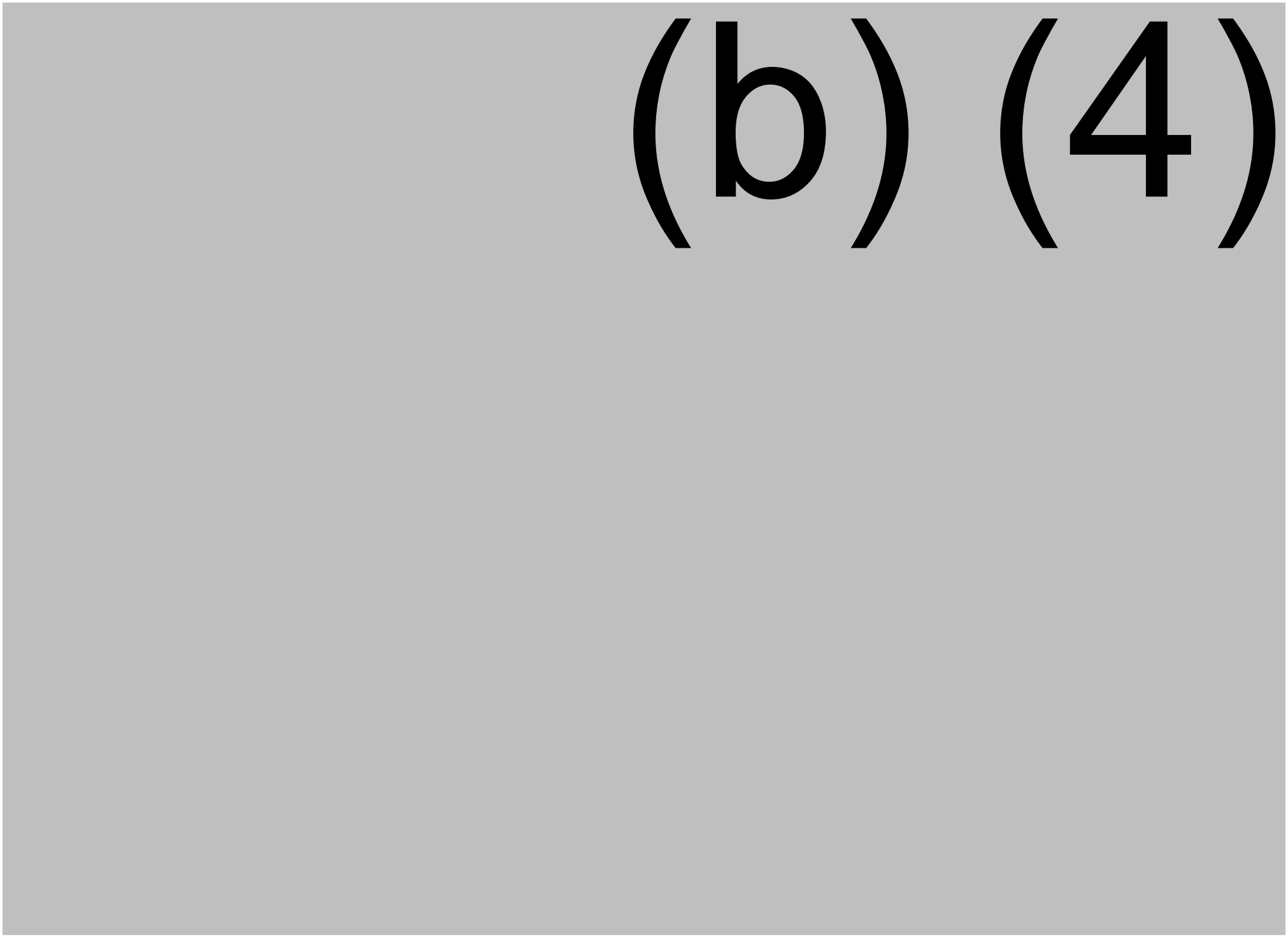

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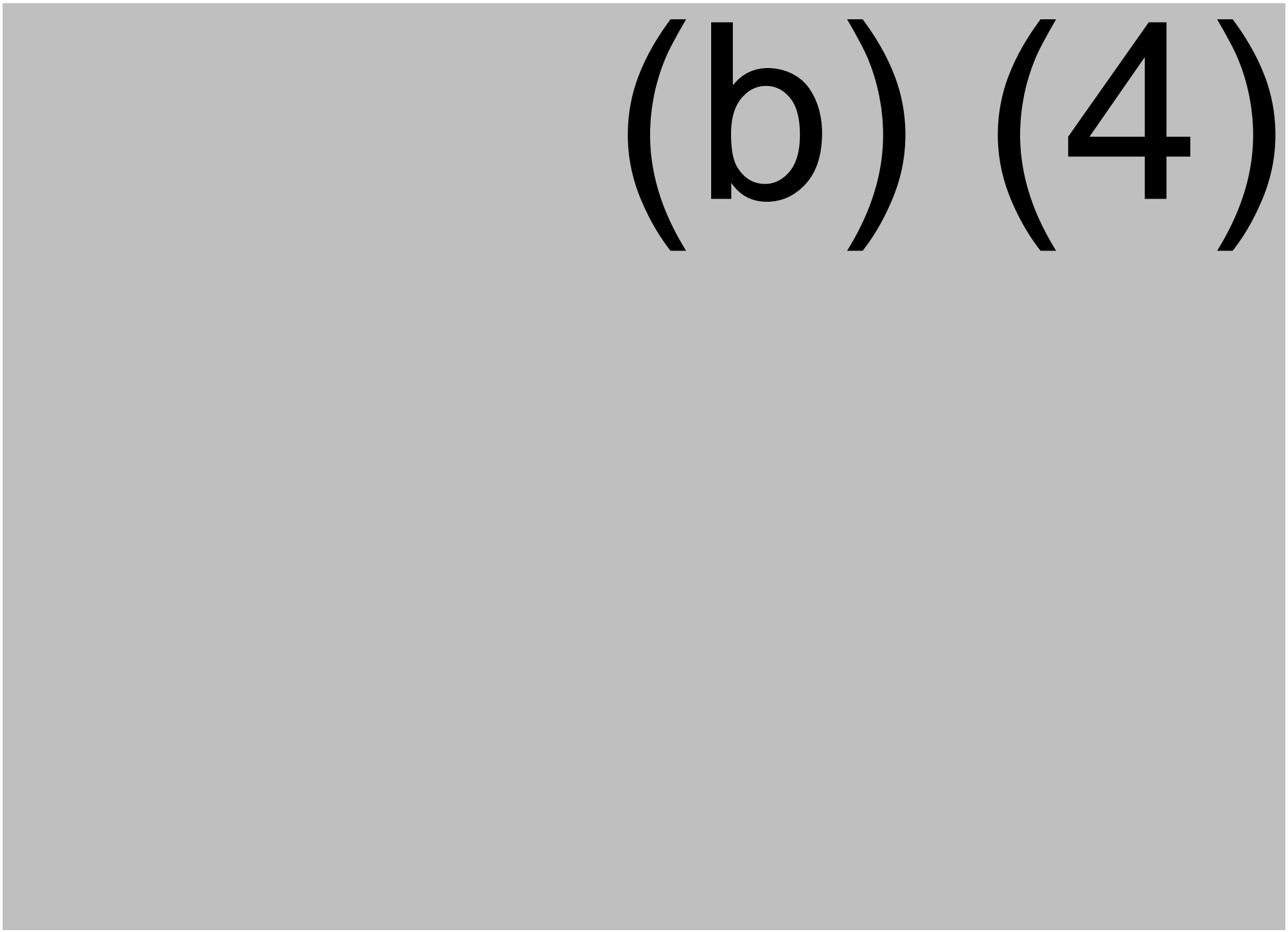

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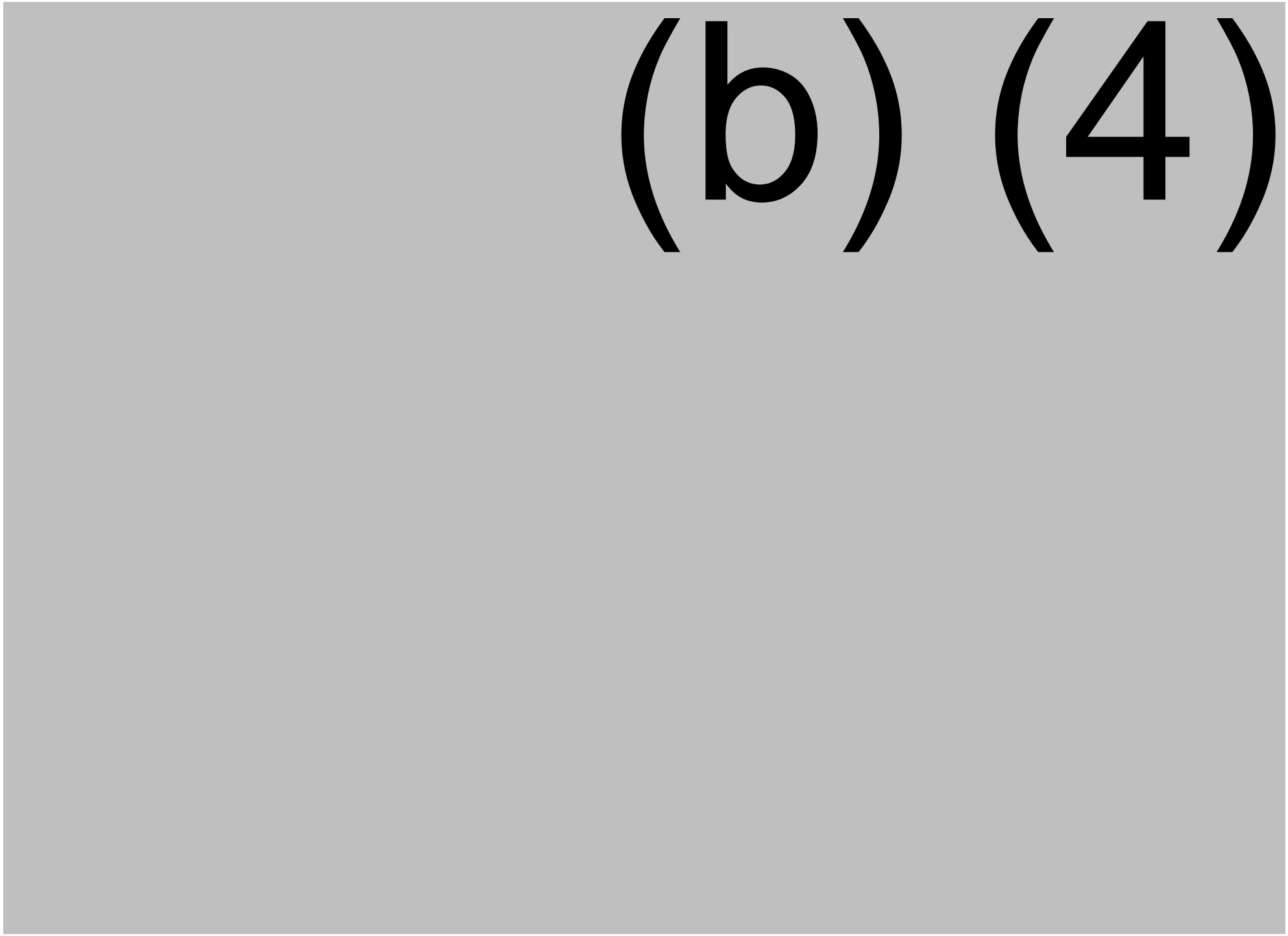

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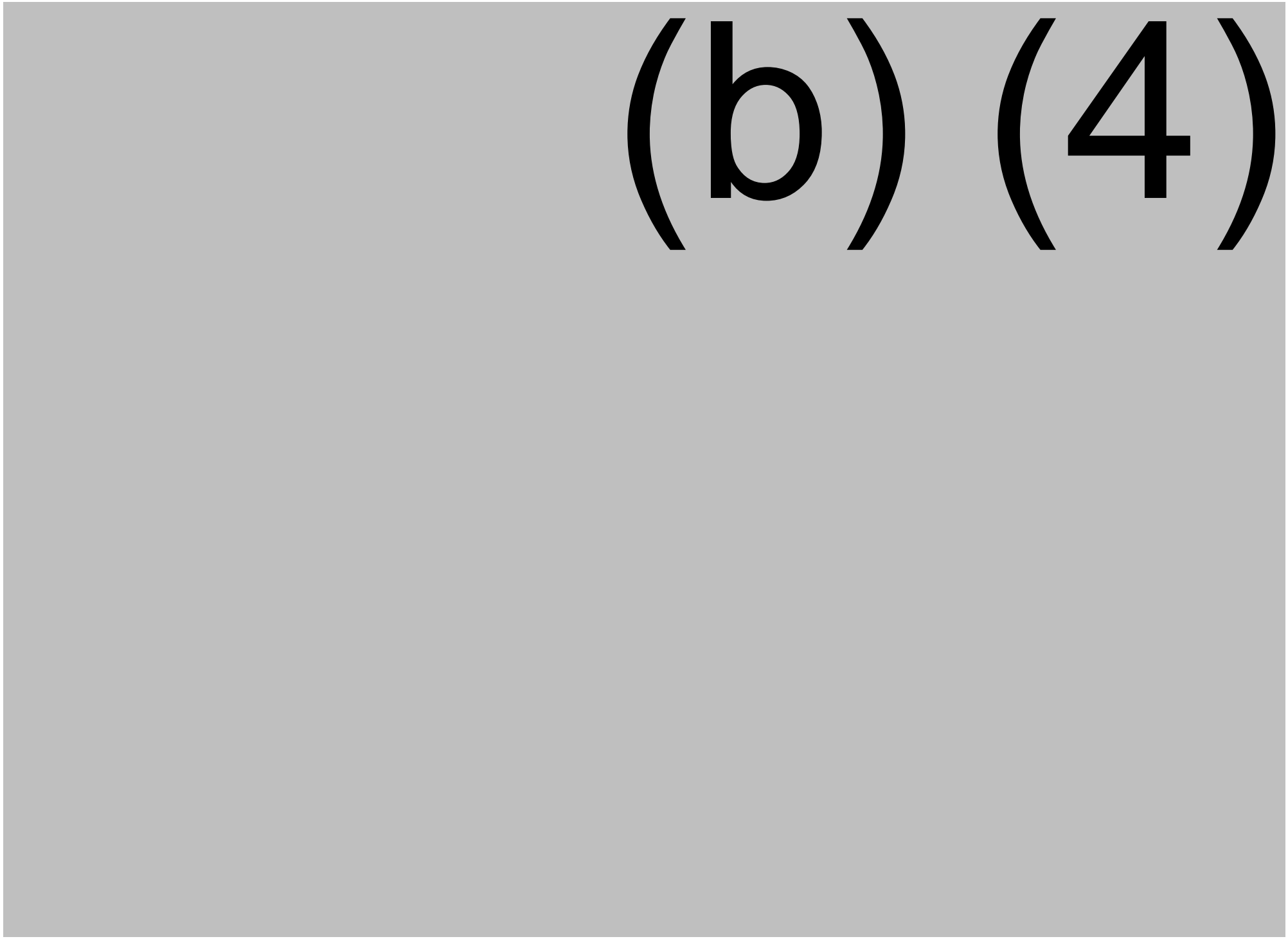

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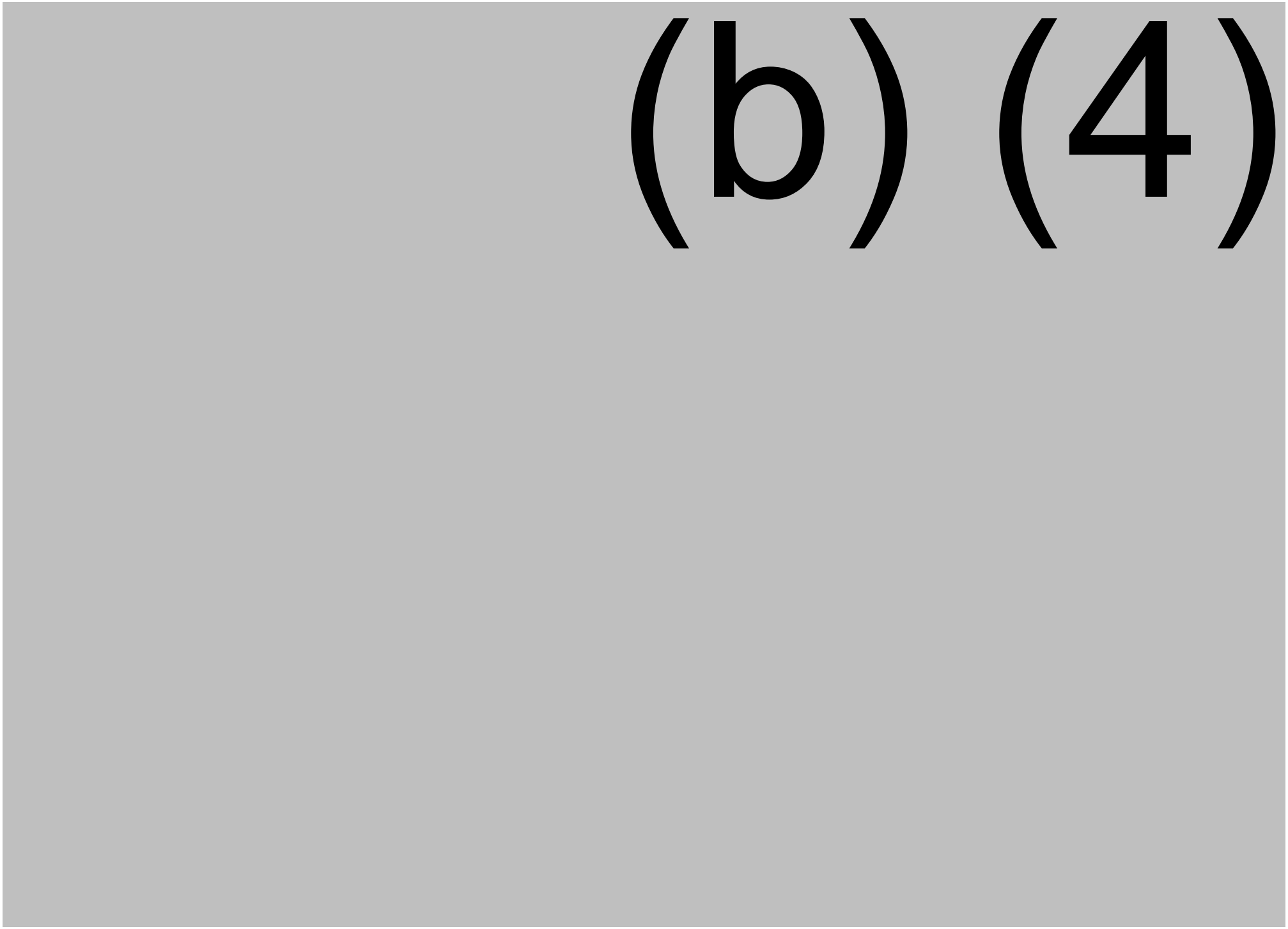

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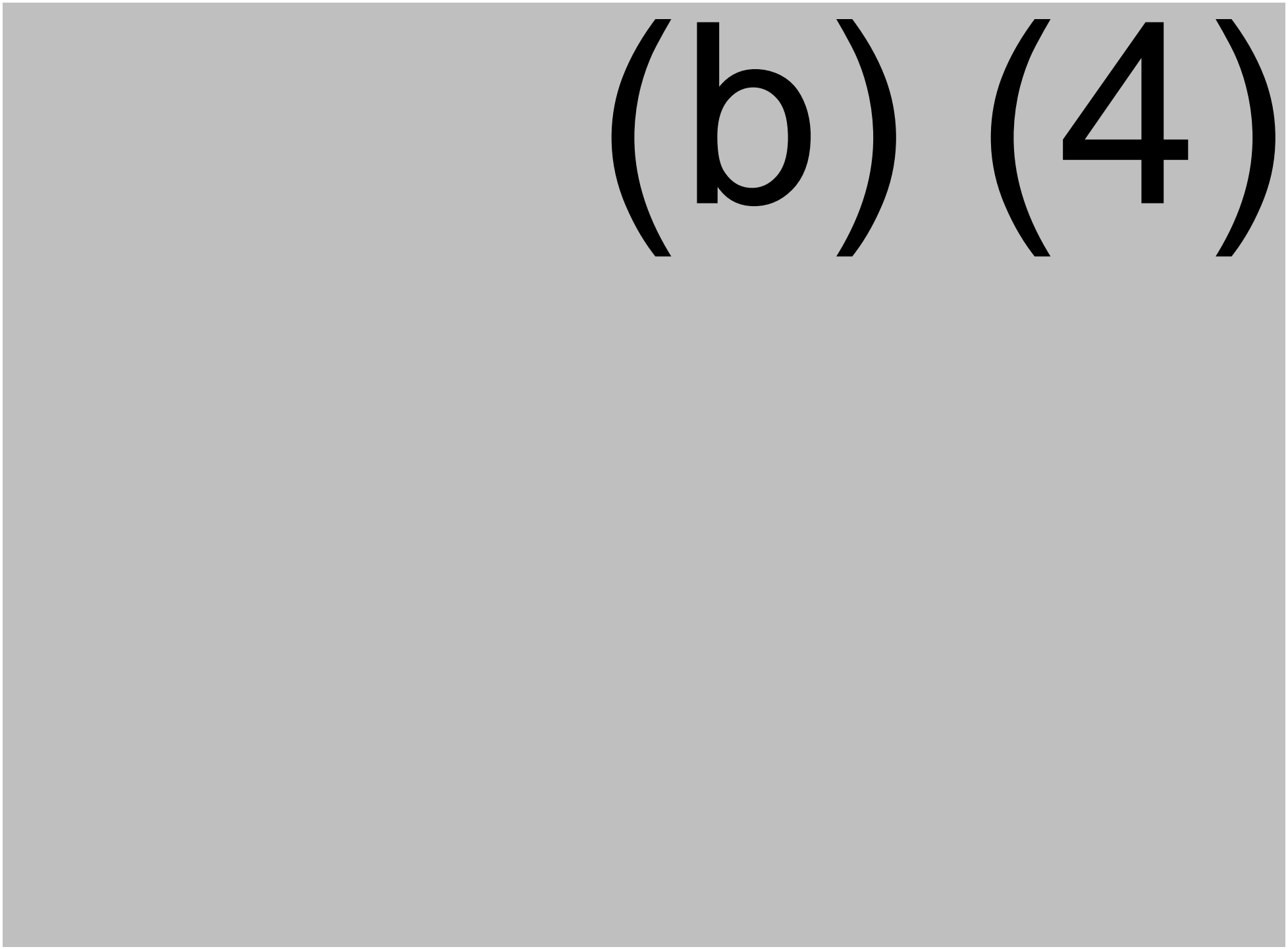

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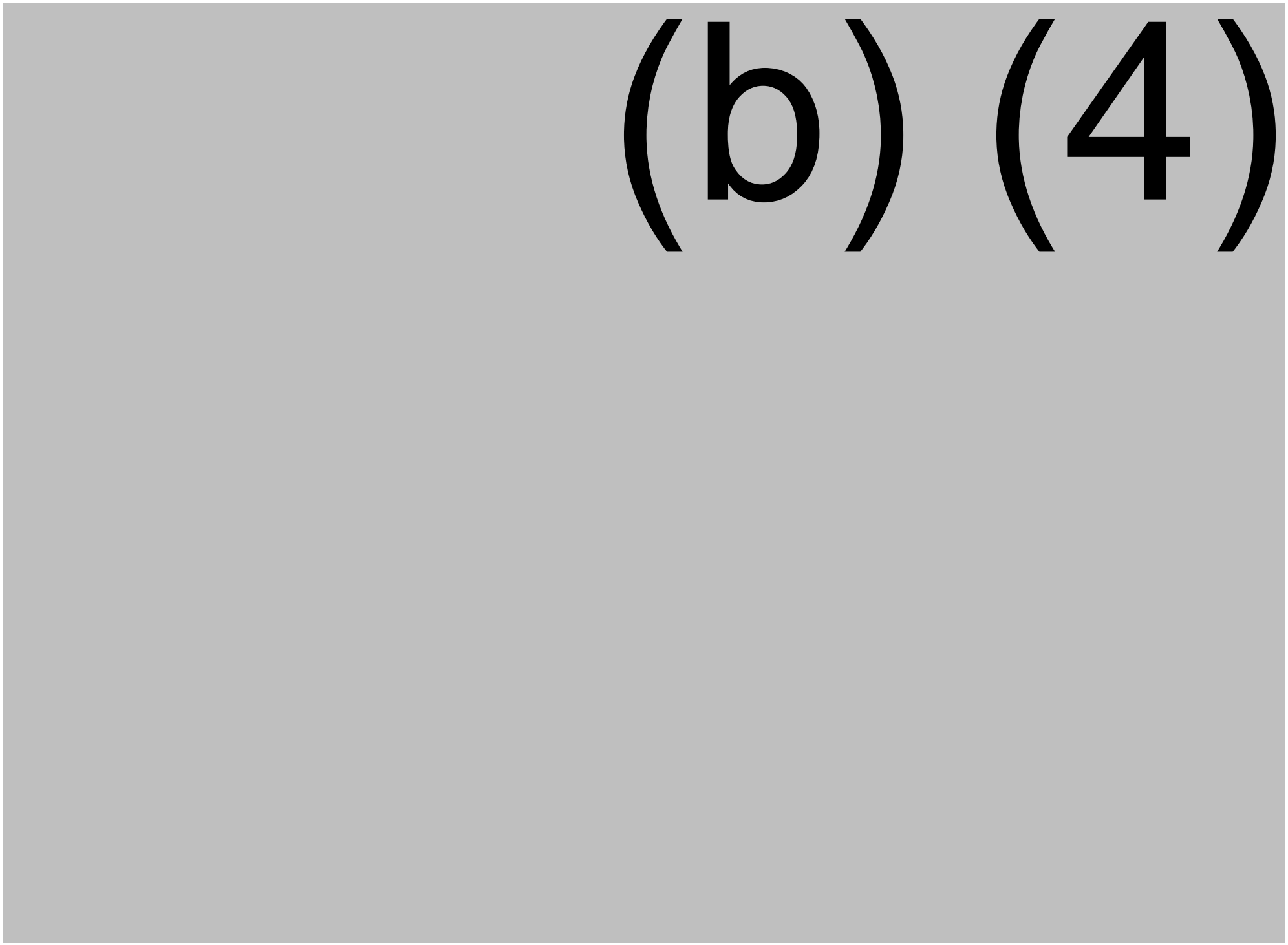

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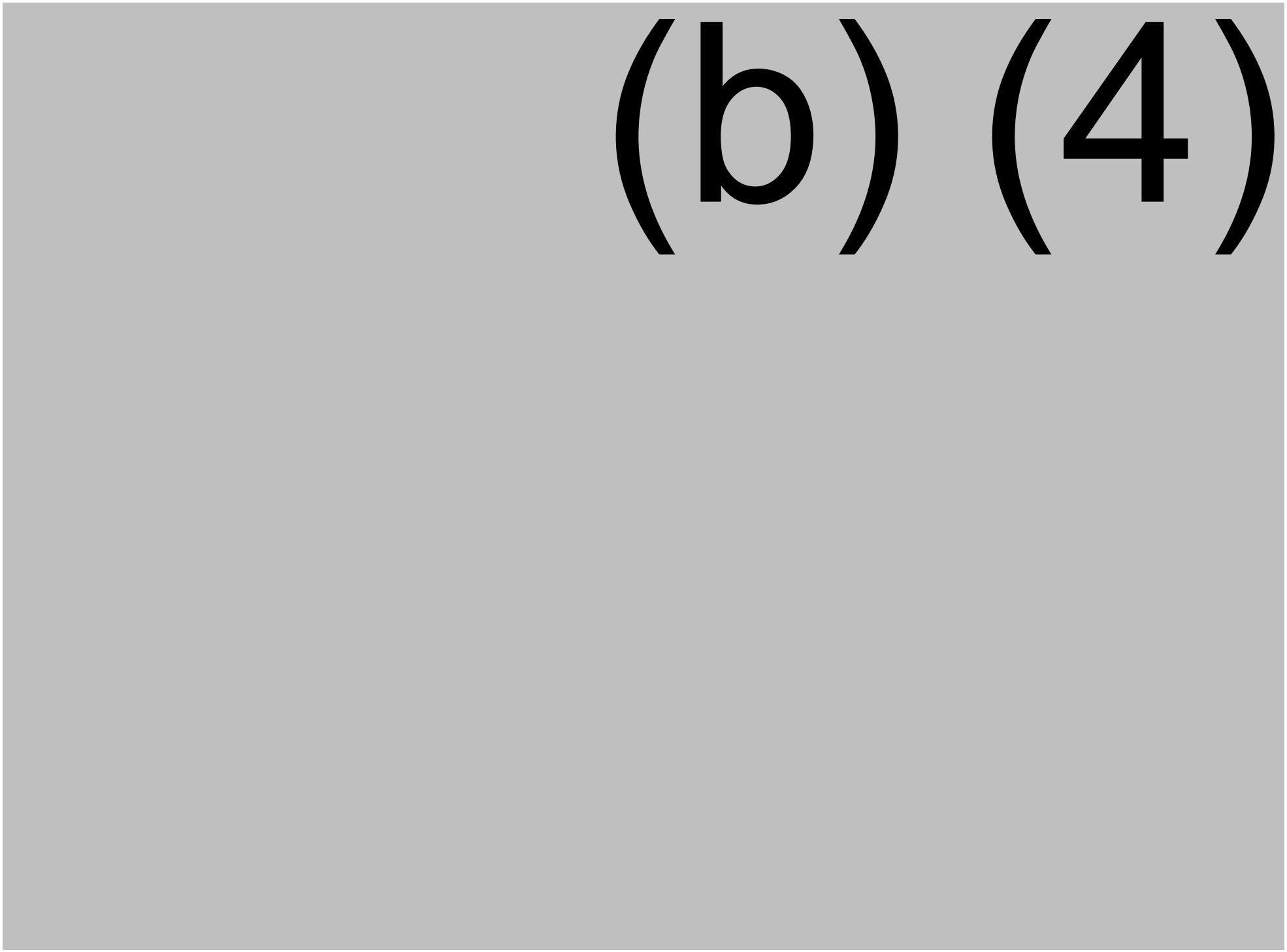

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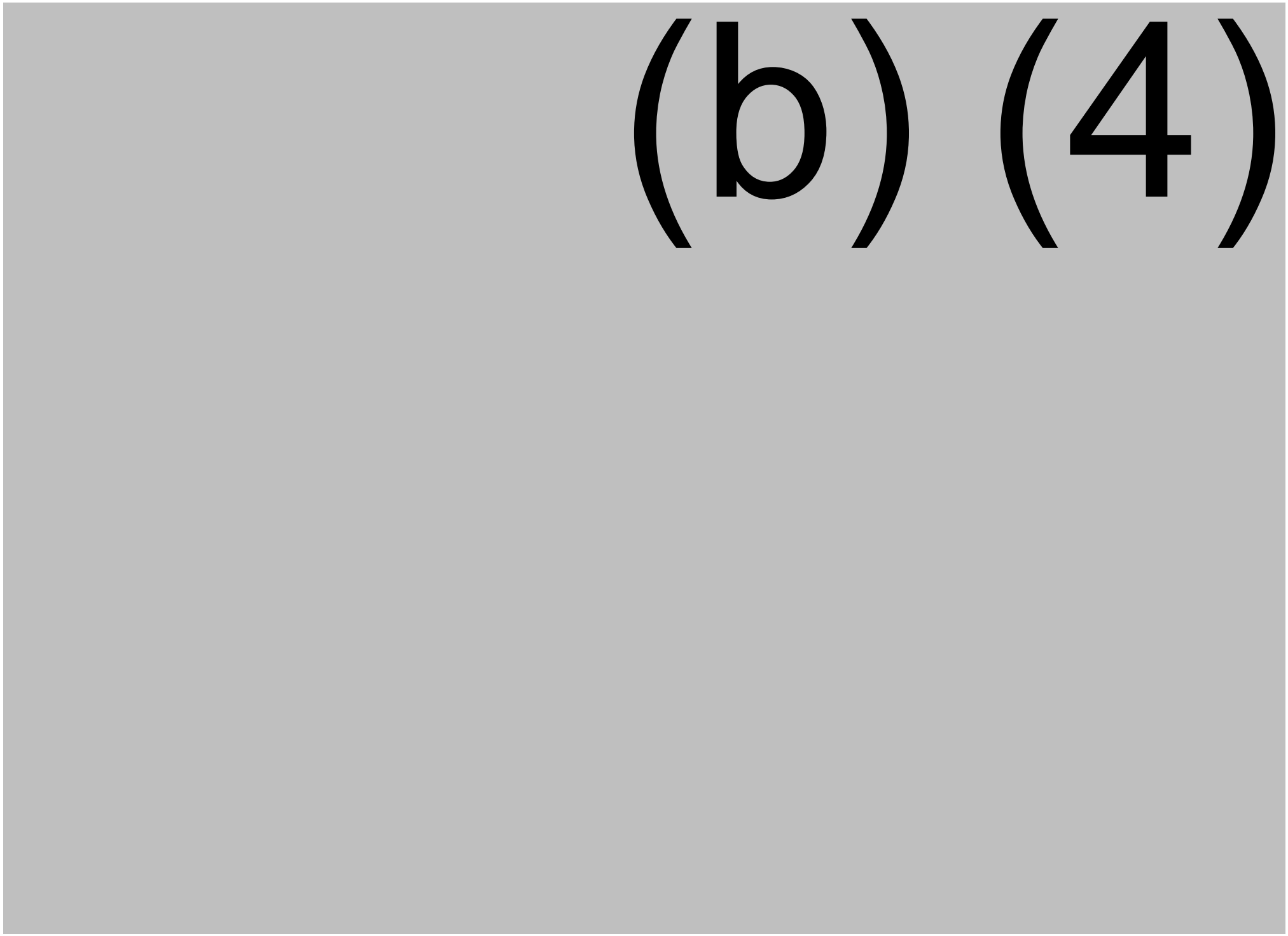

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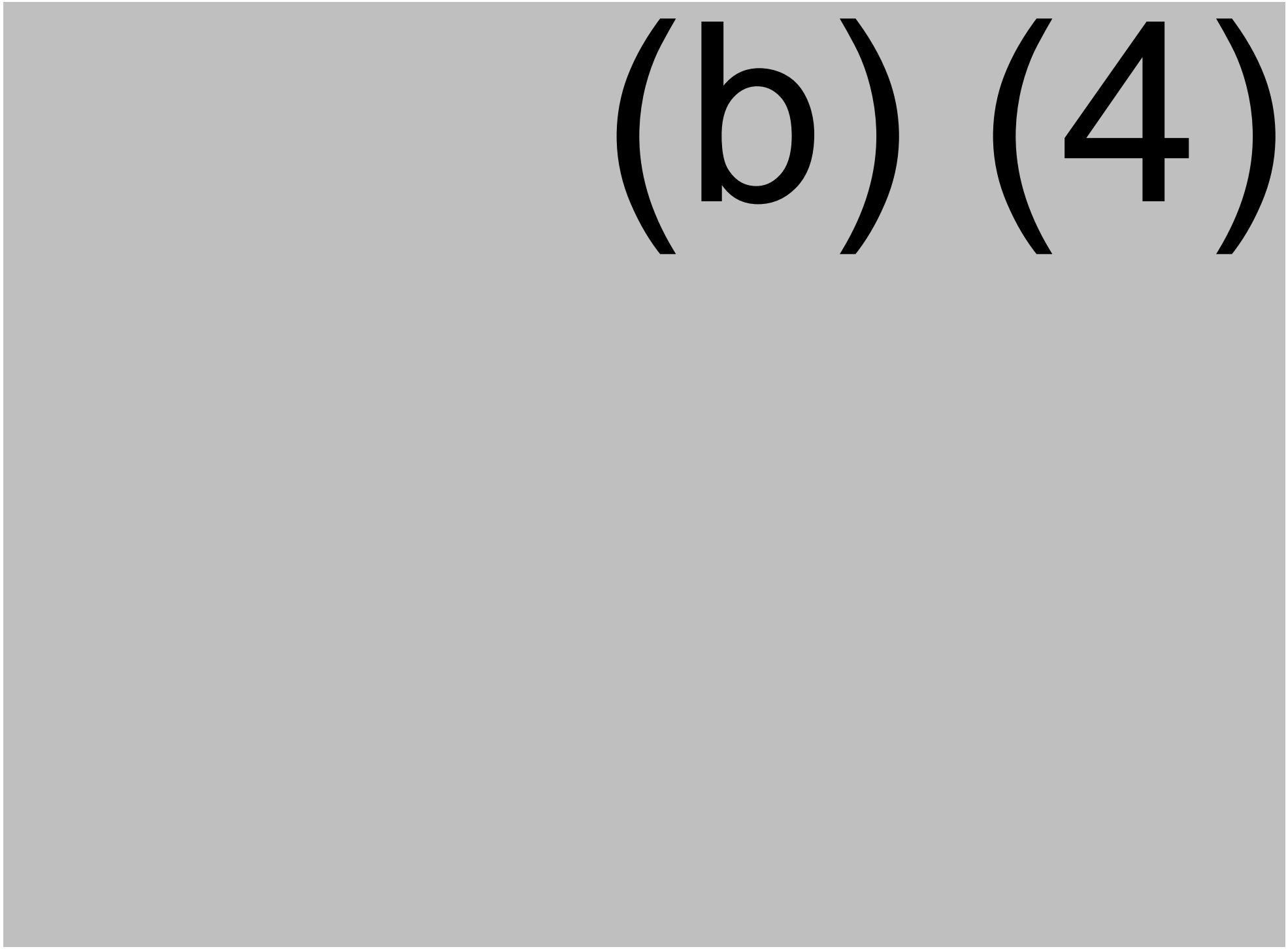

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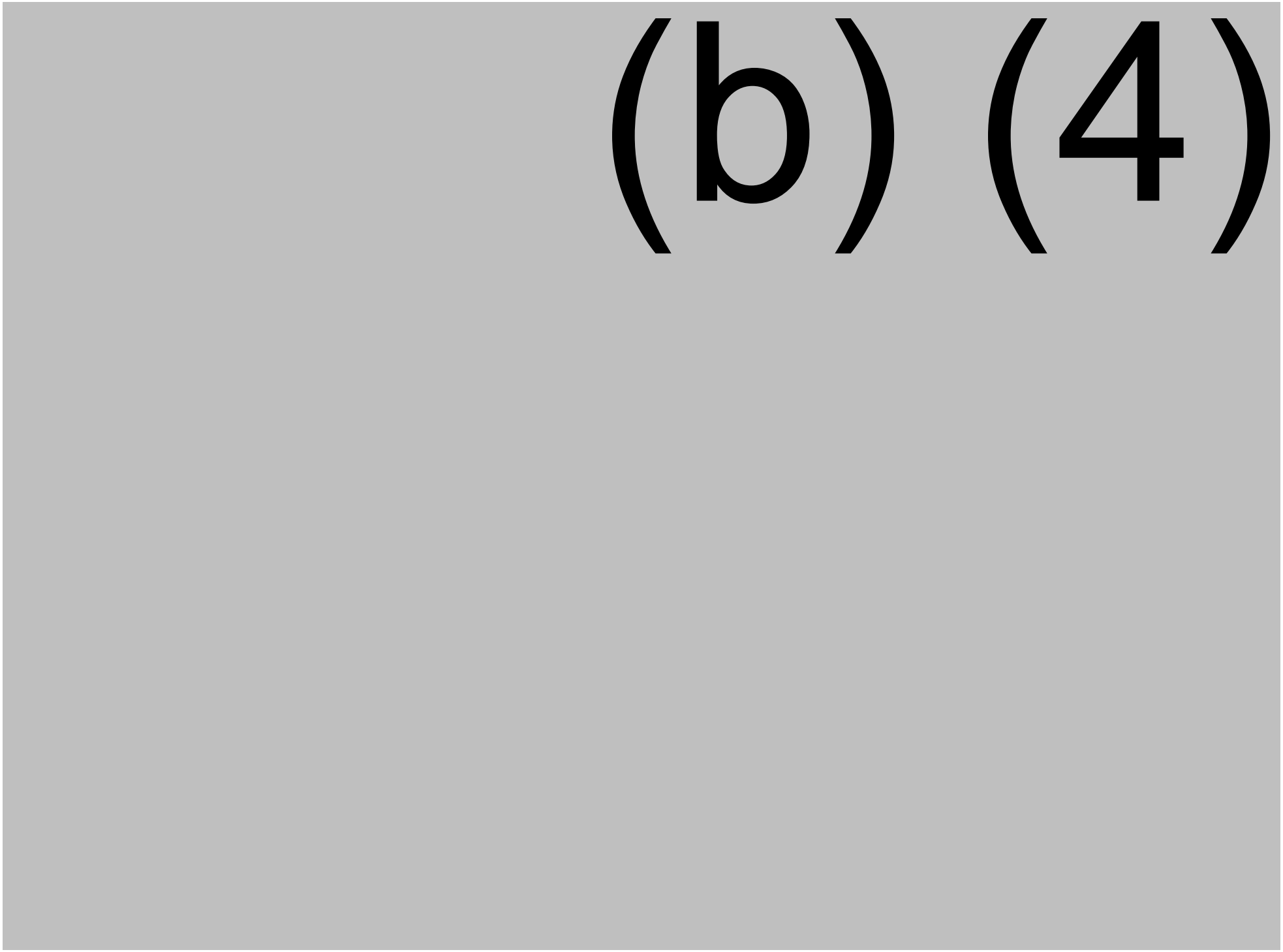

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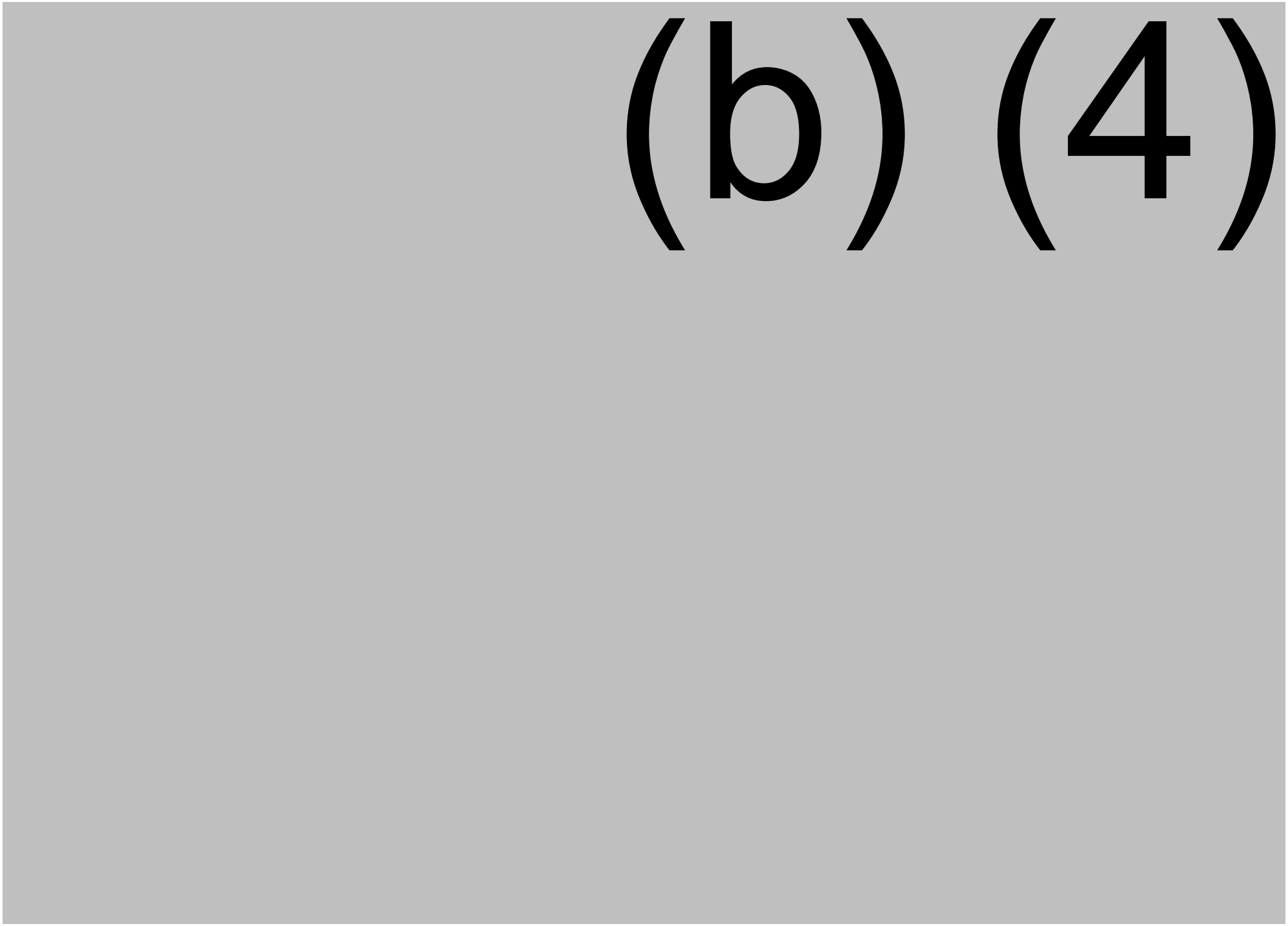

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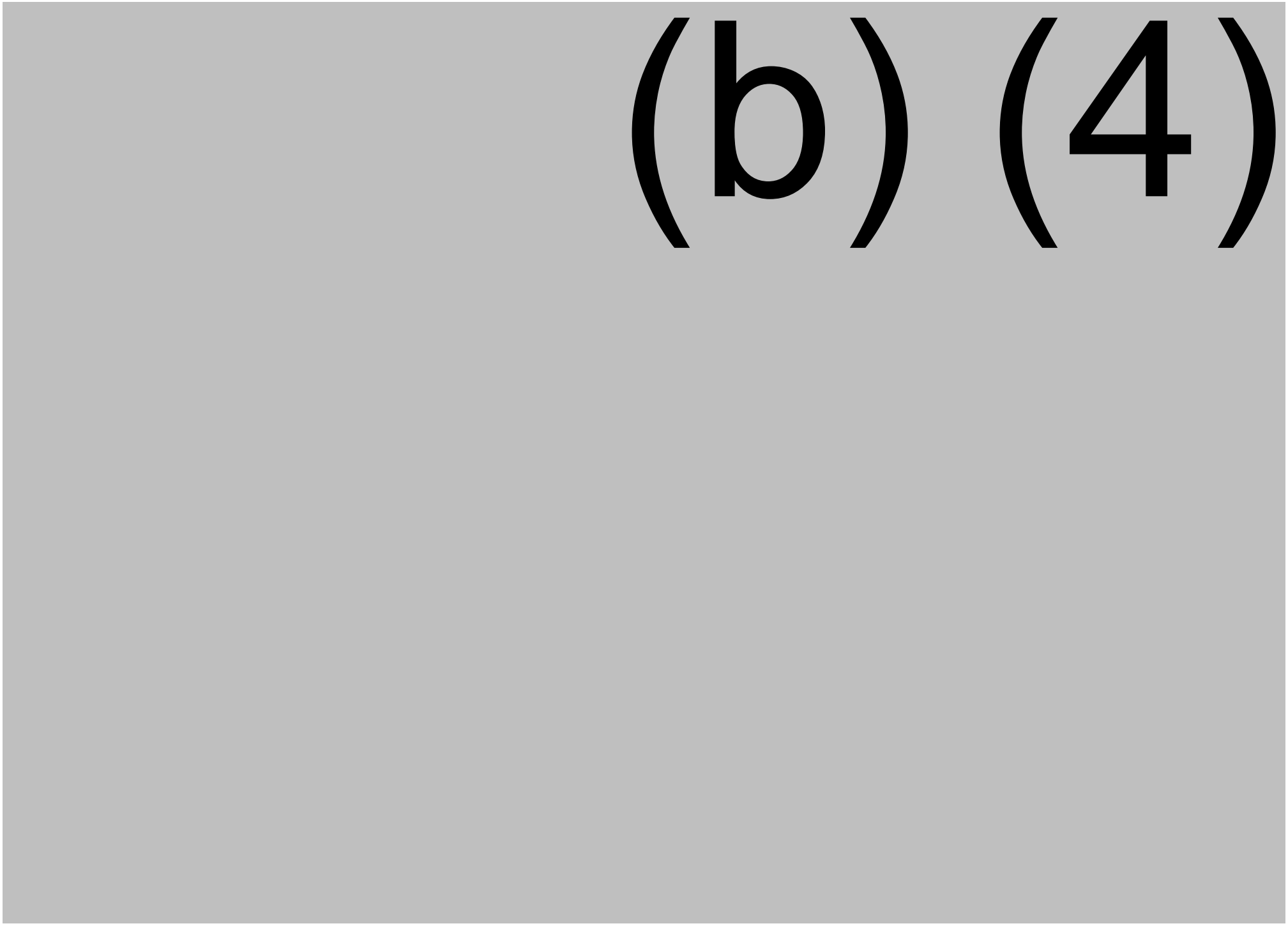

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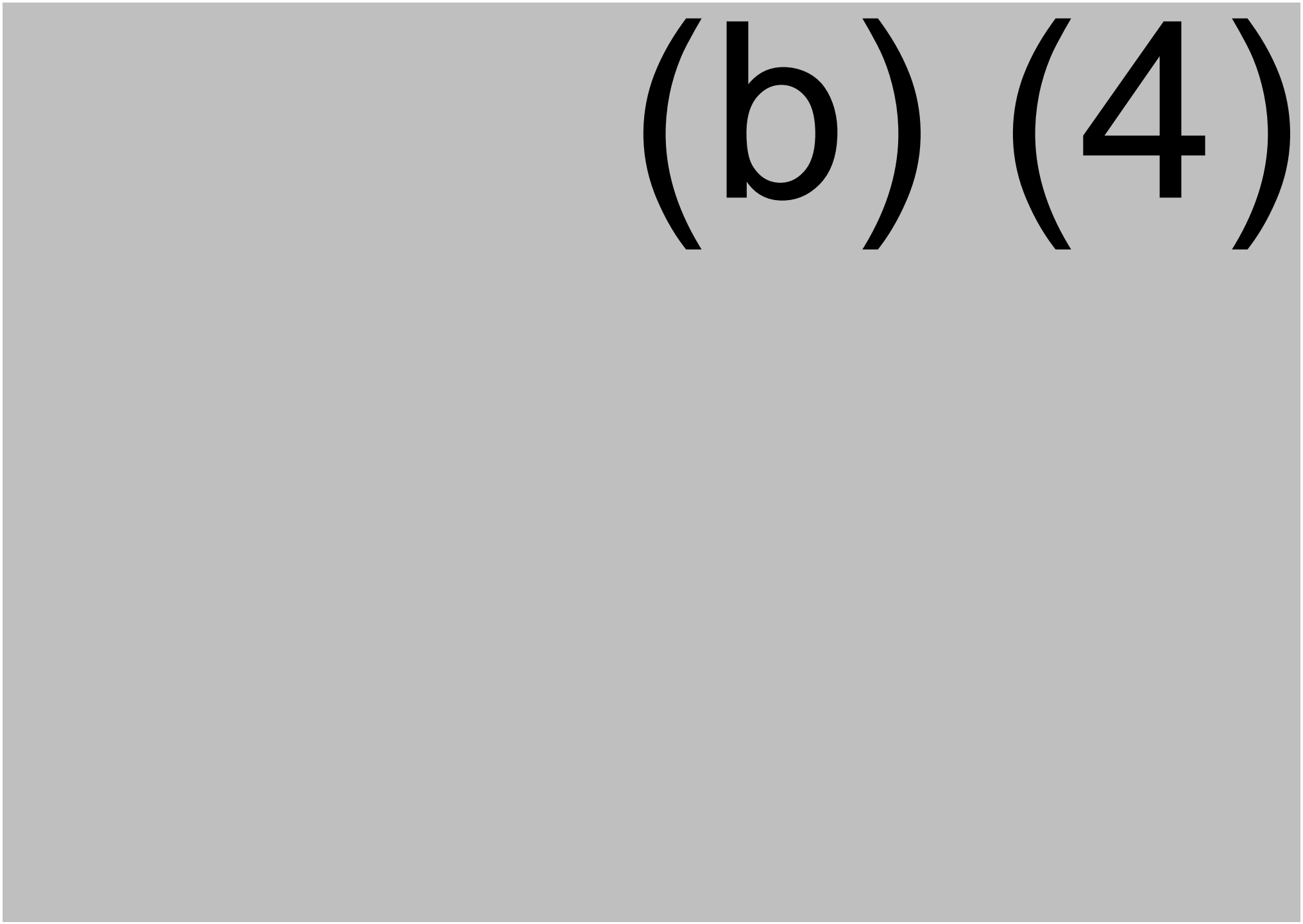

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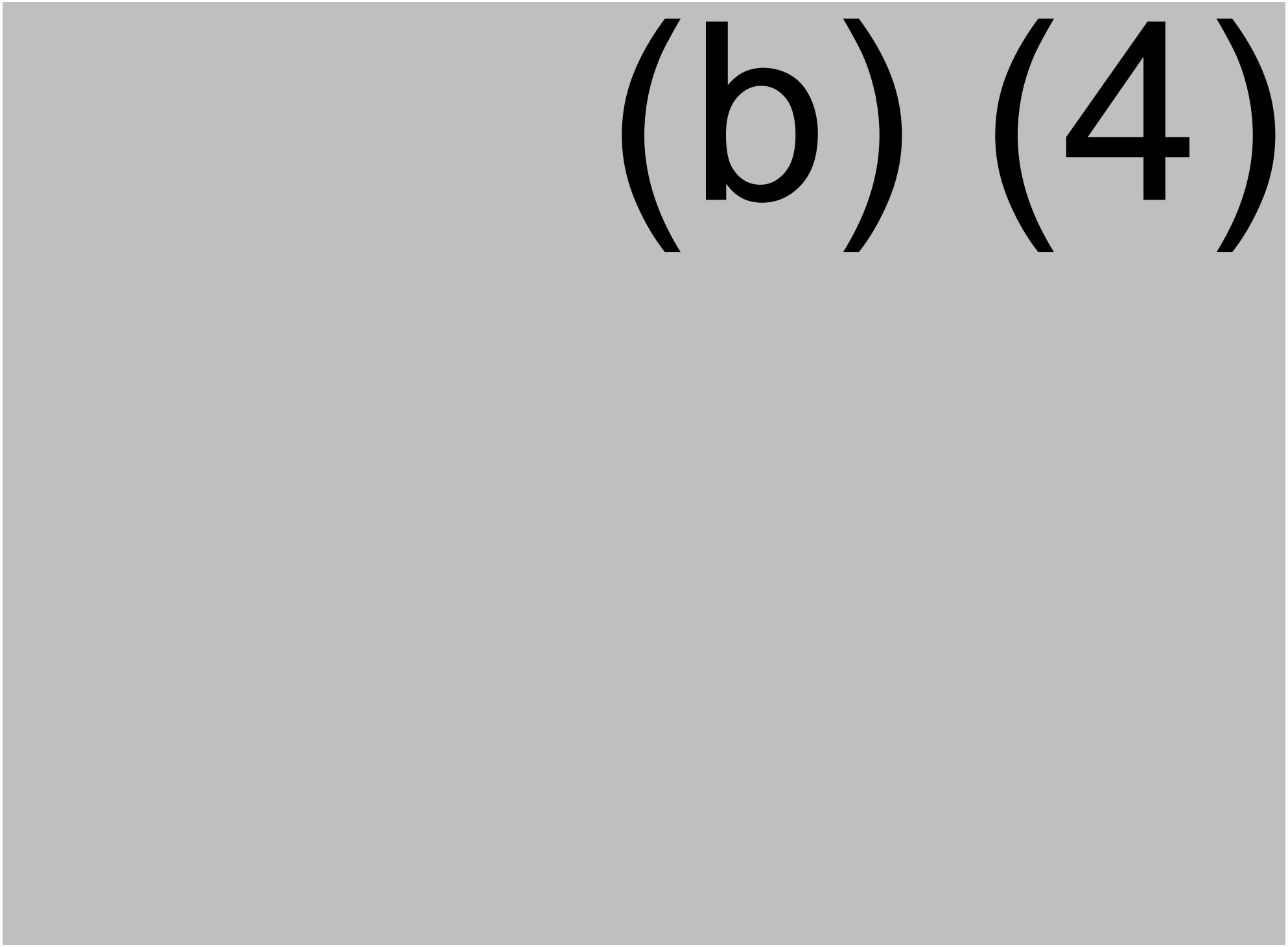

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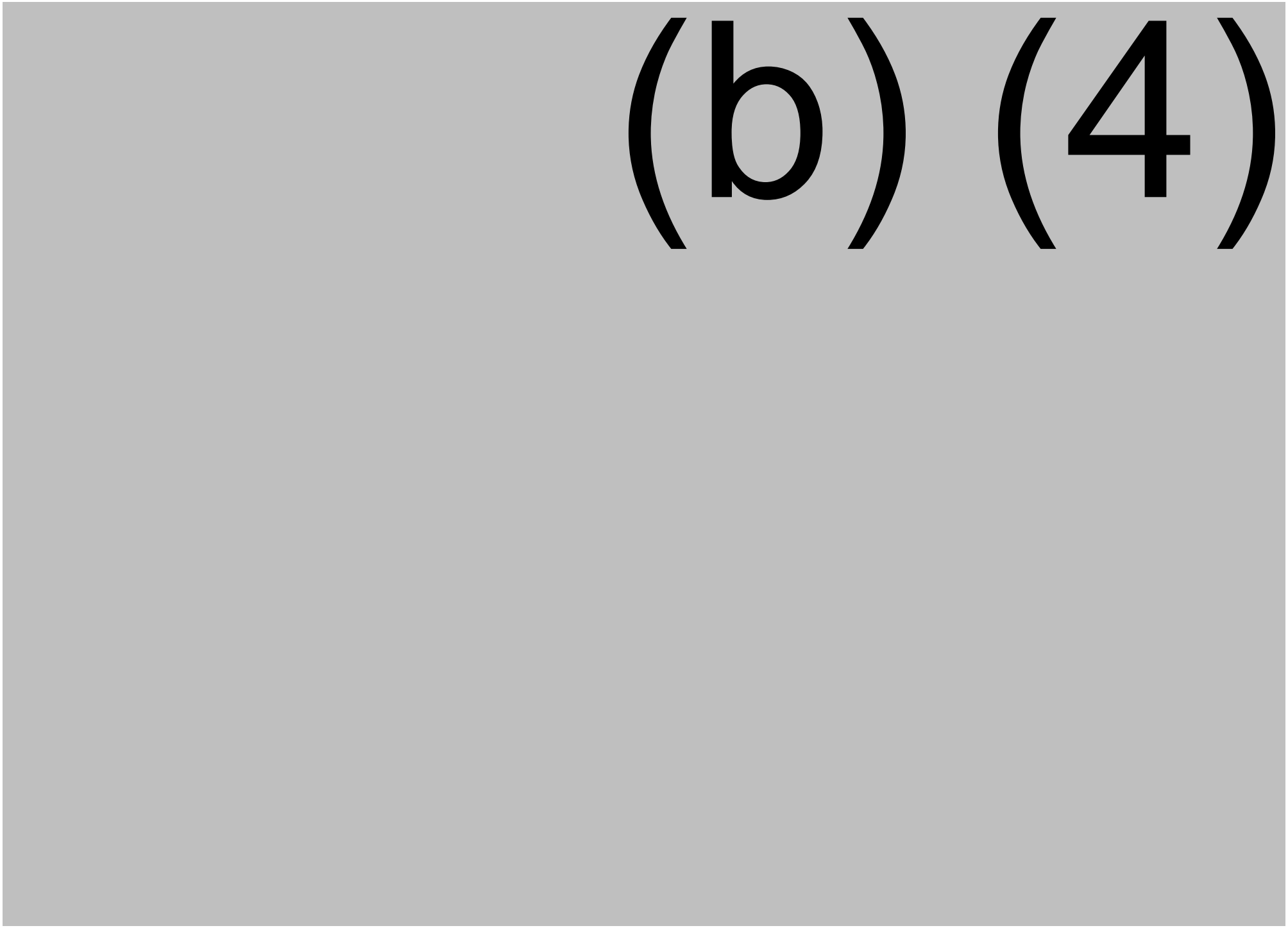

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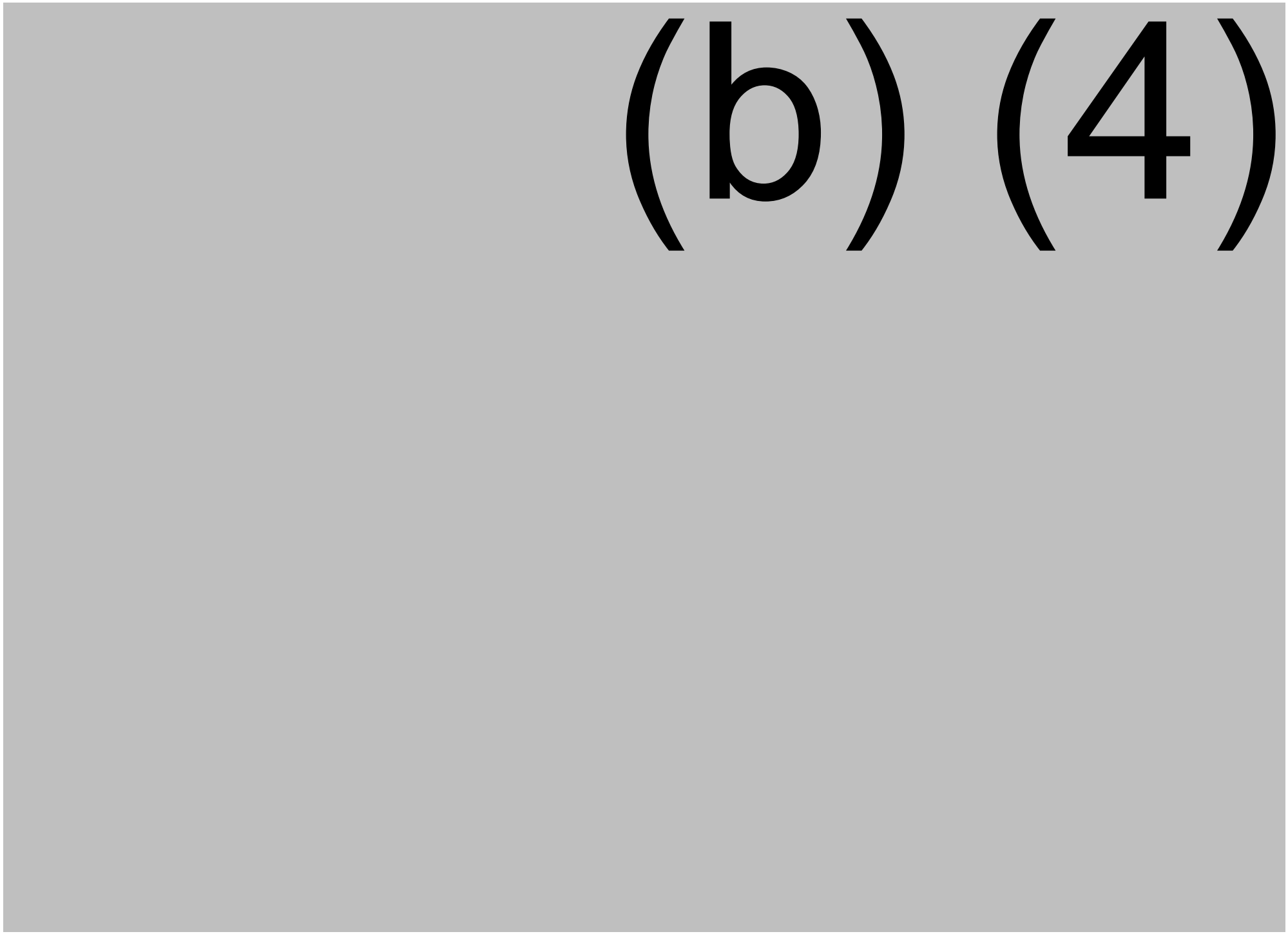

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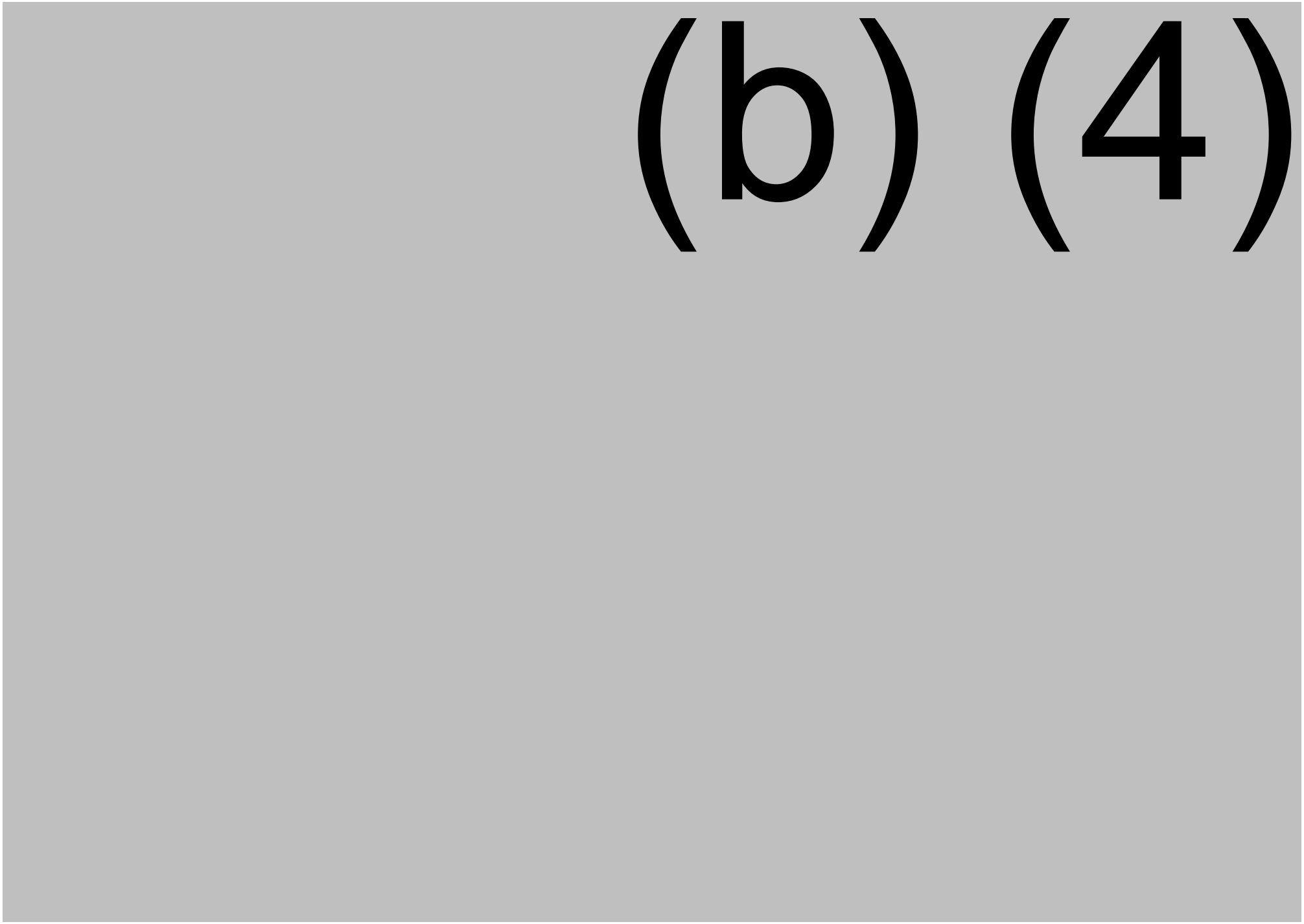

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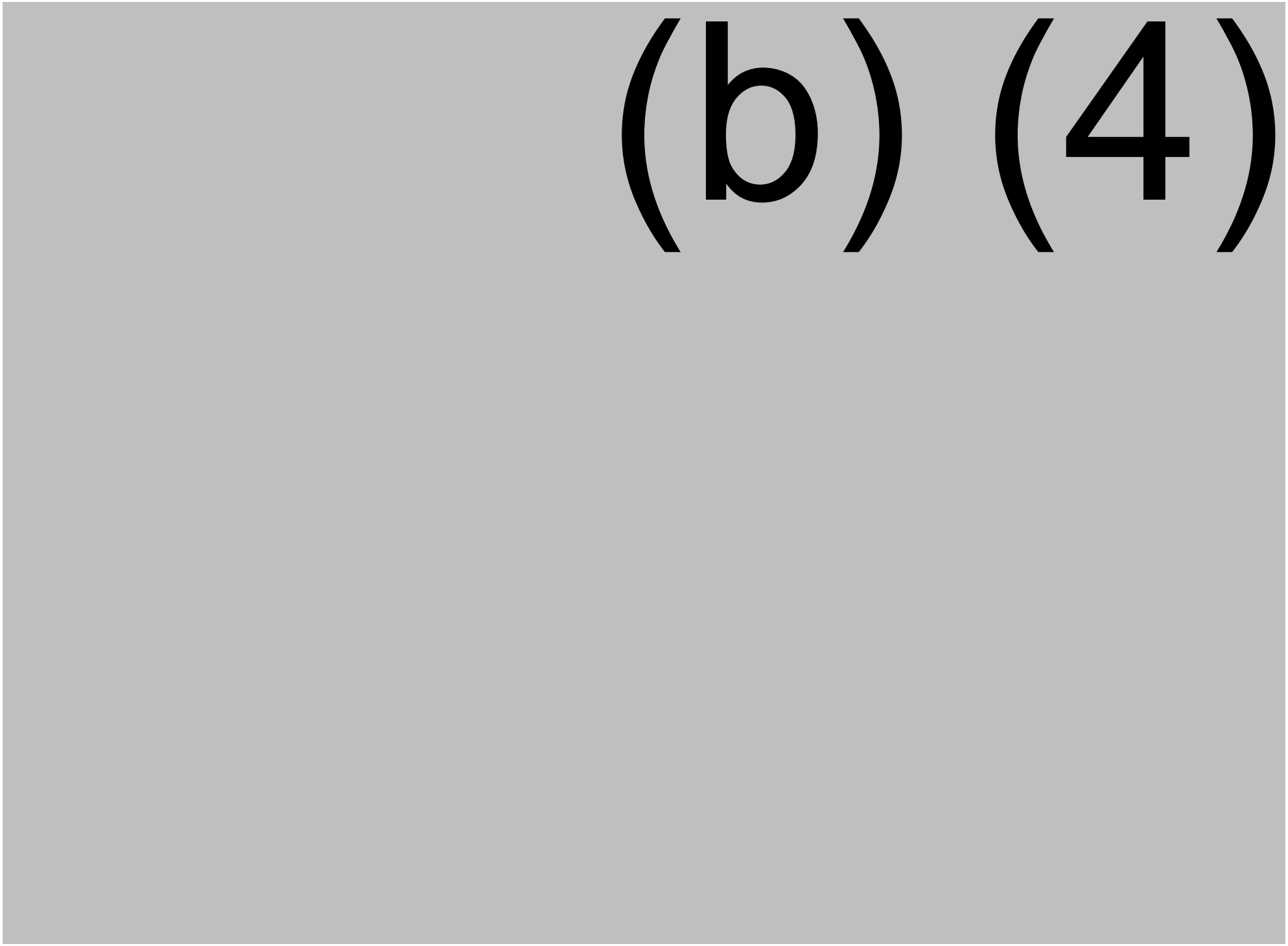

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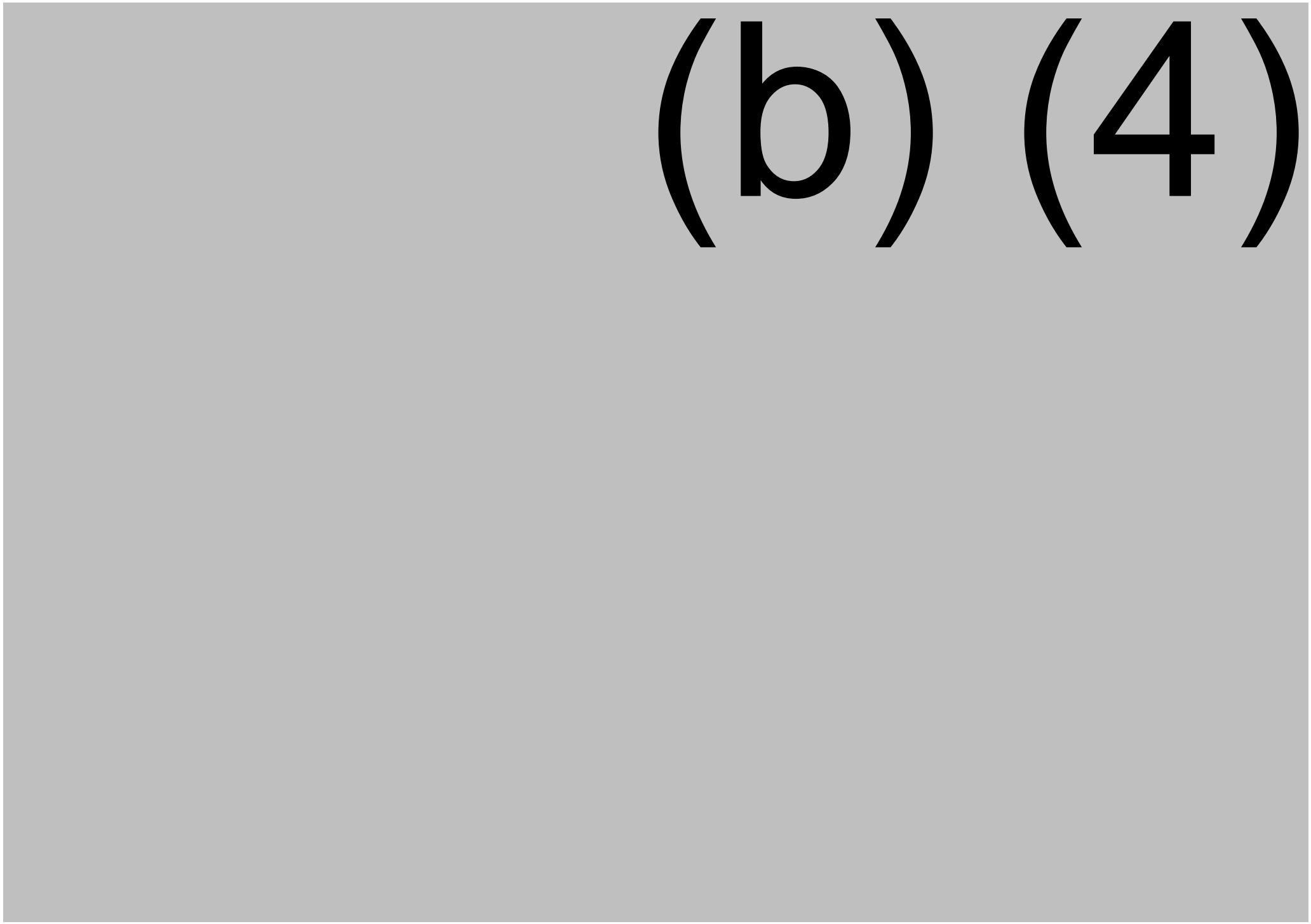

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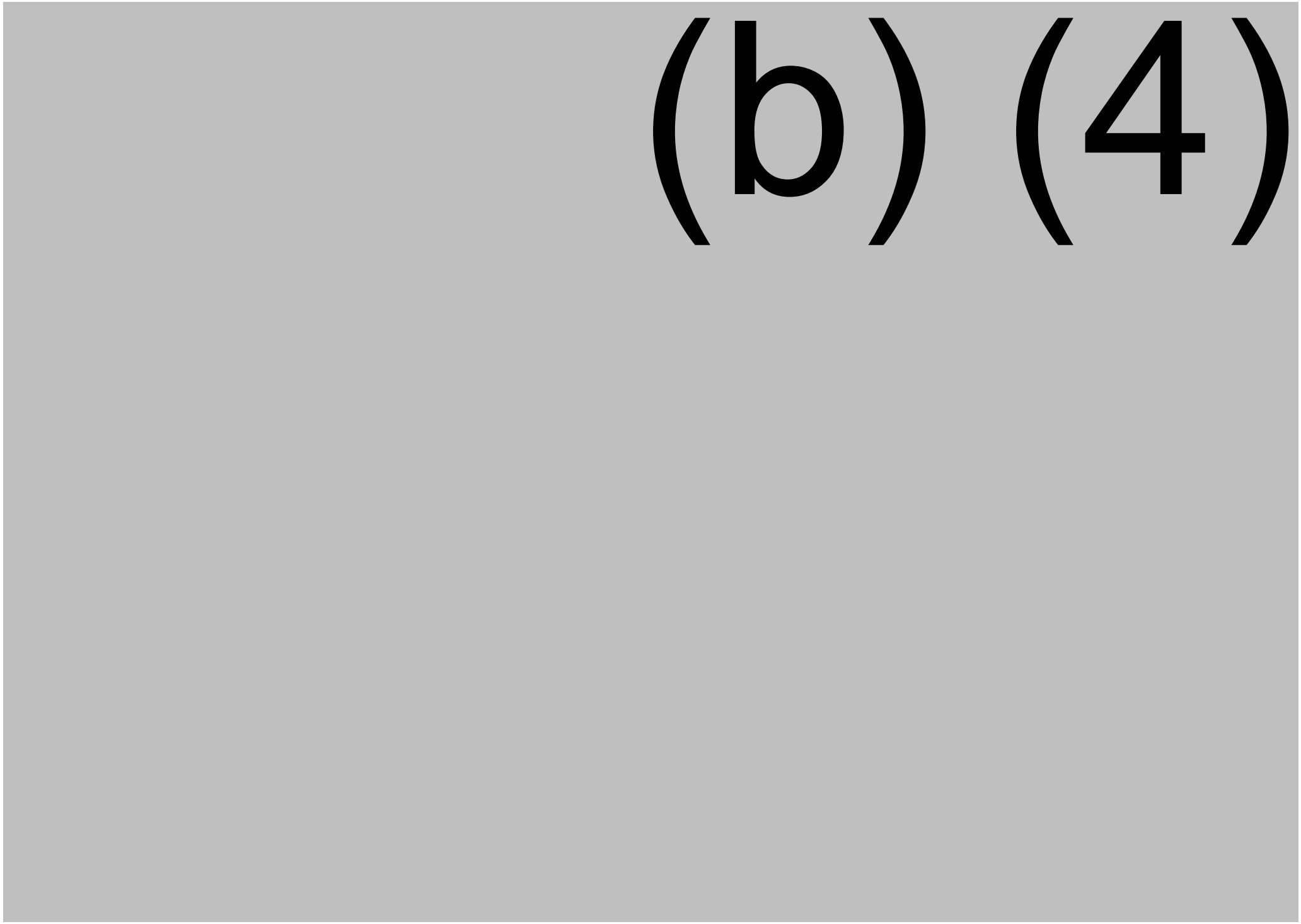

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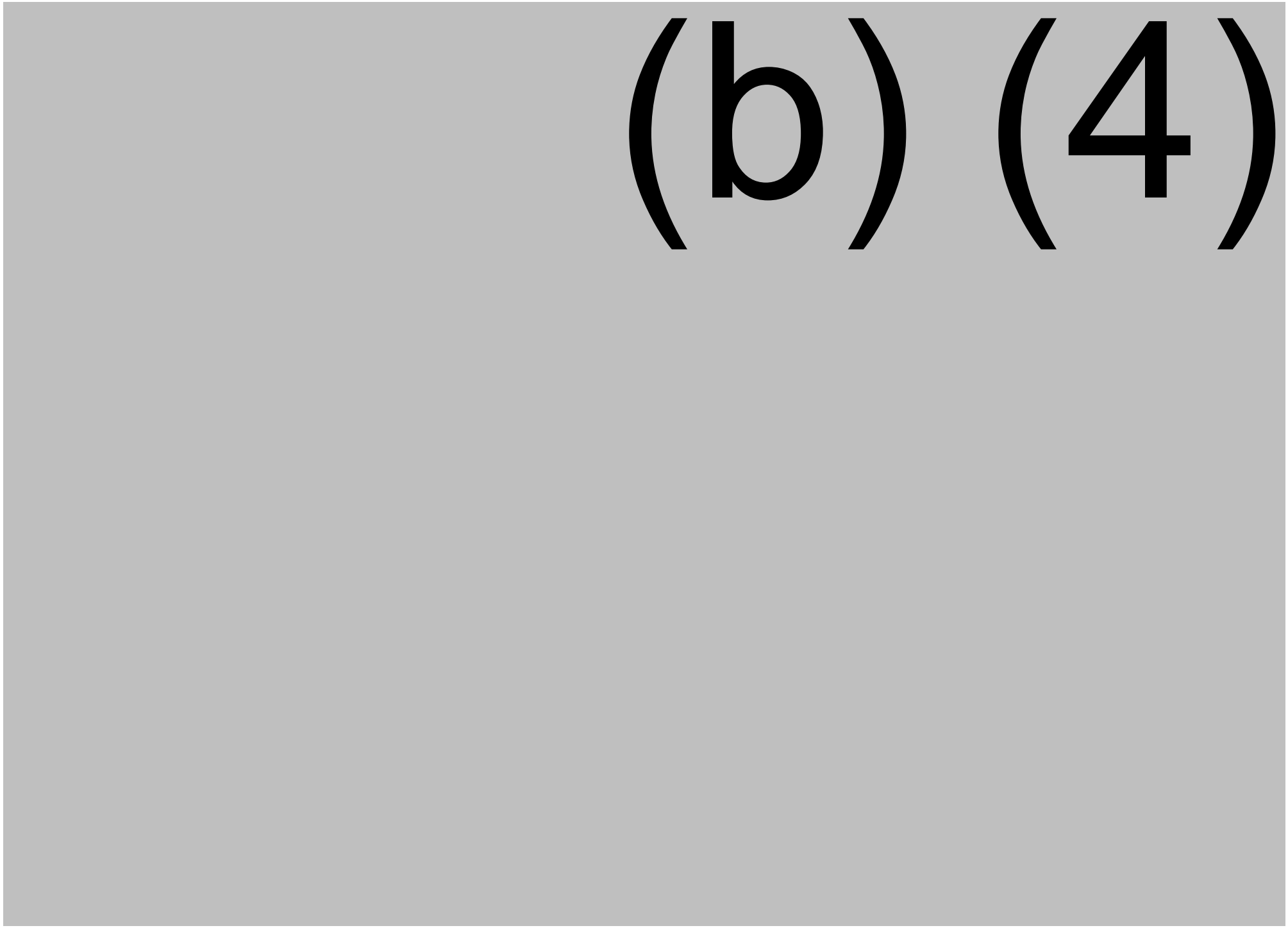

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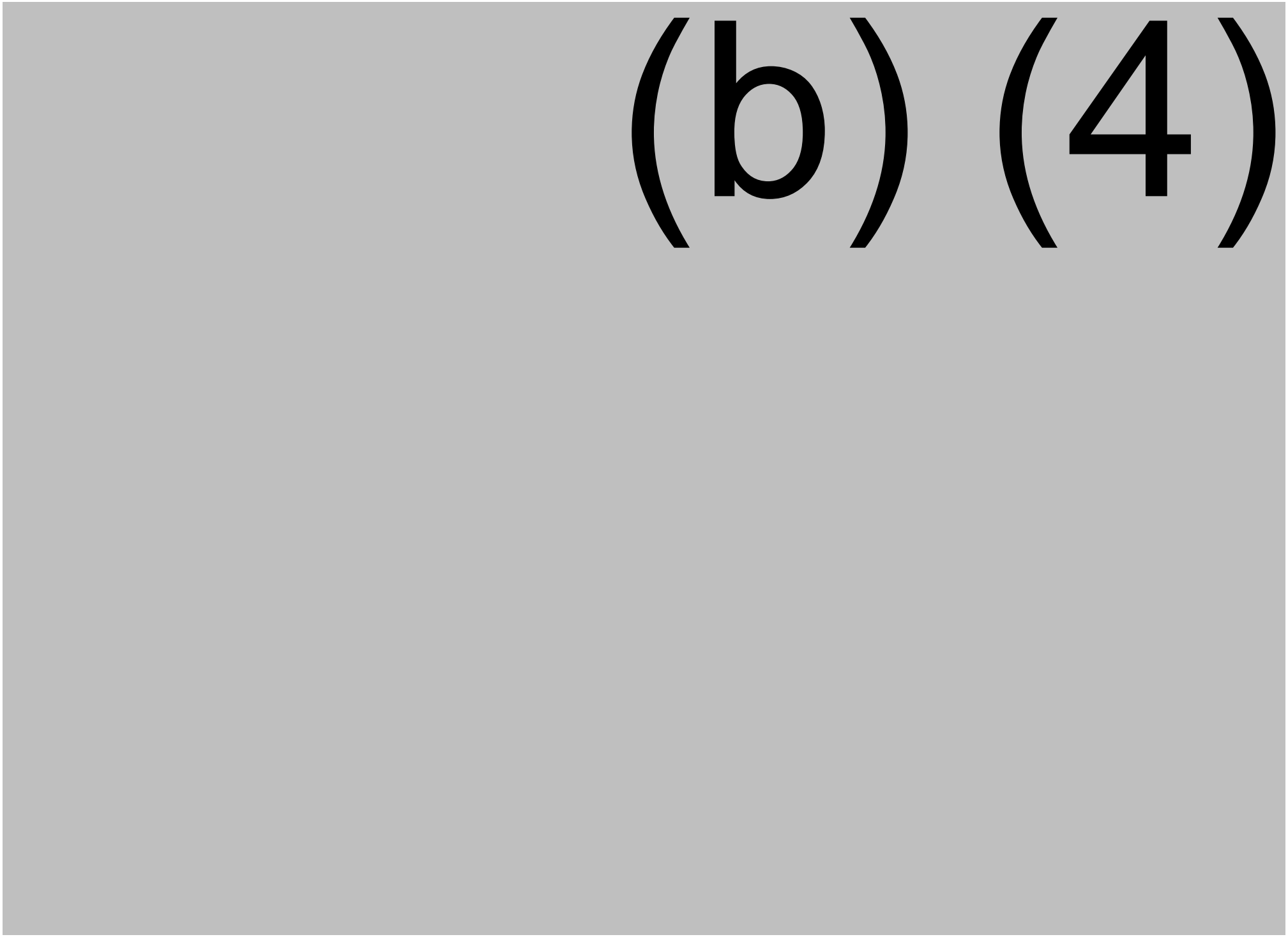

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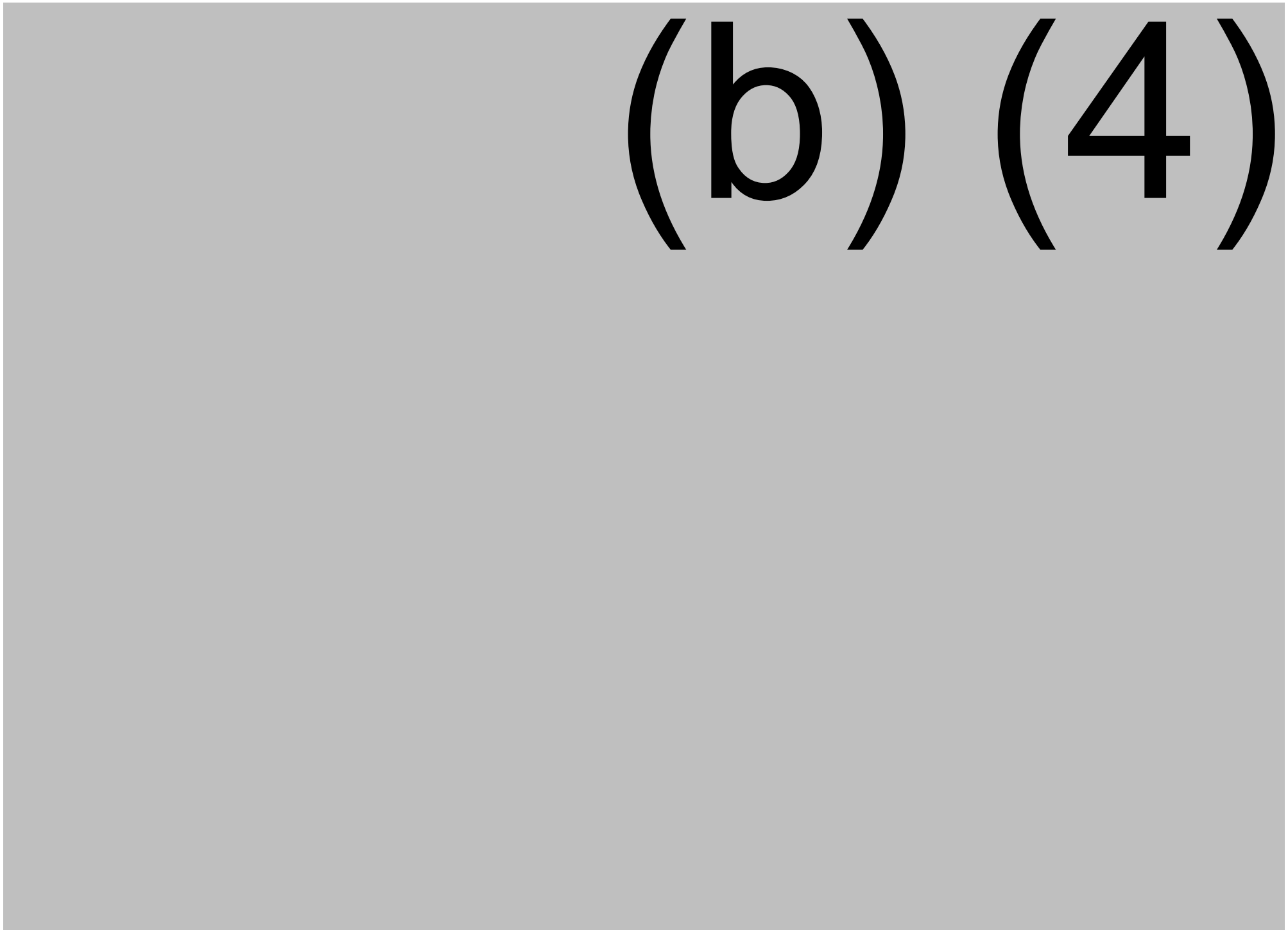

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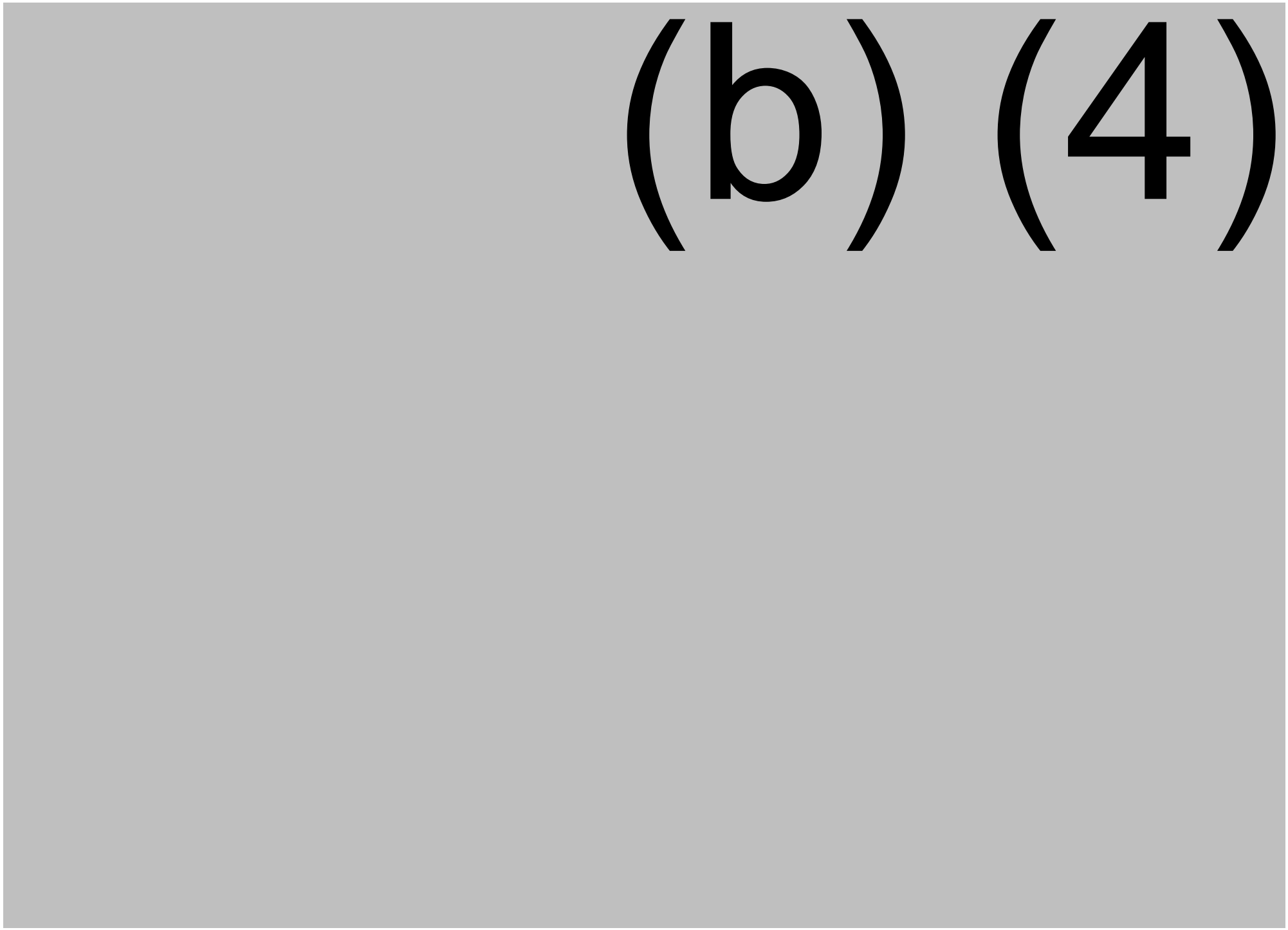

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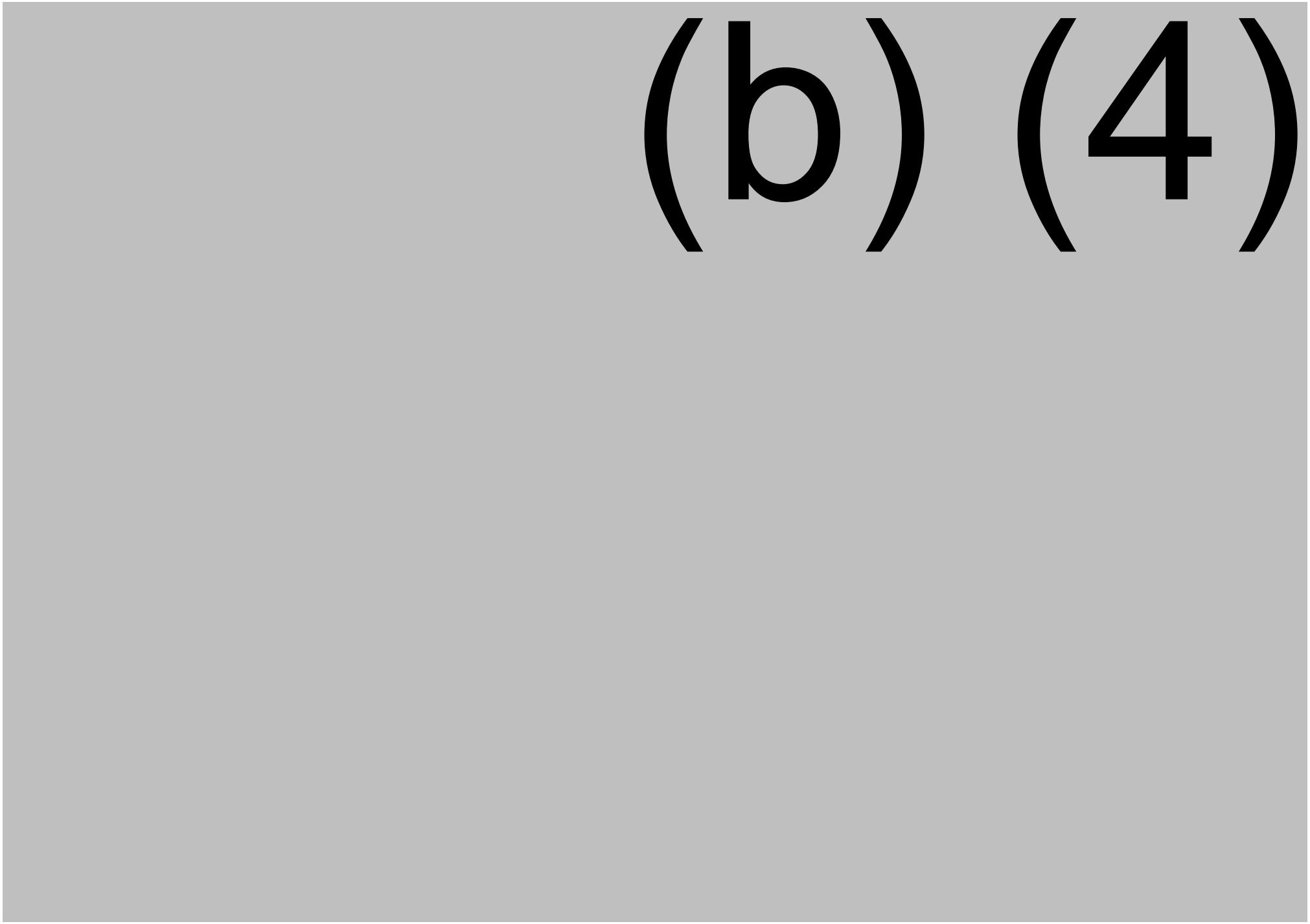

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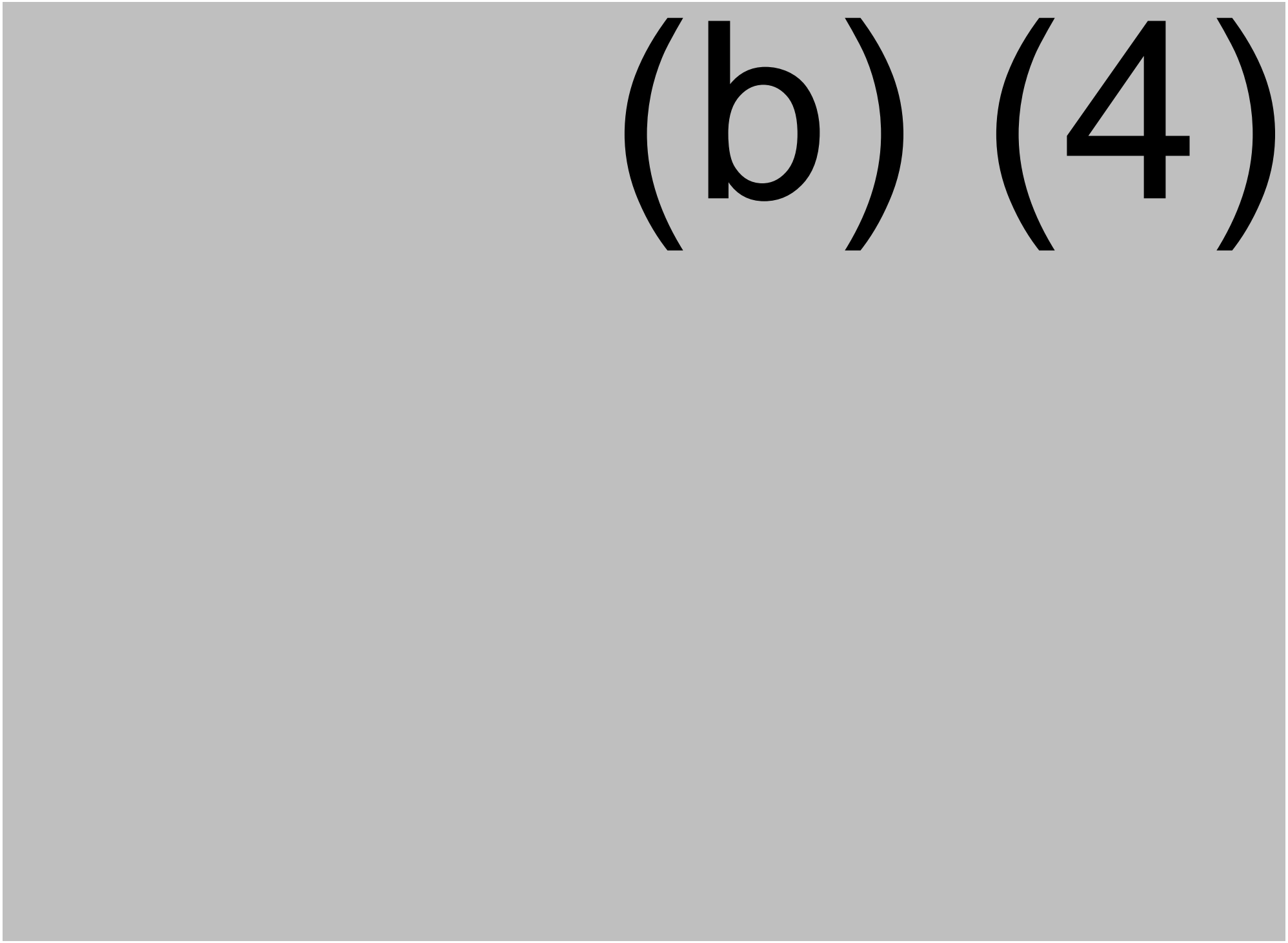

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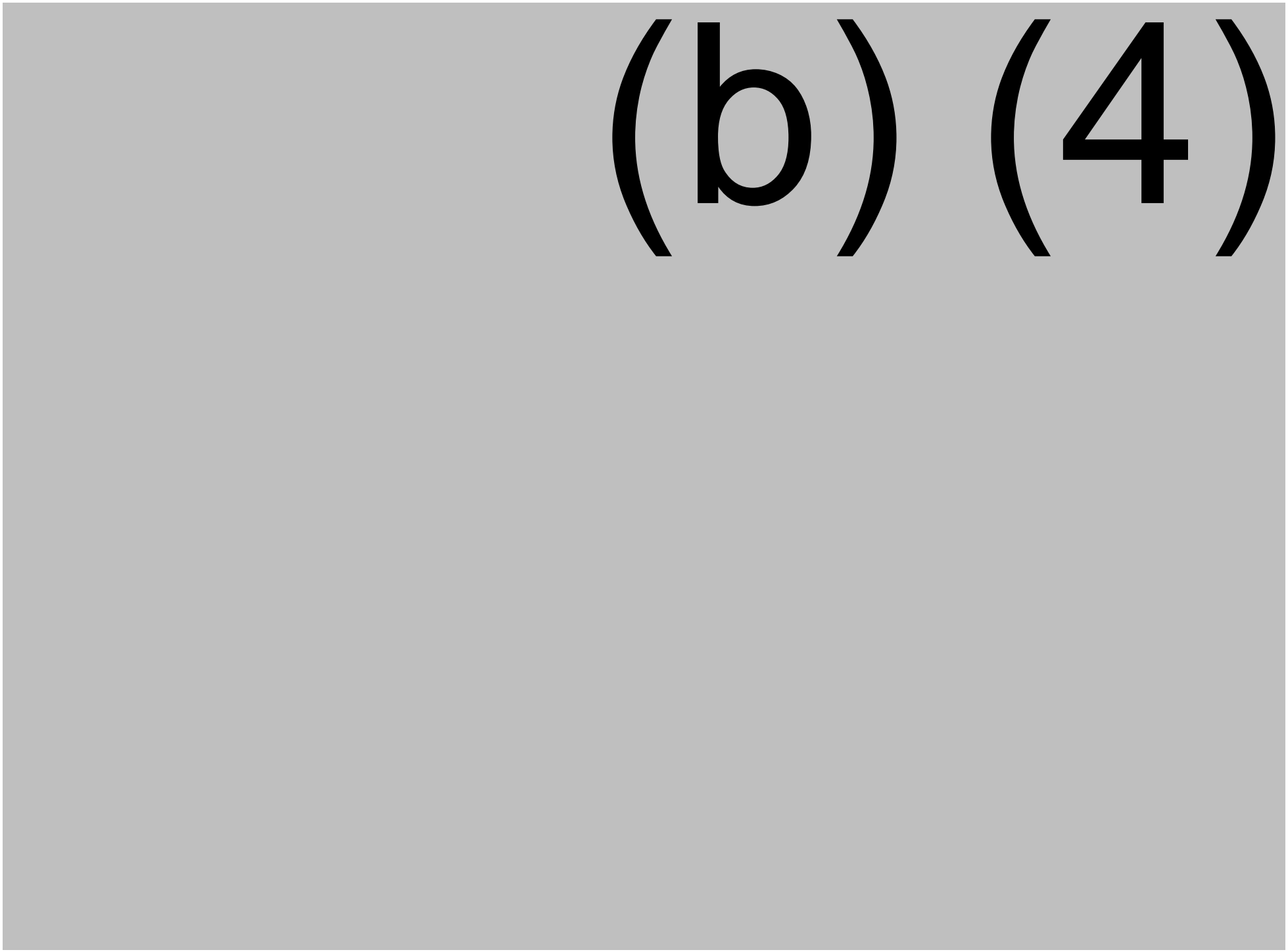

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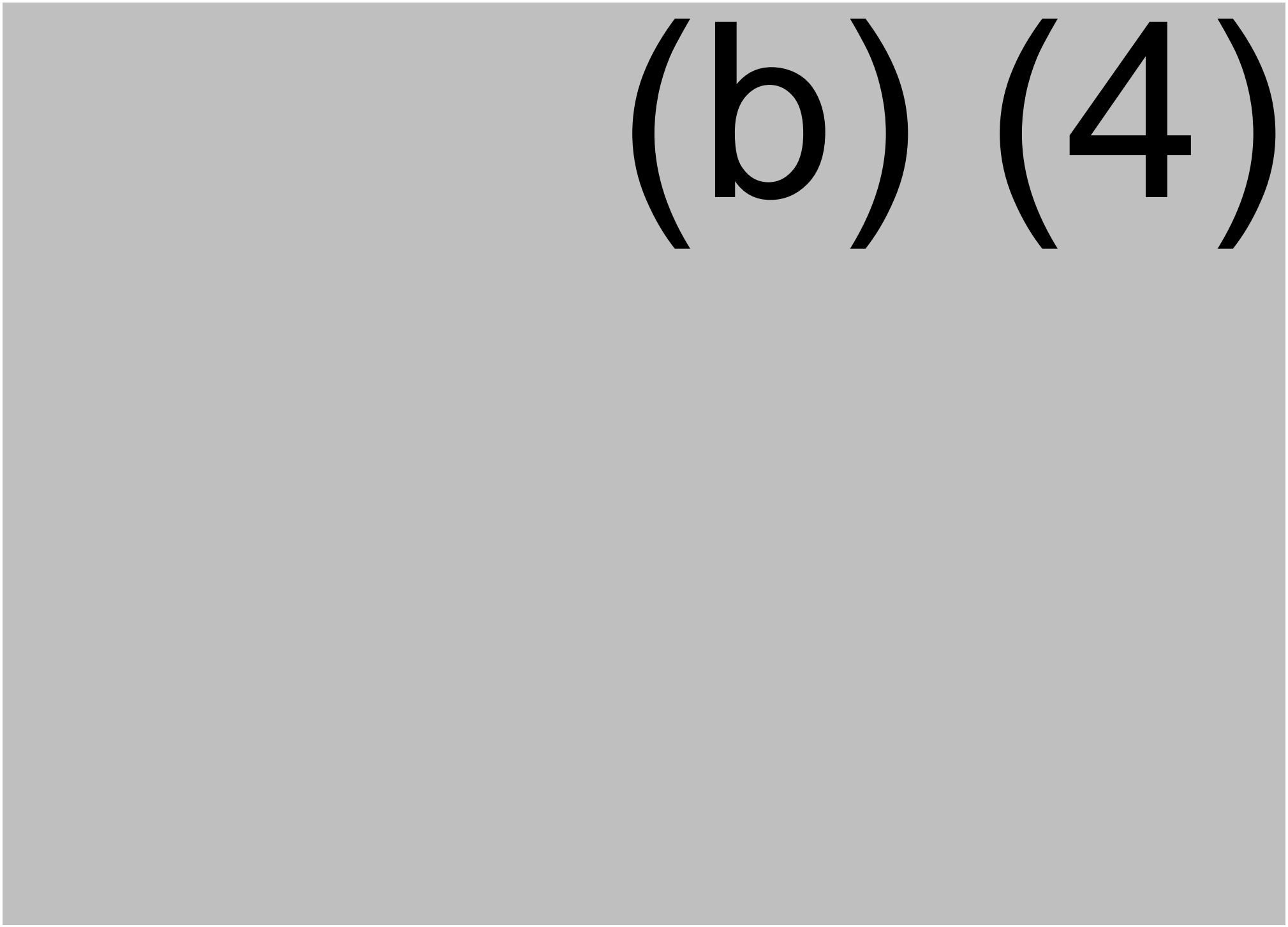

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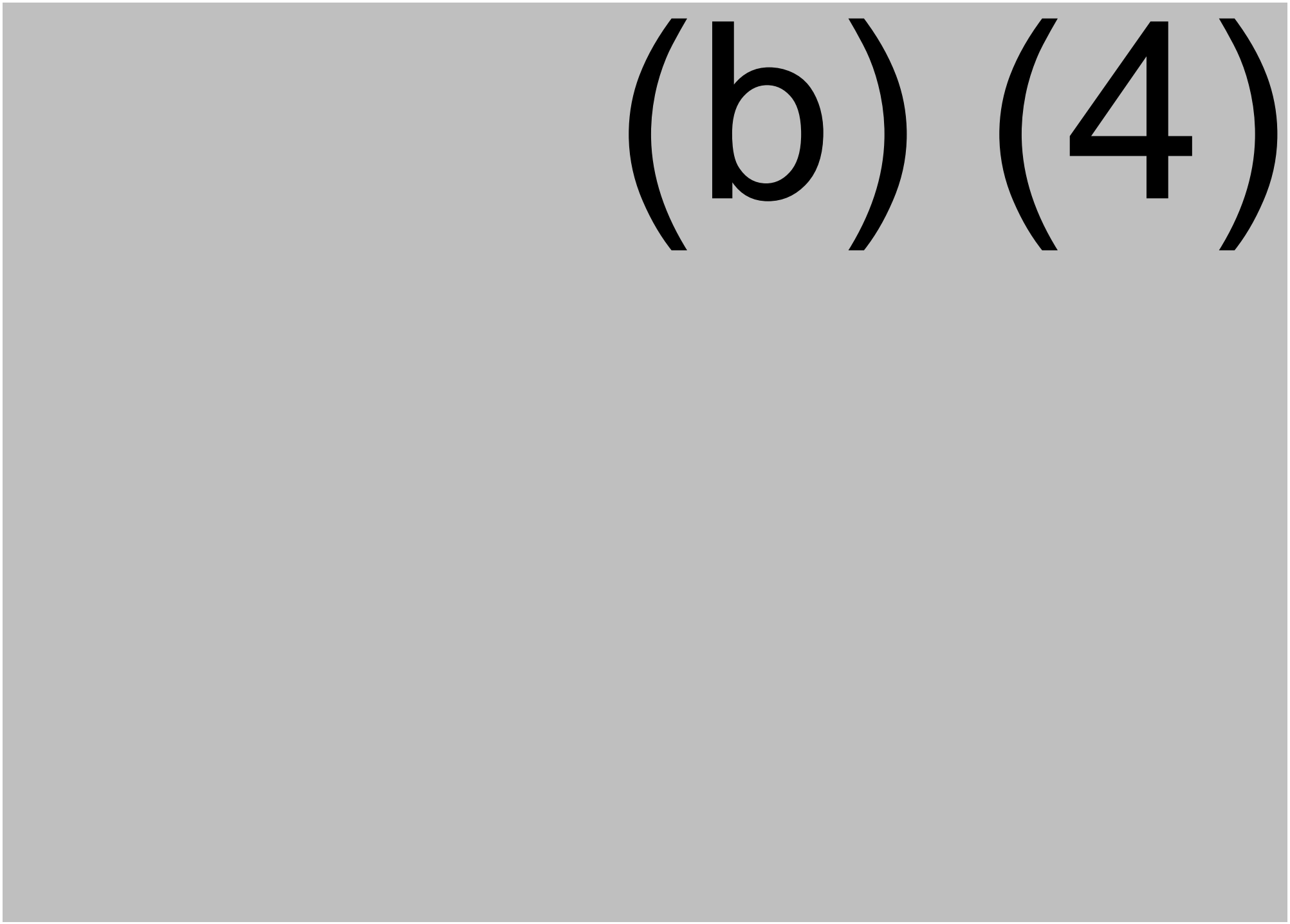

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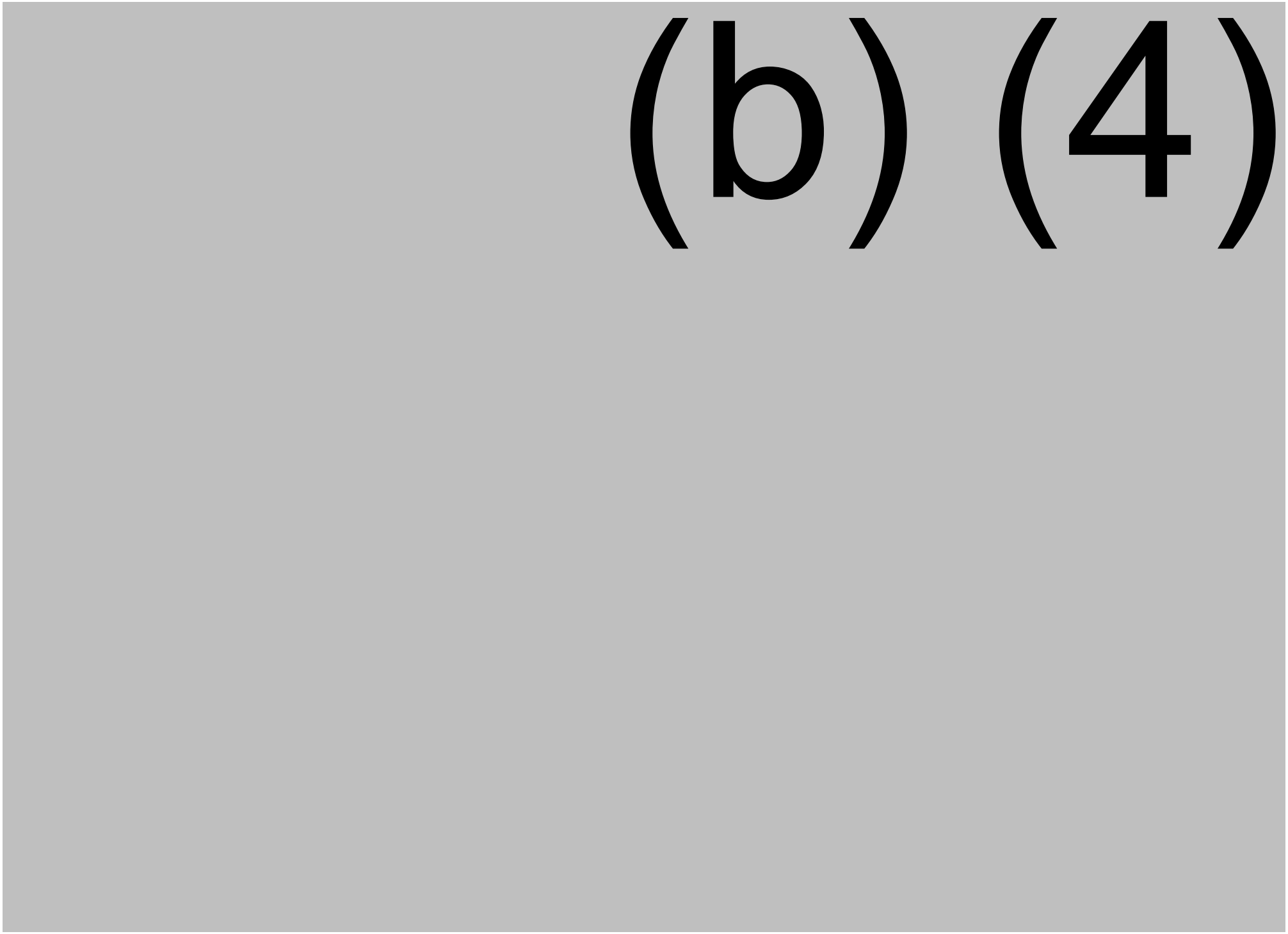

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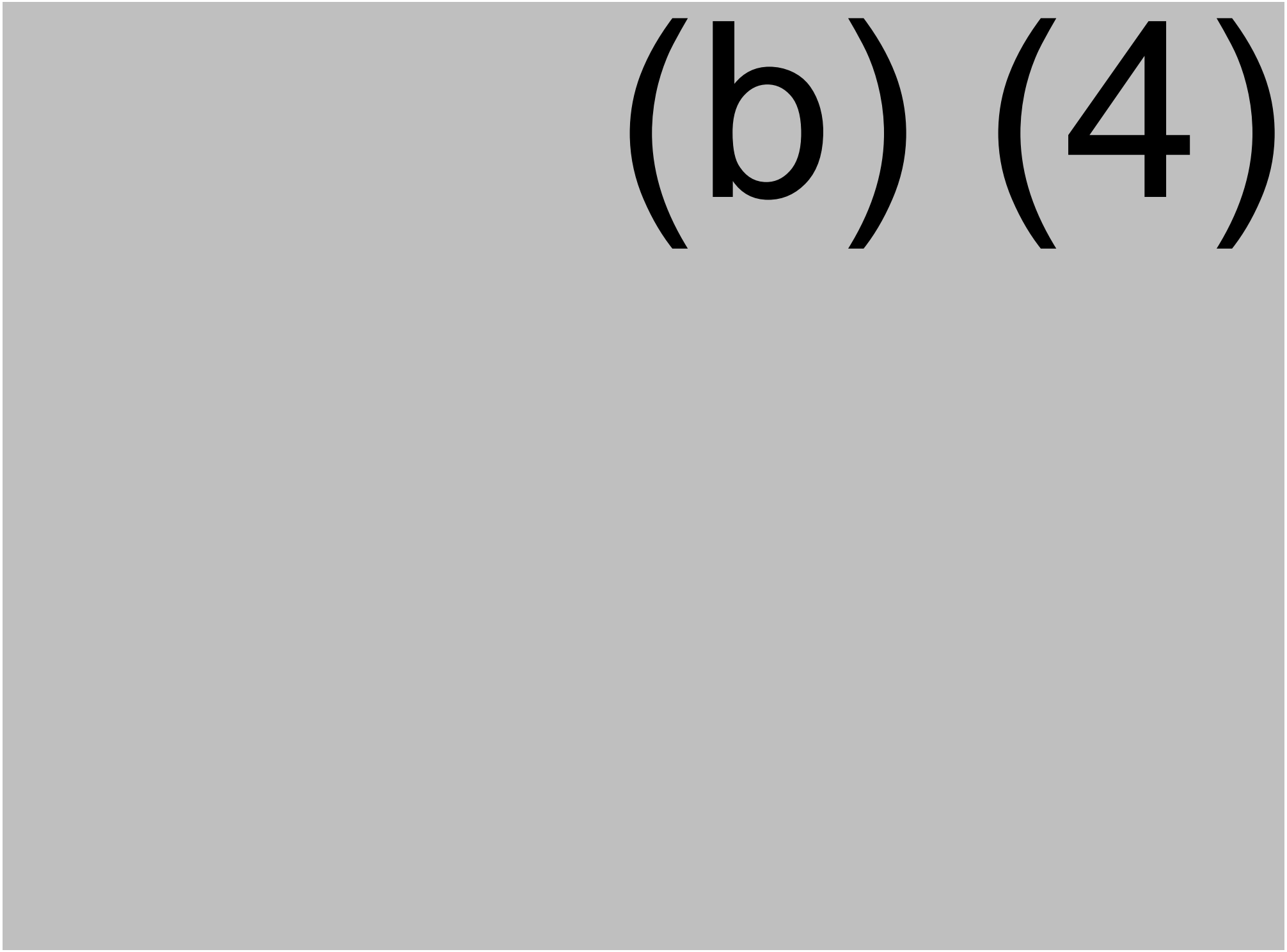

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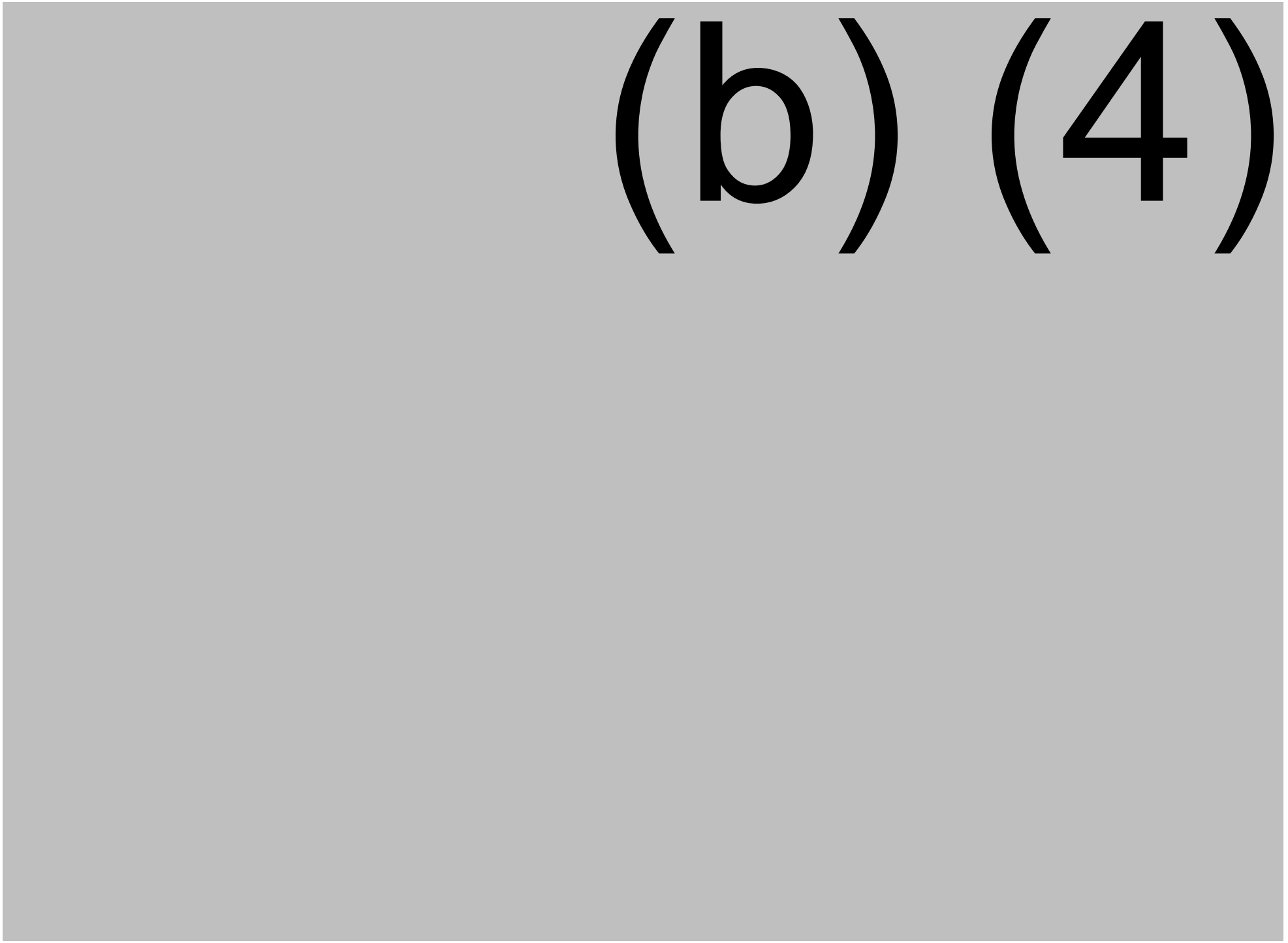

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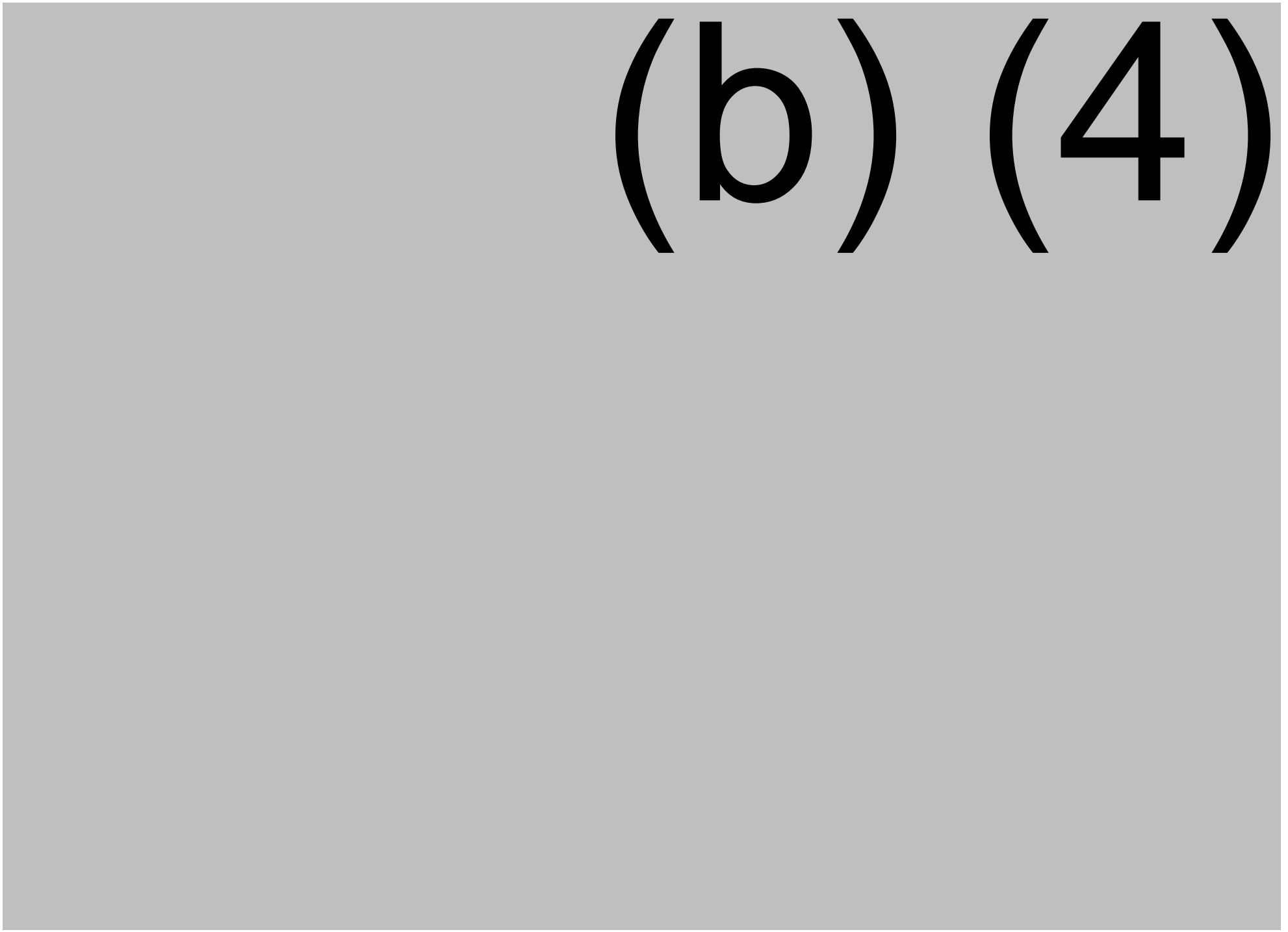

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Appendix 06: Toxigenicity Report

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Toxigenicity Report

Appendix 06: Toxigenicity Report

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Assessments of *T. thermophilus* by Regulatory Bodies

Based on the outcome of the literature search, including six Regulatory documents, *T. thermophilus* and, particularly its C1-HC strain, have been identified as non-pathogenic:

- EU Directive 2000/54/EC does not list the organism in Annex III (COMMUNITY CLASSIFICATION for agents which are known to infect humans).
- The German Committee for Biological Agents (ABAS) is responsible for the classification of biological agents into risk groups related to work activities. ABAS publishes the classifications in Technical Rules for Biological Agents (TRBA) and fungal organisms are classified in TRBA 460. The current document (updated April 14, 2020) lists *M. thermophila* /*T. thermophilus* into Risk Group 1 (**Table 1**).
    - The German Social Insurance Committee follows the ABAS classification; the current listing refers to M. thermophila as the anamorph of the teleomorph Thielavia heterothallica. Therefore T. thermophilus/M. thermophila is listed as Risk Group 1.
- The German Central Committee on Biological Safety (ZKBS) evaluated *T. thermophilus* and finalized its recommendation in May 2019 (Zentrale Kommission f die Biologische Sicherheit, 2019) as follows:

    1. Based on the long history of safe industrial use of production organisms derived from the HC strain, the derivative of the natural C1 strain, subsequent host and recipient strains in this HC safe lineage were evaluated and categorized as Risk Group 1.
    Please note: *T. thermophilus* strain DSM 33149, the production strain for mannanase 19287, and its host strain *T. thermophilus* (b) (4) , are descendants of *T. thermophilus* C1 HC and fall into Risk Group 1.
    2. Wild-type *T. thermophilus* may, on rare occasions, infect healthy humans; therefore, the committee recommended to classify the wild-type organism in Risk Group 2.

- The US FDA food branch CFSAN reviewed the GRAS Notification for a cellulase preparation produced with a *T. thermophilus* strain. The notifier Dyadic International assessed the organism as non-pathogenic and non-toxigenic. The agency issued a "No questions" letter in response to the GRAS Notification.

The listed Risk Groups and Biosafety Levels for *T. thermophilus* strains (and its taxonomic synonyms *Sporotrichum thermophile/thermophilum, Chrysosporium thermophilum,* and *Myceliophthora thermophila*) confirm the safety status of the organism. **Table 7** summarizes the findings from Regulatory agencies and cell culture depositories.

Table 1: *T. thermophilus* safety classification into Risk Groups or Biosafety Levels

Regulation/Cell Depository	Risk Group	Biosafety Level	Comment
EU Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work (ANNEX III, COMMUNITY CLASSIFICATION for agents which are known to infect humans)	N/A	N/A	*T. thermophilus* or its synonyms are not listed
ABAS (TRBA-460, Classification of Fungi)	1	N/A	Listed as *M. thermophila*
ZKBS	1 2*	N/A	*T. thermophilus* C1 strain derivatives starting with strain HC (Visser, H. *et al.*, 2011) **T. thermophilus* wild-type
GESTIS Biological Agents Database	1	N/A	Listed as *M. thermophila*
DSMZ (German Collection of Microorganisms and Cell Cultures)	1	N/A	Two *M. thermophila* strains
CBS/Westerdijk Fungal Biodiversity Institute	N/A	1	Ten *T. thermophilus / M. thermophila* strains
ATCC American Tissue Culture Collection	N/A	1 2#	16 *T. thermophilus / M. thermophila* strains # Two strains BSL 2, isolated from immunocompromised patients

History of Safe Industrial Use

Dyadic has been manufacturing C1-produced technical enzymes since 1996, including a cellulase for use in textiles; later Dyadic licensed the C1 technology to enzyme producers in biofuels (b) (4) BASF has licensed the technology from Dyadic for the production of enzymes. In 2015, (b) (4) purchased the C1 technology for enzyme production.

Dyadic developed a GM C1 strain for the production of cellulase used as a food additive in the production of beer, wine and juices ("CeluStar CL"). Their evaluation of safety included genotoxicity and oral toxicity studies of the enzyme preparation as well as a pathogenicity study of the recombinant production strain. An external group of experts reviewed the data and agreed with Dyadic's determination of safety. Following their agreement, Dyadic International notified the US Food and Drug Administration (FDA) of their determination that the enzyme preparation was generally recognized as safe for the intended use in beer, wine and juice production. FDA responded with a "No questions" letter to the GRAS notification.

In summary, Dyadic has demonstrated the safe industrial use of their proprietary C1 production strain system for over 20 years with up to 50 products for food, feed, textiles and paper/pulp.

Human Health

As mentioned in the Literature Search (Appendix 23), a total of 21 publications were progressed for detailed assessment based on full text (stage 2 evaluation). Among those publications, a recent Letter to the Editor of Clinical Microbiology and Infection (Nourrisson, C. *et al.*, 2017) reported on five cases of *T. thermophilus* infections and evaluated these cases in the context of seven previous reports in the literature. **Please note:** Our systematic literature search identified Nourrisson et al. (Nourrisson, C. *et al.*, 2017) as well as all seven previous case studies reviewed in the publication. All eight references were evaluated individually during the Stage 2 review.

The 2017 letter (Nourrisson, C. *et al.*, 2017) reported five individual cases of *T. thermophilus* infections and compared them with seven previously published case studies, covering the time span between 1990 and 2016. 5 / 12 cases involved severely immunocompromised patients, the remaining 7 / 12 cases involved patients with pre-existing health conditions or serious trauma.

Pulmonary and disseminated infections were found only in immunocompromised patients. The origin of focal infections in immunocompetent patients were related to trauma or surgery. Four of the 12 cases ended with the death of the patient, three of those in severely immunocompromised individuals. The fourth case was a patient with repeated implants of human aortic allografts. Treatments included surgery with or without antifungal therapy, with *T. thermophilus* showing low MIC values towards antifungal medications.

These results are in agreement with case studies on other filamentous fungi used as production organisms, e.g. *Aspergillus niger* (Challa, S., 2018) or *Trichoderma reesei* (Kredics, L. *et al.*, 2003), as well as other non-*Aspergillus* molds (Douglas, A.P. *et al.*, 2016; Revankar, S.G. and Sutton, D.A., 2010). Taken together, the results demonstrate that immunocompromised individuals are risk for higher morbidity from fungal infections.

In summary, healthy individuals are unlikely to be affected by *T. thermophilus*/*M. thermophila*, the organism is therefore considered non-pathogenic.

WGS Analysis for Toxins

The UniProt Toxin-Database (https://www.uniprot.org/program/Toxins) was used to identify toxins of fungi in the database. The searches identified 114 fungal toxins (as of 12/5/2023, Annex 4)), including 34 toxins in the phylum Ascomycota that *T. thermophilus* belongs to. **Table 2** lists 13 toxins from ascomycetes (see Annex 3 for the listing of all 114 fungal toxins). The DSM 33149 genome was queried against all 114 sequences of identified fungal toxins to evaluate the potential for expressing toxins found in fungal species.

The BLASTP analyses showed no hits to 108 of 114 fungal toxins. Only 6 toxins generated homology hits in the DSM 33149 genome (**Table 2)**.

Table 2: Fungal toxins listed in UniProt

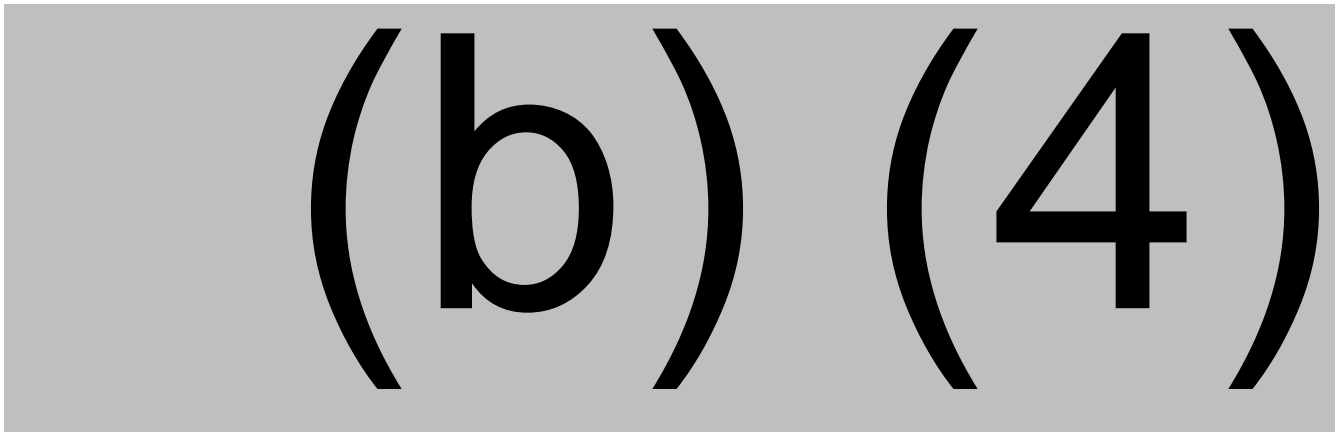
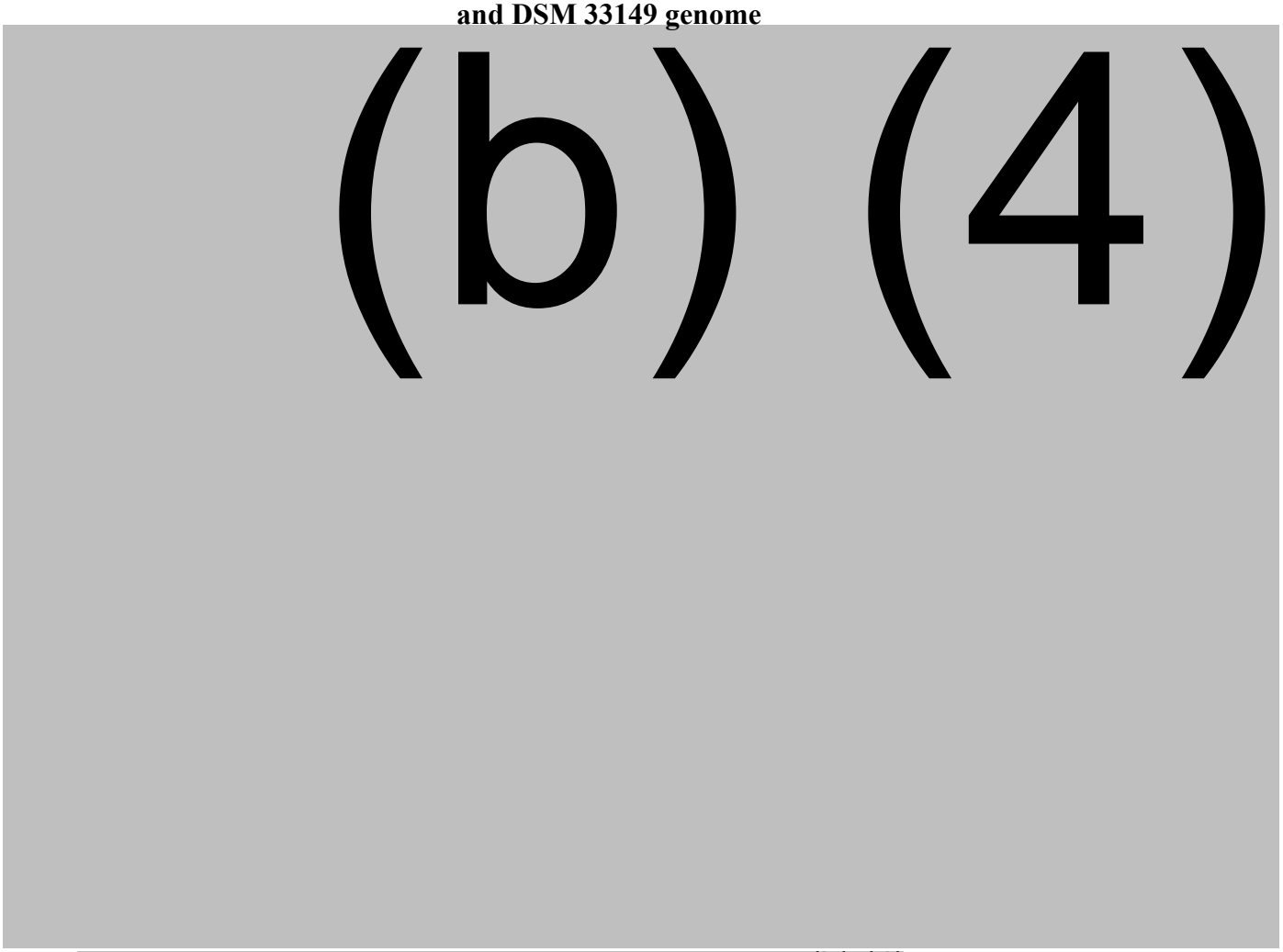


Table 3: BLAST P search results for homologies identified between fungal toxins and DSM 33149 genome



(b) (4) is composed of three components/subunits: alpha, beta and gamma. All homologies have been found between the alpha subunit of the toxin and the production strain genome. The alpha subunit codes for a chitinase and

is not toxic in itself. No alignment is found with either beta or gamma subunit. The gamma subunit is the only true toxin that requires both alpha and beta subunits to cross the cell membrane of the target organism and block mitosis in the target organism (Butler, A.R. *et al.*, 1991). In addition, the reference genome *T. thermophilus* (strain ATCC 42464) assembly site lists 39 potential chitinase genes (https://mycocosm.jgi.doe.gov/Spoth2/Spoth2.home.html), which provides a rationale for the found homology.

(b) (4) is a phytotoxin and belongs to a group of proteins widely distributed among fungi, in particular in Ascomycetes and Basidiomycetes. It exhibits both phytotoxic activities and can function to elicit plant defense responses when expressed in beneficial fungi, including *Trichoderma* sp. Cell-wall as well as chitin-binding activities have also been described for CP proteins (Pazzagli, L. *et al.*, 2014). Due to the proteinaceous nature of the substance, the phytotoxin will be degraded in the target animals digestive system.

In summary, none of the identified toxins show significant homologies to proteins in the genome of *T. thermophilus* DSM 33149.

Mycotoxins

Experimental results demonstrate the absence of mycotoxins (aflatoxins, deoxynivalenol/vomitoxin, fuminosins, zearalenone, ochratoxin A) from DSM 33149 fermentation supernatants, Mannanase CC and (b) (4). Two Natupulse® TS test articles demonstrated the presence of Deoxynivalenol/Vomitoxin (DON) and zearalenone (ZEA) at low levels, likely from its main ingredient, the feed material wheat bran. After changes to the raw material supply, no additional mycotoxin findings were recorded. Three Natupulse® TS production batches have been analyzed and all were found to be below the LOD/LOQ of these mycotoxins (Appendix 16).

In addition, no publications have been identified that would indicate the production of mycotoxins (aflatoxins, deoxynivalenol/vomitoxin, fuminosins, zearalenone, ochratoxin A) by *T. thermophilus/M. thermophila*. Lastly, a GRAS Notification to CFSAN evaluated a cellulase preparation produced by a recombinant production strain of the progenitor C1-HC lineage for the intended use as an enzyme in the production of wine, beer and fruit juices. The notice demonstrated the absence of mycotoxins in the enzyme preparation (Dyadic International (USA) Inc, 2009; FDA Center for Food Safety and Applied Nutrition (CFSAN), 2009)}. Please note: the *T. thermophilus* production strain DSM 33149 is also derived from the progenitor strain T. thermophilus C1-HC.

Emerging mycotoxins / secondary metabolites – Tenuazonic Acid and Myceliothermophins

Tenuazonic Acid (TeA) is a secondary metabolite produced primarily by *Alternaria ssp.* and a few other ascomycetes, including *Epicoccum sorghinum* and *Pyricularia oryzae* (previously identified as *Magnaporthe oryzae*). TeA biosynthesis is catalyzed by the enzyme TeA synthetase (TAS1), which in turned is regulated in *P. oryzae* by a Zn(II)2-Cys6-type transcription factor, now identified as TAS2. When searching for TAS2 homologs, the *T. thermophilus* genome was shown to include TAS1 and TAS2 homologs (Yun, C.-S. *et al.*, 2017).

Preliminary studies using a commercial TeA standard for spiking into Mannanase CC enzyme

concentrates indicated that TeA cannot be identified or quantitated in the BASF laboratory method described below for myceliothermophins. Instead, Mannanase CC production batches and final Natupulse TS and TS L product batches derived from them were sent to an external lab accredited for the analysis of TeA in food samples. Given the comparable nature of the feed additives to food components, the laboratory agreed to test the samples for the presence of TeA using the accredited test method. This method is derived from the published detection method (Siegel, D. *et al.*, 2009)

Table 4 lists the results for two commercial Mannanase CC, three commercial Natupulse TS and three Natupulse TS batches. Please see Annex 3 for the analytical reports.

Table 4: TeA content of Mannanase CC, Natupulse TS and Natupulse TS L commercial production samples (Assay LOQ 10 µg/kg)

	Mannanase CC			Natupulse TS			Natupulse TS L		
Lot #	(b) (4)								
TeA [µg/kg]	12	<10		11	<10	<10	<10	<10	<10

The systematic literature search identified two references reporting the isolation of a class of secondary metabolites from *T. thermophilus*, named myceliothermophins, in organic extracts of the organism (Gao, Y.-L. *et al.*, 2019; Yang, Y.L. *et al.*, 2007). Using a commercially available Myceliothermophin E (MT E) standard, all three Mannanase CC production batches were tested by LC-MS with an analytical method developed at BASF SE for this purpose. Samples of the concentrate, either in the absence or the presence of spiked MT E, were mixed with MeOH (1 volume sample + 10 vol MeOH). This treatment precipitates proteins and other interfering substances. After centrifugation, the supernatant was tested directly by LC/MS.

Analysis 2020

Peaks corresponding to the retention time established for the purified standard were detected in CC samples spiked with 10 or 1 µg/L (or ppb) MT E. No corresponding peaks were found in samples not spiked with the metabolite. In addition, the manufacturing process for Mannanase 19287 separates all production organisms (rendered non-viable) from the fermentation broth during the first steps of downstream processing and no organic solvents or cell disruption are used during the entire manufacturing process. As demonstrated during the 90-day subchronic oral toxicity study of mannanase 19287, no toxicological or pathogenic activities were identified in the concentrate. Finally, given that the IC_{50} for MT E towards cancer cell lines has been determined at 0.25 – 0.41 µg/mL (ppm) (Yang, Y.L. *et al.*, 2007), the absence of a signal at the 250-fold lower concentration (1 ppb vs 250ppb) in the Mannanase CC concentrate further substantiates the safety argument.

Analysis 2023

Using commercially available MT standards for MT A/B, a racemic mix of both stereoisomers, MT C and MT E, Mannanase CC production batch (b) (4) (BASF internal ID (b) (4) and batch (b) (4) ((b) (4) were analyzed according to the above mentioned laboratory method. Concentrate samples with or without MT spikes (at 10 µg/L or ppb) were extracted with MeOH or EtOH and tested for the presence of the known MT cyto/mycotoxins. No substantial

differences were observed between the chromatograms analyzing the respective alcohol extracts.

Specific peaks of MT substances were observed in spiked Mannanase CC samples, whereas non-spiked samples did not exhibit specific peaks at the identified run times. These results from this study demonstrate that myceliothermophins A/B, C and E can be detected when spiked into two mannanase 19287 concentrates from large-scale fermentations produced with the *T. thermophilus* production strain DSM 33149. This confirms and expands on our previous results for myceliothermophin E spiked into three previous commercial concentrates.

In summary, the emerging MT mycotoxins are detectable when spiked into Mannanase CC enzyme concentrates but not in non-spiked samples. These results support our evaluation of the mannanase 19287 enzyyme preparation being GRAS for their intended use to reduce intestinal viscosity.

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Annex 1 – Report *Myceliothermophin* Analysis in Mannanase CC

Report Myceliothermophin Analysis in Mannanase CC

Analysis Performed By

(b) (6) and Martin Penkert
March 2020

Final Report

Andreas Kern, PhD
Sr. Regulatory Affairs Manager Animal Nutrition

July 6, 2023

Report Myceliothermophin Analysis in Mannanase CC

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Technical Expert:

(b) (6) 17.08.2023

Dr. Martin Penkert Date

Team Lead R & D
Bio Mass Spec/Proteomics

Myceliothermophin Analysis in Mannanase CC

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Myceliothermophin Analysis in Mannanase CC

Summary

(b) (4)

Introduction

Filamentous fungi produce secondary metabolites (SMs), that, in contrast to primary metabolites, are not directly involved in the nutrition, growth and proliferation of the organism. SMs fulfill separate functions, including infection, defense or tissue degradation. Among fungi, well-known SMs include penicillin, mycotoxins and phytotoxins. The fungus *Thermothelomyces thermophilus* and its naturally occurring strain C1 have been developed into a group of host organisms that can serve as microbial production systems for large-scale manufacture of enzymes and other proteins. The production strain (DSM 33149) for the mannanase 19287 enzyme preparation, with the intended use as an animal feed additive, is a derivative strain tracing its lineage back to C1.

Yang et al., (Chem. Eur. J. 2007, 13, 6985 – 6991), identified a group of secondary metabolites, specifically polyketides, produced by *T. thermophilus* (at the time classified as *Myceliophthora thermophila*) and named this group Myceliothermophins (MTs). The paper identified five family members (MTs A, B, C, D and E), three of which exhibited cytotoxic activity against various cancer cell lines with IC_{50} values between 0.25 and 1.3 µg/mL (MTs A, C, E).

More recently, a novel member of this family of polyketides, MT F (Gao et al, Nat Prod Res 2019, DOI:10.1080/14786419.2019.1641810), has been identified demonstrating a similar cytotoxicity (IC50) as MT C.

It is therefore necessary to evaluate whether these potentially cytotoxic MTs are present in the mannanase enzyme preparation produced with *T. thermophilus* strain DSM 33149.

Materials and Methods

1. **Mannanase CC**

Mannanase CC (Mannanase 19287 concentrate) production lots (b) (4) were generated during commercial scale fermentation of the production strain DSM 33149 and subsequent downstream processing of the fermentation broth; the production organism was killed and removed from the supernatant. Subsequent filtration and purification steps resulted in the commercial Mannanase CC concentrates.

(b) (4)

2. **Reference Materials**

(b) (4)

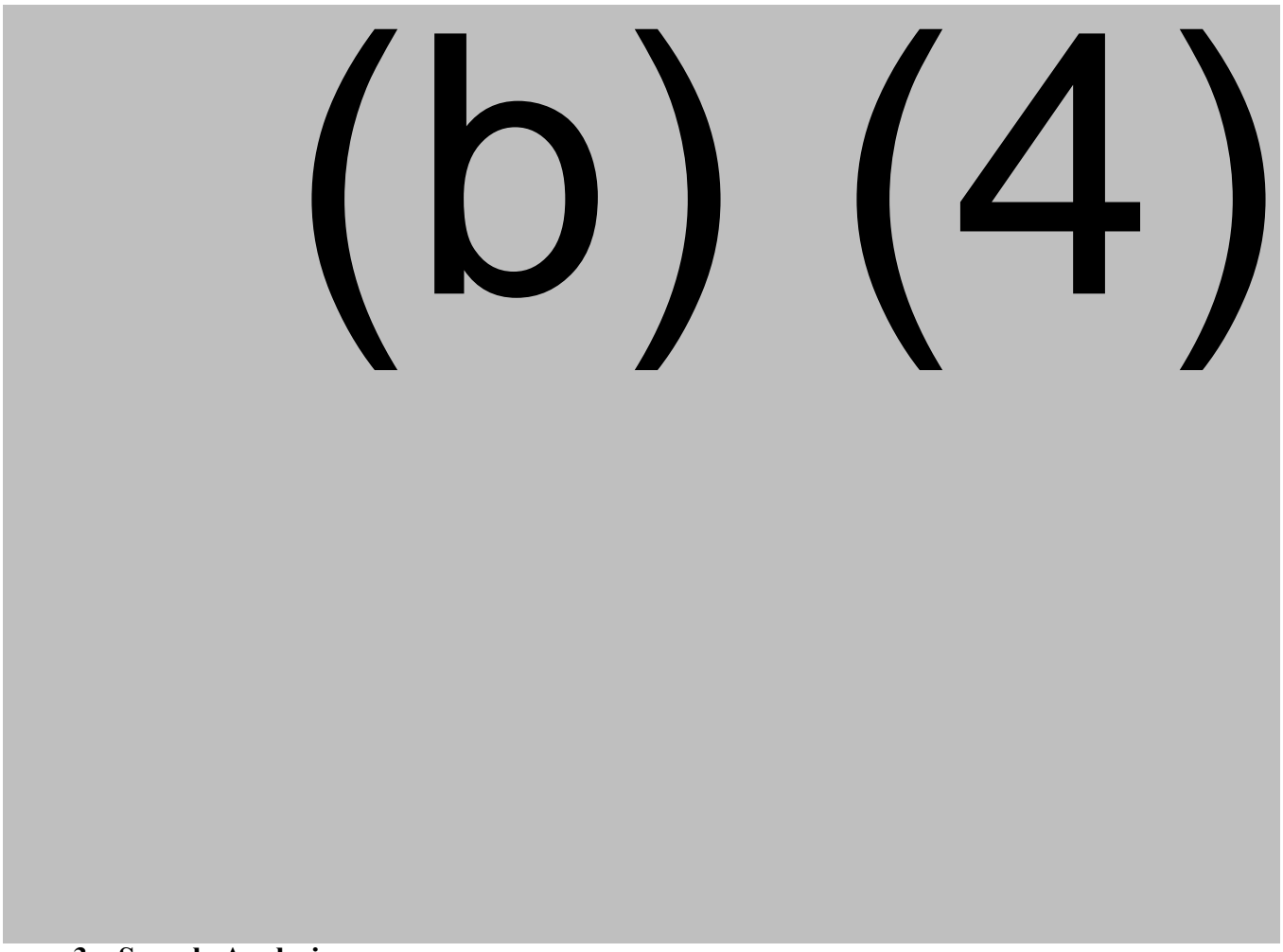
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Results

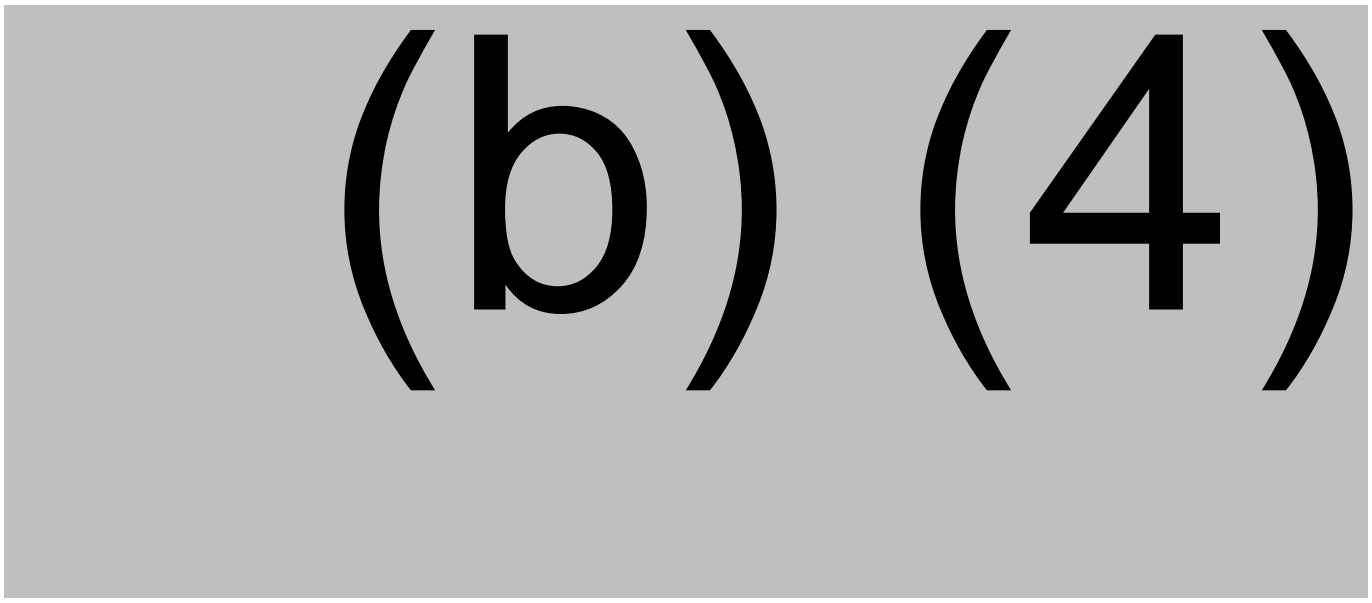
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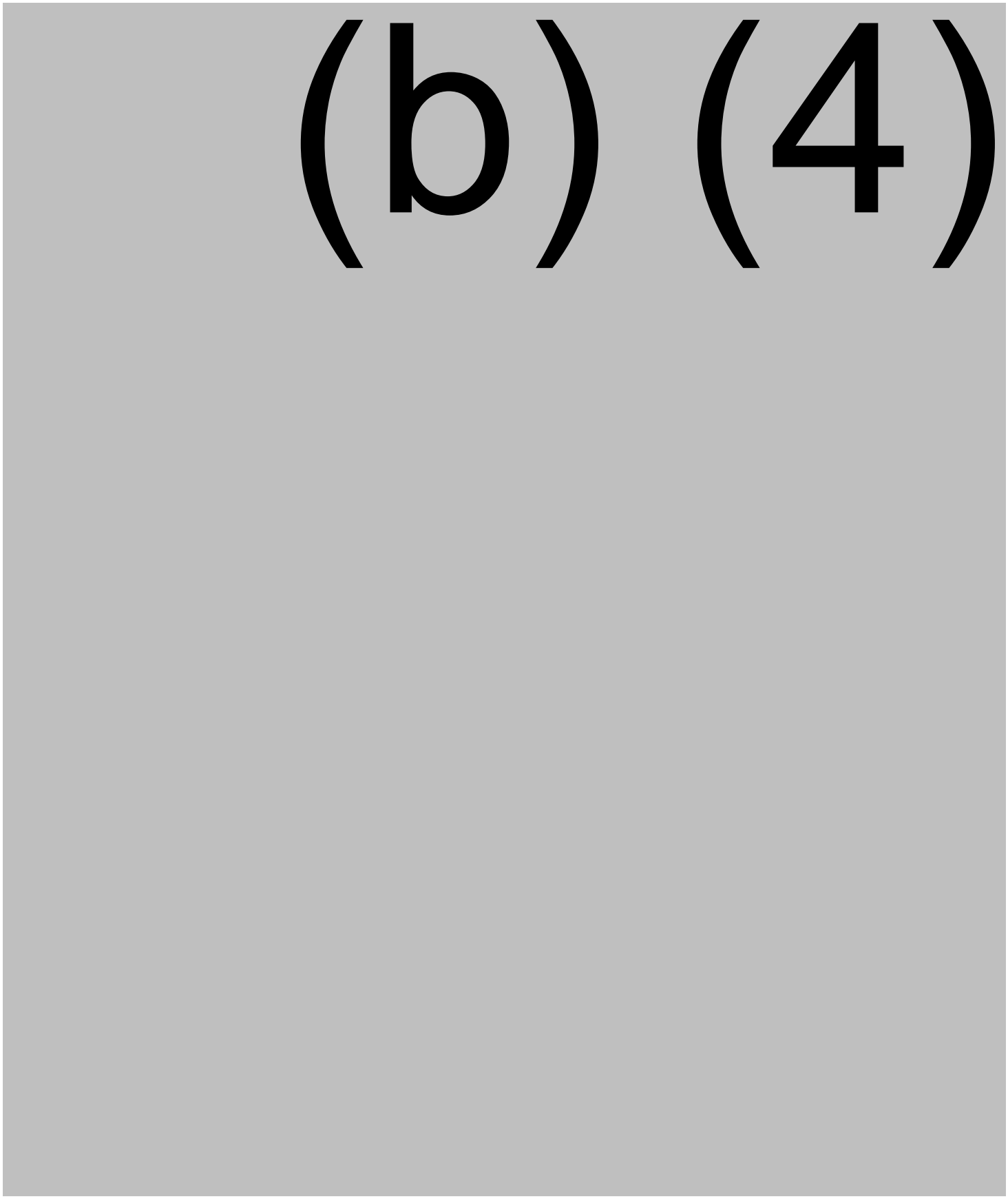
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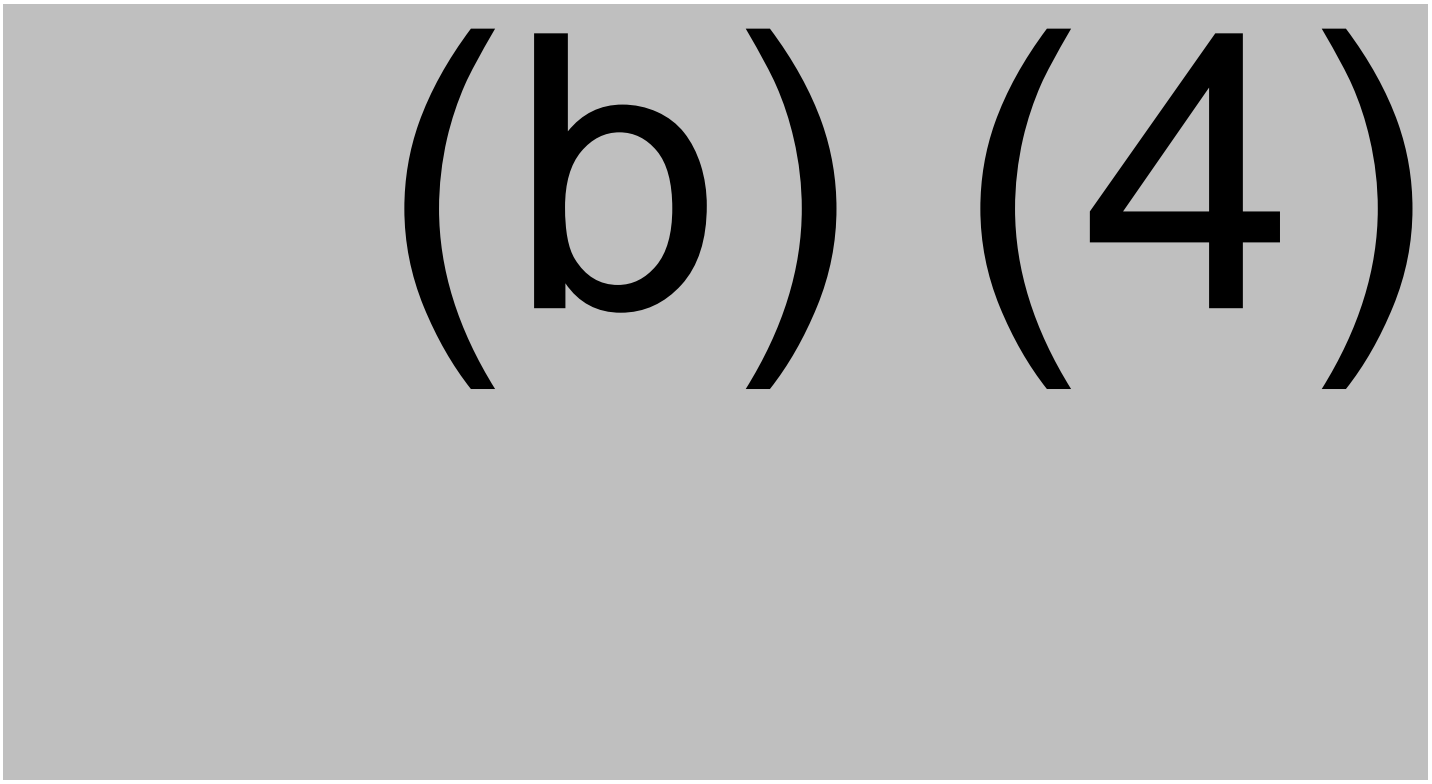
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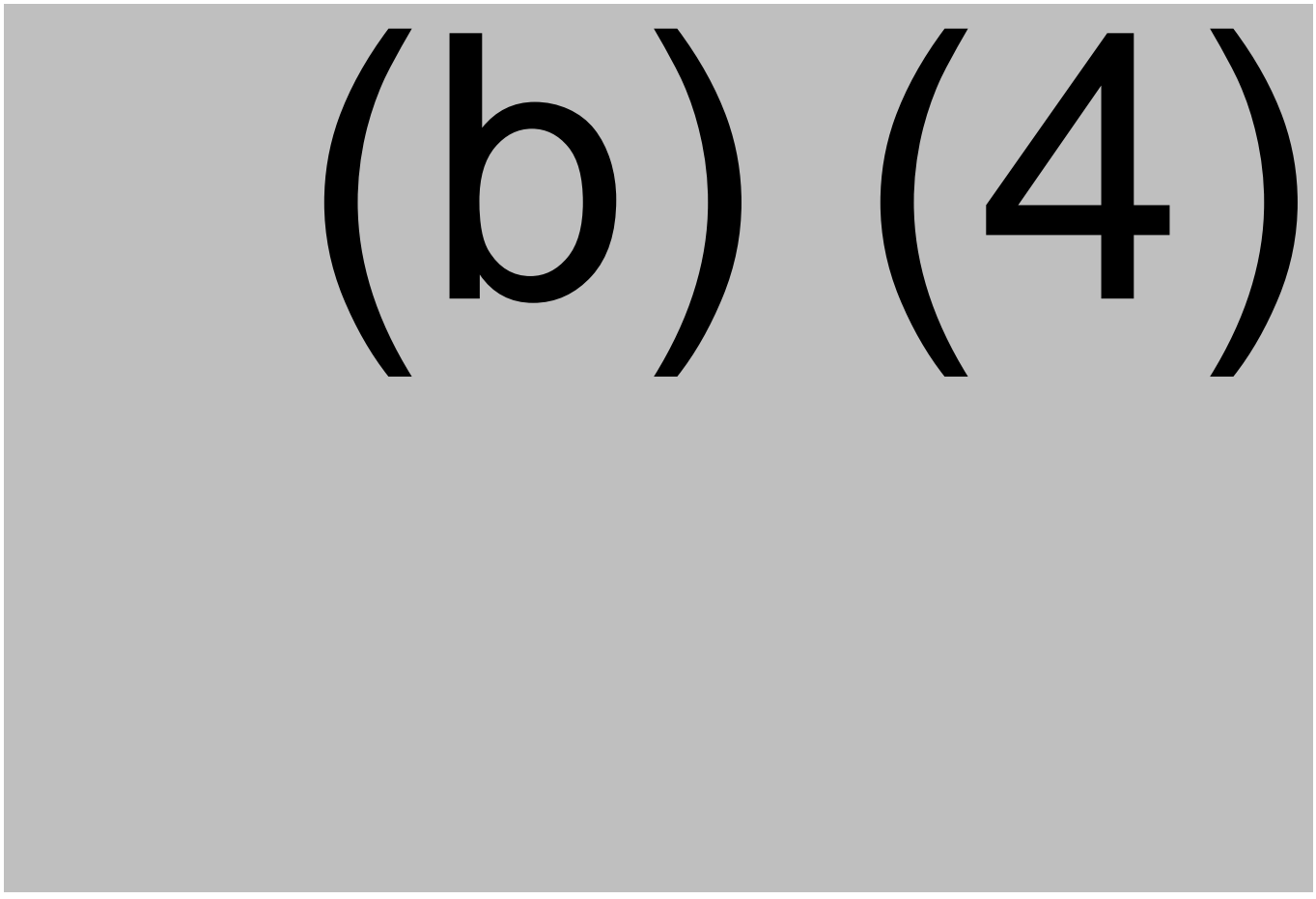
3. **Sample Analysis**



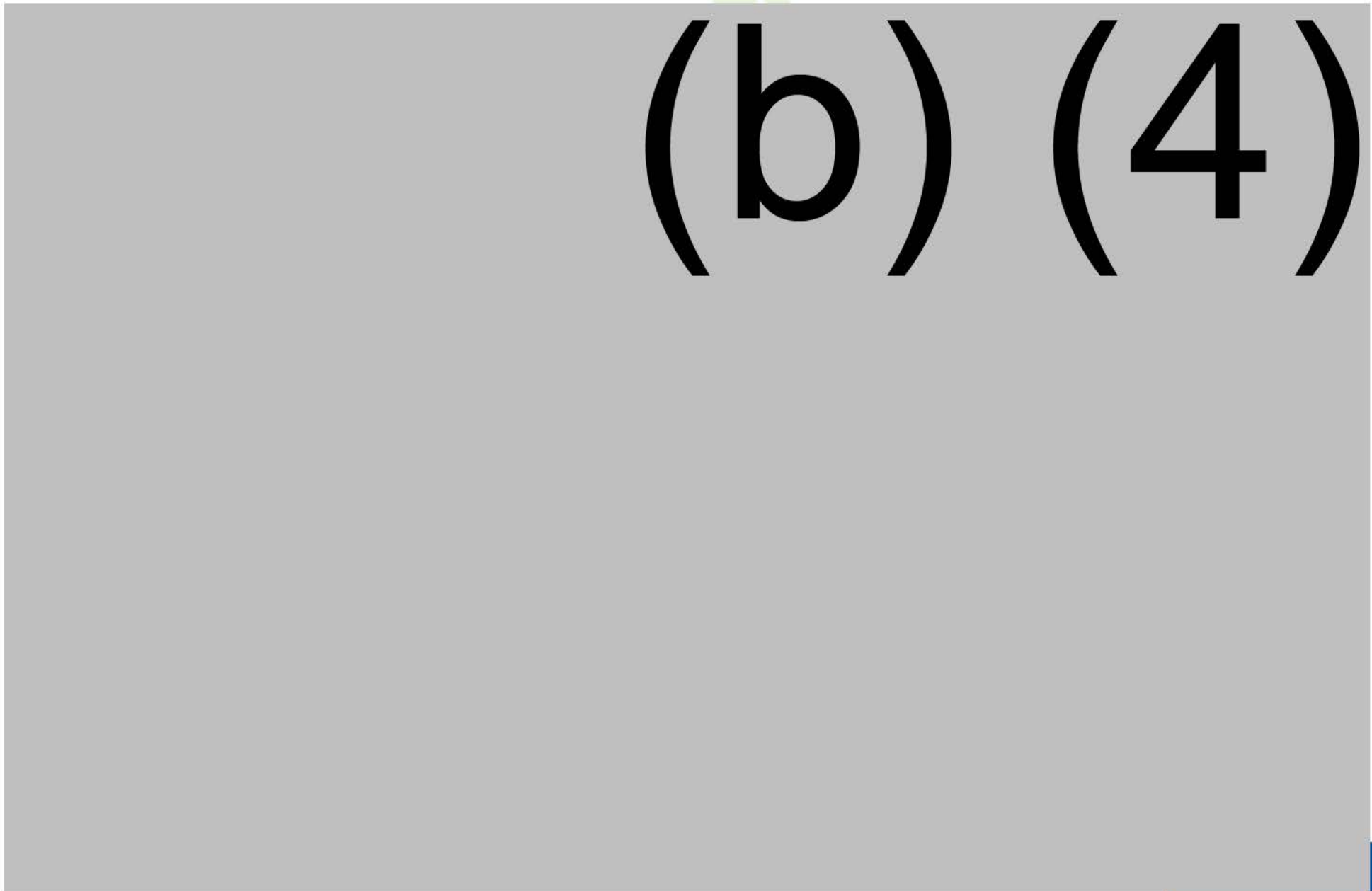

(b) (4)



Conclusions:



Myceliothermophin Analyses 20200310 - Chromatograms


(b) (4)

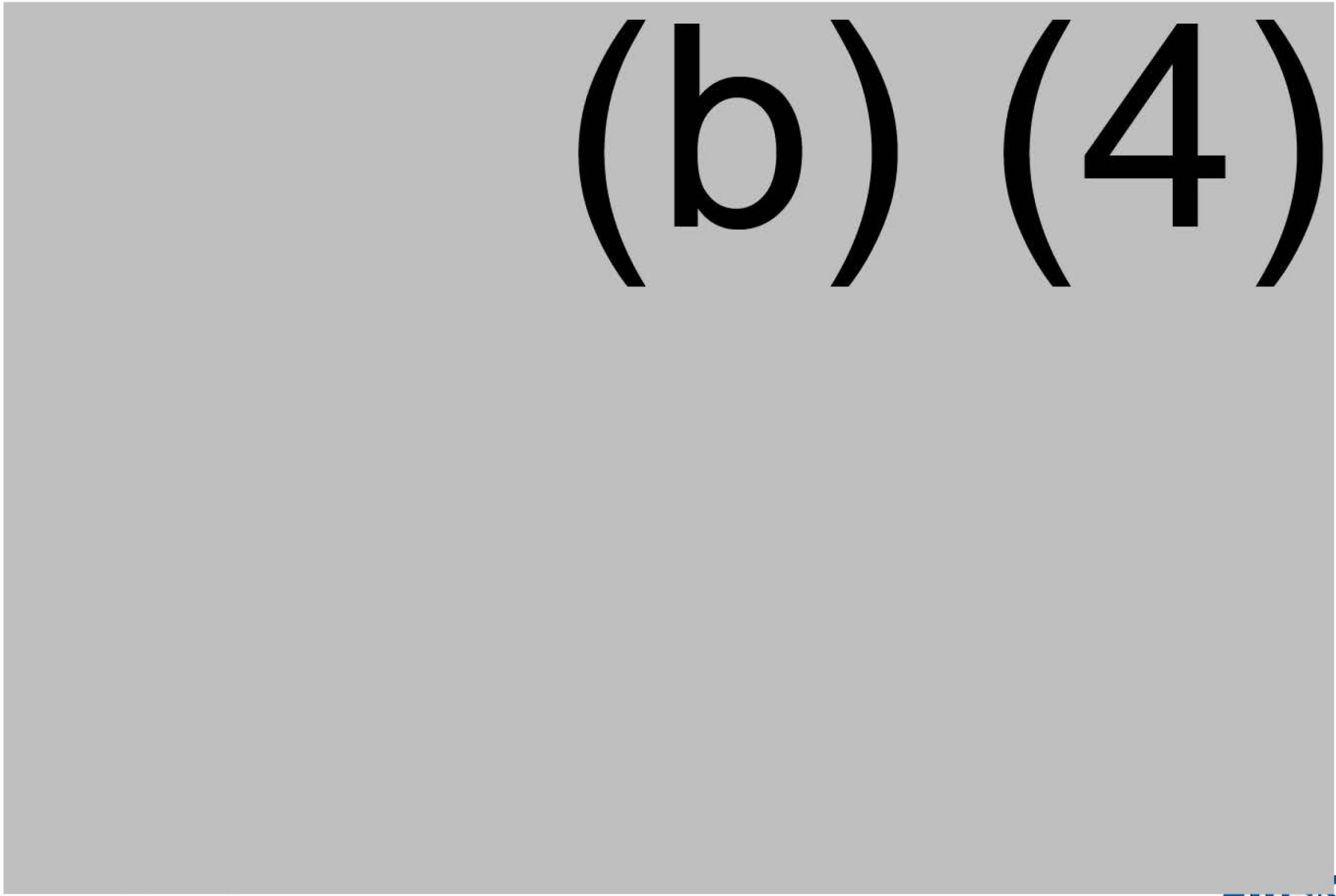


Figure 2A

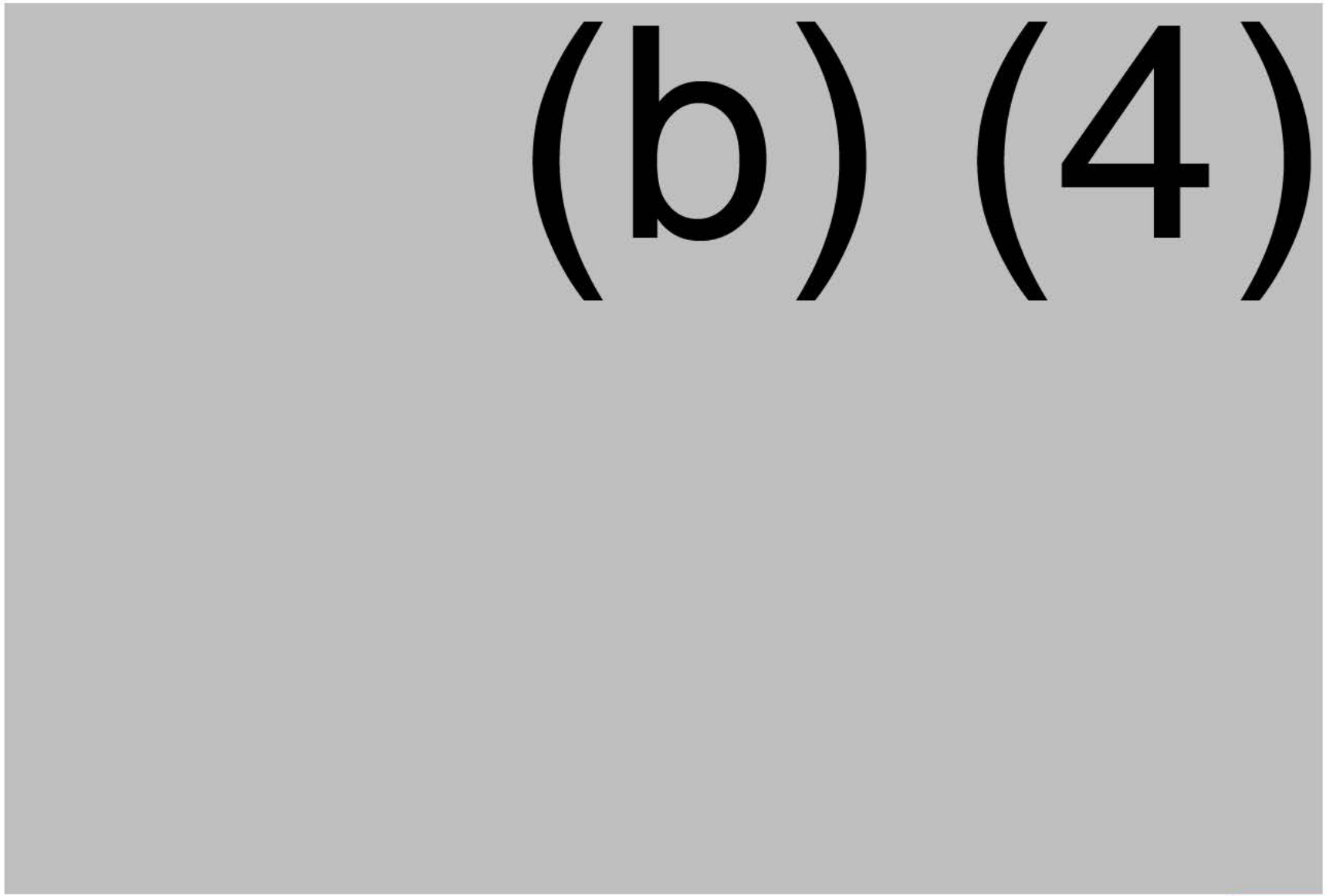


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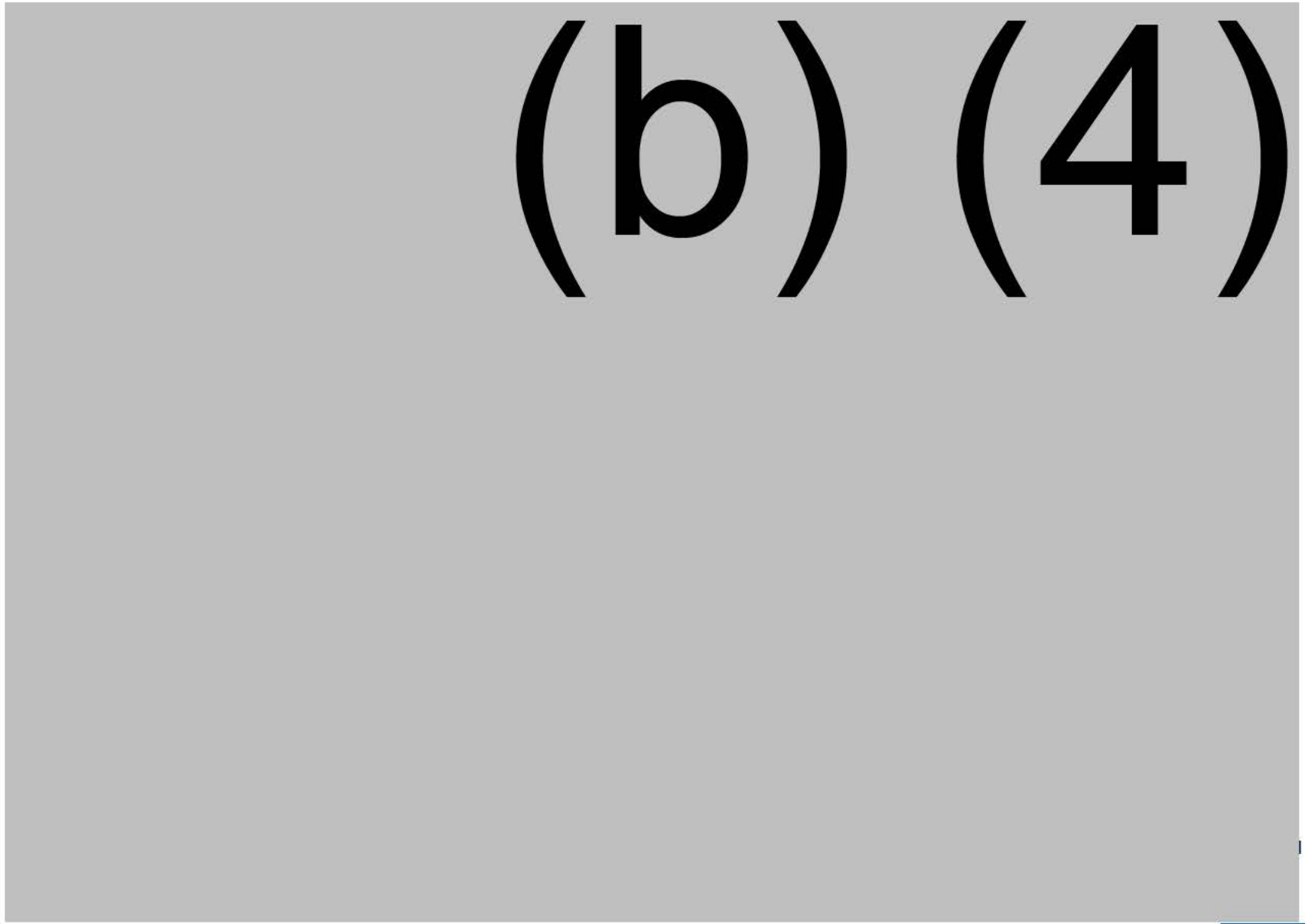


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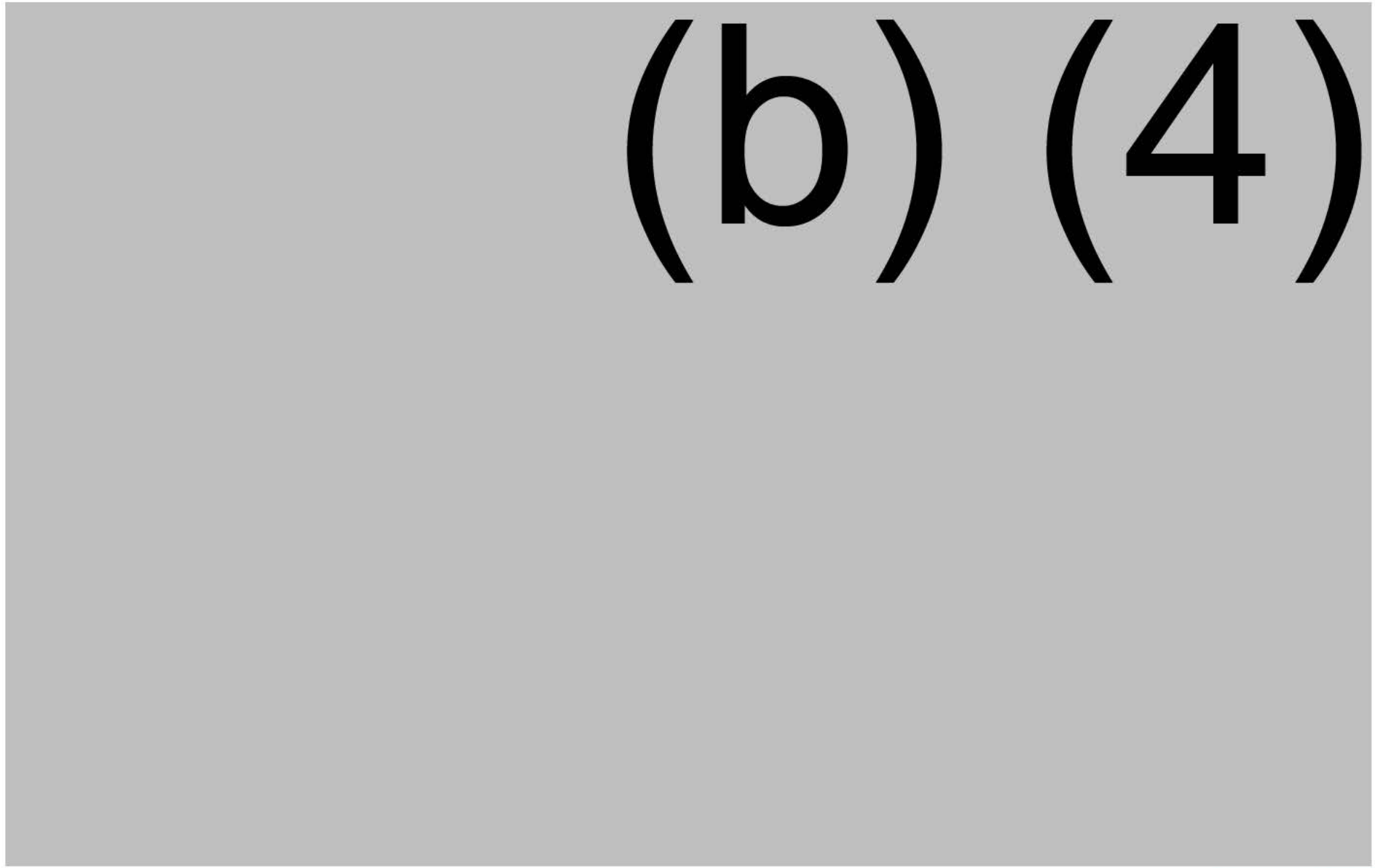


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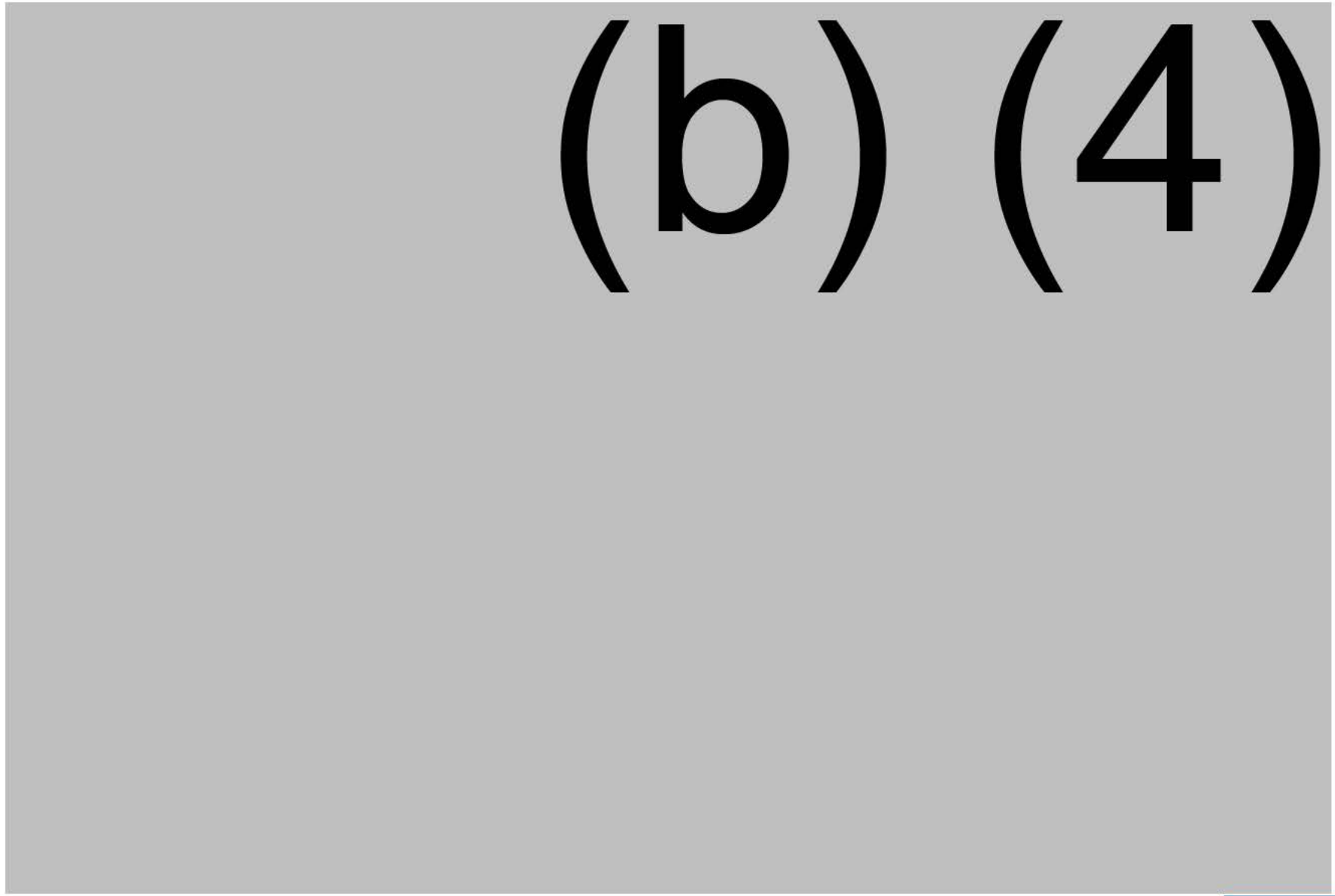


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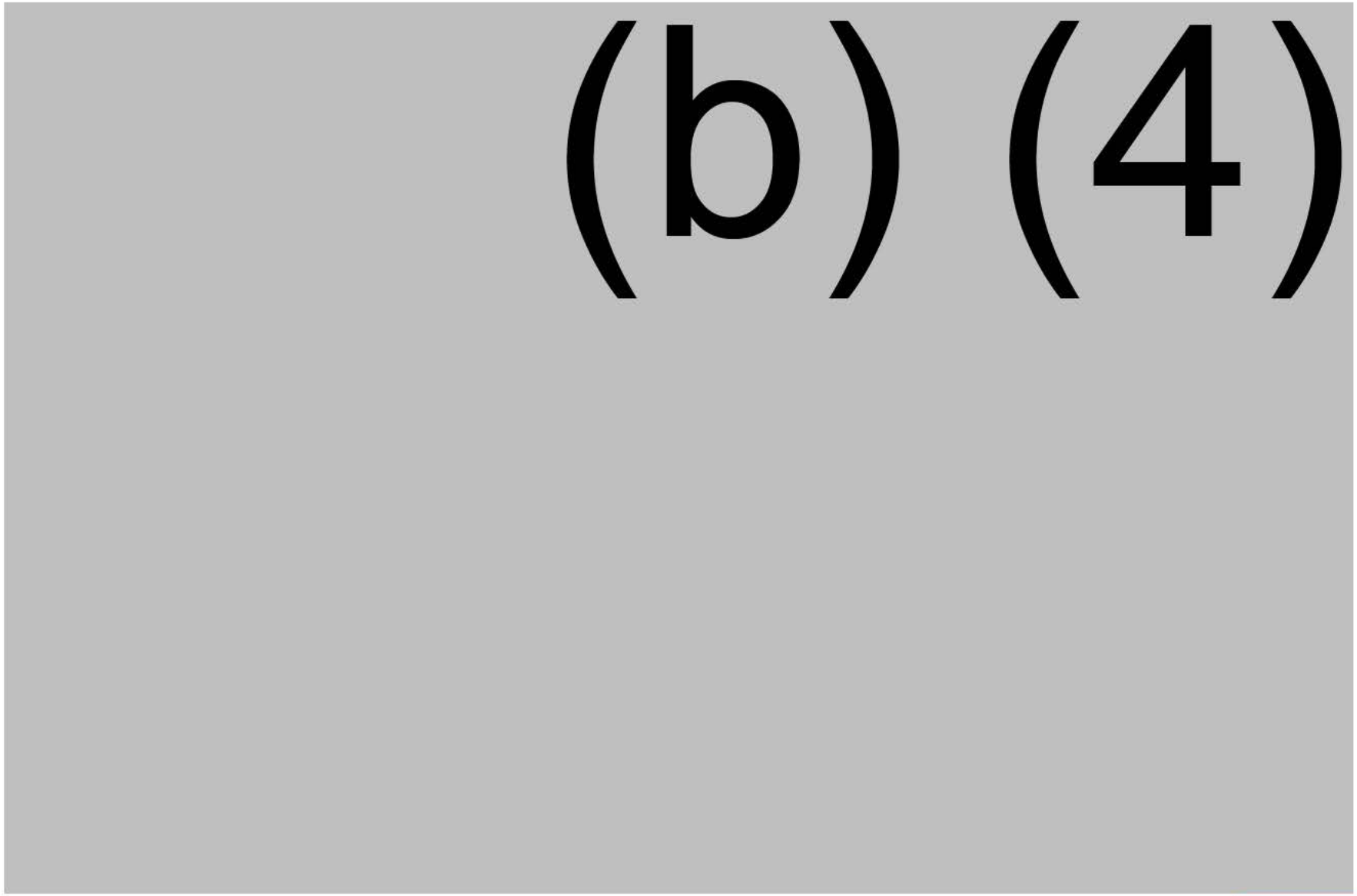


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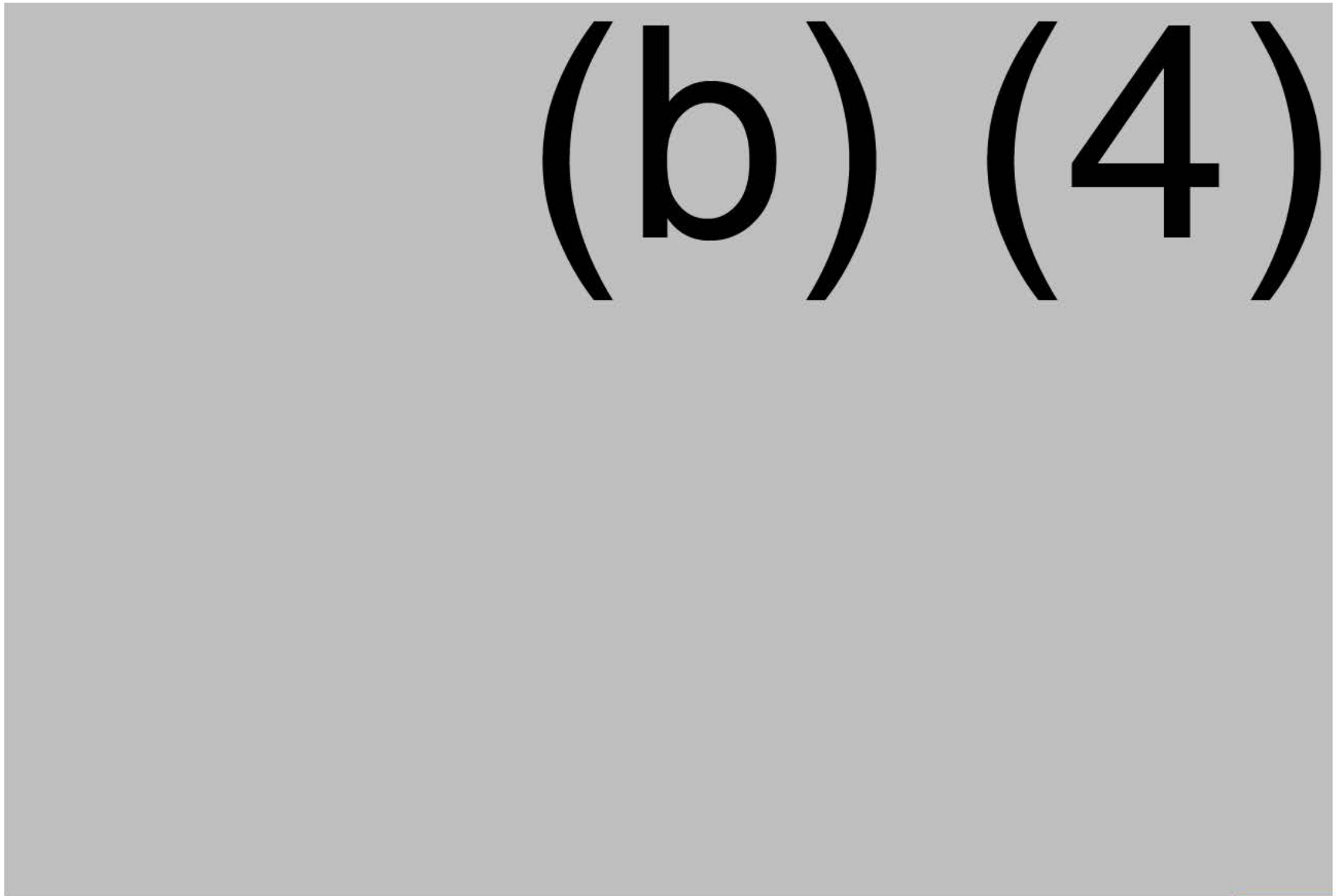


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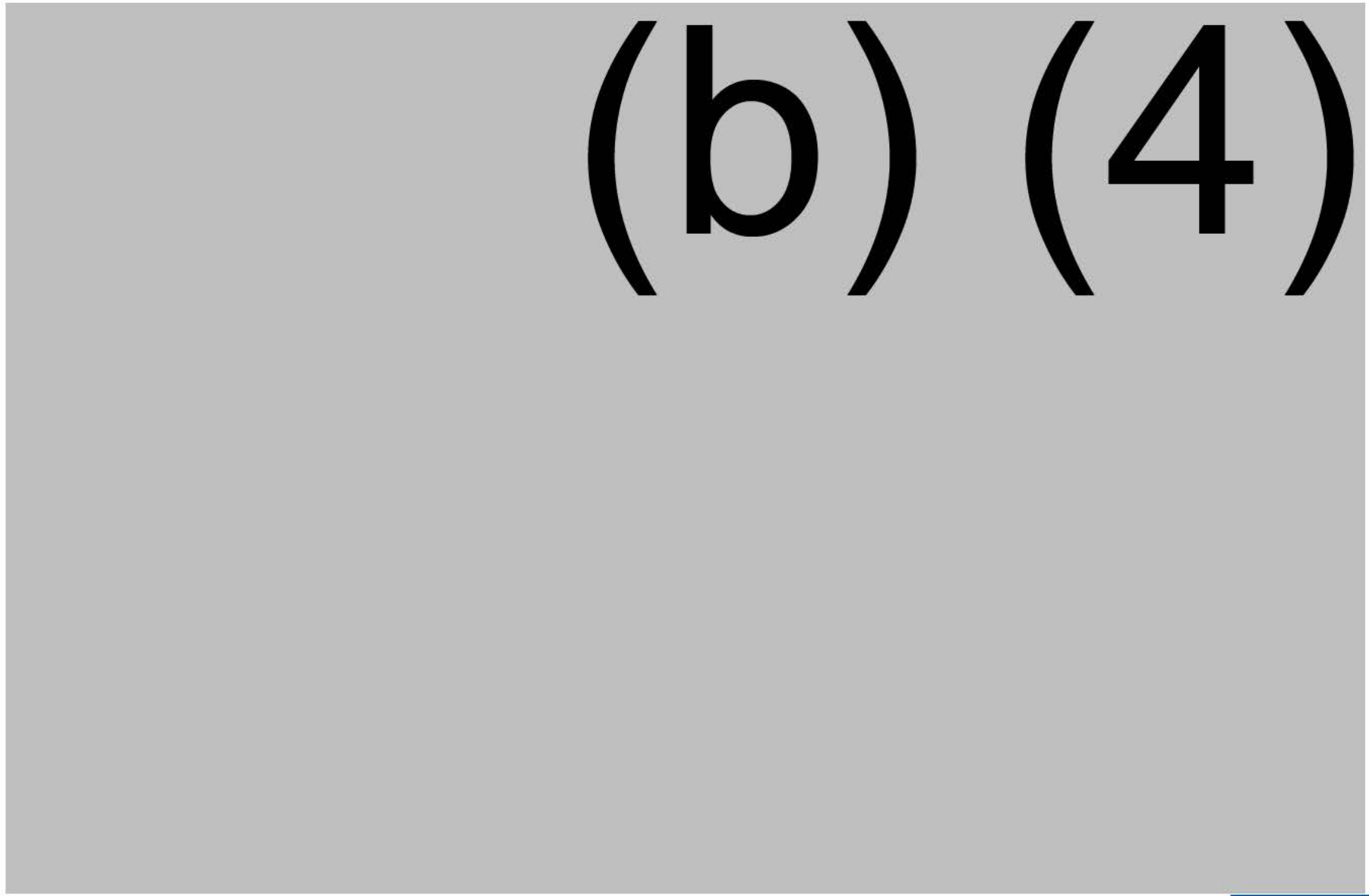


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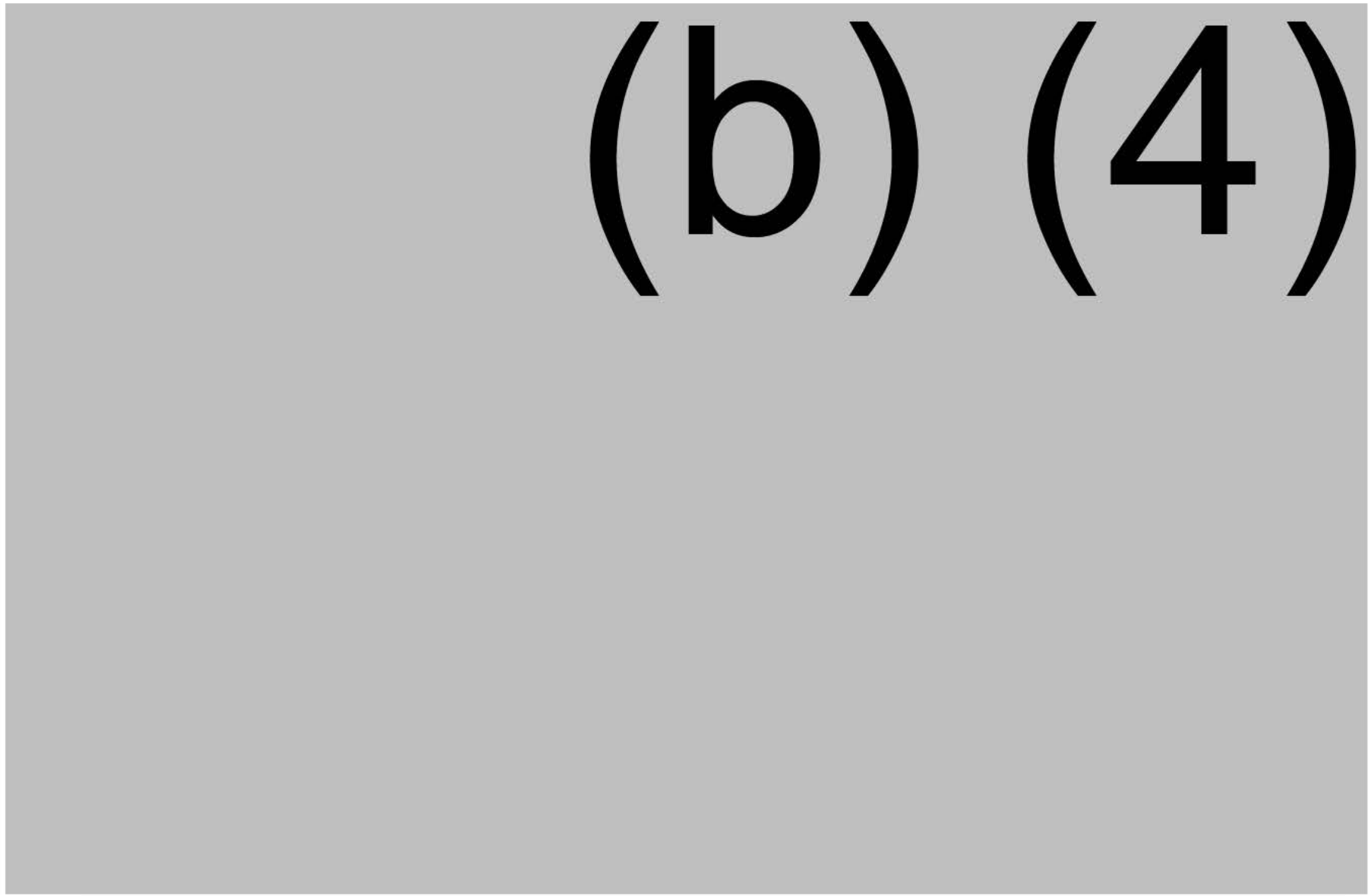


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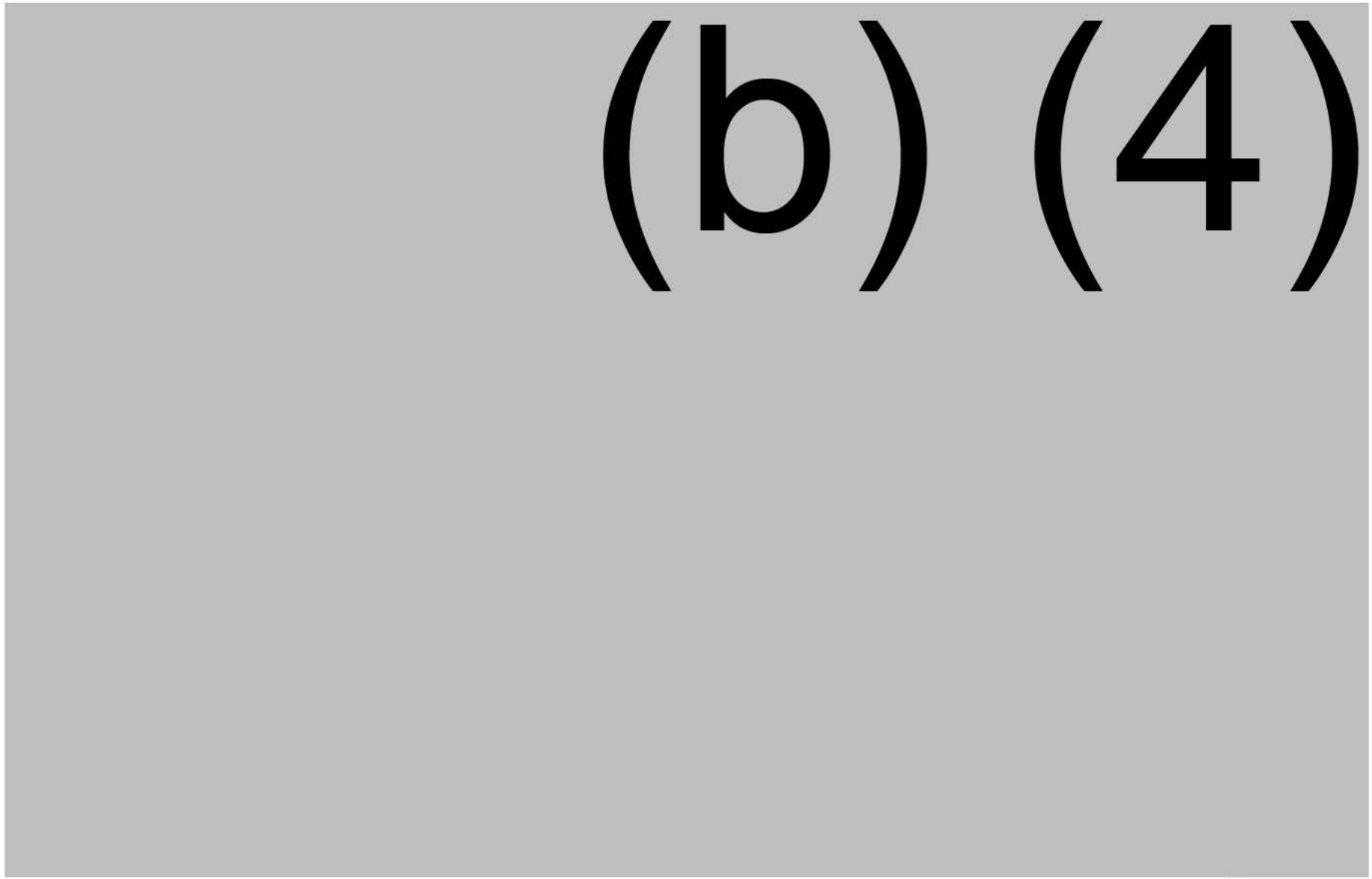


Figure 5B

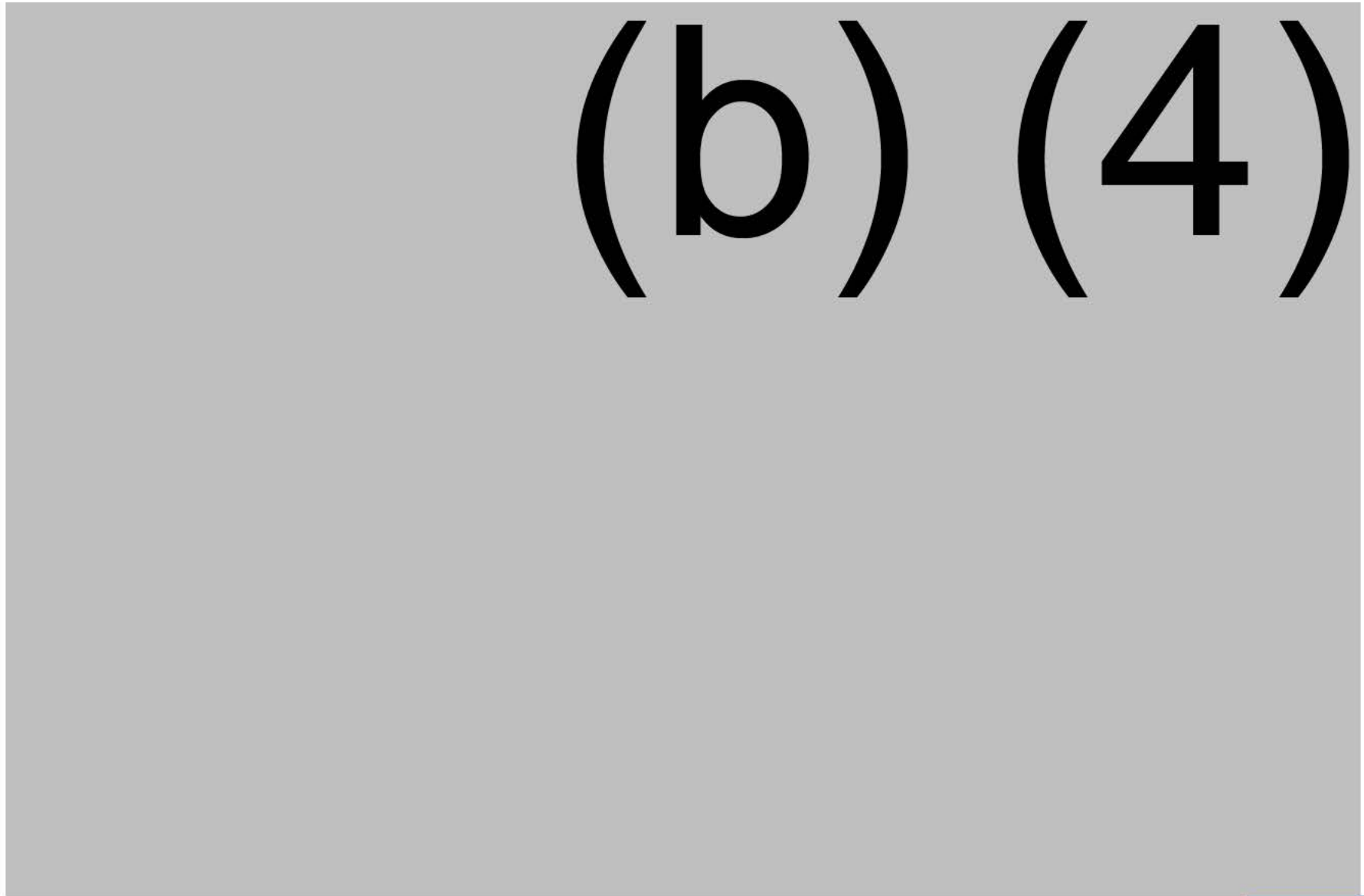


Figure 5C

Annex 2 – Detection of Novel Mycotoxins in Mannanase CC

Detection of Novel Mycotoxins in Mannanase CC

- Myceliothermophins A, C and E
- Tenuazonic Acid

(b) (6)
Andreas Kern, PhD

December 6, 2023

BASF
We create chemistry

Detection of Novel Mycotoxins in Mannanase CC

- Myceliothermophins A, C and E
- Tenuazonic Acid

SIGNATURE PAGE

Author:

KernA14

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Andreas Kern, Ph.D.

Date

Sr. Regulatory Affairs
Manager Animal Nutrition

Technical Expert:

(b) (6)

12/07/2023

Date

BASF Enzymes, LLC

Detection of Novel Mycotoxins in Mannanase CC

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Novel Mycotoxin Analysis in Natupulse® CC

Introduction

Filamentous fungi produce secondary metabolites (SMs), that, in contrast to primary metabolites, are not directly involved in the nutrition, growth and proliferation of the organism. SMs fulfill separate functions, including infection, defense or tissue degradation. Among fungi, well-known SMs include penicillin, mycotoxins and phytotoxins. The naturally occurring strain C1 of the filamentous fungus *Thermothelomyces thermophilus* has been developed into a group of host organisms that can serve as microbial production systems for large-scale manufacture of enzymes and other proteins. The production strain DSM 33149 for the mannanase 19287 enzyme preparation, with the intended use as an animal feed additive, is a derivative strain tracing its lineage back to C1.

A 2007 publication (Yang, Y.L. *et al.*, 2007) identified a group of secondary metabolites, specifically polyketides, produced by *T. thermophilus* (at the time classified as *Myceliophthora thermophila*) and named this group Myceliothermophins (MTs). The paper identified five family members (MTs A, B, C, D and E), three of which exhibited cytotoxic activity against various cancer cell lines with IC_{50} values between 0.25 and 1.3 µg/mL (MTs A, C, E). Interestingly, MTs B and D are diastereomers of MTs A and C, respectively; however, only A and C demonstrate cytotoxic activities. A novel related polyketide, MT F, has been identified demonstrating a similar cytotoxicity (IC50) as MT C (Gao, Y.-L. *et al.*, 2019). Structurally, MT F is an oxidized form of MT A.

Tenuazonic Acid (TeA) is a secondary metabolite produced primarily by *Alternaria ssp.* and a few other ascomycetes, including *Epicoccum sorghinum* and *Pyricularia oryzae*, previously identified as *Magnaporthe oryzae*. TeA biosynthesis is catalyzed by the enzyme TeA synthetase (TAS1), which in turned is regulated in *P. oryzae* by a Zn(II)2-Cys6-type transcription factor, now identified as TAS2. When searching for TAS2 homologs, the *T. thermophilus* genome was shown to include TAS1 and TAS2 homologs (Yun, C.-S. *et al.*, 2017).

It is therefore necessary to evaluate whether TeA or cytotoxic MTs are present in the mannanase enzyme preparation produced with *T. thermophilus* strain DSM 33149.

Materials and Methods

1. **Mannanase CC**

(b) (4)

2. **Reference Materials**

(b) (4)

3. **Sample Preparation**

(b) (4)

4. **Spiking Experiments**

(b) (4)

5. **LC-MS/MS**

(b) (4)

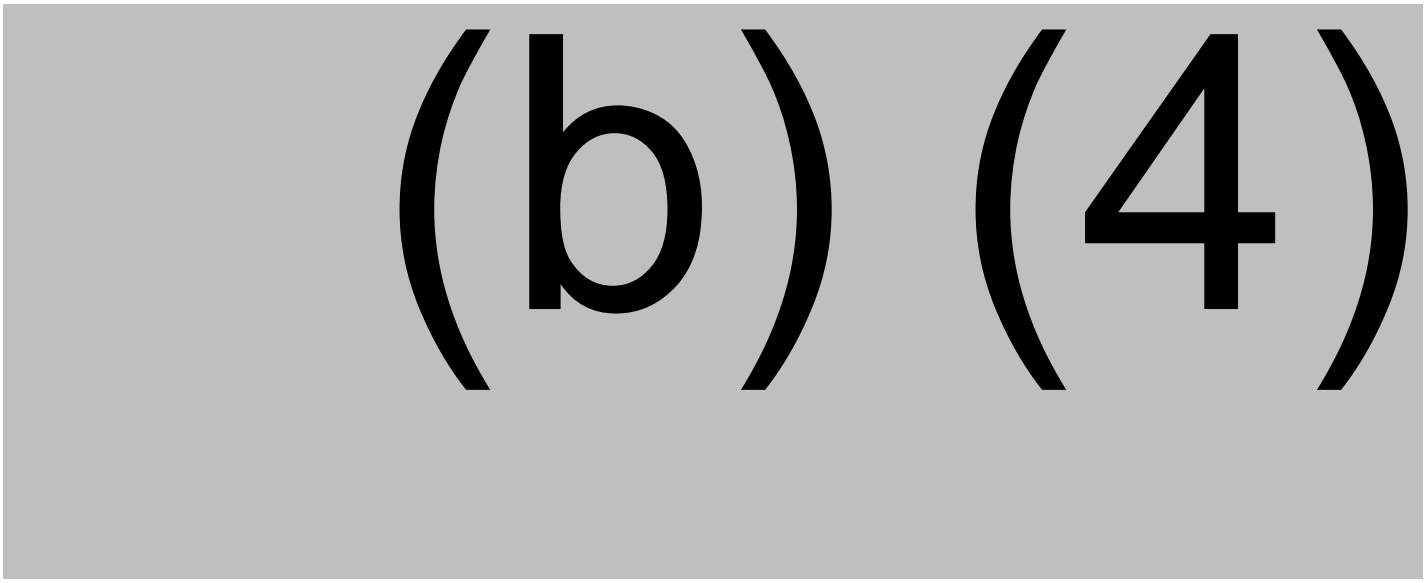
6. **Method Development**

(b) (4)

(b) (4)

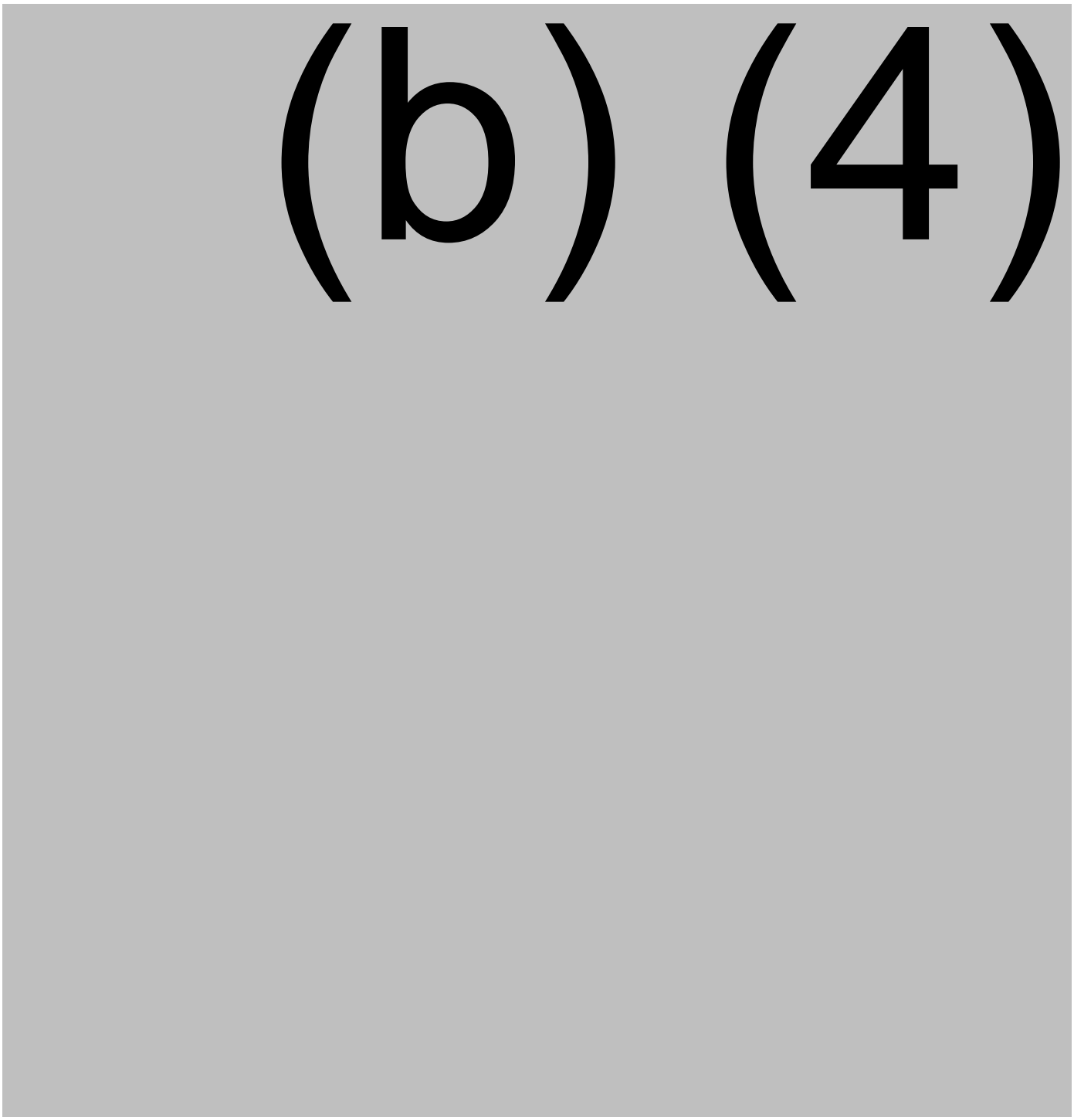
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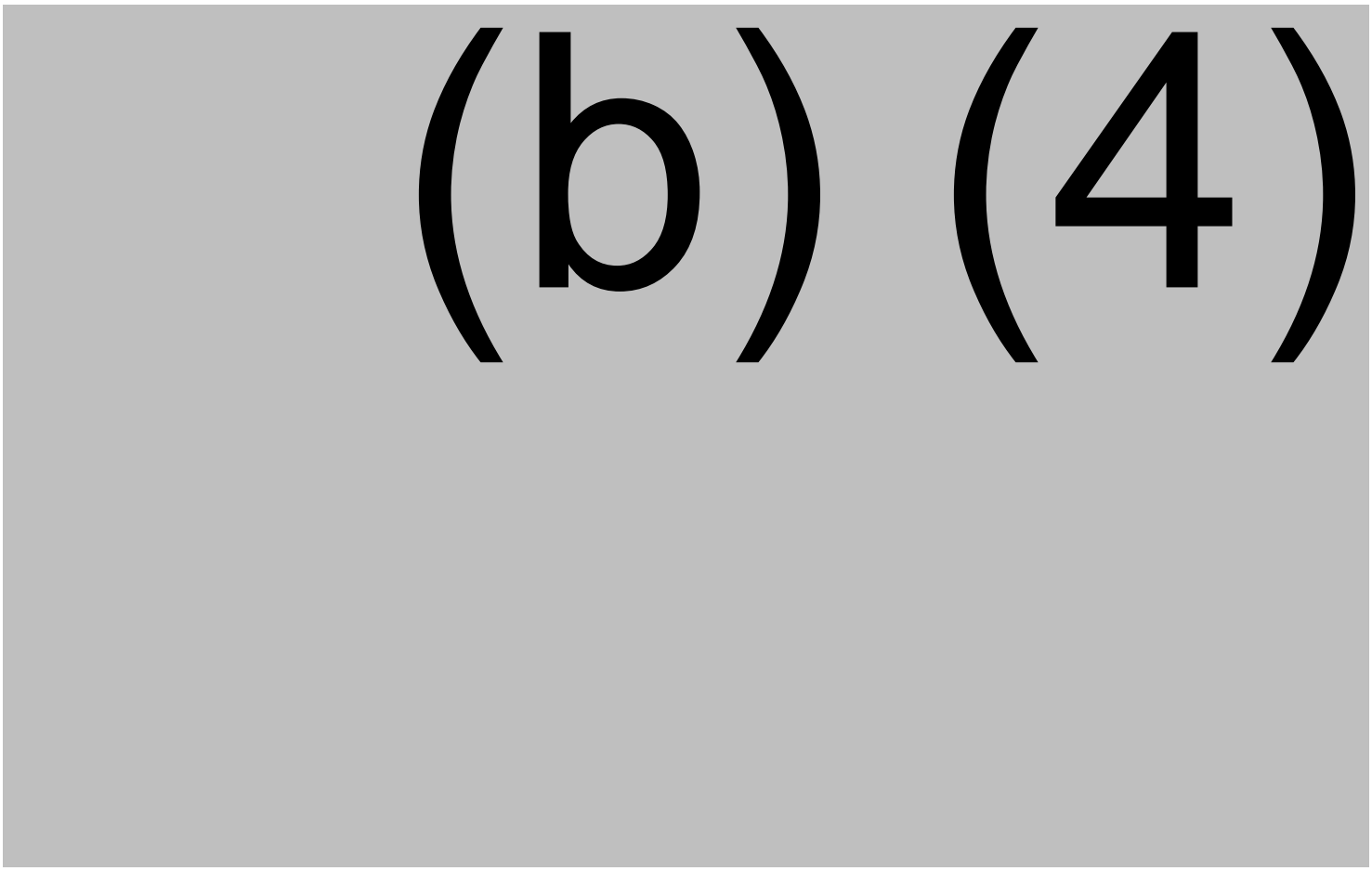
(b) (4)

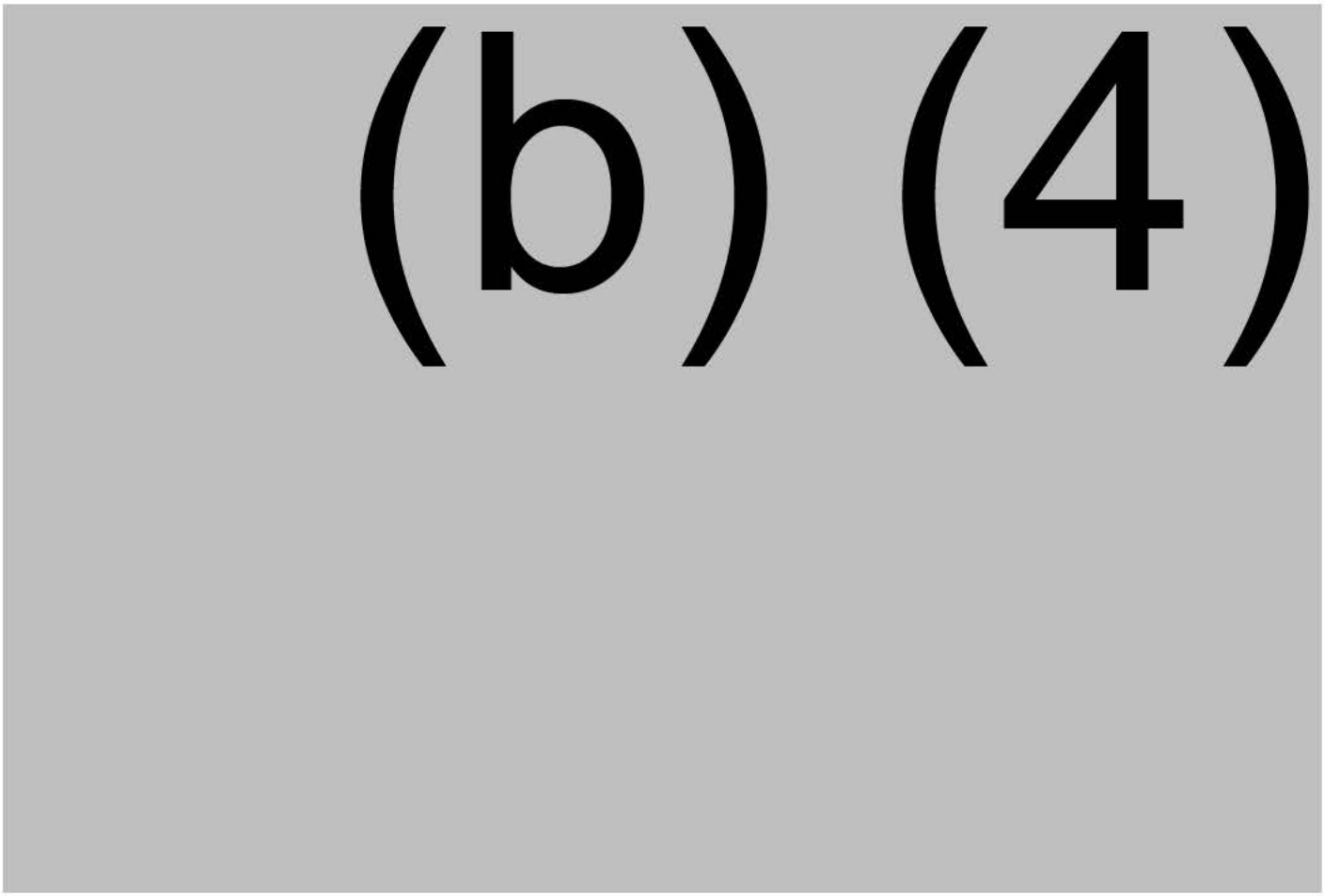


In summary, the results of this study demonstrate that myceliothermophins A/B, C and E can be detected when spiked into two mannanase 19287 concentrates from large-scale fermentations produced with the *T. thermophilus* production strain DSM 33149. This confirms and expands on our previous results for myceliothermophin E spiked into three previous commercial concentrates (Appendix # signed report). The current method is not adequate for the detection of TeA at low spiking concentrations. Instead, potential presence of the mycotoxin has been analyzed in an accredited external laboratory (see separate report).

Tables and Figures




(b) (4)


(b) (4)

References

Gao, Y.-L., Zhang, M.-L., Wang, X., Zhang, H.-D. et al. *Isolation and characterization of a new cytotoxic polyketide–amino acid hybrid from Thermothelomyces thermophilus ATCC 42464*. Natural Product Research, pp. 1-7 (2019).

Yang, Y.L., Lu, C.P., Chen, M.Y., Chen, K.Y. et al. *Cytotoxic Polyketides Containing Tetramic Acid Moieties Isolated from the Fungus Myceliophthora Thermophila: Elucidation of the Relationship between Cytotoxicity and Stereoconfiguration*. Chemistry A European Journal 13 (24), pp. 6985-6991 (2007).

Yun, C.-S., Motoyama, T. and Osada, H. *Regulatory Mechanism of Mycotoxin Tenuazonic Acid Production in Pyricularia oryzae*. ACS Chemical Biology 12 (9), pp. 2270-2274 (2017).

Annex 3 – (b) (4) Test Reports

(b) (6), (b) (4)

BASF SE
Carl-Bosch-Straße 38

67056 Ludwigshafen am Rhein
Deutschland

Analysis Report No.: **234368**

Product:	Mannanase CC
Date of reception:	01.08.2023 parcel service (TNT)
Order of:	26.07.2023 written by (b) (6)
Assignment:	Tenuazonic acid
Labelling:	Mannanase CC Art.: 83012427 Batch: (b) (4)
Quantity:	0,5 kg
Packaging:	PE-HD-bottle
Sampling date:	
Temperature at reception / storage:	2,3°C / <= -18°C
Start / End of analysis:	03.08.2023 / 07.08.2023

Results of Analysis:

Tenuazonic acid

Parameter	Result	Unit
Tenuazonic acid	12	µg/kg

Methods applied:
Determination of tenuazonic acid in feedstuff with LC-MS/MS (PAW 029, 2022-11). Limit of quantification: 10 µg/kg. #

(b) (6)

The results refer solely to the samples analysed.
An uncomplete copy of this report requires in advance our written approval.
The methods labelled with # are non-accredited test methods.

(b) (6), (b) (4)

(b) (6), (b) (4)

BASF SE
Carl-Bosch-Straße 38

67056 Ludwigshafen am Rhein
Deutschland

Date: 07.08.2023

Analysis Report No.: **234369**

Product:	Mannanase CC
Date of reception:	01.08.2023 parcel service (TNT)
Order of:	26.07.2023 written by (b) (6)
Assignment:	Tenuazonic acid
Labelling:	Mannanase CC Art.: 83012427 Batch: 46226977L0
Quantity:	0,5 kg
Packaging:	PE-HD-bottle
Sampling date:	
Temperature at reception / storage:	2,3°C / <= -18°C
Start / End of analysis:	03.08.2023 / 07.08.2023

(b) (6)

Results of Analysis:

Tenuazonic acid

Parameter	Result	Unit
Tenuazonic acid	< 10	µg/kg

Methods applied:
Determination of tenuazonic acid in feedstuff with LC-MS/MS (PAW 029, 2022-11). Limit of quantification: 10 µg/kg. #

(b) (6)

The results refer solely to the samples analysed.
An uncomplete copy of this report requires in advance our written approval.
The methods labelled with # are non-accredited test methods.

(b) (4), (b) (6)

(b) (6)

BASF SE
Carl-Bosch-Straße 38

67056 Ludwigshafen am Rhein
Deutschland

Analysis Report No.: **233890-A**

This Analysis Report replaces Analysis Report No. 233890; Change: Report language

Product:	Natupulse TS (Feed additive)
Date of reception:	04.07.2023 parcel service (FedEx)
Order of:	30.06.2023 written by (b) (6)
Assignment:	Tenuazonic acid
Labelling:	Natupulse TS Lot: 78708624U0
Quantity:	0,5 kg
Packaging:	PE-HD-bottle
Sampling date:	Sampling was carried out by customer
Temperature at reception / storage:	Room temperature / Room temperature
Start / End of analysis:	05.07.2023 / 17.07.2023

(b) (6)

Results of Analysis:

Tenuazonic acid

Parameter	Result	Unit
Tenuazonic acid	11	µg/kg

Methods applied:
Determination of tenuazonic acid in feedstuff with LC-MS/MS (PAW 029, 2022-11). Limit of quantification: 10 µg/kg.#

(b) (6)

The results refer solely to the samples analysed.
An uncomplete copy of this report requires in advance our written approval.
The methods labelled with # are non-accredited test methods.

(b) (6), (b) (4)

BASF SE
Carl-Bosch-Straße 38

67056 Ludwigshafen am Rhein
Deutschland

Date: 18.07.2023

Analysis Report No.: **233891-A**
This Analysis Report replaces Analysis Report No. 233891; Change: Report language

Product:	Natupulse TS (Feed additive)
Date of reception:	04.07.2023 parcel service (FedEx)
Order of:	30.06.2023 written by
Assignment:	Tenuazonic acid
Labelling:	Natupulse TS Lot: 07589975L0
Quantity:	0,5 kg
Packaging:	PE-HD-bottle
Sampling date:	Sampling was carried out by customer
Temperature at reception / storage:	Room temperature / Room temperature
Start / End of analysis:	05.07.2023 / 17.07.2023

(b) (6)

Results of Analysis:

Tenuazonic acid

Parameter	Result	Unit
Tenuazonic acid	< 10	µg/kg

Methods applied:

Determination of tenuazonic acid in feedstuff with LC-MS/MS (PAW 029, 2022-11). Limit of quantification: 10 µg/kg.#

(b) (6)

The results refer solely to the samples analysed.
An uncomplete copy of this report requires in advance our written approval.
The methods labelled with # are non-accredited test methods.

(b) (4), (b) (6)

BASF SE
Carl-Bosch-Straße 38

67056 Ludwigshafen am Rhein
Deutschland

Date: 18.07.2023

Analysis Report No.: **233892-A1**
This Analysis Report replaces Analysis Report No. 233892-A; Change of customer address

Product:	Natupulse TS (Feed additive)
Date of reception:	04.07.2023 parcel service (FedEx)
Order of:	30.06.2023 written by (b) (6)
Assignment:	Tenuazonic acid
Labelling:	Natupulse TS Lot: 99457956P0
Quantity:	0,5 kg
Packaging:	PE-HD-bottle
Sampling date:	Sampling was carried out by customer
Temperature at reception / storage:	Room temperature / Room temperature
Start / End of analysis:	05.07.2023 / 17.07.2023

(b) (6)

Results of Analysis:

Tenuazonic acid

Parameter	Result	Unit
Tenuazonic acid	< 10	µg/kg

Methods applied:

Determination of tenuazonic acid in feedstuff with LC-MS/MS (PAW 029, 2022-11). Limit of quantification: 10 µg/kg.#

(b) (4)

The results refer solely to the samples analysed.
An uncomplete copy of this report requires in advance our written approval.
The methods labelled with # are non-accredited test methods.

(b) (6), (b) (4)

BASF SE
Carl-Bosch-Straße 38

67056 Ludwigshafen am Rhein
Deutschland

Date: 18.07.2023

Analysis Report No.: **233893-A**
This Analysis Report replaces Analysis Report No. 233893; Change: Report language

Product:	Natupulse TS L (Feed additive)
Date of reception:	04.07.2023 parcel service (FedEx)
Order of:	30.06.2023 written by
Assignment:	Tenuazonic acid
Labelling:	Natupulse TS L Lot: 46618524U0
Quantity:	0,5 kg
Packaging:	PE-HD-bottle
Sampling date:	Sampling was carried out by customer
Temperature at reception / storage:	Room temperature / Room temperature
Start / End of analysis:	05.07.2023 / 17.07.2023

(b) (6)

Results of Analysis:

Tenuazonic acid

Parameter	Result	Unit
Tenuazonic acid	< 10	µg/kg

Methods applied:
Determination of tenuazonic acid in feedstuff with LC-MS/MS (PAW 029, 2022-11). Limit of quantification: 10 µg/kg.#

(b) (6)

The results refer solely to the samples analysed.
An uncomplete copy of this report requires in advance our written approval.
The methods labelled with # are non-accredited test methods.

(b) (4), (b) (6)

(b) (4)

BASF SE
Carl-Bosch-Straße 38

67056 Ludwigshafen am Rhein
Deutschland

Date: 18.07.2023

Analysis Report No.: **233894-A**
This Analysis Report replaces Analysis Report No. 233894: Change: Report language

Product:	Natupulse TS L (Feed additive)
Date of reception:	04.07.2023 parcel service (FedEx)
Order of:	30.06.2023 written by (b) (6)
Assignment:	Tenuazonic acid
Labelling:	Natupulse TS L Lot: 016445-L.5
Quantity:	0,5 kg
Packaging:	PE-HD-bottle
Sampling date:	Sampling was carried out by customer
Temperature at reception / storage:	Room temperature / Room temperature
Start / End of analysis:	05.07.2023 / 17.07.2023

(b) (6)

Results of Analysis:

Tenuazonic acid

Parameter	Result	Unit
Tenuazonic acid	< 10	µg/kg

Methods applied:
Determination of tenuazonic acid in feedstuff with LC-MS/MS (PAW 029, 2022-11). Limit of quantification: 10 µg/kg.#

(b) (6)

The results refer solely to the samples analysed.
An uncomplete copy of this report requires in advance our written approval.
The methods labelled with # are non-accredited test methods.

BASF SE
Carl-Bosch-Straße 38

67056 Ludwigshafen am Rhein
Deutschland

Analysis Report No.: **233895-A**
This Analysis Report replaces Analysis Report No. 233895: Change: Report language

Product:	Natupulse TS L (Feed additive)
Date of reception:	04.07.2023 parcel service (FedEx)
Order of:	30.06.2023 written by
Assignment:	Tenuazonic acid
Labelling:	Natupulse TS L Lot: 016445-L.6
Quantity:	0,5 kg
Packaging:	PE-HD-bottle
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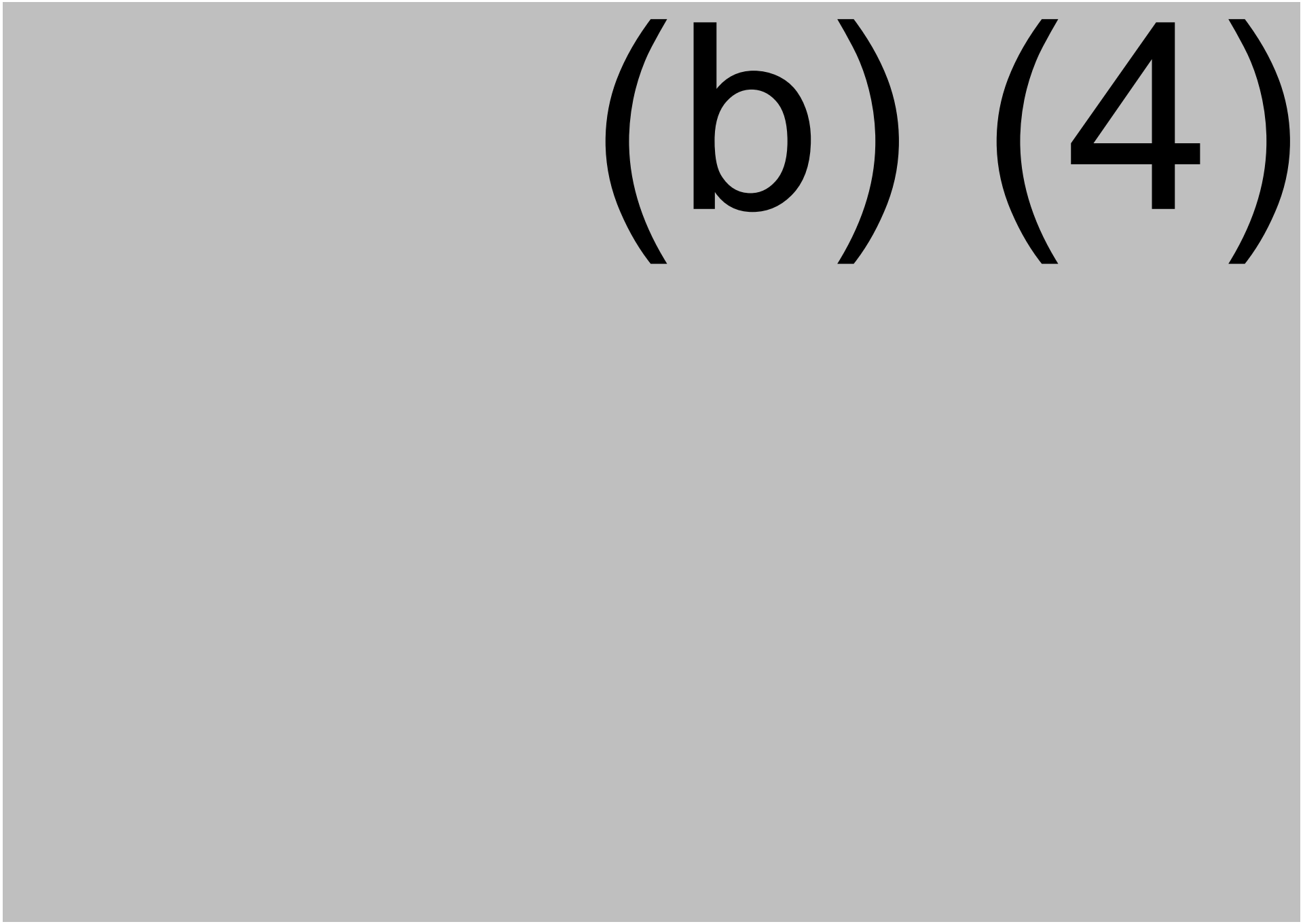
Tenuazonic acid

Parameter	Result	Unit
Tenuazonic acid	< 10	µg/kg

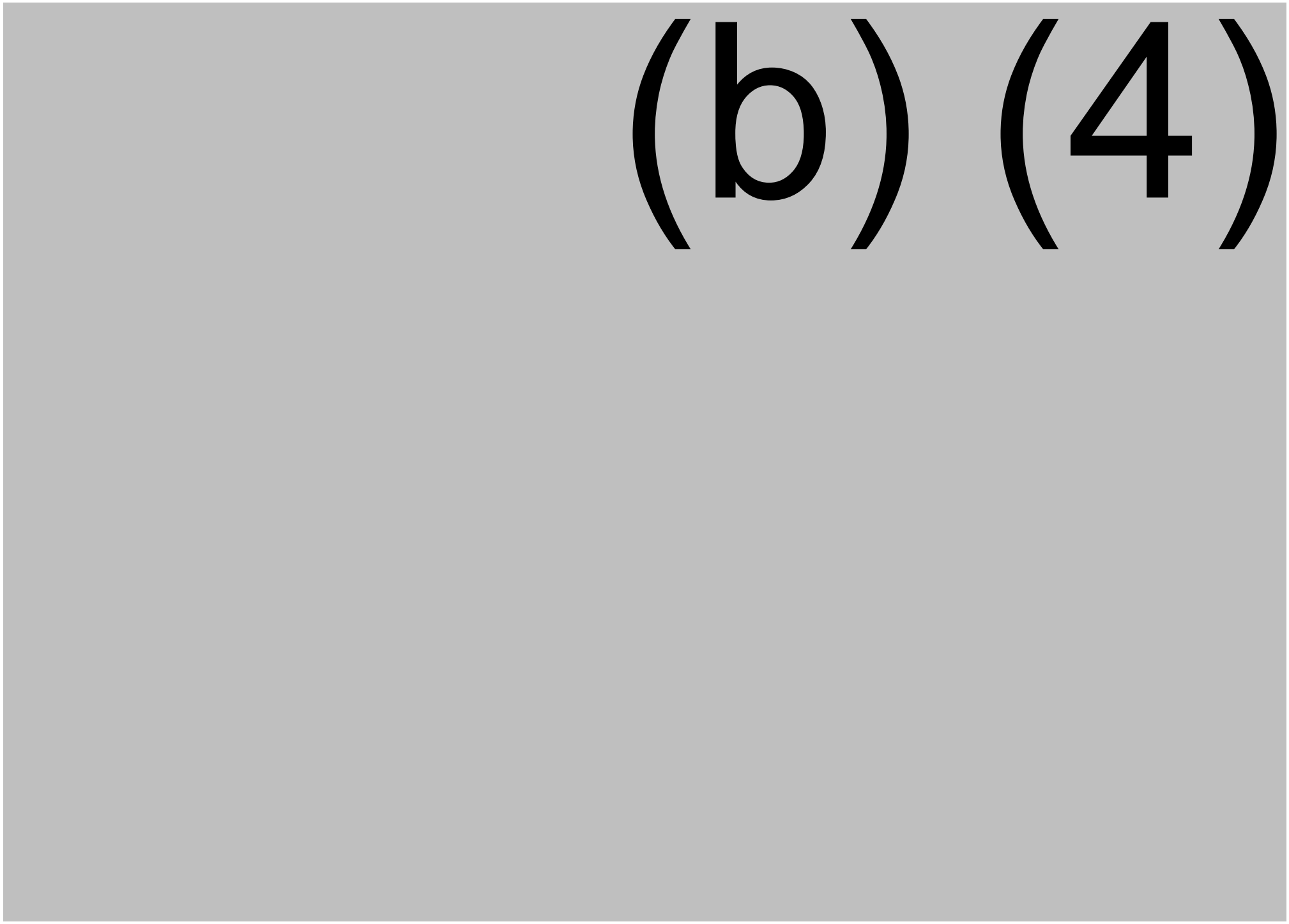
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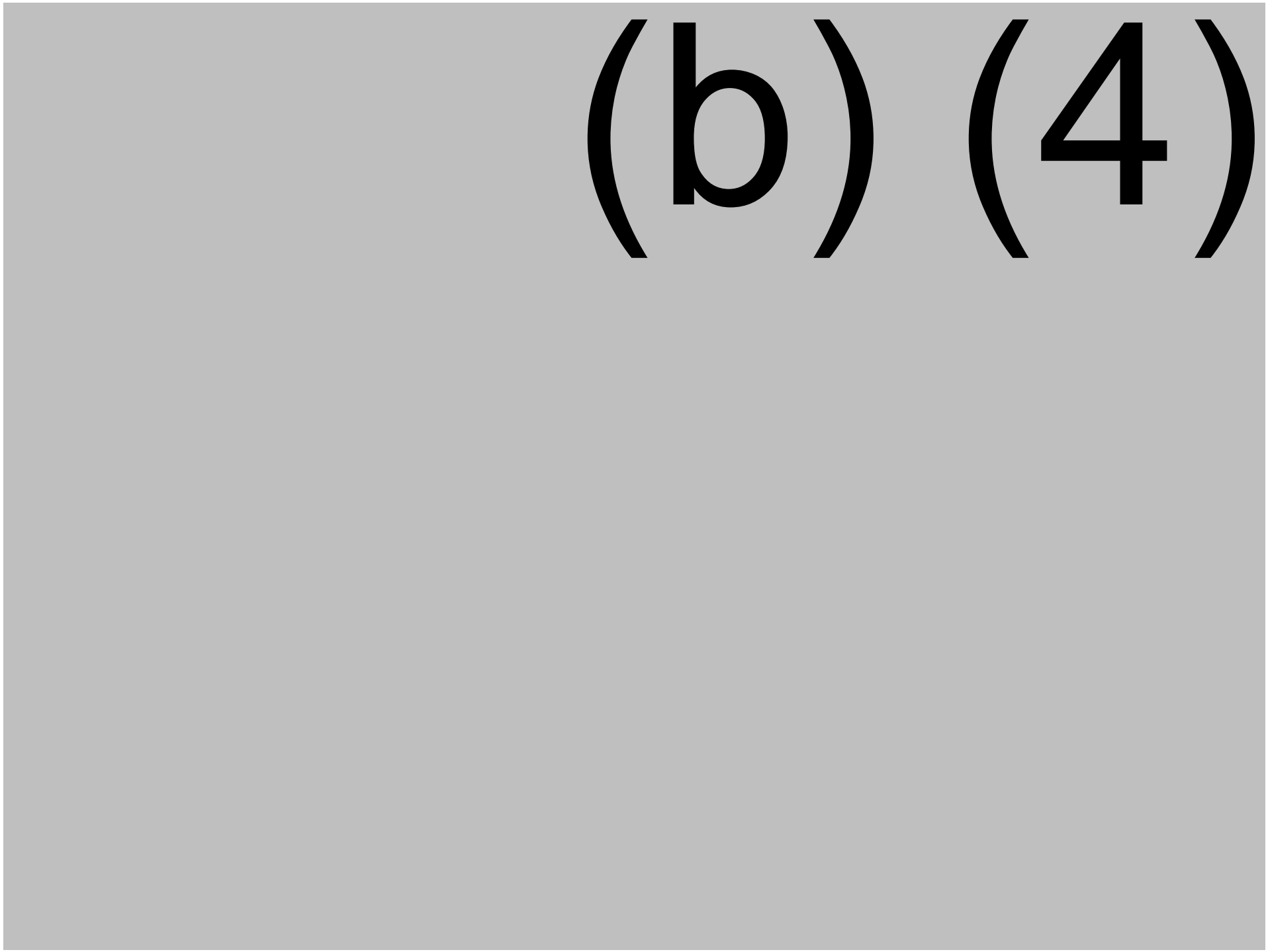
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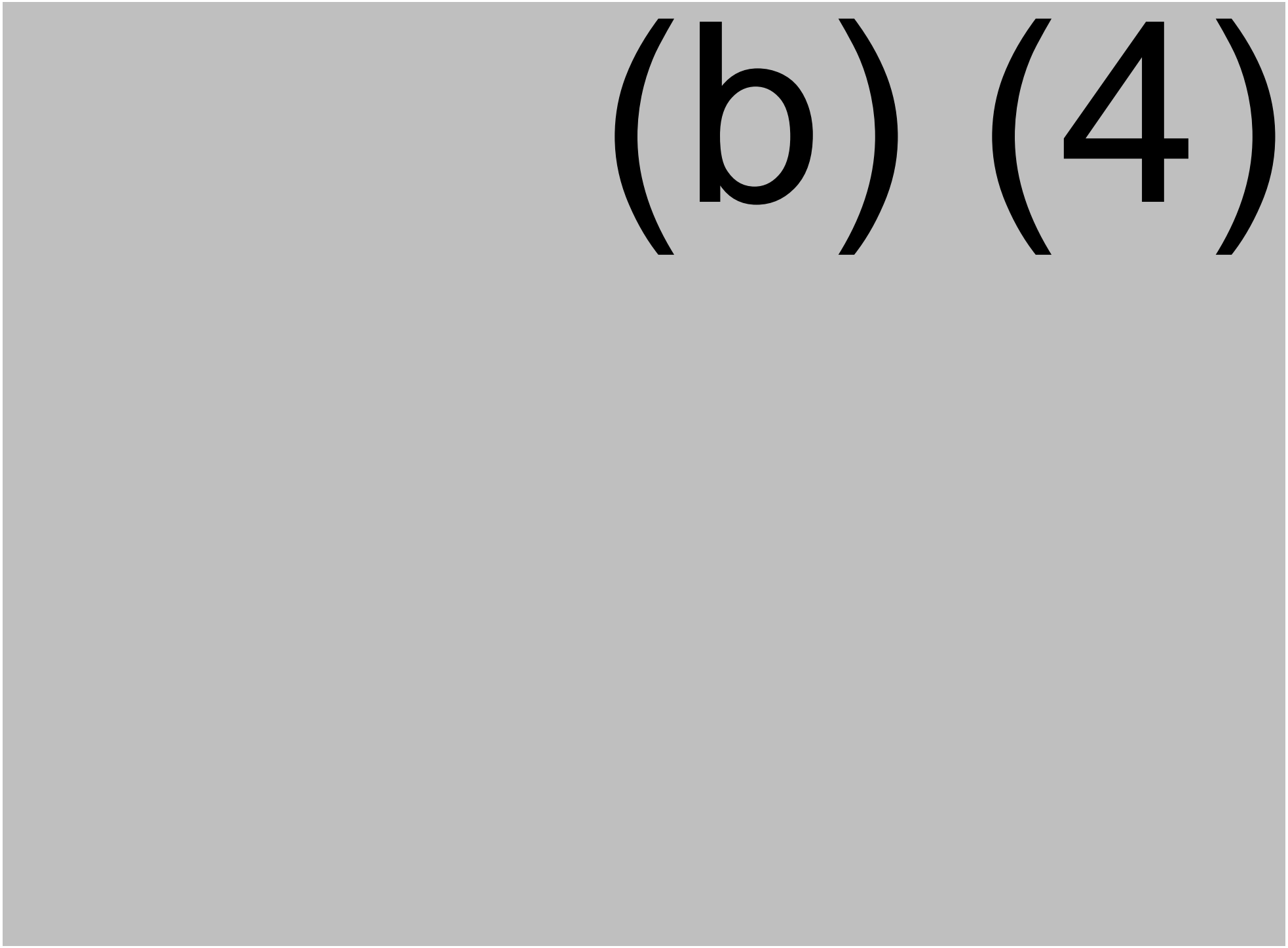
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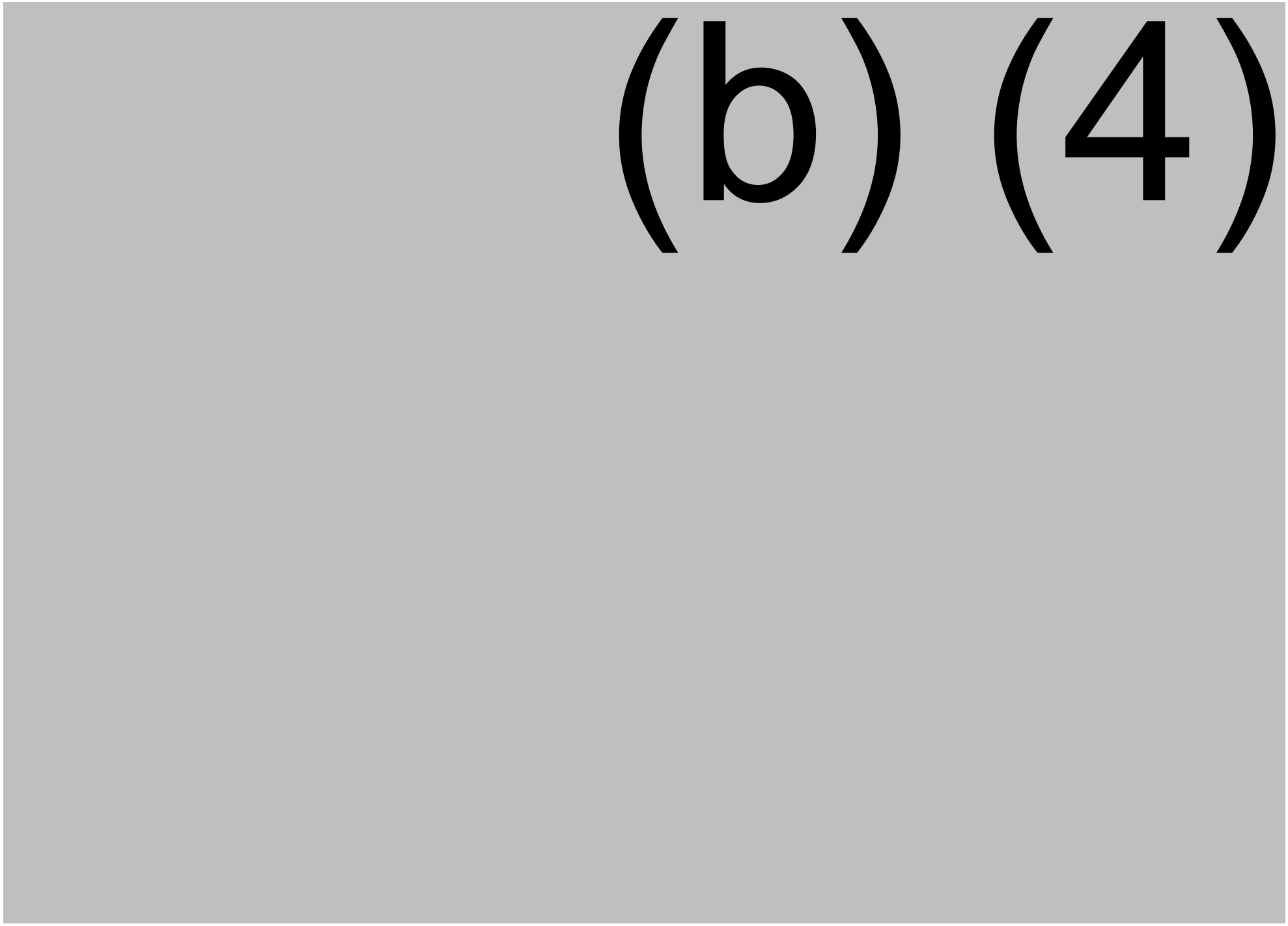

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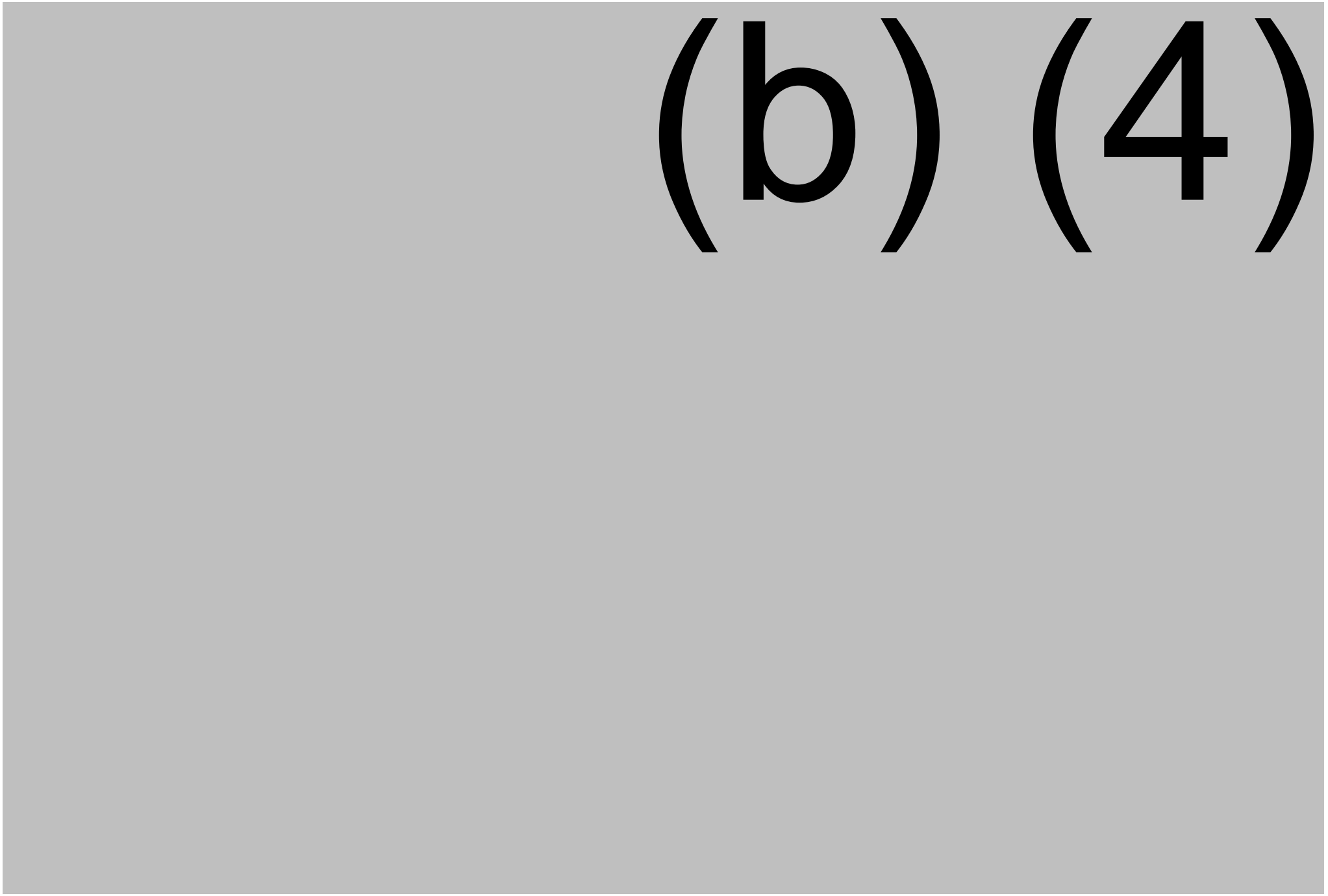
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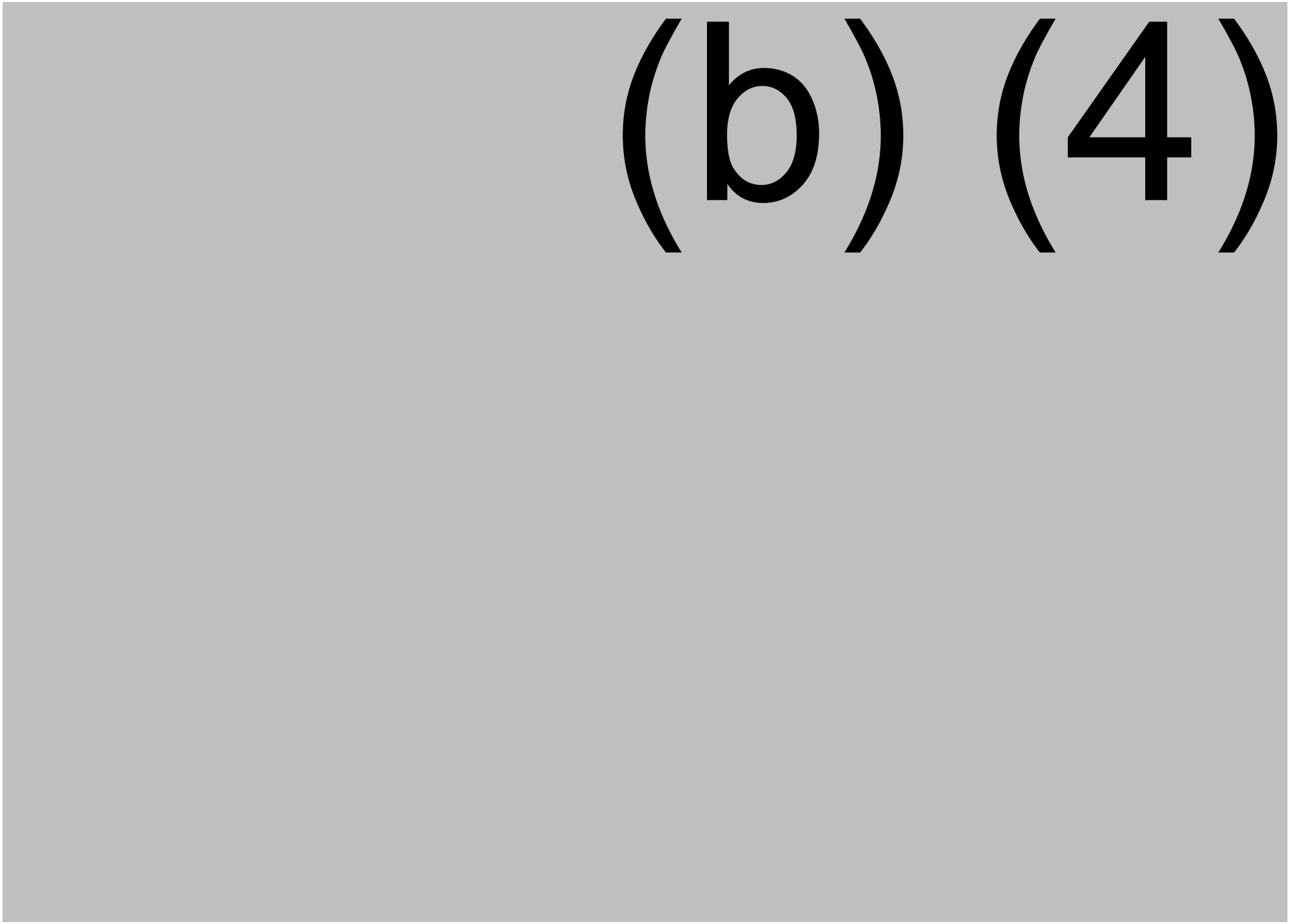

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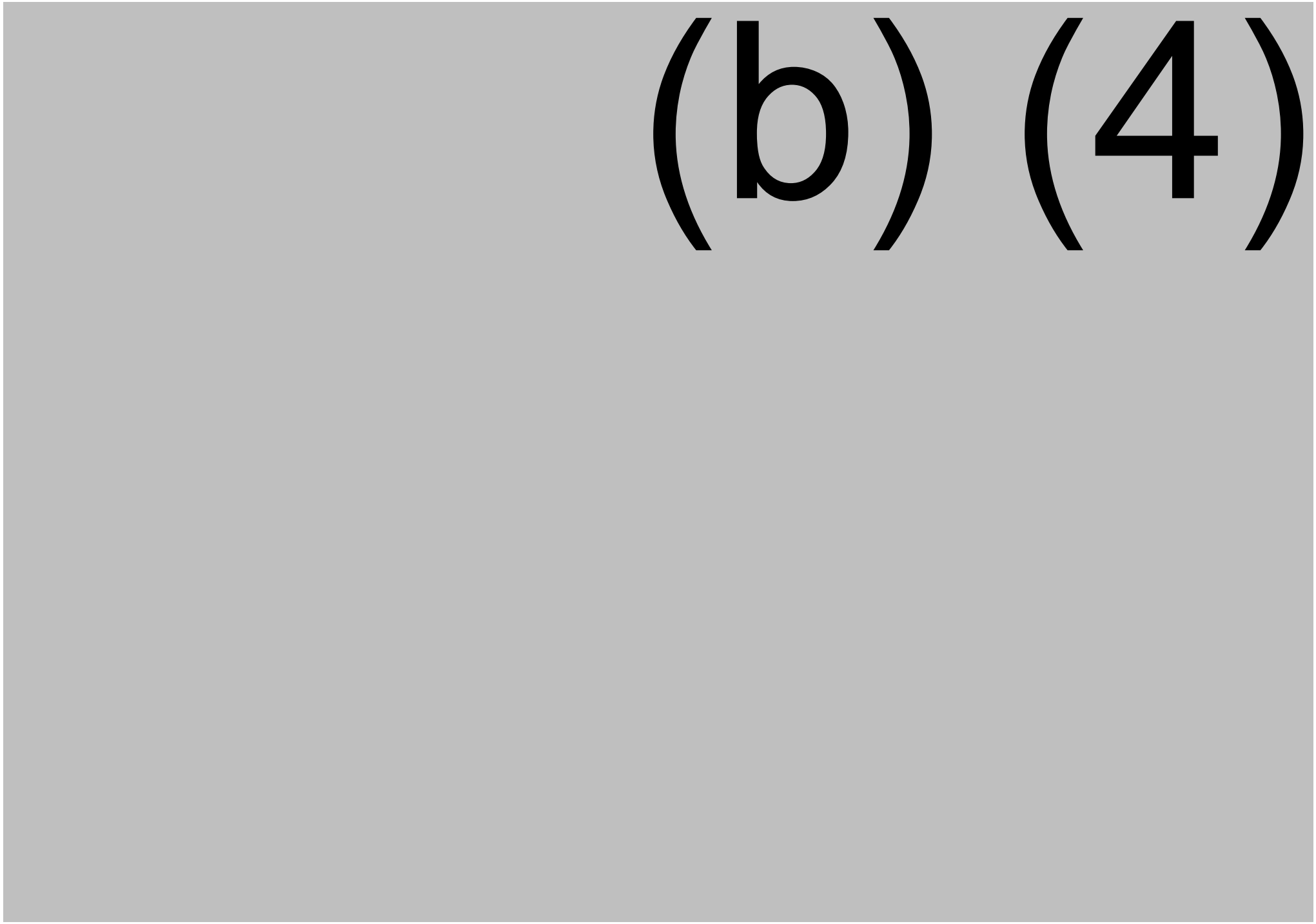

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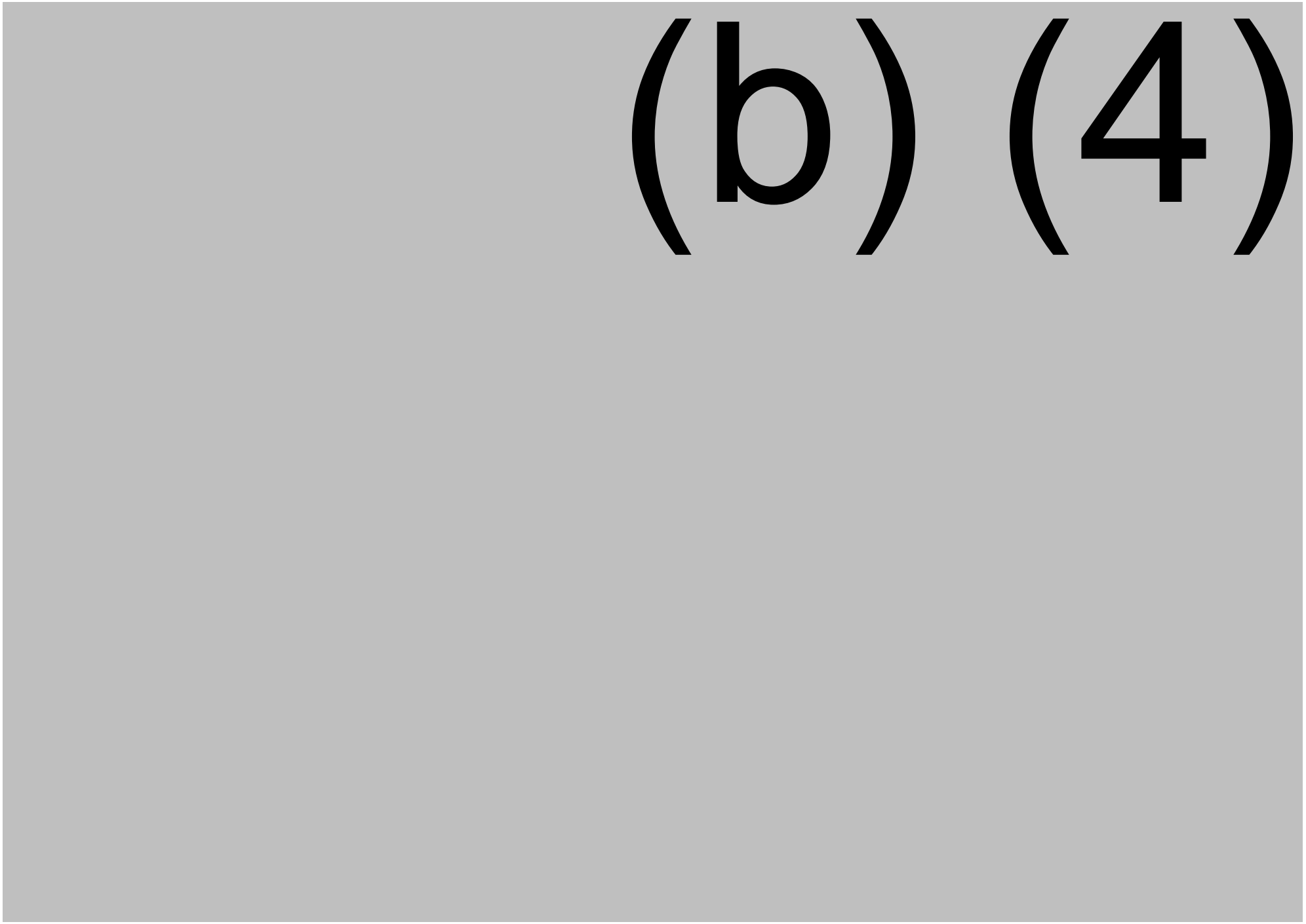

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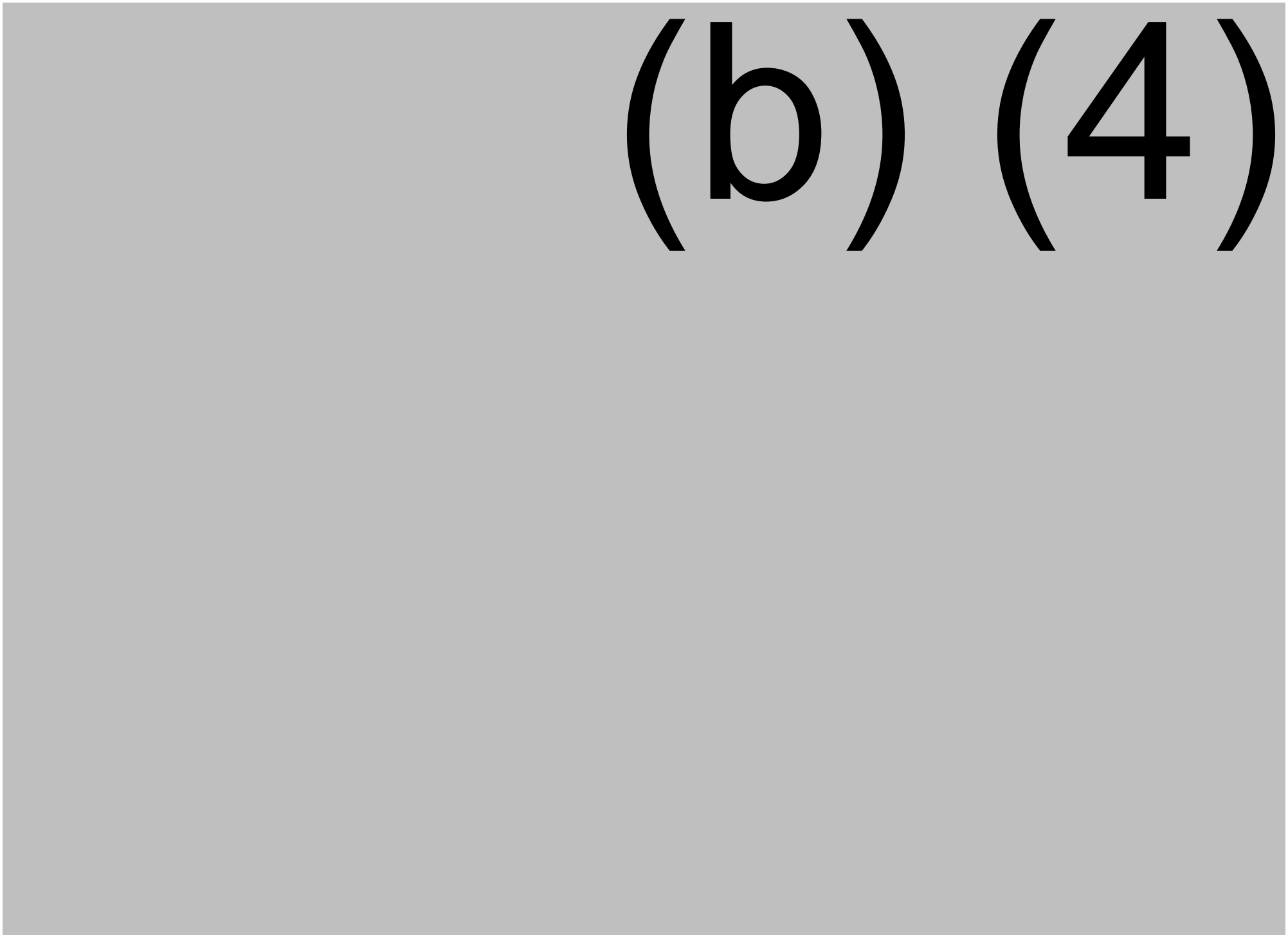

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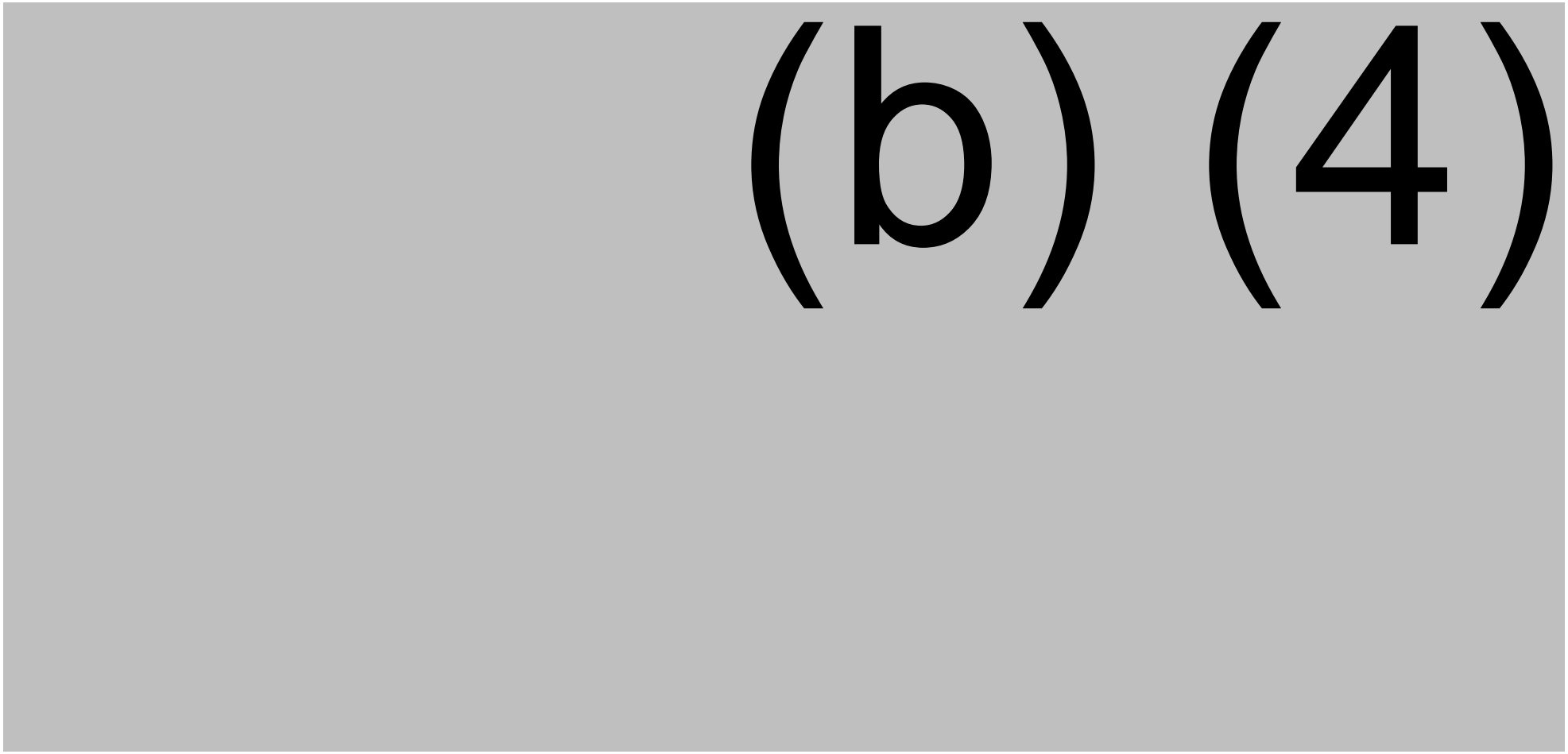

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(b) (4)


(b) (4)


(b) (4)

Appendix 07: Protein Characterization Report

BASF
We create chemistry

Mannanase 19287

Protein Characterization Report

Mannanase 19287

Protein Characterization Report

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KernA14 Digitally signed by KernA14
Date: 2024.02.14 07:34:25 -08'00'

Andreas Kern, Ph.D. **Date**
Sr. Regulatory Affairs Manager
Animal Nutrition

Technical Reviewer:

(b) (6)

Enzymes Process and Strain

Production Strain DSM 33149
Protein Characterization Report

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Introduction

The filamentous fungus *T. thermophilus* is able to secrete large quantities of enzymes and may serve as an alternative to similar well-known production hosts, including *Aspergillus niger* or *Trichoderma reesei* (Singh, B., 2016). A natural strain of *T. thermophilus* named C1 was isolated from (b) (6) and has been deposited at the All-Russian Collection of Microorganisms (VKM, accession number VKM F-3500-D). Initially classified as *Chrysosporium lucknowense*, it was later identified as a strain of *Myceliophthora thermophila*, the former name of *T. thermophilus*. C1 has been developed into an expression platform for industrial-scale quantities of endogenous and recombinant enzymes and other proteins (Visser, H. et al., 2011). BASF has licensed this platform to manufacture enzymes for food, feed and technical applications.

Dyadic selected host strain (b) (4) based on its ability to secrete a highly enriched enzyme preparation with a very low background of secreted endogenous *T. thermophilus* proteins, including proteases that may degrade the target protein in the fermentation supernatant. The purpose of transforming *T. thermophilus* host strain (b) (4) with the BASF-proprietary synthetic (b) (4) gene is to generate a genetically modified production strain carrying multiple copies for the large-scale manufacture of the mannanase 19287 preparation. Mannanase 19287 (b) (4) is classified as an endo-β-mannanase (E.C. 3.2.1.78), and tolerance of higher temperatures is designed into the mannanase enzyme to maintain activity after exposure to heat conditions during the pelleting process of feed. In addition, mannanase 19287 is also optimized for enzyme activity at an acidic pH environment and resistance to pepsin digestion to allow for enzyme activity in the digestive system of the target species.

Sequence Characteristics of Mannanase 19287

Several bacterial and fungal species express enzymes that degrade the hemicellulosic components of plant cell walls, including mannanases(Bhat, M.K., 2000; Chauhan, P.S. *et al.*, 2012; Dhawan, S. and Kaur, J., 2007; Moreira, L.R.S. and Filho, E.X.F., 2008; van Zyl, W.H. *et al.*, 2010). The biotech company (b) (4) developed genetically modified enzymes by introducing point mutations into the *T. reesei (Hypocrea jecorina)* wild-type mannanase (b) (4) in a protein-engineering approach (Kensch, O., 2015; Kensch, O. *et al.*, 2010). BASF acquired synthetic enzyme sequences from (b) (4) that were optimized for specific characteristics. Mannanase 19287, the subject of this application, is a feed enzyme with improved thermotolerance and high enzymatic activity in acidic pH conditions, expressed in the production strain DSM 33149 from the synthetic (b) (4) gene (**Table 1**).

Thermotolerance is designed into the synthetic (b) (4) gene product mannanase 19287 enzyme to maintain activity after exposure to high temperature during the pelleting process of animal feed. In addition, manF is also optimized for enzyme activity at an acidic pH optimum, to allow for enzyme activity in the digestive tract of the non-ruminant target species.

Additional modifications were introduced into the (b) (4) DNA sequence to improve expression and secretion in the fungal organism *T. thermophilus*. The modifications included the (b) (4) of *T. thermophilus*. Neither modification changes the secreted mature mannanase 19287 enzyme protein; all modifications are described below.

Table 1: Amino acid sequences of mannanase 19287 and wild-type *T. reesei* (b) (4) mature proteins

Mannanase 19287 ((b) (4) unpublished and proprietary)
(b) (4)
(b) (4) ***T reesei (Hypocrea jecorina)* – GenBank** (b) (4)
(b) (4)

The respective signal peptide sequences for both proteins are listed in *italics* but are not present in the secreted proteins.

Detailed mannanase 19287 sequence analysis

The endo-mannanase (b) (4) is expressed by the native *Trichoderma reesei* strain RUT C-30 expresses and belongs to the GH5 family of glycohydrolases (Aspeborg, H. *et al.*, 2012). The (b) (4) gene has been sequenced and characterized (Stalbrand, H. *et al.*, 1995). The sequence alignment between the mature mannanase 19287 protein sequence ((b) (4) gene product) and the highly homologous *T. reesei* (b) (4) endo-β-mannanase contain (b) (4)s. The modifications result in the optimized thermostability, necessary during the high temperatures encountered during the production of pelleted feeds; the activity improvement at acidic pH conditions optimizes hemicellulose degradation in the digestive track of the target animals.

T. thermophilus DSM 33149 expresses the synthetic mannanase gene (b) (4) **Table 2** shows the outcome of the homology analysis between the secreted mature mannanase 19287 and the mature (b) (4) protein. The secreted mannanase 19287 is classified as a mannan endo-1,4-β-mannosidase with the IUBMB code 3.2.1.78 and has the CAS Registry Number 37288-54-3.

Table 2: Outcome of homology analysis for T. reesei (b) (4) and mannanase 19287 expressed by T. thermophilus DSM33149

Aligned_sequences: 2
1: (b) (4)_T_reesei_no_signal_sequence, Length: 410
2: (b) (4)_Protein_no_signal_sequence, Length: 410

(b) (4)

Prog

Mannanase 19287 BLASTP against UniProt database

The Basic Local Alignment Search Tool (BLAST; https://blast.ncbi.nlm.nih.gov), (Altschul, S.F. *et al.*, 1990; Schäffer, A.A. *et al.*, 2001) modified for protein homology searches against non-redundant sequences (BLASTP) was used to identify the alignment between the mannanase 19287 protein sequence produced by DSM 33149 and other protein sequences in the UniProt database. The search was limited to 1000 hits/alignments. **Table 3** lists the top ten sequence alignments of the BLAST search; all 1000 hits are listed in Annex 1 Homology Search Report Mannanase.

Table 3: Top 10 BLASTP results for mannanase 19287 ((b) (4) gene product, mature secreted protein, 410 amino acids) against UniProt database

Database ID	Score [bits]	E-value	Identities	Positives	Gaps
(b) (4)					

The ten most significant alignments are listed with scores, E-values, identities, positives and gaps.

As expected, the sequence of the *T. reesei* (*Hypocrea jecorina*) (b) (4) protein showed the highest level of sequence homology with mannanase 19287; as mentioned above, the synthetic (b) (4) sequence was optimized from the *T. reesei* wild-type mannanase using a protein-engineering approach (Kensch, O., 2015; Kensch, O. *et al.*, 2010). The BLASTP search was limited to 1000 hits/alignments; all remaining 990 hits represent other fungal glycohydrolases, including the

endogenous *T. thermophilus* β-mannanase. The E-values of all 1000 alignments range from 0.0 to 7e-64 and demonstrate the high degree of homology of mannanase 19287 with fungal endo-1,4-β-mannanases (Annex 1).

Evaluation of Amino Acid Modifications in Mannanase 19287 (Endo-β-Mannanase)

Strain DSM 33149 of the filamentous fungus *Thermothelomyces thermophilus* is an endo-β-mannanase production strain and was developed for BASF by Dyadic NL. *T. thermophilus* was previously classified as *Myceliophthora thermophila*, a species name still frequently used in publications (Marin-Felix, Y. et al., 2015). BASF has licensed the patented *T. thermophilus* C1 strain platform for homologous and heterologous protein production (internal BASF cell bank (b) (4)). For the generation of DSM 33149, (b) (4) *T. thermophilus* (*M. thermophila*) (b) (4) (Visser, H. et al., 2011) (b) (4) expression cassette.

Trichoderma reesei is a fungal organism known for the production of many enzymes that degrade plant- and fungal non-starch polysaccharides (NSPs). The thermotolerant organism secretes an endo-β-mannanase, (b) (4) that has been classified as a member of the glycohydrolase family GH5-7. Like other members of the family, the enzyme contains a conserved (βα)8 barrel structure of the hydrolase domain (Sabini, E. et al., 2000). Eight amino acids are strictly conserved in these hydrolases, with two glutamate residues forming the catalytic center of the enzyme (Aspeborg, H. et al., 2012).

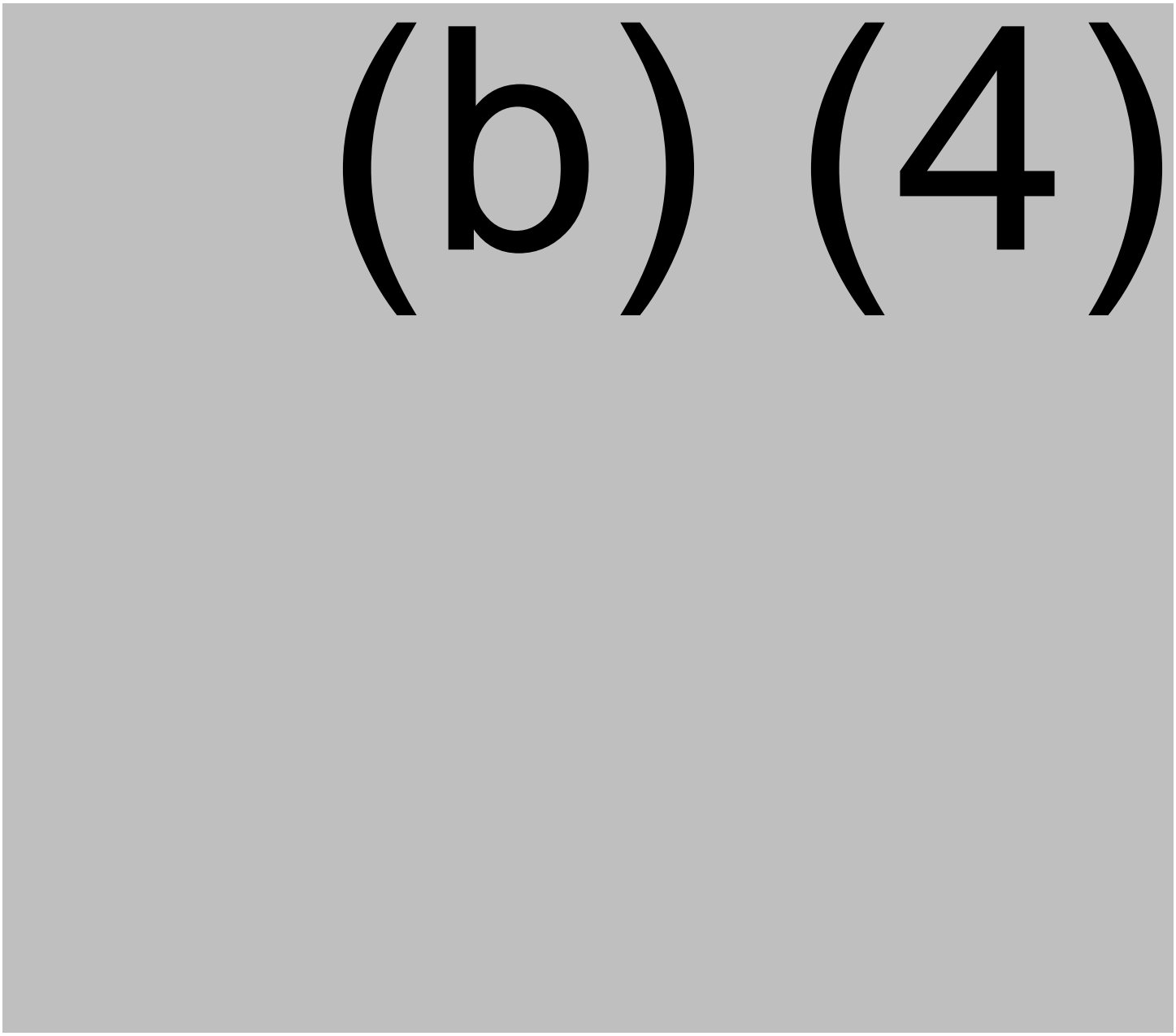
Two patents describe the effects of amino acid modifications in (b) (4) US7846705 (Kensch, O. et al., 2010) and US9040277 (Kensch, O., 2015). Modified (b) (4) proteins were heterologously expressed in Saccharomyces cerevisiae and tested for thermostability, pH-dependent activity and pepsin digestibility. This section of the report summarizes the homology of the synthetic mannanase 19287 expressed in T. thermophilus with the native T. reesei (b) (4) enzyme and the variants described in the patents.

Homology Alignment

Protein gap analysis was performed using the NEEDLE application within the EMBOSS Suite (http://emboss.open-bio.org/; Rice et al. 2000, Trends in Genetics. 16-276 – EMBOSS: The European Molecular Biology Open Software Suite): "NEEDLE reads two input sequences and writes their optimal global sequence alignment to file. It uses the Needleman-Wunsch alignment algorithm to find the optimum alignment (including gaps) of two sequences along their entire length. The algorithm uses a dynamic programming method to ensure the alignment is optimum, by exploring all possible alignments and choosing the best. A scoring matrix is read that contains values for every possible residue or nucleotide match. Needle finds the alignment with the maximum possible score where the score of an alignment is equal to the sum of the matches taken from the scoring matrix, minus penalties arising from opening and extending gaps in the aligned sequences. The substitution matrix and gap opening, and extension penalties are user-specified."

The comparison of the (b) (4) sequence with other GH5-7 family glucohydrolases is a summary of separate alignments the by MULTICOMPARE application of the above-mentioned EMBOSS Suite.

Heterologous Expression of T. reesei (b) (4) Amino Acid Variants in *S. cerevisiae*



Endo-β-Mannanase 19287 Protein Sequence and Homology Analysis with *T. reesei* (b) (4)

Table 2 lists the amino acid sequences of the native *T. reesei* (b) (4) and (b) (4) (mannanase 19287), expressed from the synthetic gene (b) (4) in *T. thermophilus*. The outcome of the NEEDLE analysis is listed in **Table 3**, demonstrating a 96.3 % amino acid identity, a 98.3 % similarity and the complete absence of gaps between the two sequences.

Figure 1 provides the detailed alignment of both sequences, including a) the consensus sequence of the homology, b) the identity of strictly conserved amino acids within the GH5 family of

glycohydrolases, c) the modified amino acids in mannanase 19287 when compared to the native *T. reesei* (b) (4) enzyme, and d) the secondary structure elements of (b) (4) based on the 3D structure determination of the enzyme (Sabini, E. *et al.*, 2000). The alignment demonstrates that mannanase 19287 maintains all the strictly conserved amino acids and is therefore classified as a member of the GH5 family and 5-7 subfamily. No modifications of the strictly conserved residues have occured. Seven of fifteen modifications occur in known α-helices and β-structures, the remaining changes are not associated with defined secondary structures.

As outlined previously (Hilge, M. *et al.*, 1998) and listed in **Table 4** below, the eight residues play an important role in the positioning of the mannose polysaccharide and the lysis of the glycoside bond by the catalytic amino acids. **Figure 2** demonstrates the alignment of four GH5 family β-mannanases, namely *Thermobifida fusca* (THEFU), *T. reesei* ((b) (4) *Aspergillus niger* (MANA_ASPNC), and *T. thermophilus* DSM 33149 ((b) (4) as shown in (Sabini, E. *et al.*, 2000).

Impact of modified amino acids on characteristics of heterologously expressed Man5A variants

Two patents (Kensch, O., 2015; Kensch, O. *et al.*, 2010) describe the impact of modifications to the *T. reesei* GH5-7 mannanase, (b) (4) after heterologous expression of the variants in *S. cerevisiae*. A total of (b) (4) have been listed in US7846705 that demonstrate beneficial outcomes in thermostability, either as single substitutions and/or in combination. The mannanase 19287 enzyme (or (b) (4) expressed by *T. thermophilus* strain DSM 33149 (b) (4) s. The characteristics of a large number of variants, including thermostability and activity under pH and pepsin conditions encountered during digestion of animal food, are disclosed in both patents.

Amino acid variants included in (b) (4) either as single modifications or combinations up to three residues are listed in **Table 4** together with the impact on thermostability or pepsin conditions. The data for these variants are disclosed in US7846705.

Further mannanase variants prepared from the modified amino acids of US7846705 were tested containing (b) (4) in mannanase 19287; their outcomes are disclosed in US9040277. **Table 5** lists the (b) (4) and their impact on the enzyme characteristics. The variant expressed in *T. thermophilus* strain DSM33149 has been included for comparison. **Please note:** (b) (4)) were not included in US9040277; their impact on thermostability has been disclosed in US7846705 (see **Table 4**).

The results in **Table 5** demonstrate that a full-length (b) (4) variant with the native C-terminal CBD module and (b) (4)) shows improved thermostability from 74.6 to 82.6 C. Similarly, the combination of (b) (4)) increases thermostability of a truncated Man5A variant (having a His-tag replacing the native CBD domain) by 9.3°C over the S3R variant, from 75.7 to 85 °C. The four missing modifications in the full-length (b) (4) variant as well as (b) (4) in the truncated mannanase demonstrated improvements on inactivation temperatures and pepsin resistance in **Table 4**.

In summary, the disclosures in both US7846705 and US9040277 demonstrate improvements in inactivation temperatures and retention of enzyme activities after pepsin treatment. The thermotolerance of the (b) (4) variants expressed heterologously in *Saccharomyces* matches well with the data for mannanase 19287, the (b) (4) variant expressed heterologously in *T. thermophilus* DSM 33149. In lab studies at BASF, thermotolerance of mannanase 19287 was shown at temperatures up to 90 °C. See Biochemical Characterization Mannanase 19287 report for the details on the residual enzyme activity after exposure elevated temperatures.

Table 4: Strictly conserved amino acids in GH5 β-mannanases and their proposed functions

T. fusca	(b) (4)	Function
	(b) (4)	
	(b) (4)	

Figure 1: Alignment of mannanase 19287 ((b) (4) protein sequence with T. reesei endo-β-mannanase ((b) (4)

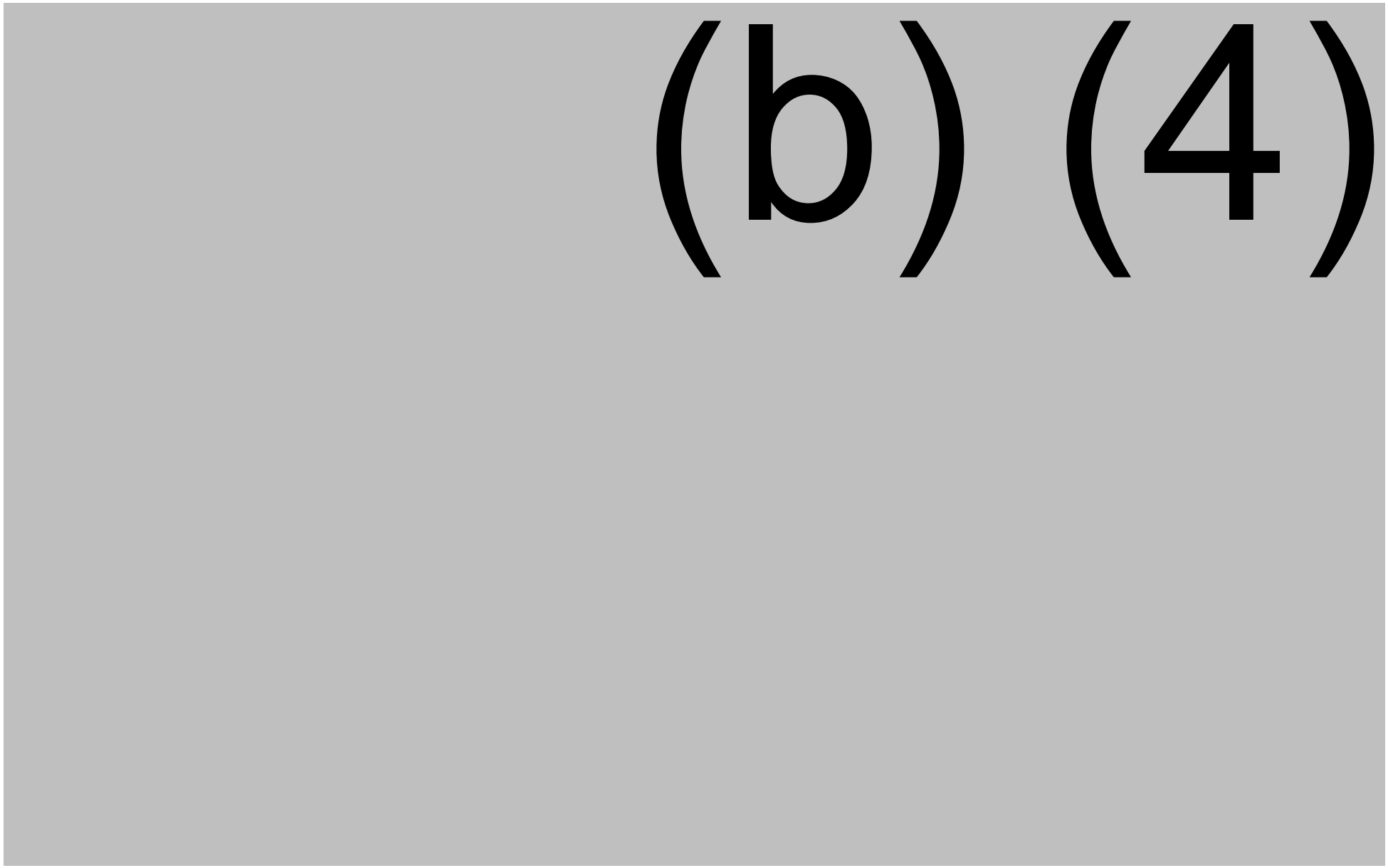


Figure 2: Alignment of GH5 Family β-Mannanases THEFU-Man5A-MANA_(b) (4)

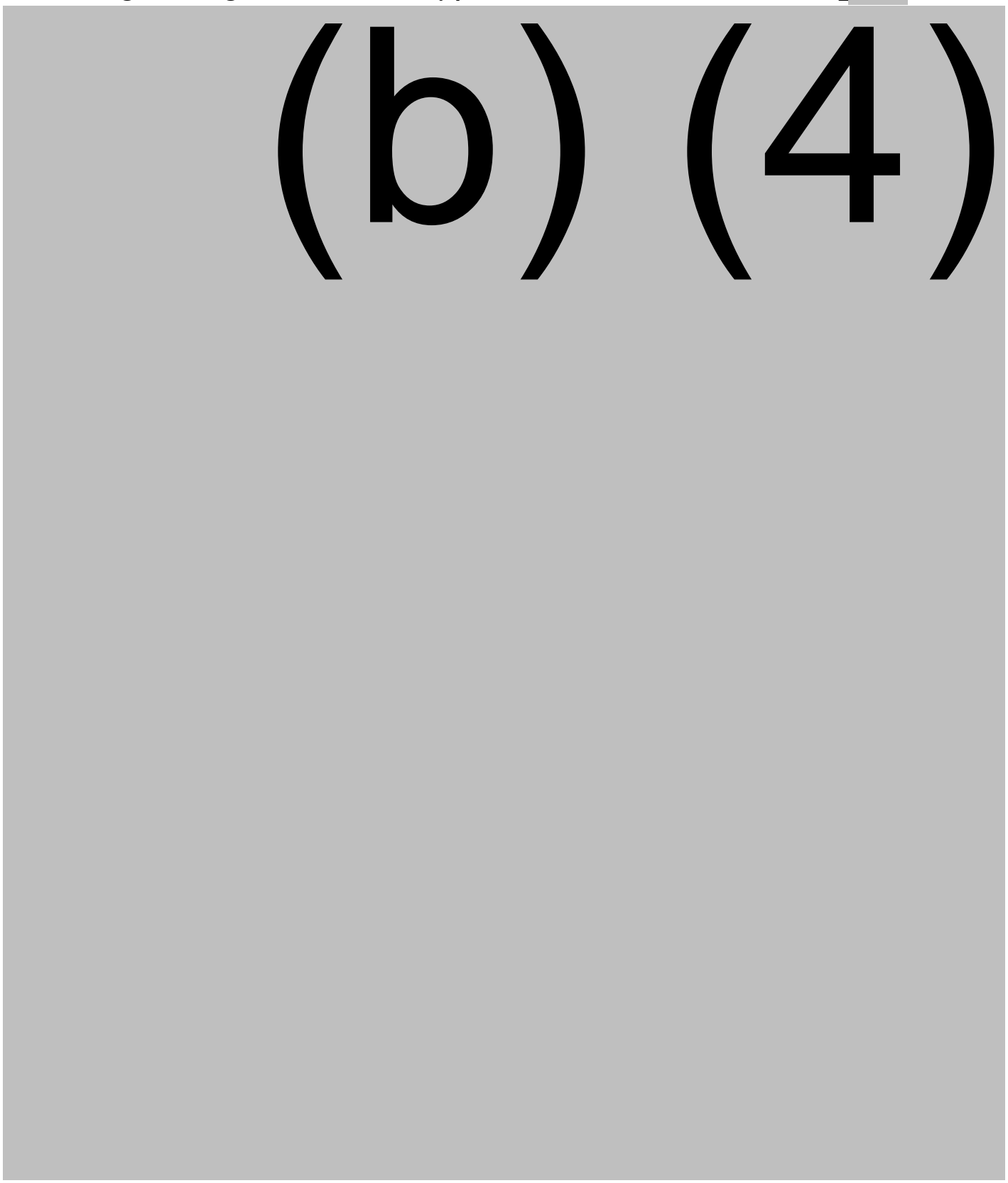


Table 5: Impact of amino acid modifications on thermostability, pH and proteolytic inactivation (Kensch, O. et al., 2010)

T. reesei (b) (4) Variants	IT [°C]	pH 2.65 w/o Pepsin	pH 2.65 + 0.25 mg/ml Pepsin [% resid. activ]
(b) (4)			

Table 6: Impact of combined amino acid modifications on thermostability (Kensch, O., 2015)

															C-term	IT50 [°C]	Pepsin [% resid. activ]
(b) (4)																	

Table 7: Summary of combined amino acid modifications on thermostability and pH/pepsin stability

T. reesei Man5A variants	C-terminus	TD	pH/pepsin stability
(b) (4)			

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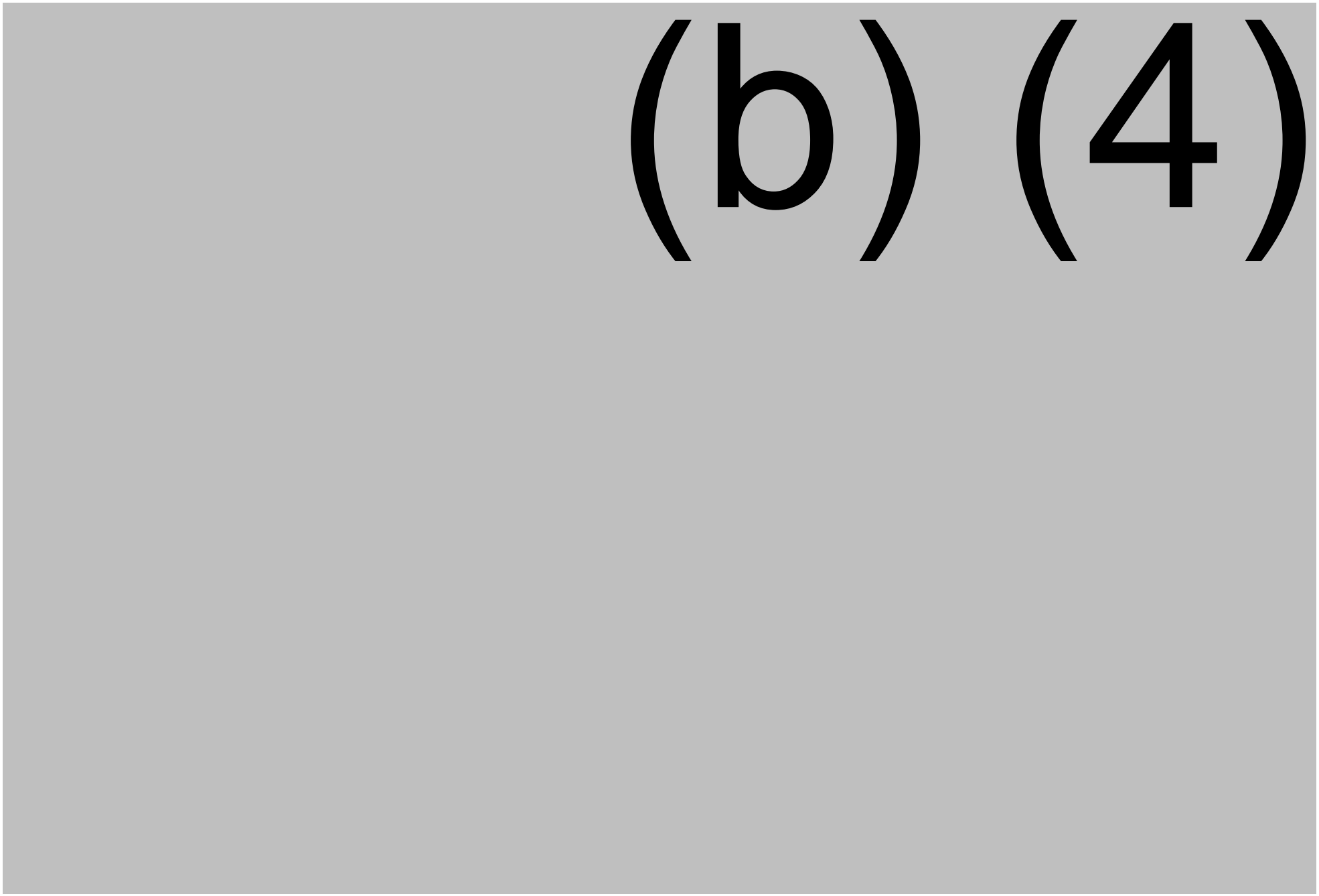
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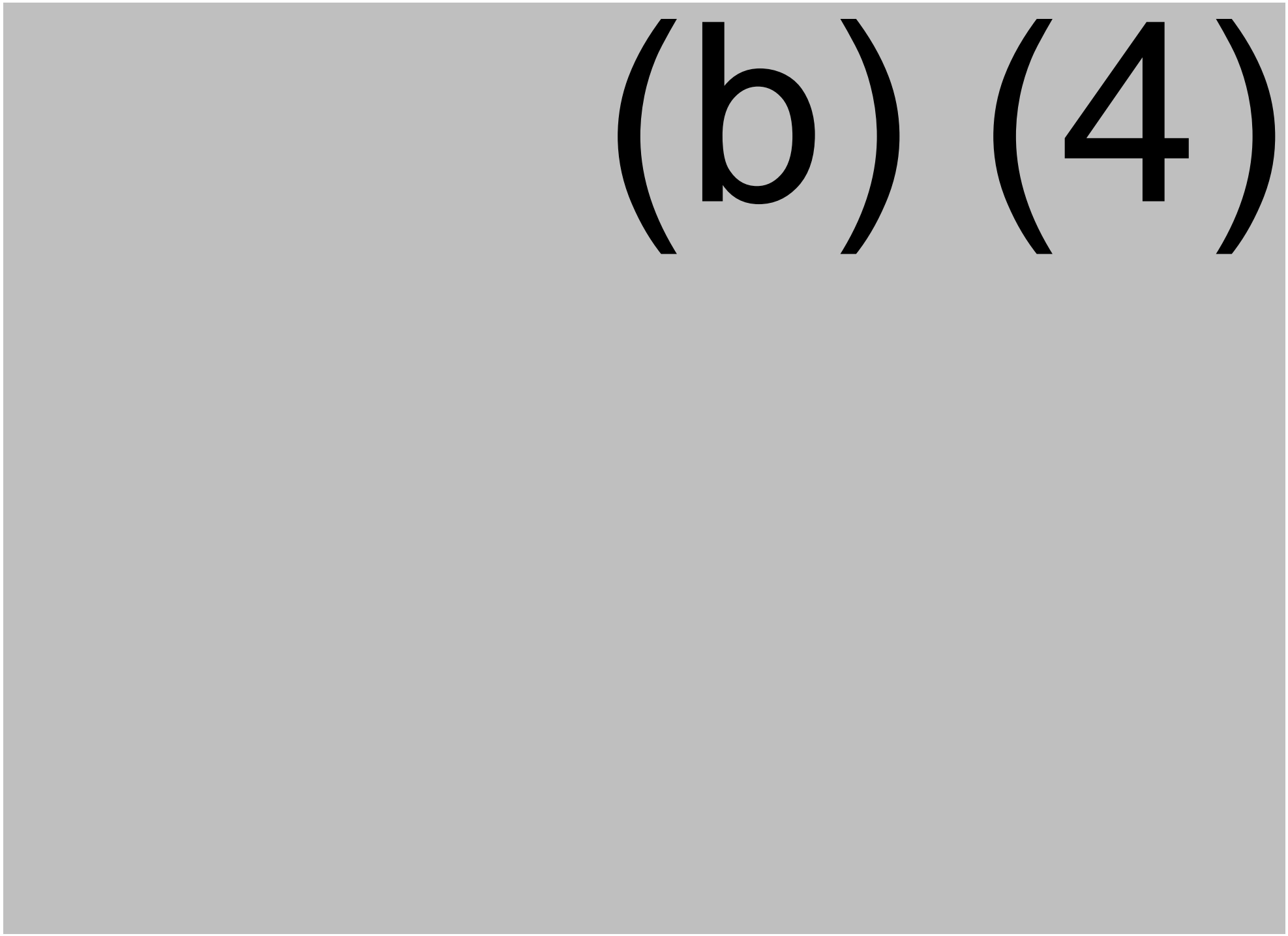
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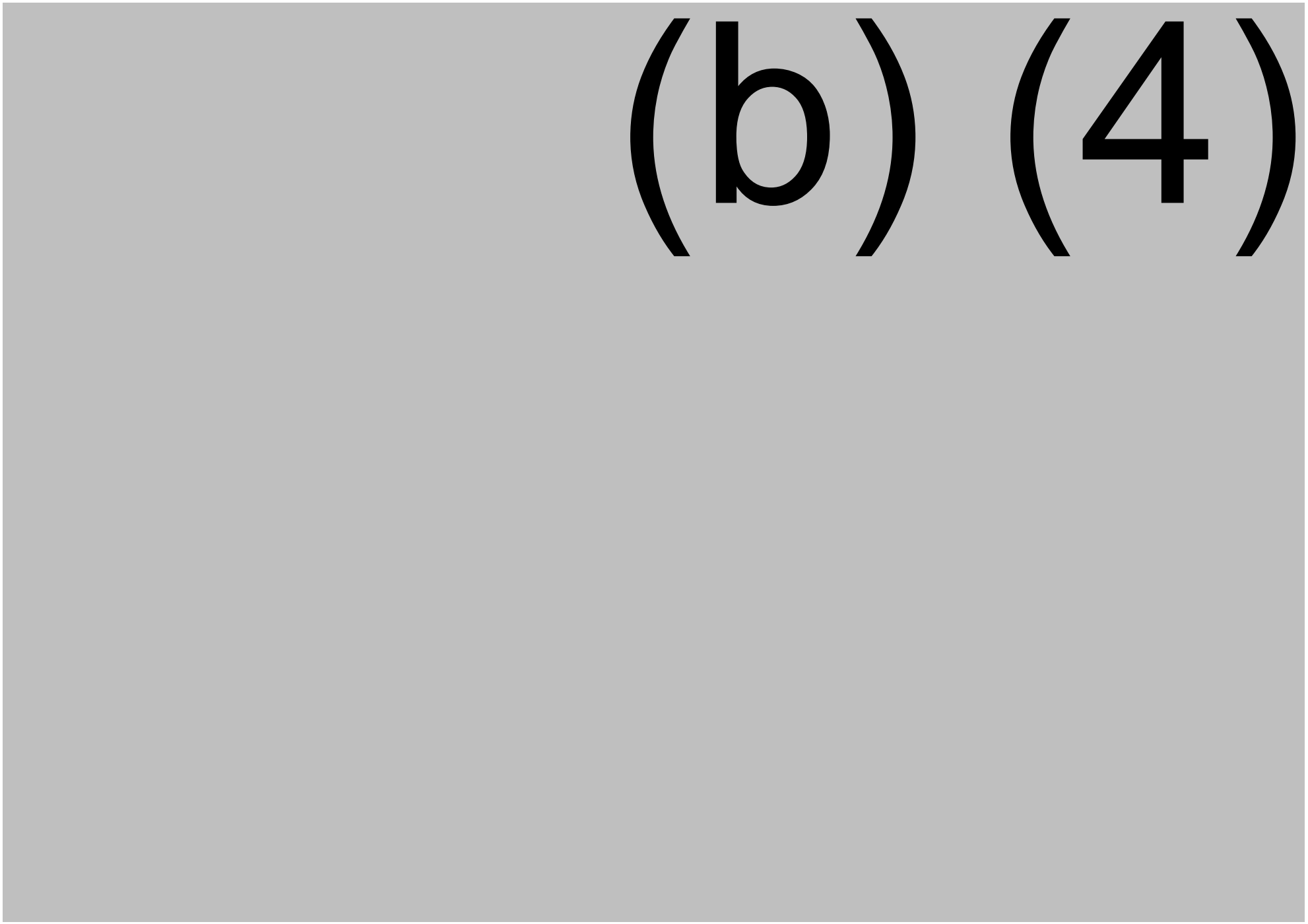
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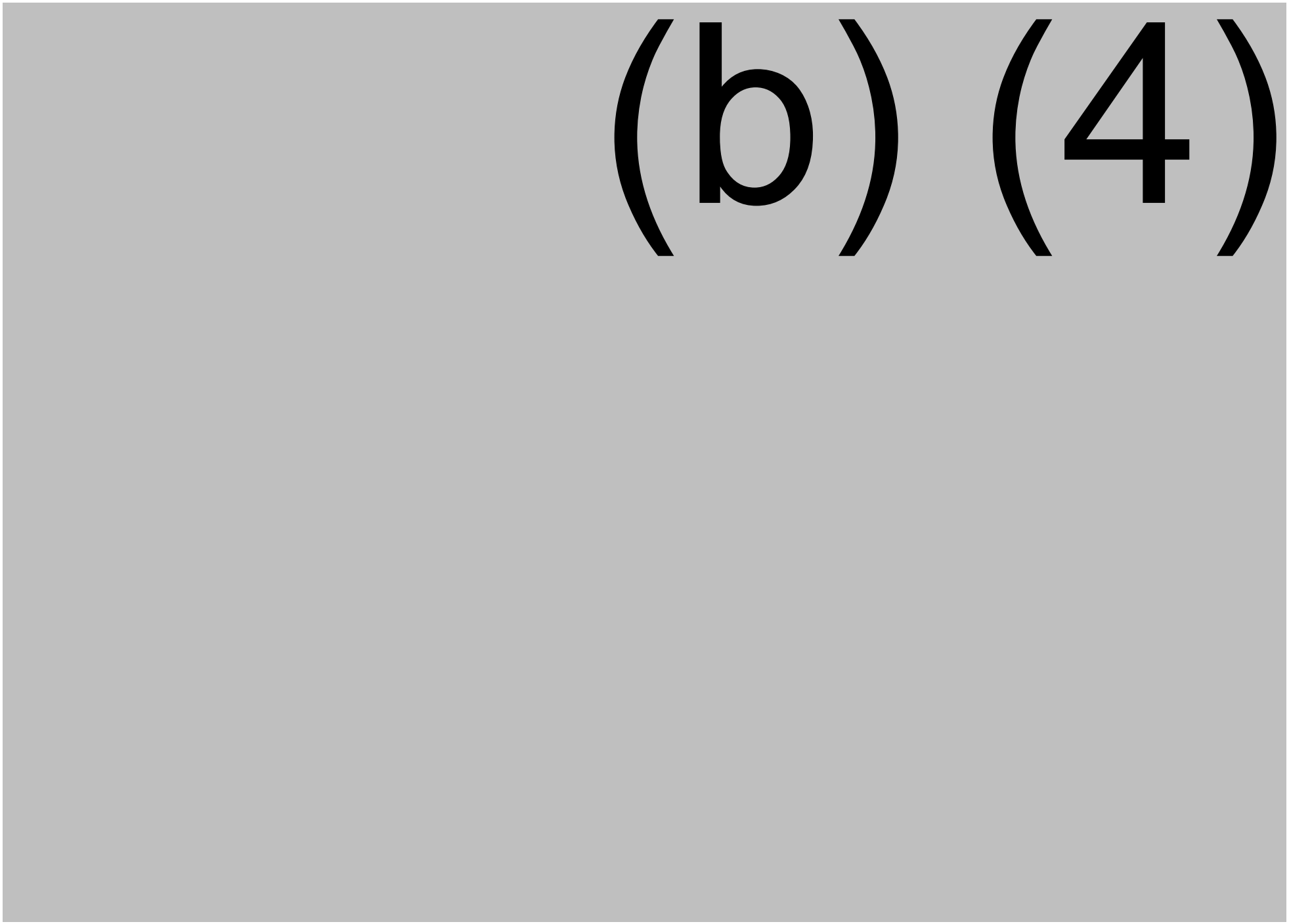
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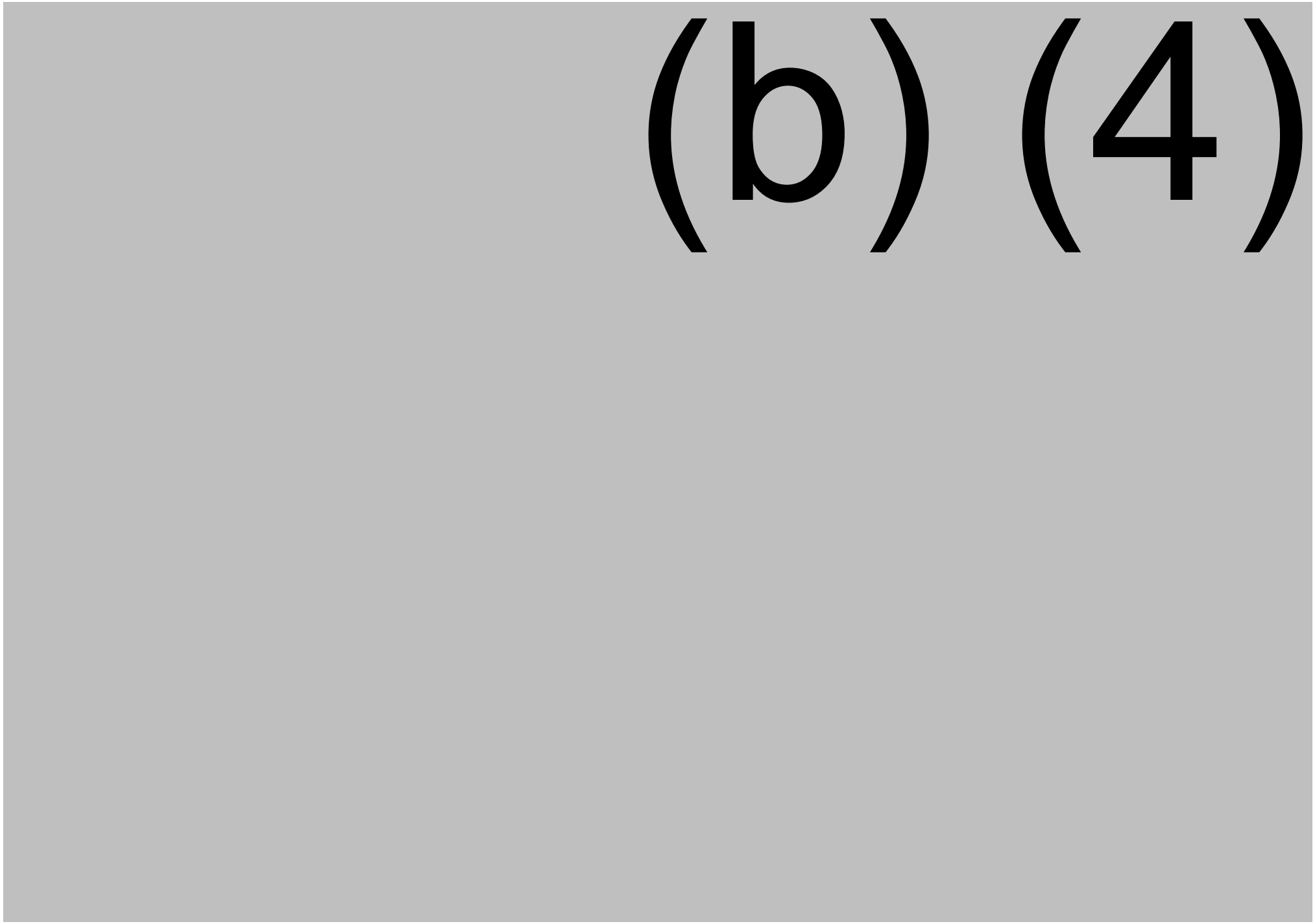
Annex 1: Homology Search Mannanase 19287 ((b) (4) – 1000 hits

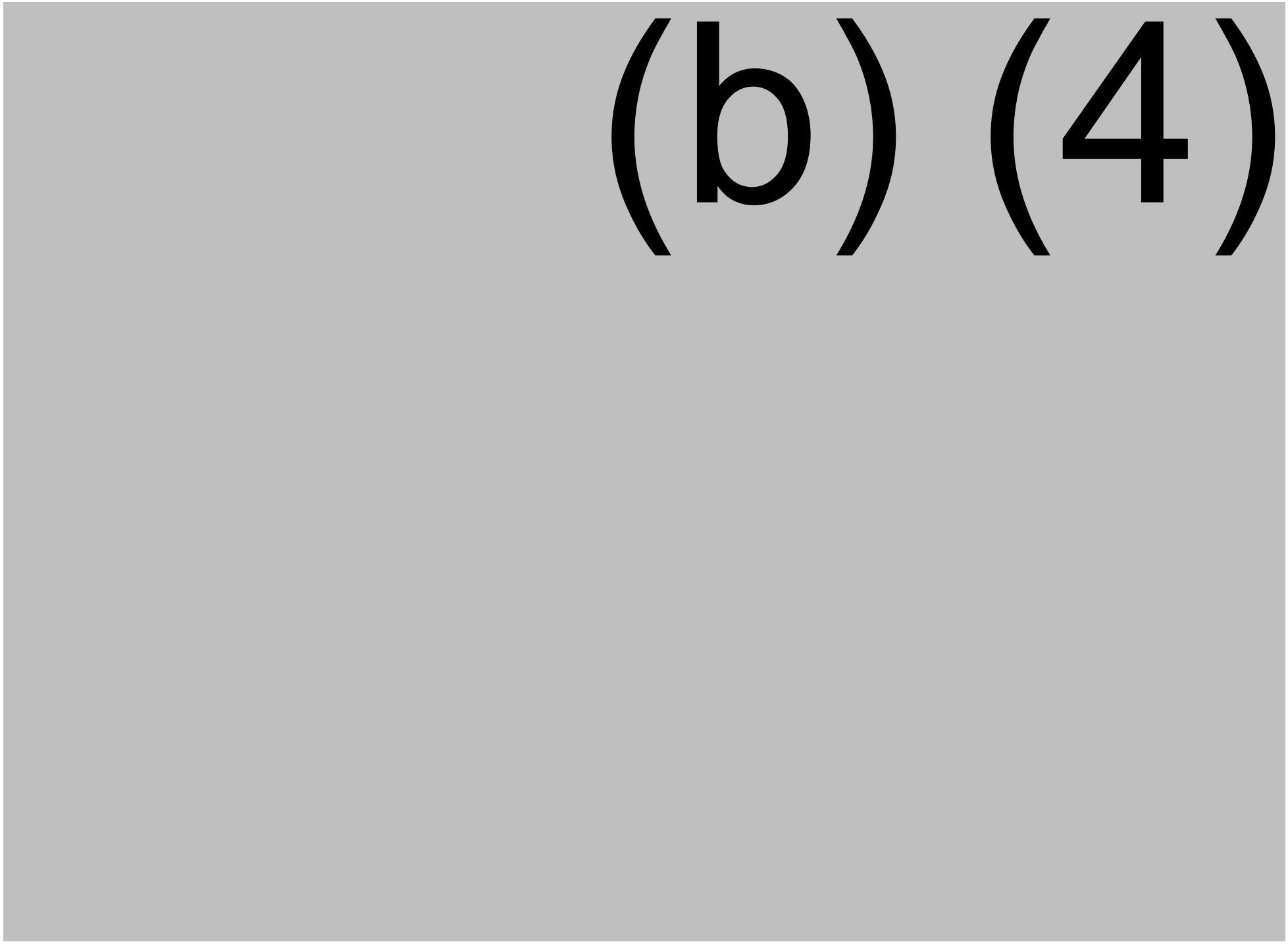


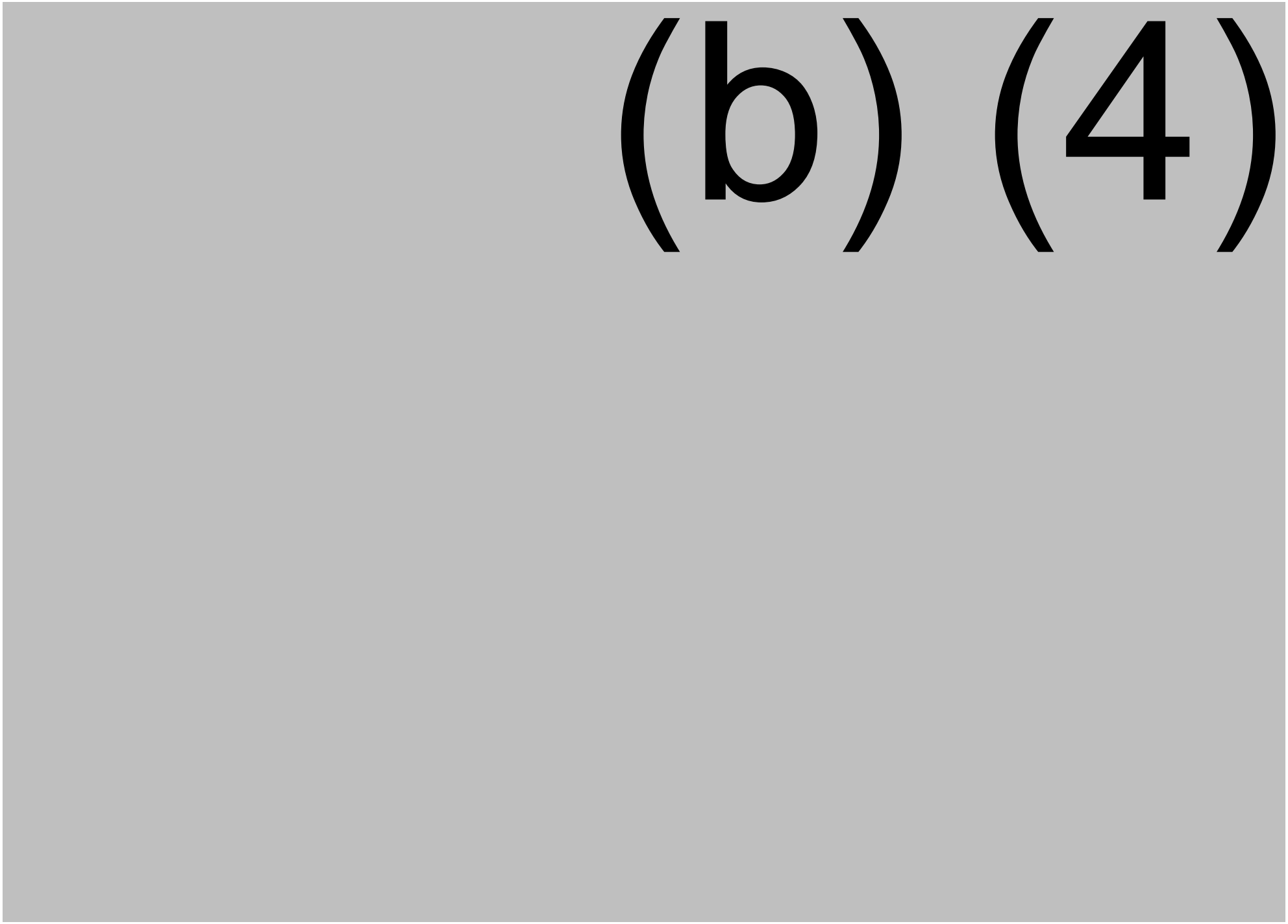

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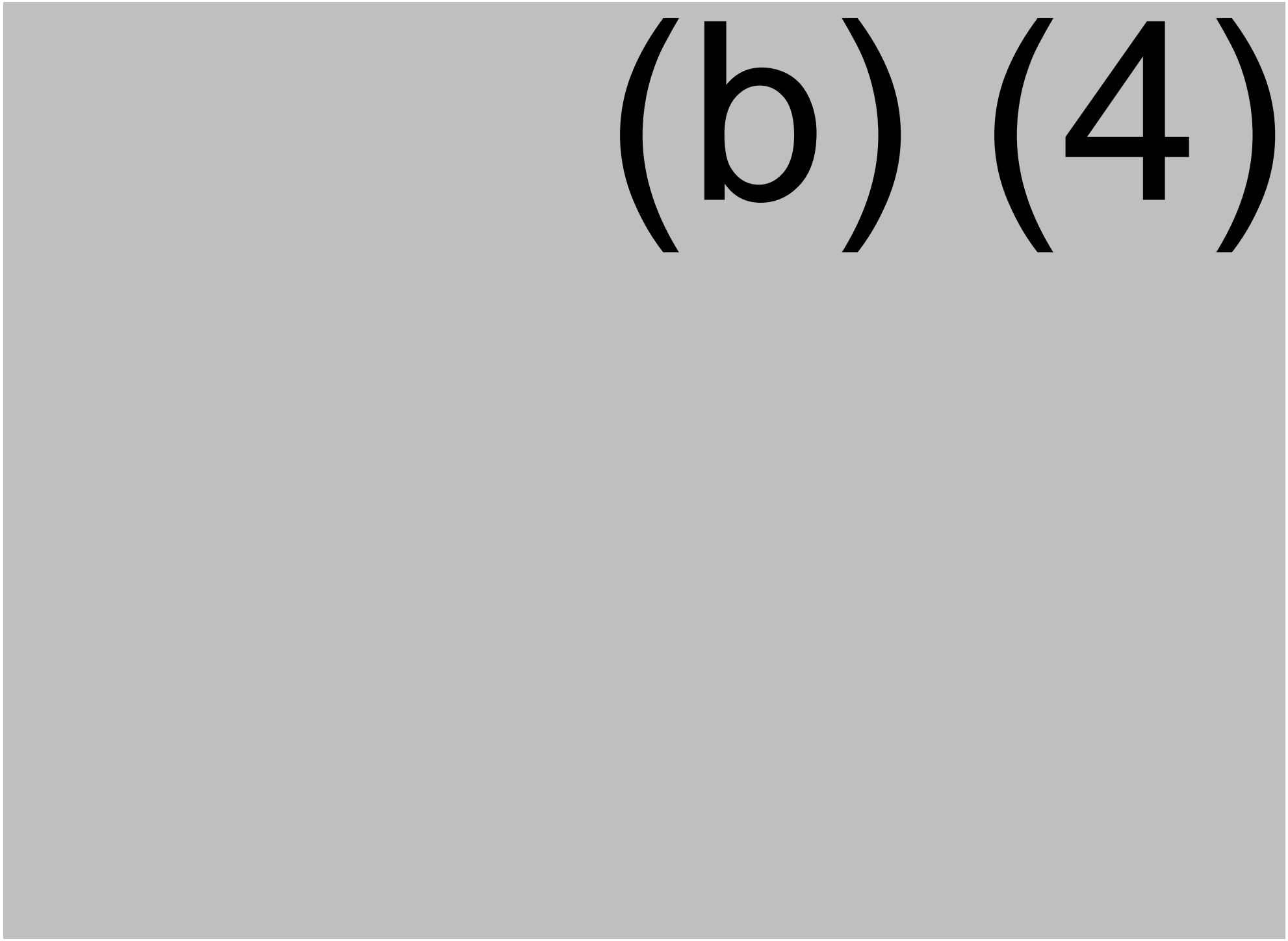

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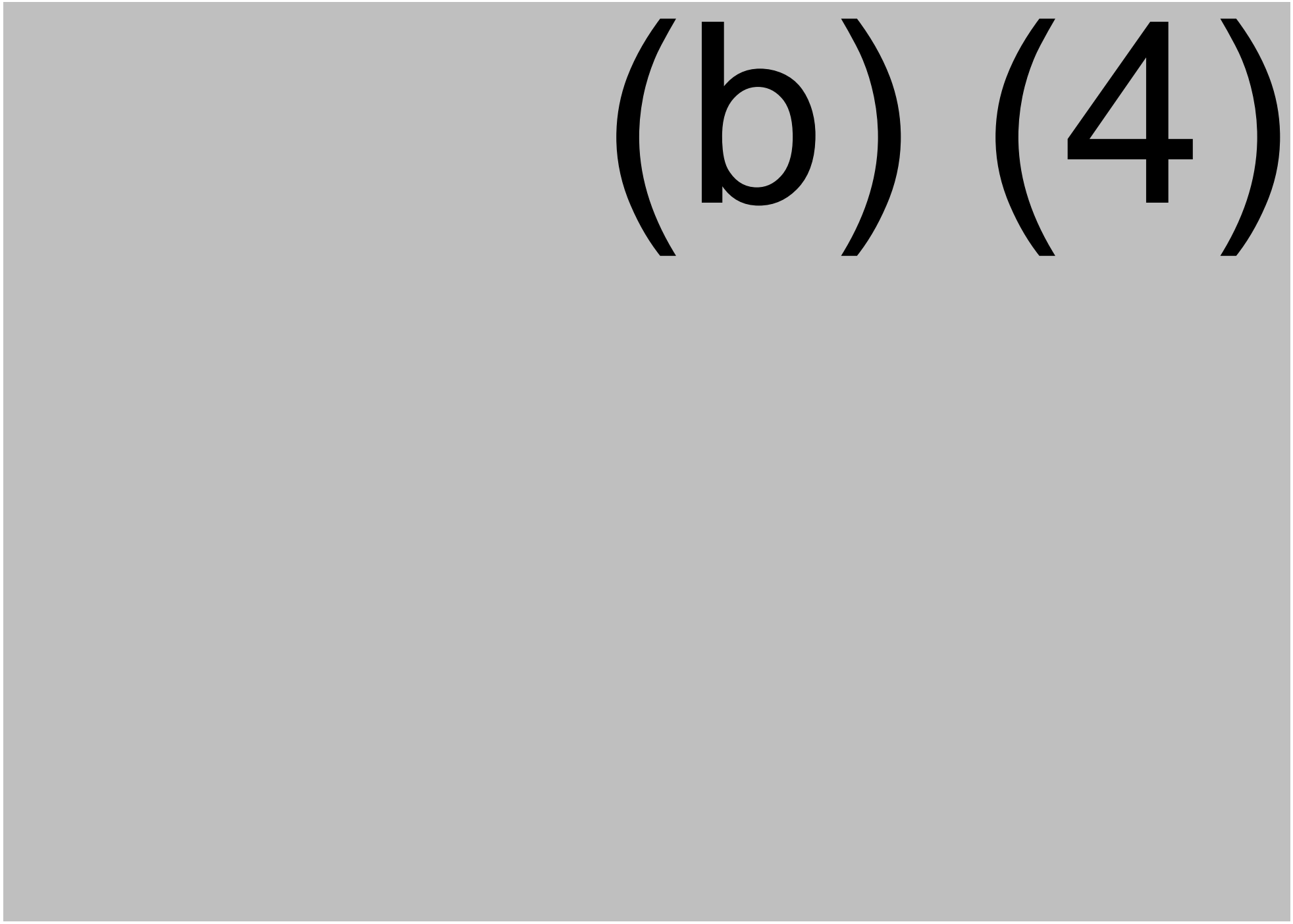

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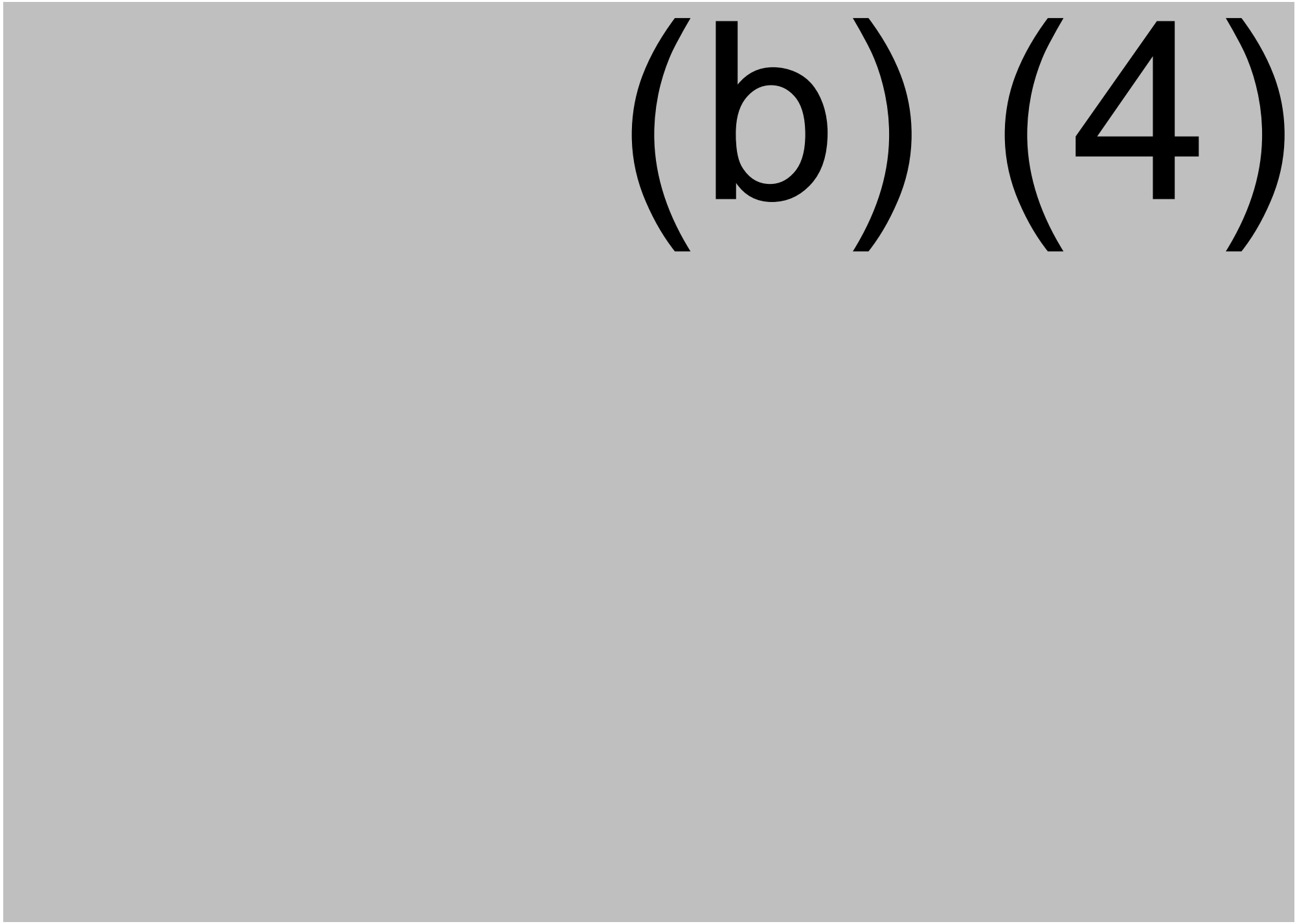

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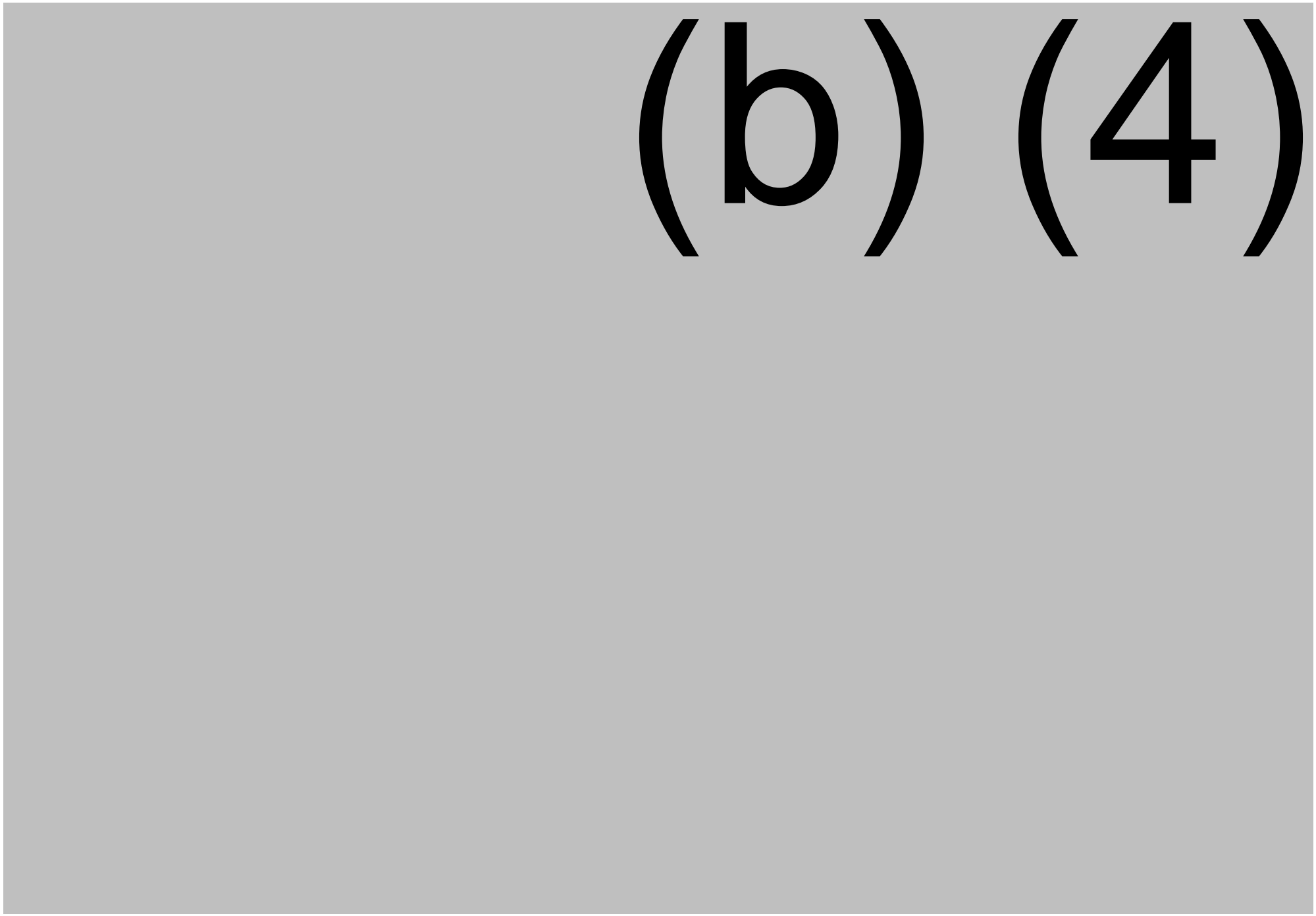

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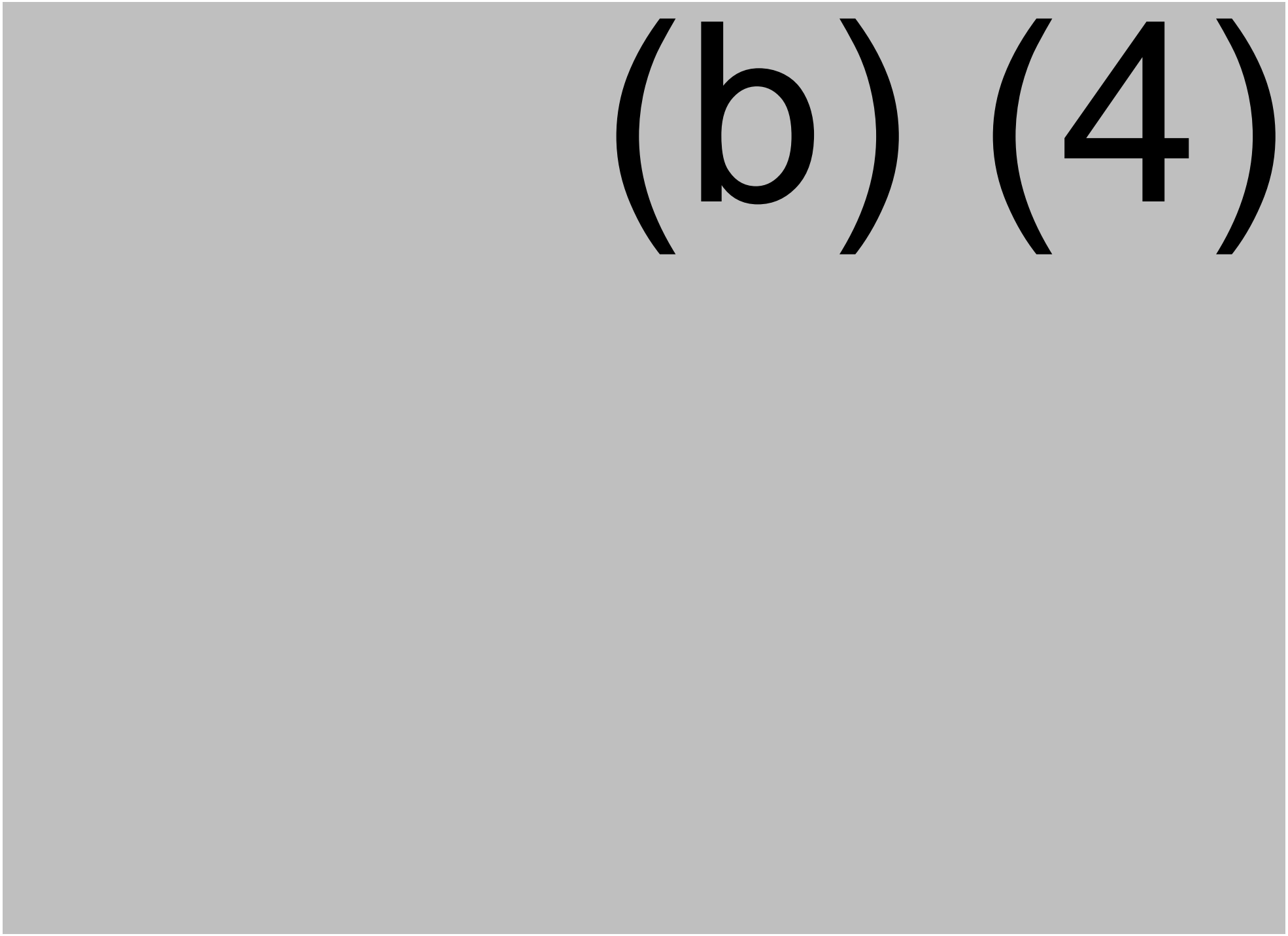

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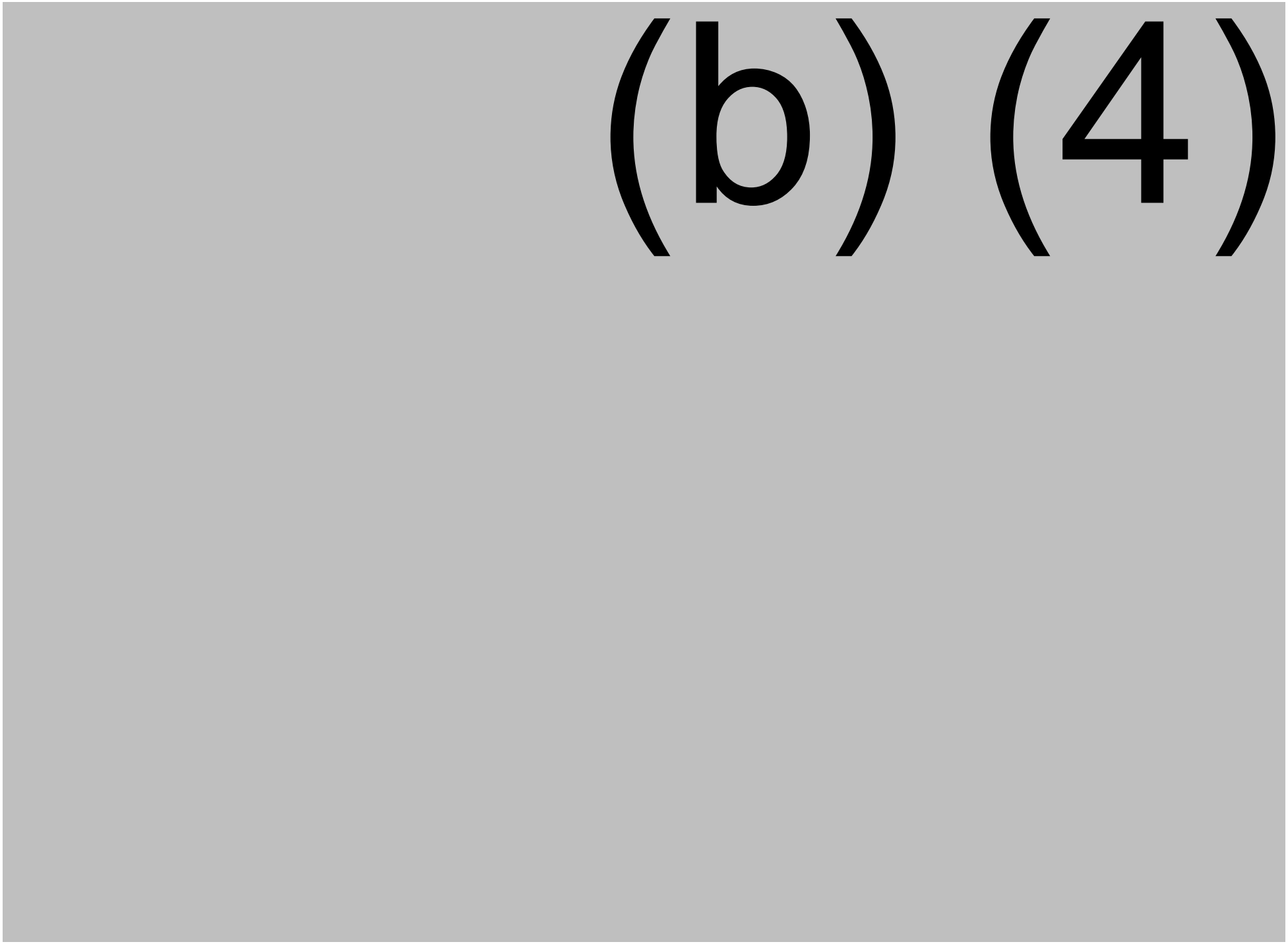

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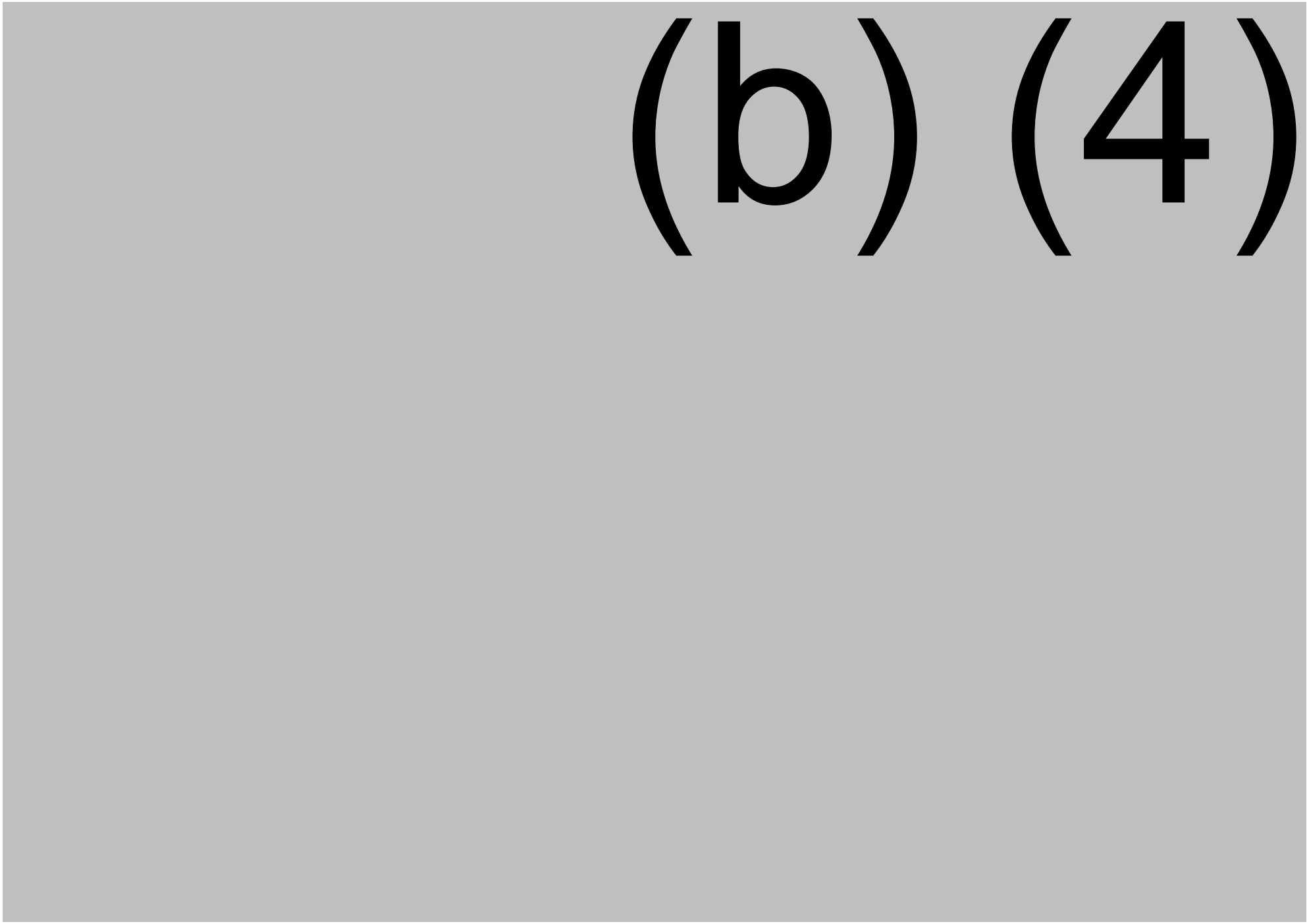

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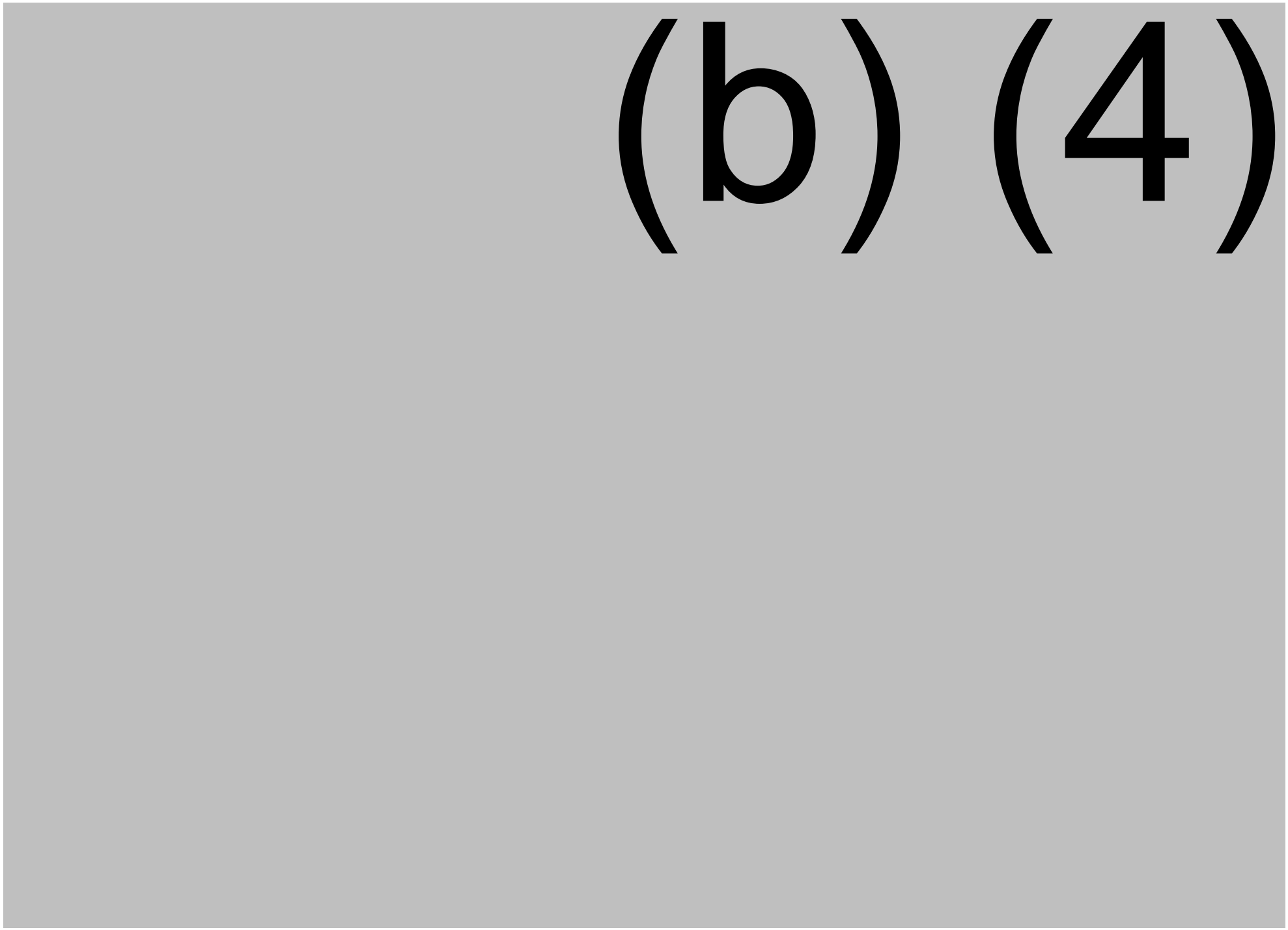

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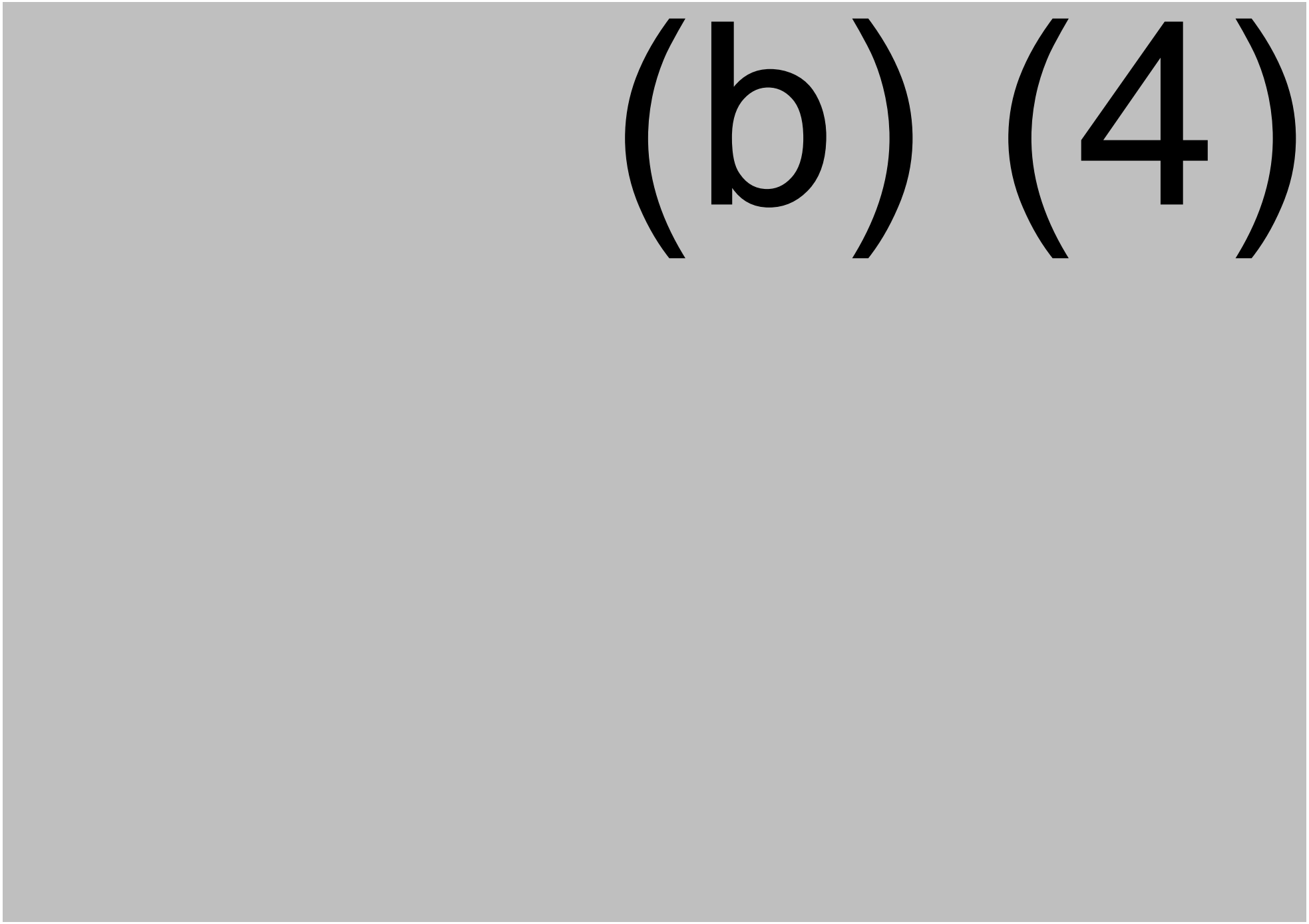

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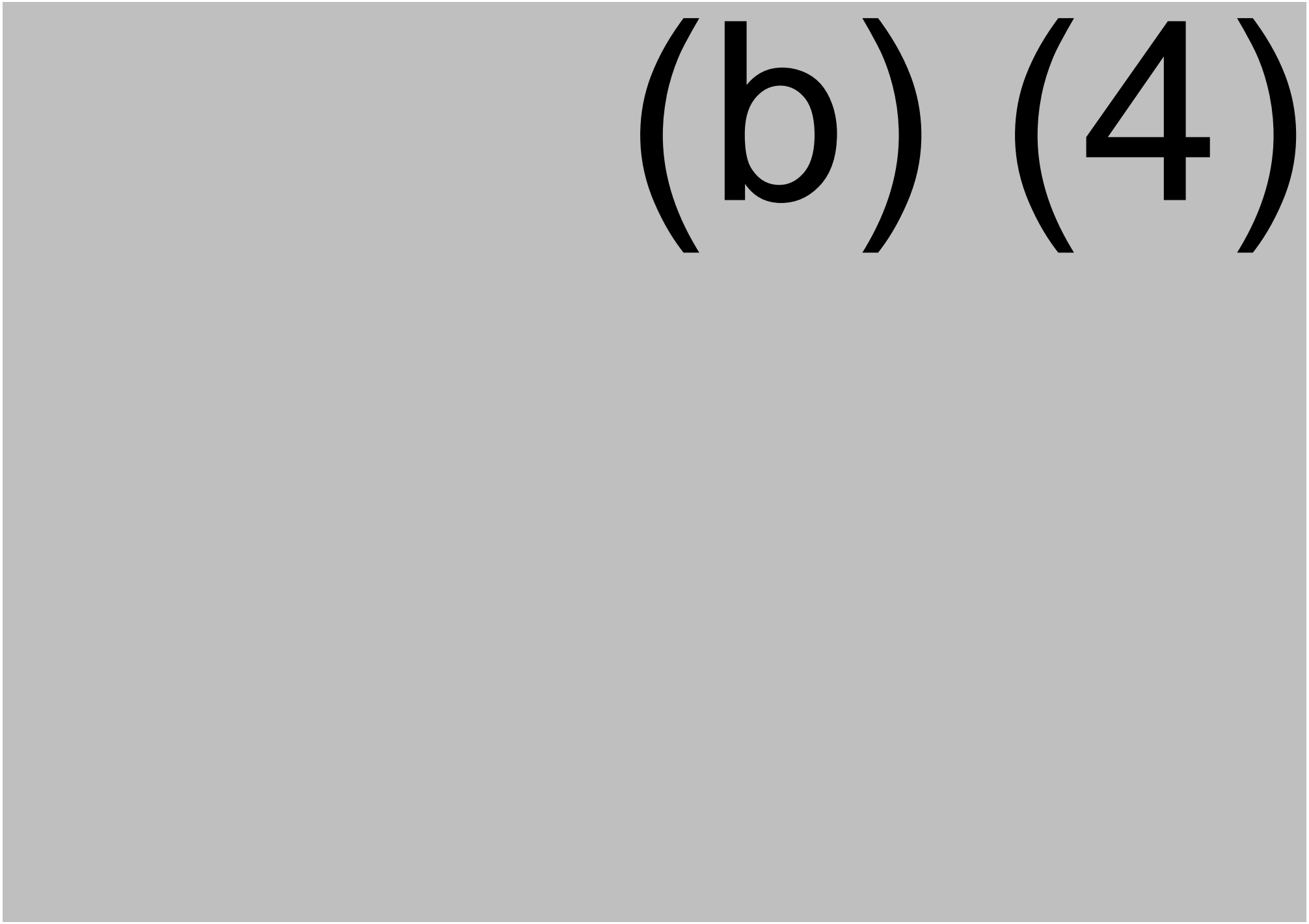

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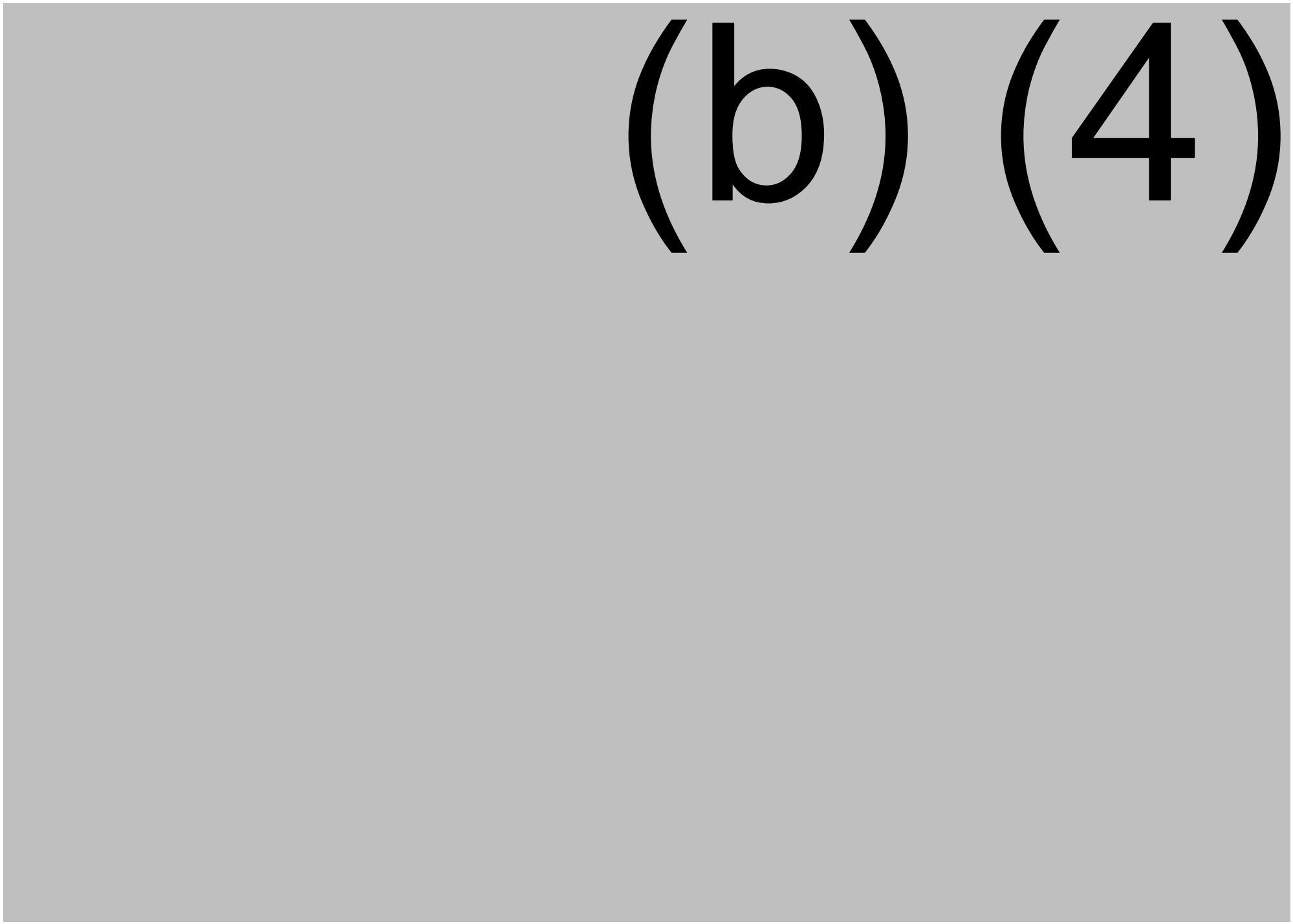

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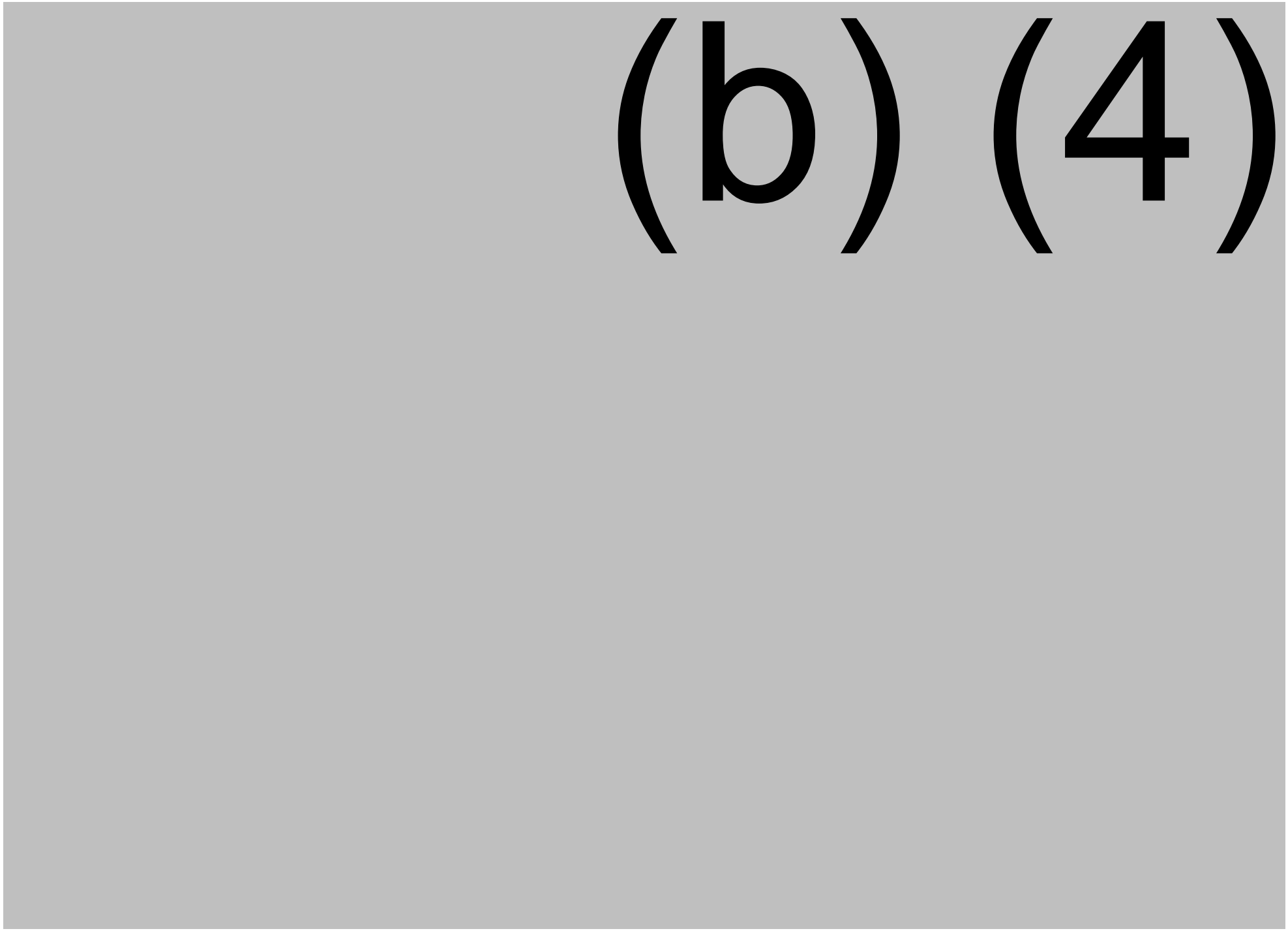

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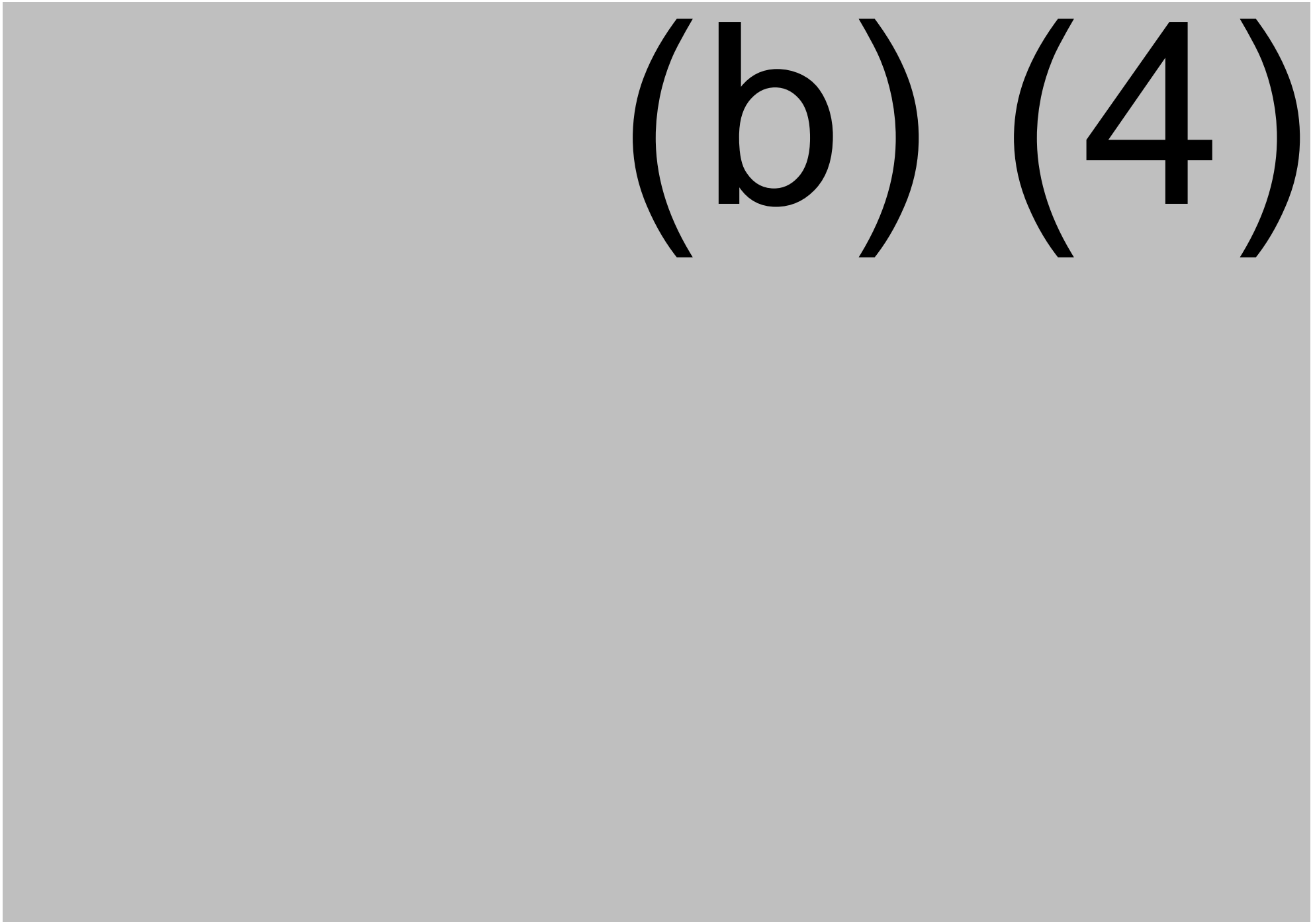

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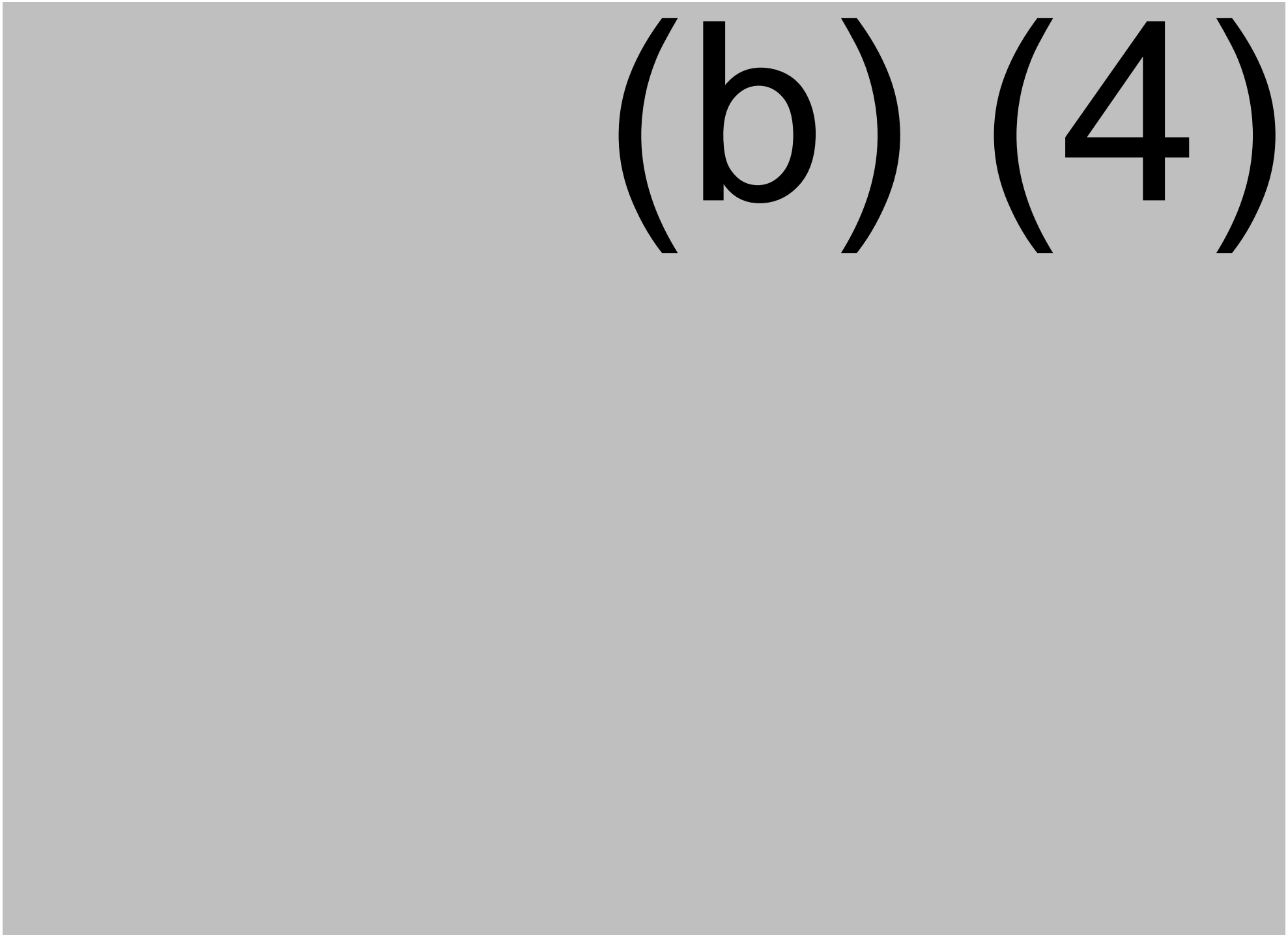

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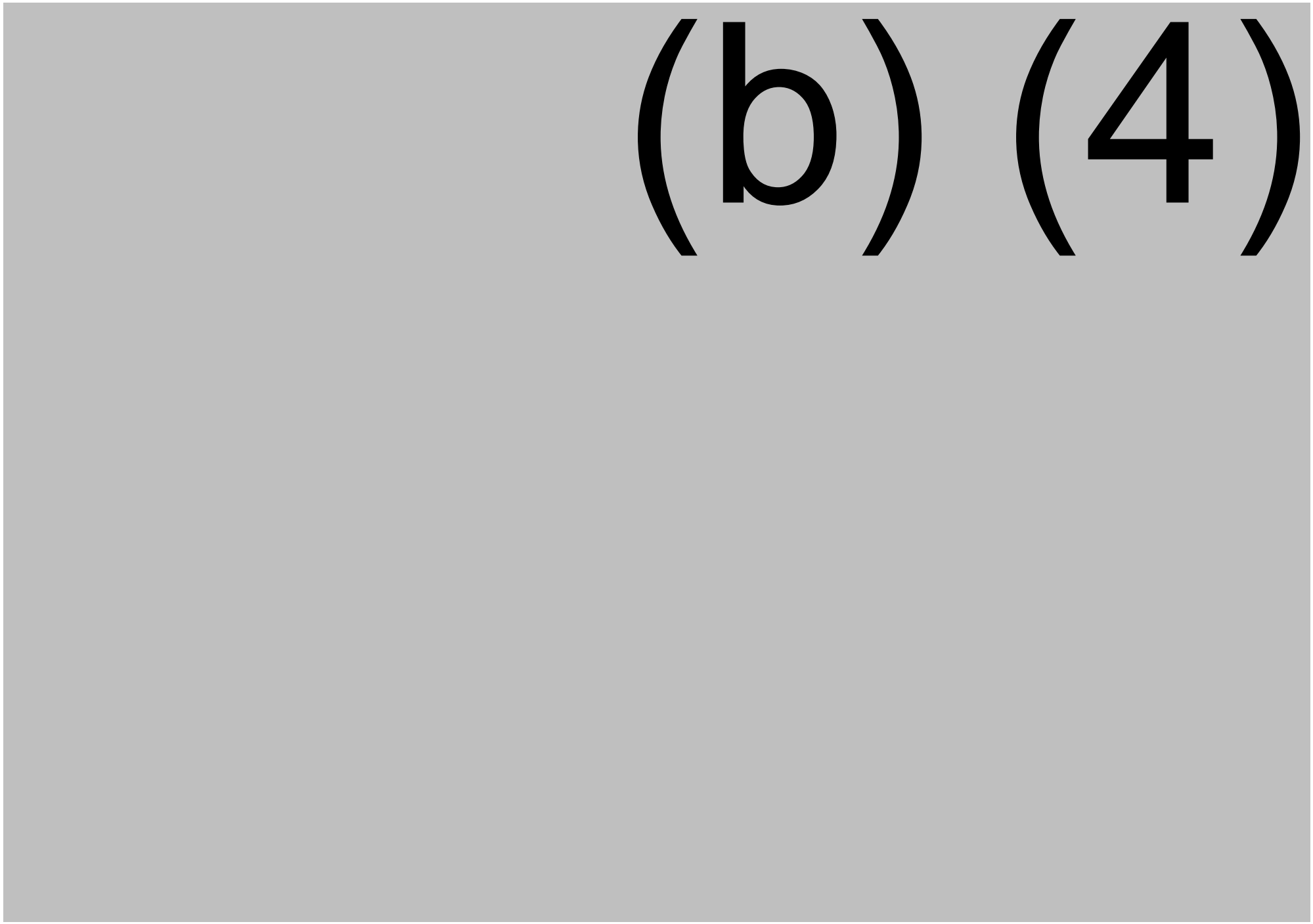

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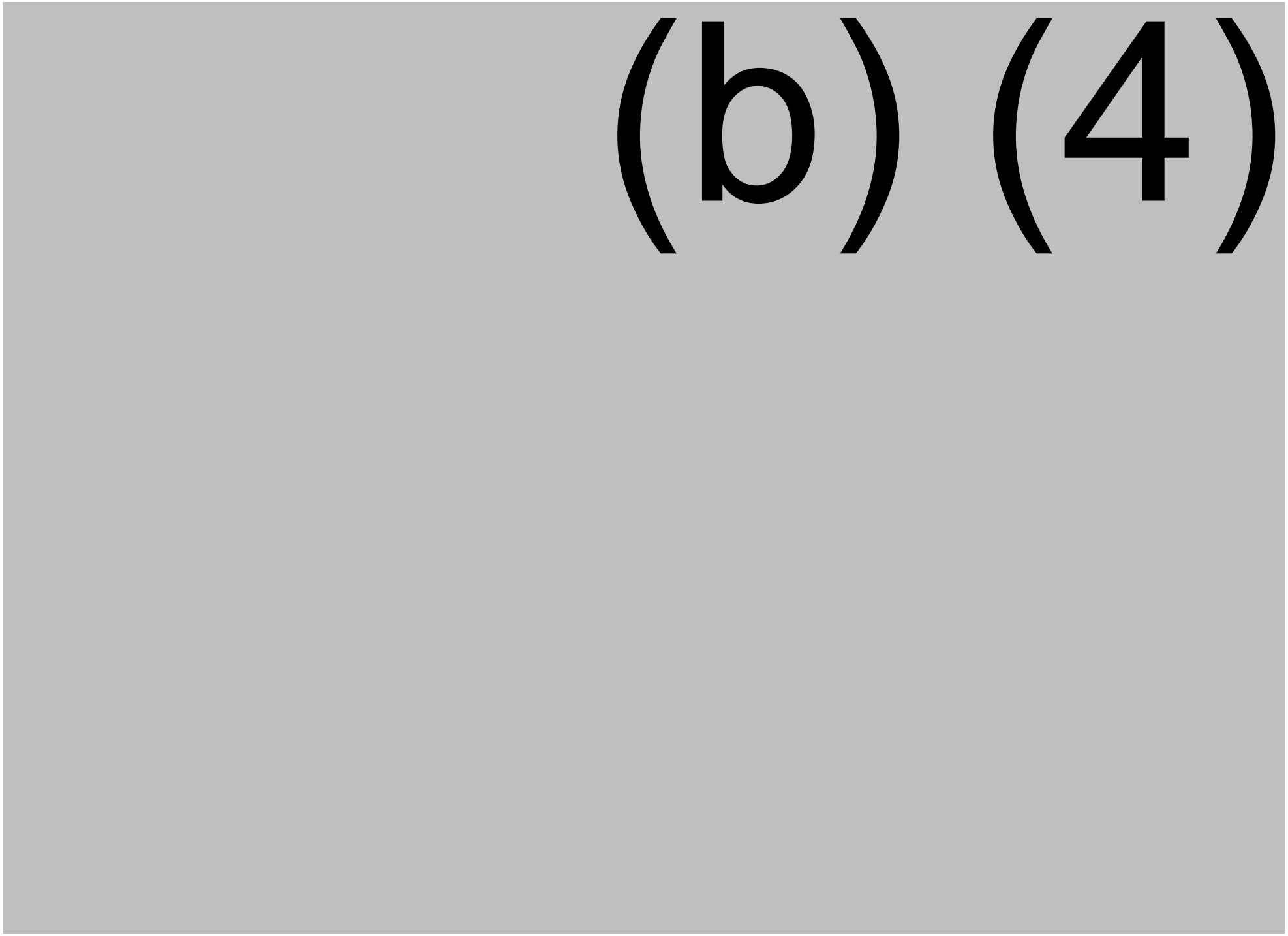

(b) (4)


(b) (4)


(b) (4)


(b) (4)


(b) (4)


(b) (4)

Appendix 08: Strain Development Report

BASF
We create chemistry

Development Report *T. thermophilus* C1

Host Strain (b) (4)

BASF
We create chemistry

Development Report *T. thermophilus* C1 Host Strain (b) (4)

Author:

KernA14 Digitally signed by KernA14
Date: 2024.02.14 06:27:11 -08'00'

Andreas Kern, Ph.D. **Date**
Sr. Regulatory Affairs Manager
Animal Nutrition

Technical Reviewer:

(b) (6)

Enzymes Process and Strain

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Plasmid Sequence 14

ORF Listings, minimum length 50 codons ((b) (4) start codons) 18

Annex 2 – (b) (4) Knock-out 30

Plasmid: (b) (4) circular 30

Plasmid Sequence 31

ORF Listings, minimum length 50 codons ((b) (4) start codons) 35

Homology Analyses of (b) (4)-based ORFs in the alp1 knockout locus post (b) (4) removal .. 37

Annex 3 – (b) (4) Knock-out 42

Plasmid (b) (4) 42

Plasmid Sequence 43

ORF Listings, minimum length 50 codons ((b) (4) start codons) 46

Mutagenic Development of C1 Strains

The filamentous fungus *Thermothelomyces thermophilus* (*T. thermophilus)* is able to secrete large quantities of enzymes and may serve as an alternative to similar well-known production hosts, including *Aspergillus niger* or *Trichoderma reesei* (Singh, B., 2016). A natural strain of *T. thermophilus,* named C1, was isolated from (b) (6) and has been deposited at the All-Russian Collection of Microorganisms (VKM, accession number VKM F-3500-D). Initially classified as *Chrysosporium lucknowense*, it was later identified as a strain of *Myceliophthora thermophila*, the former name of *T. thermophilus*. C1 has been developed into an expression platform for industrial-scale quantities of endogenous and recombinant enzymes and other proteins (Visser, H. *et al.*, 2011).

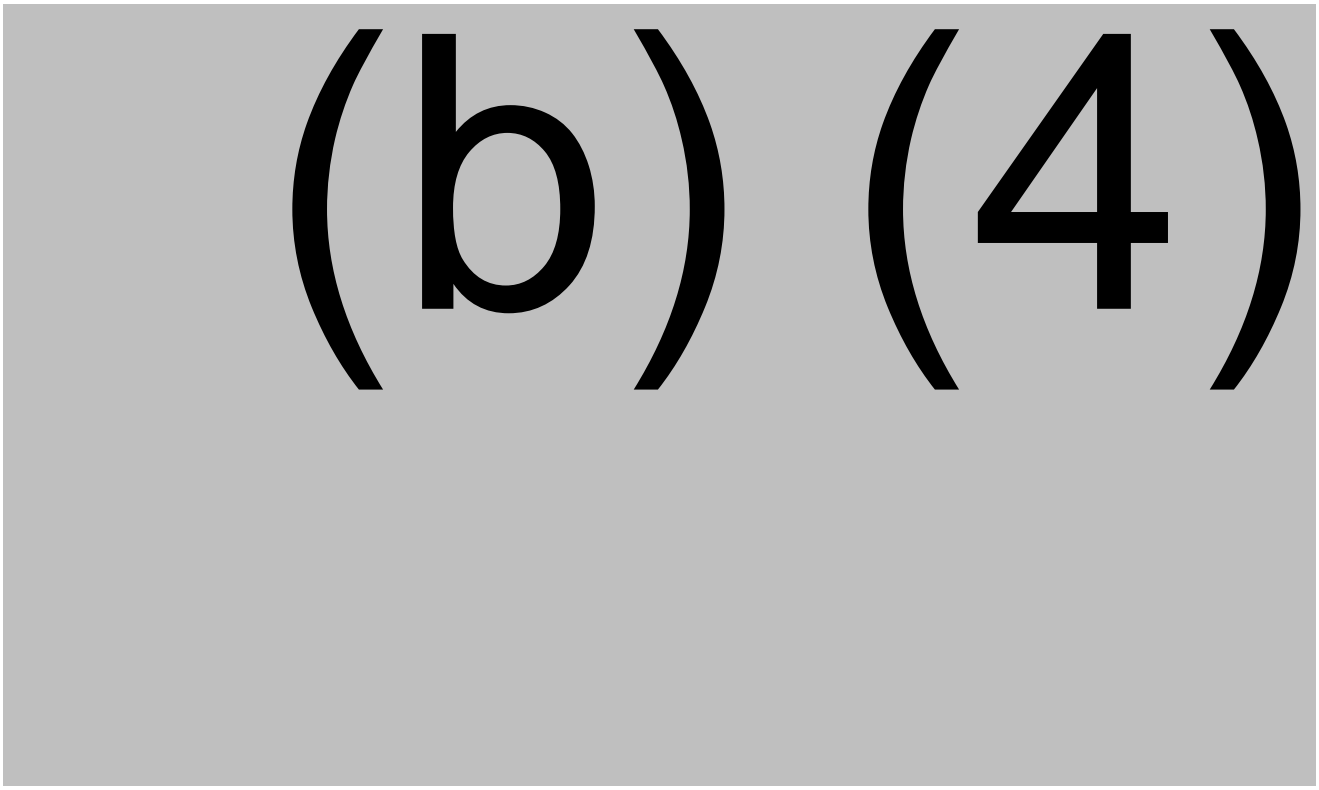
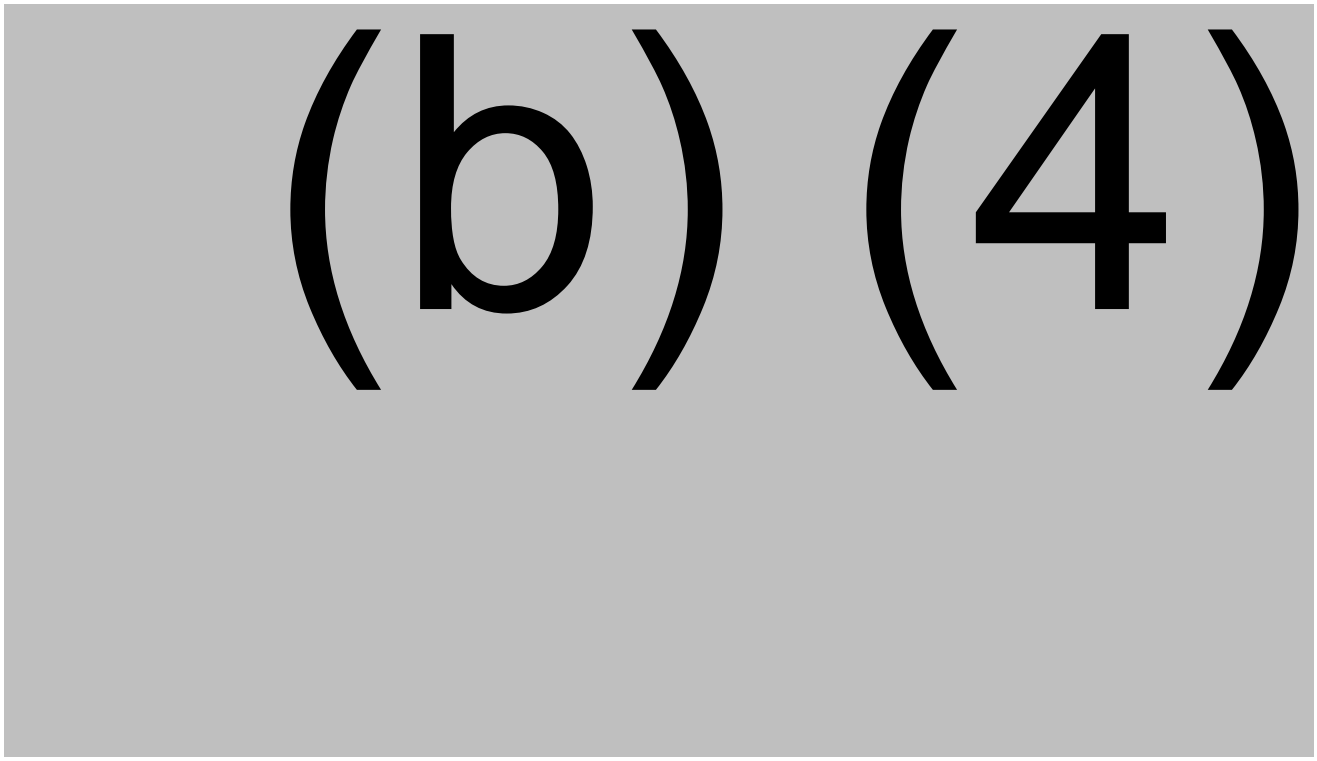


Table 1: Dyadic mutagenic strain optimization of wild-type isolate *T. thermophilus* C1



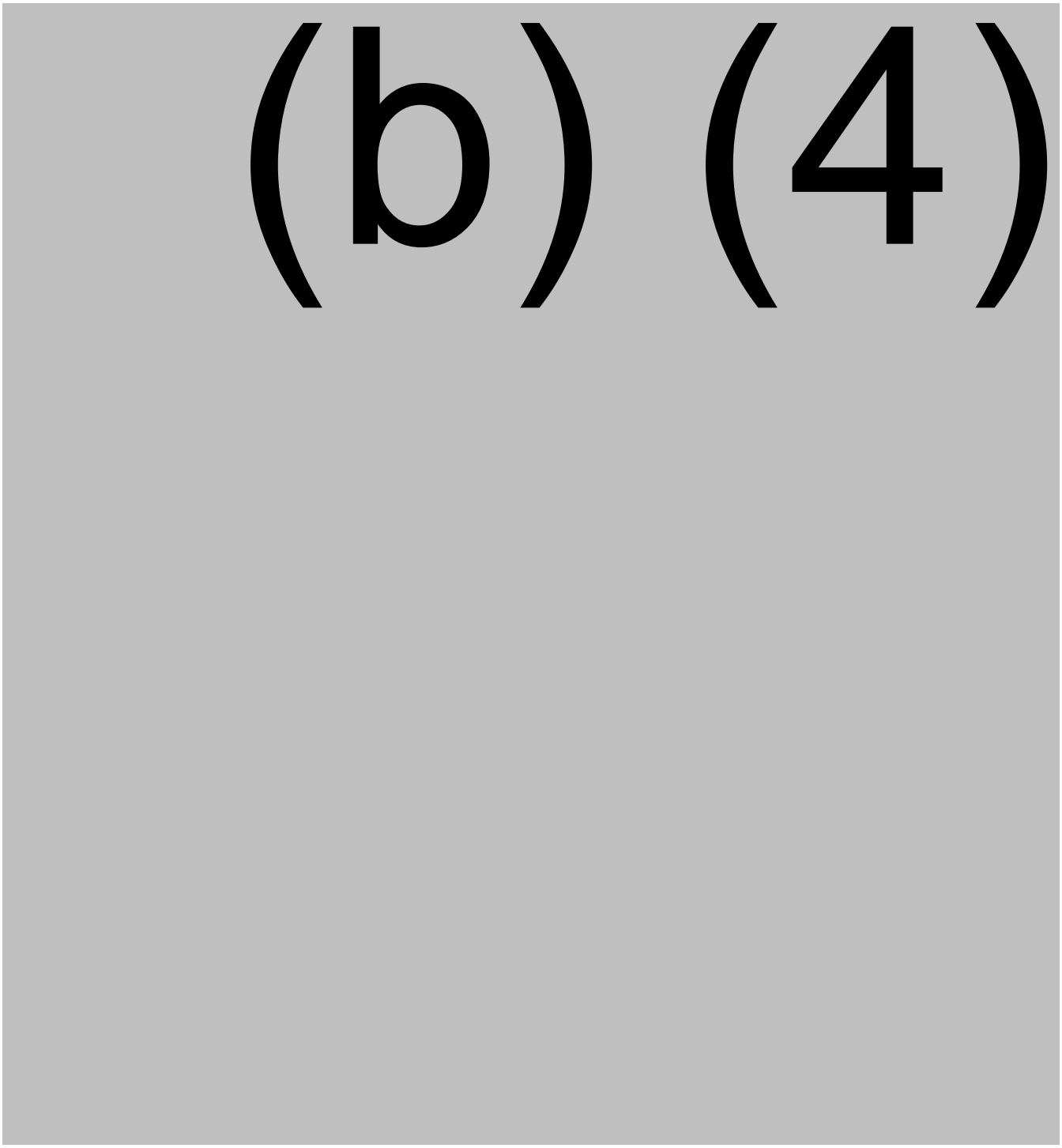

(b) (4)

Figure 1: Schematic representation of homologous recombination for deletion or

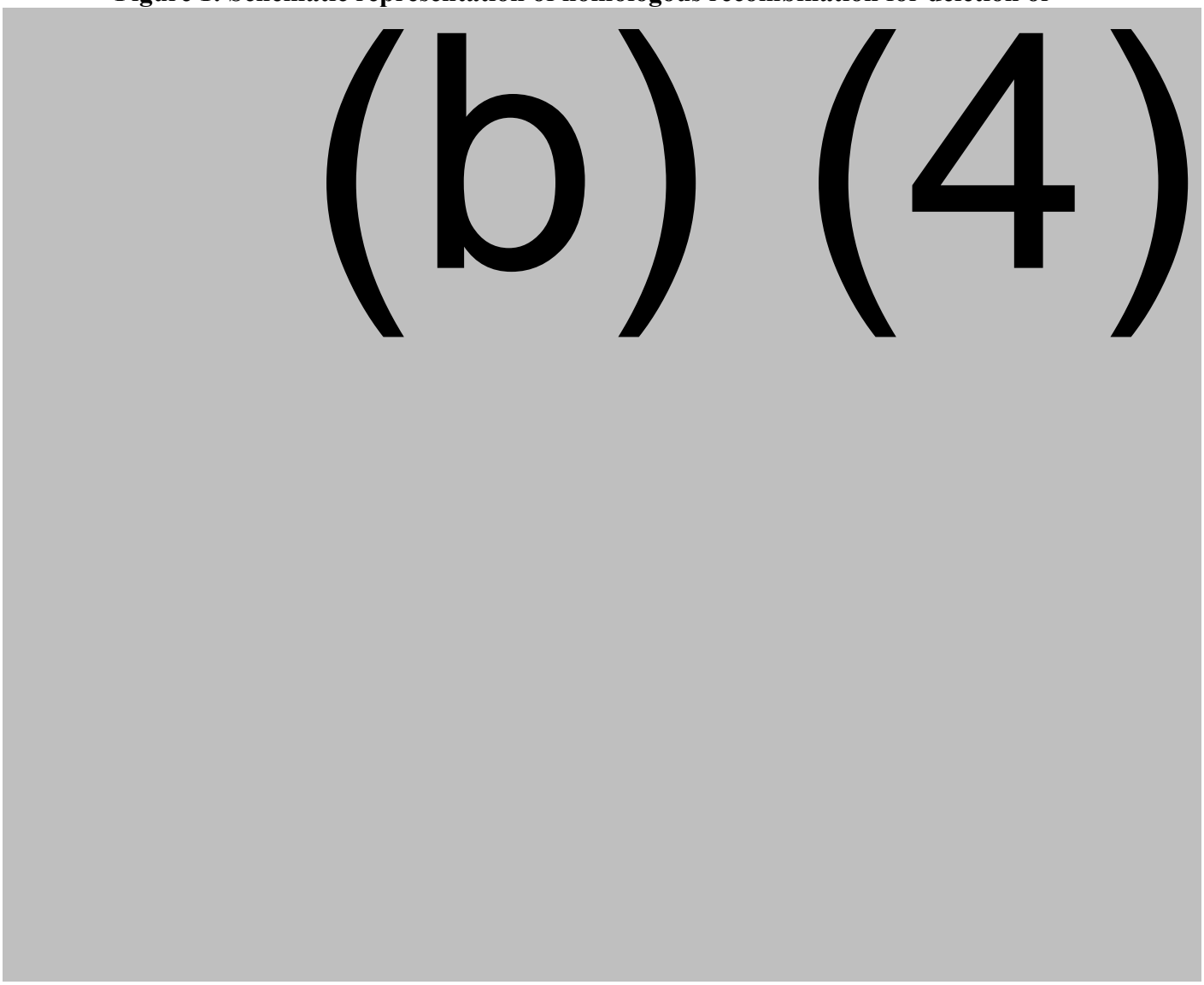
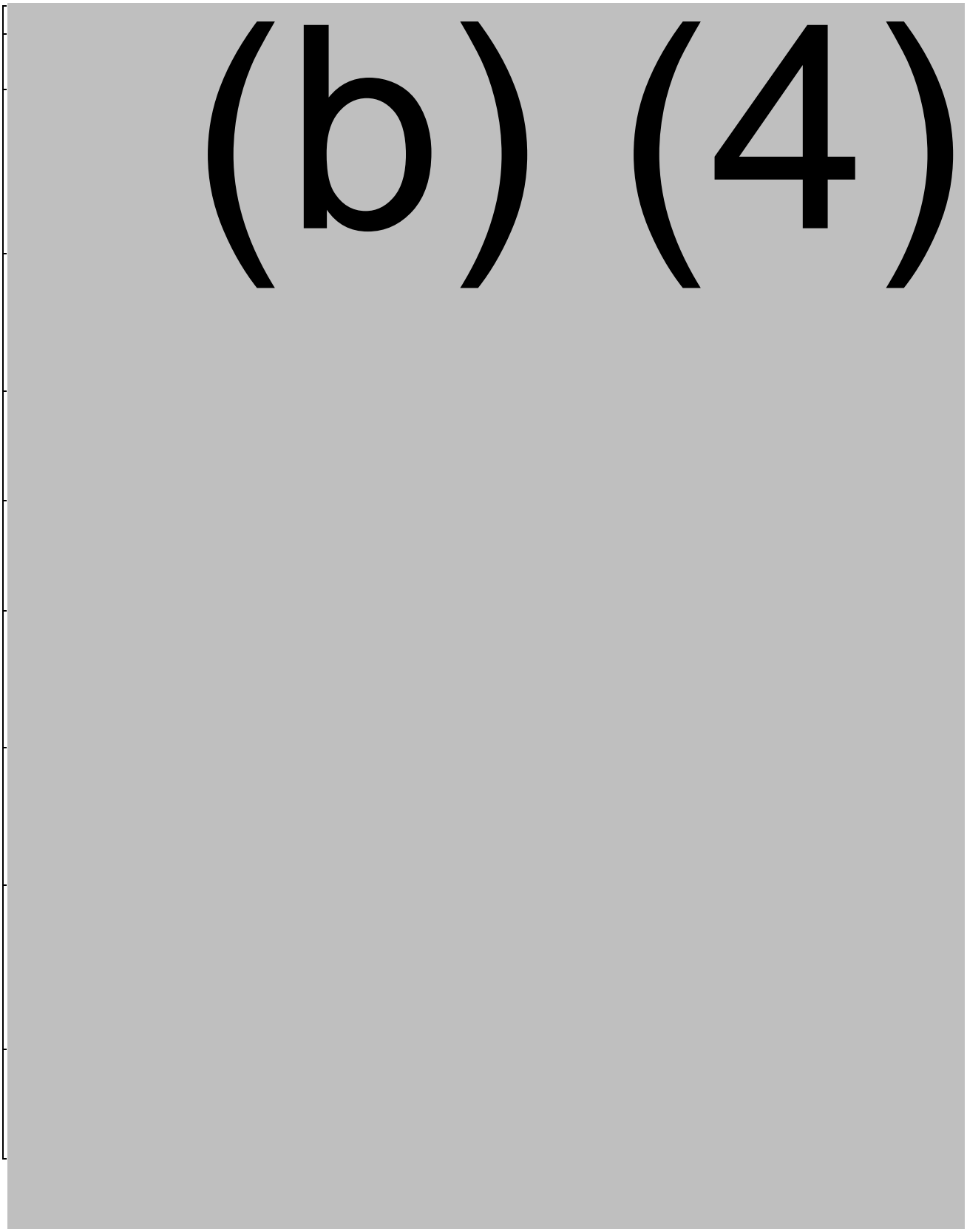
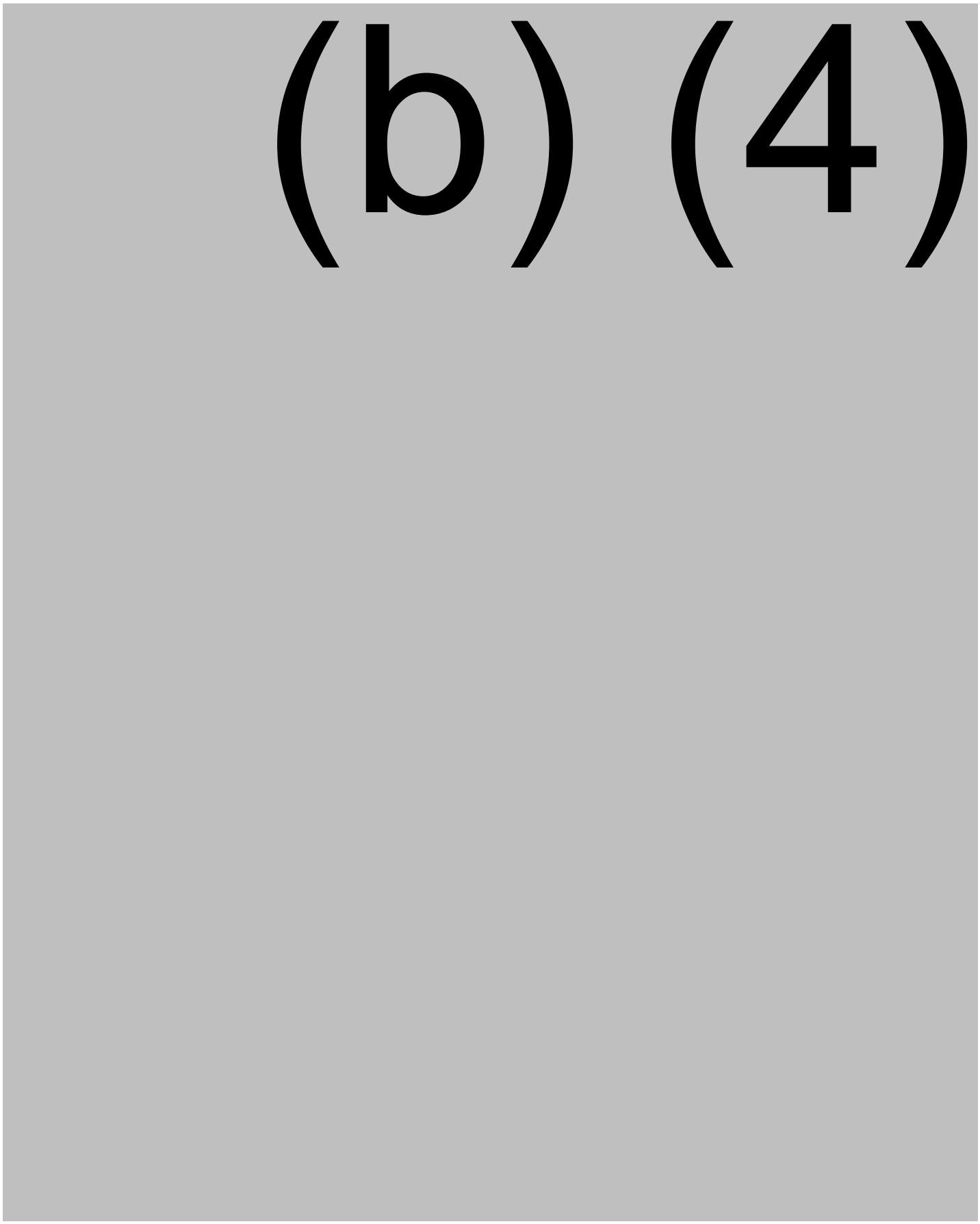
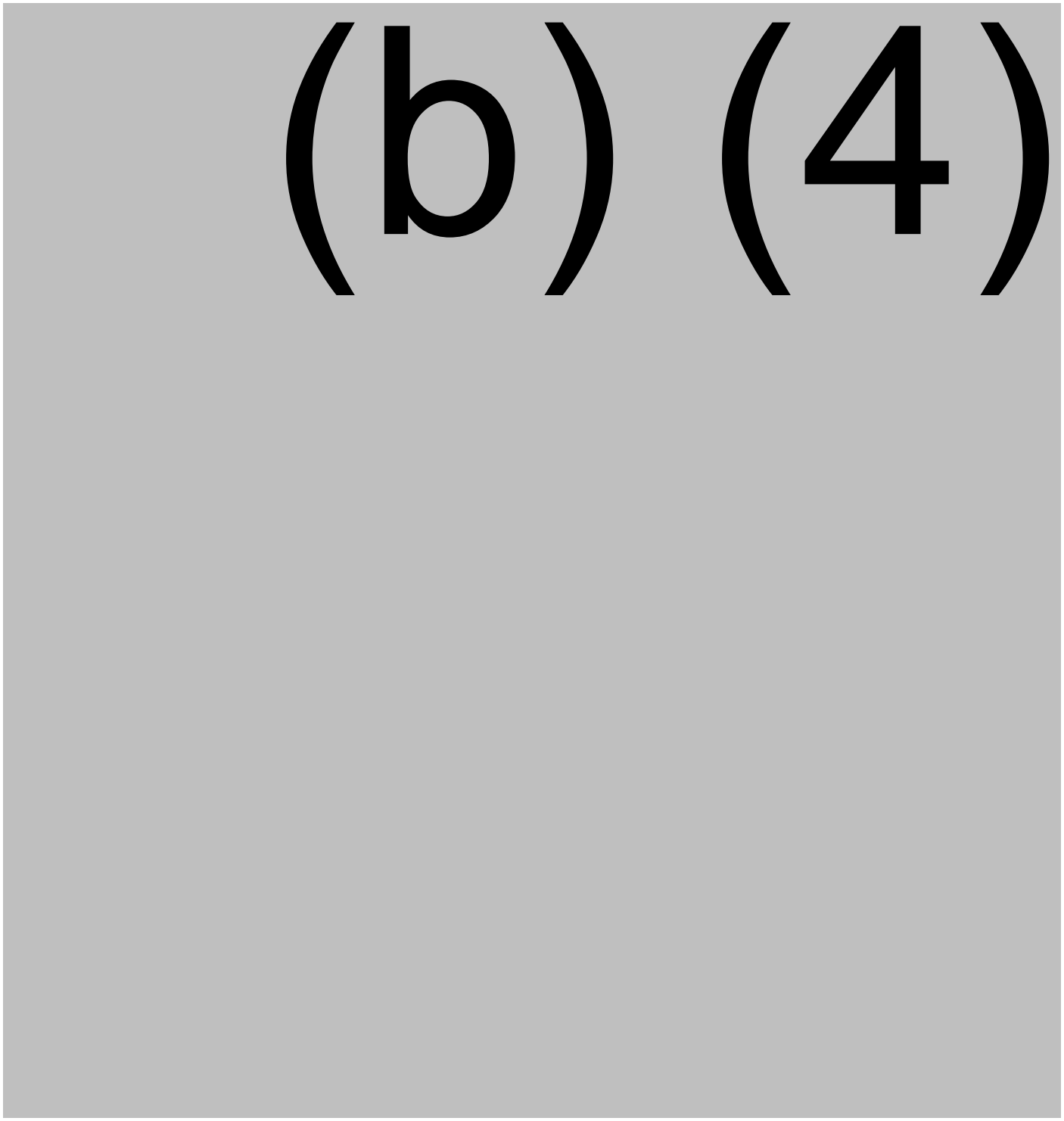


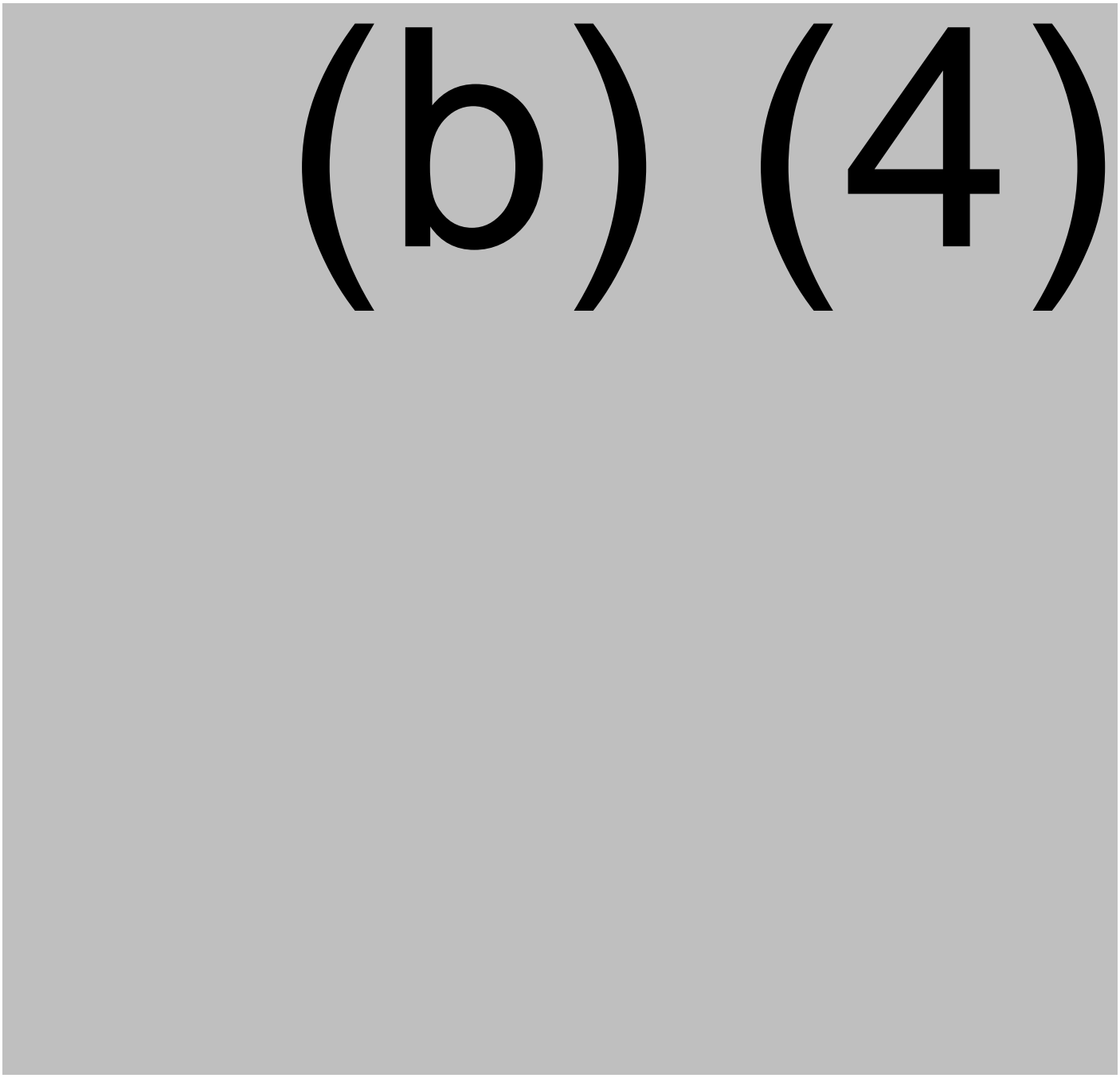
Table 2: Recombinant DNA approaches during development of *T. thermophilus* LC strain

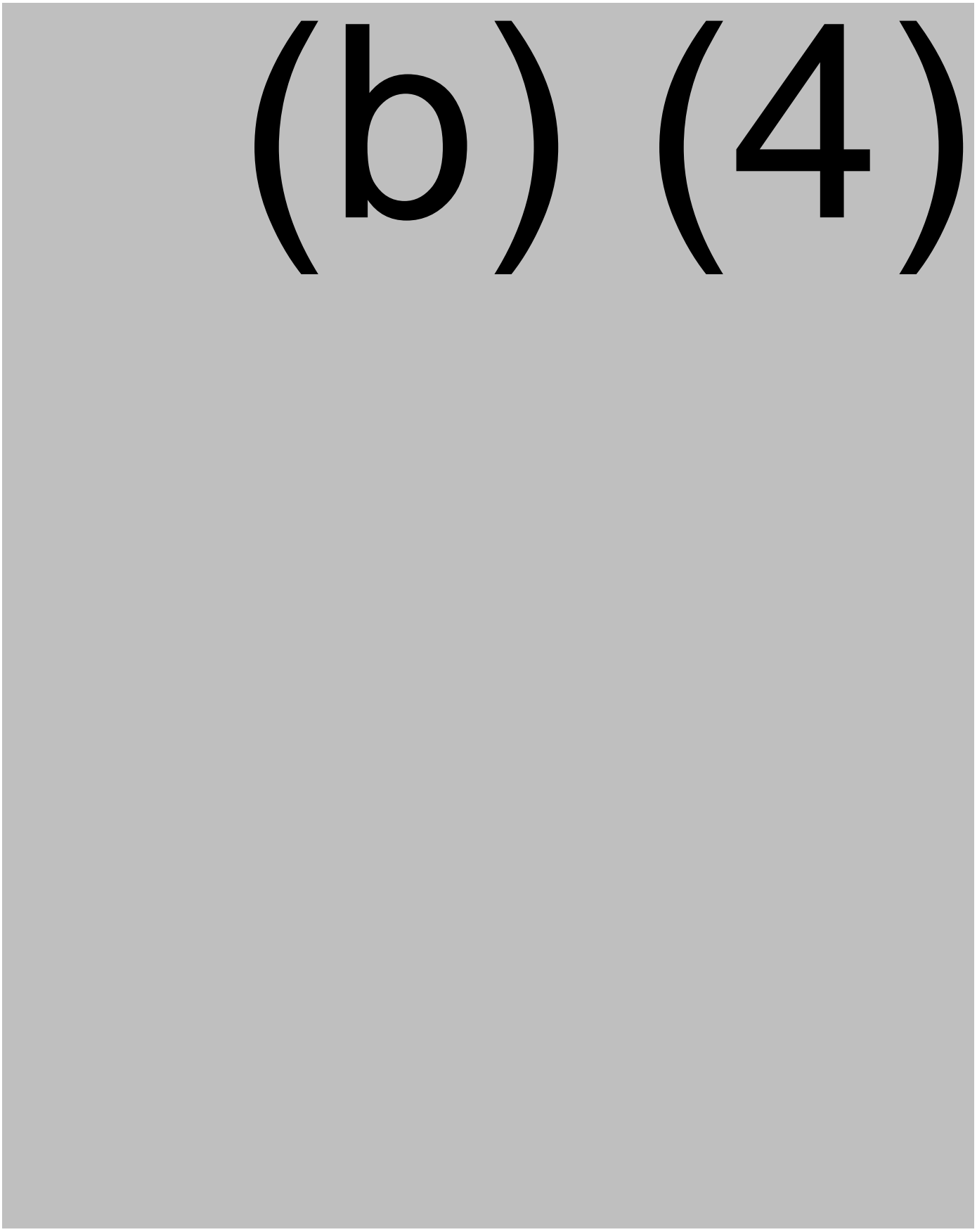
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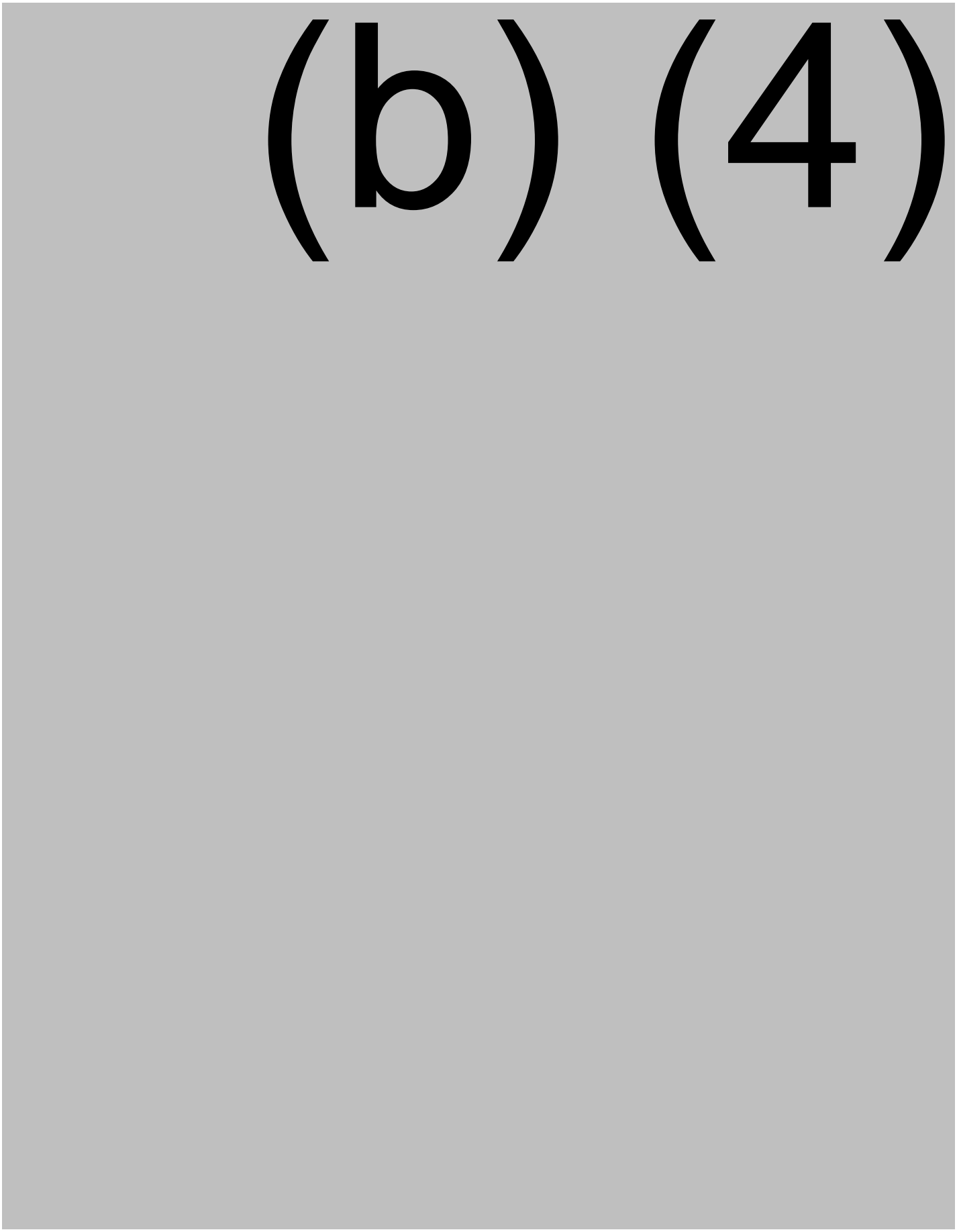


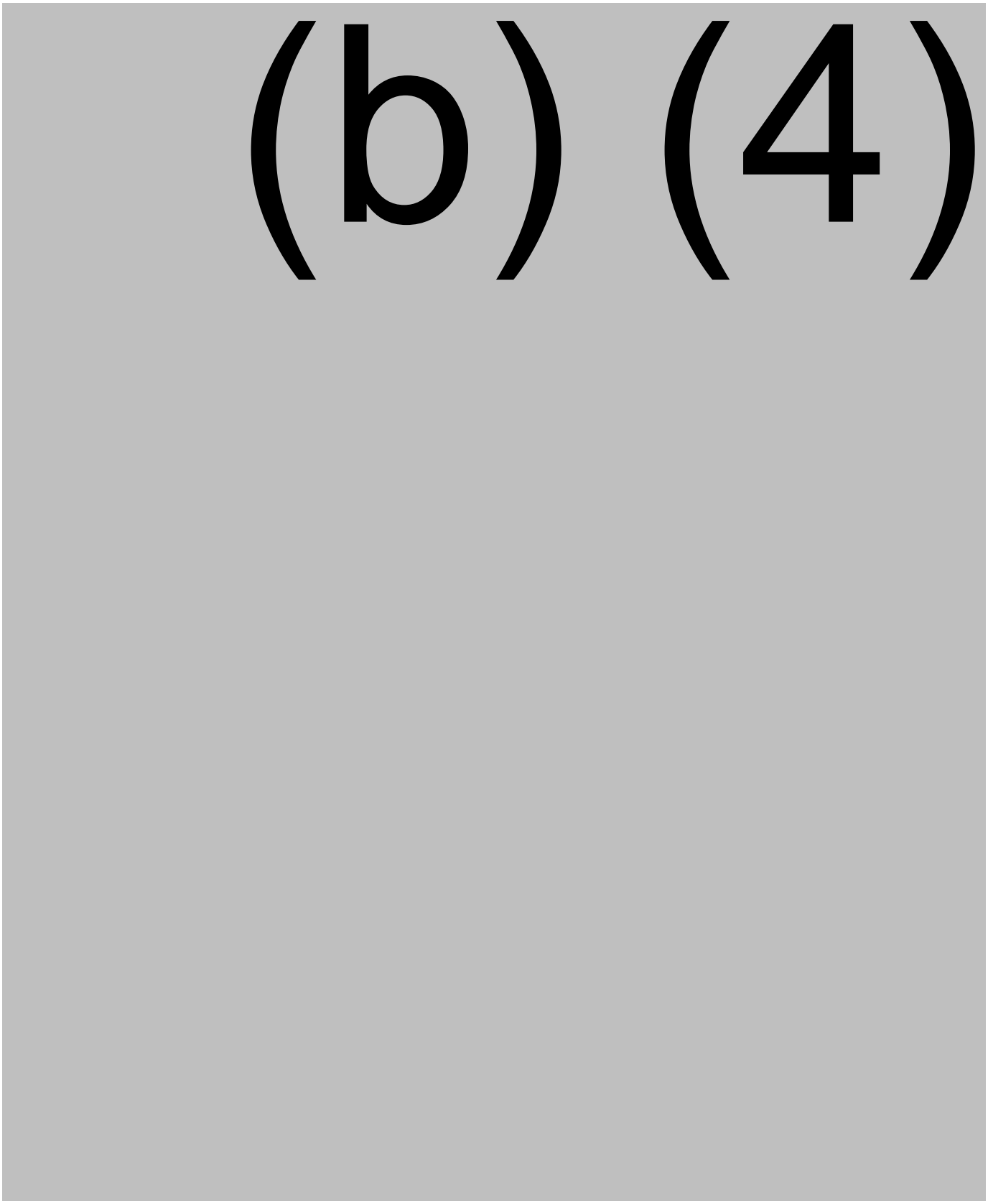

(b) (4)


(b) (4)


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(b) (4)

References:

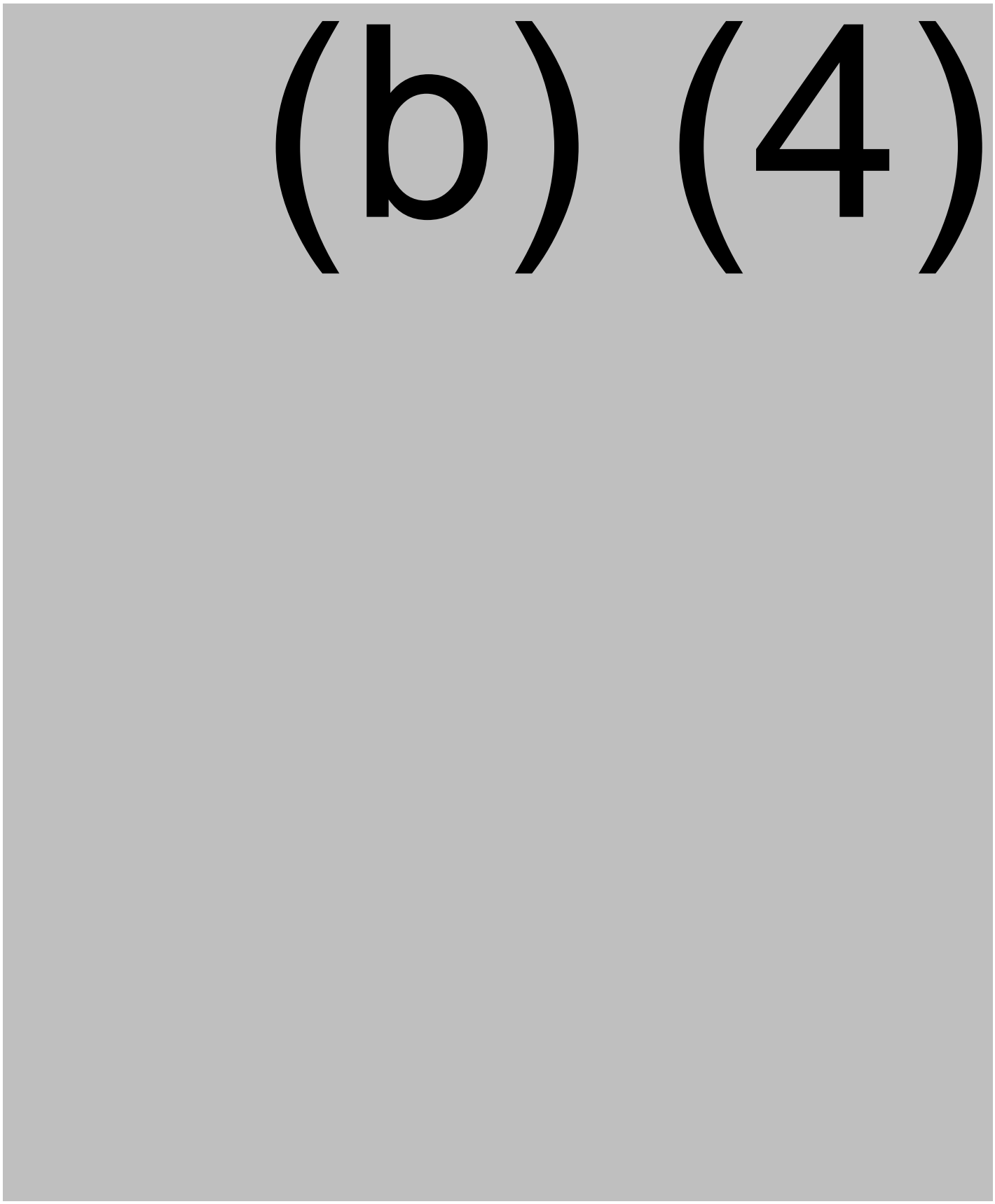
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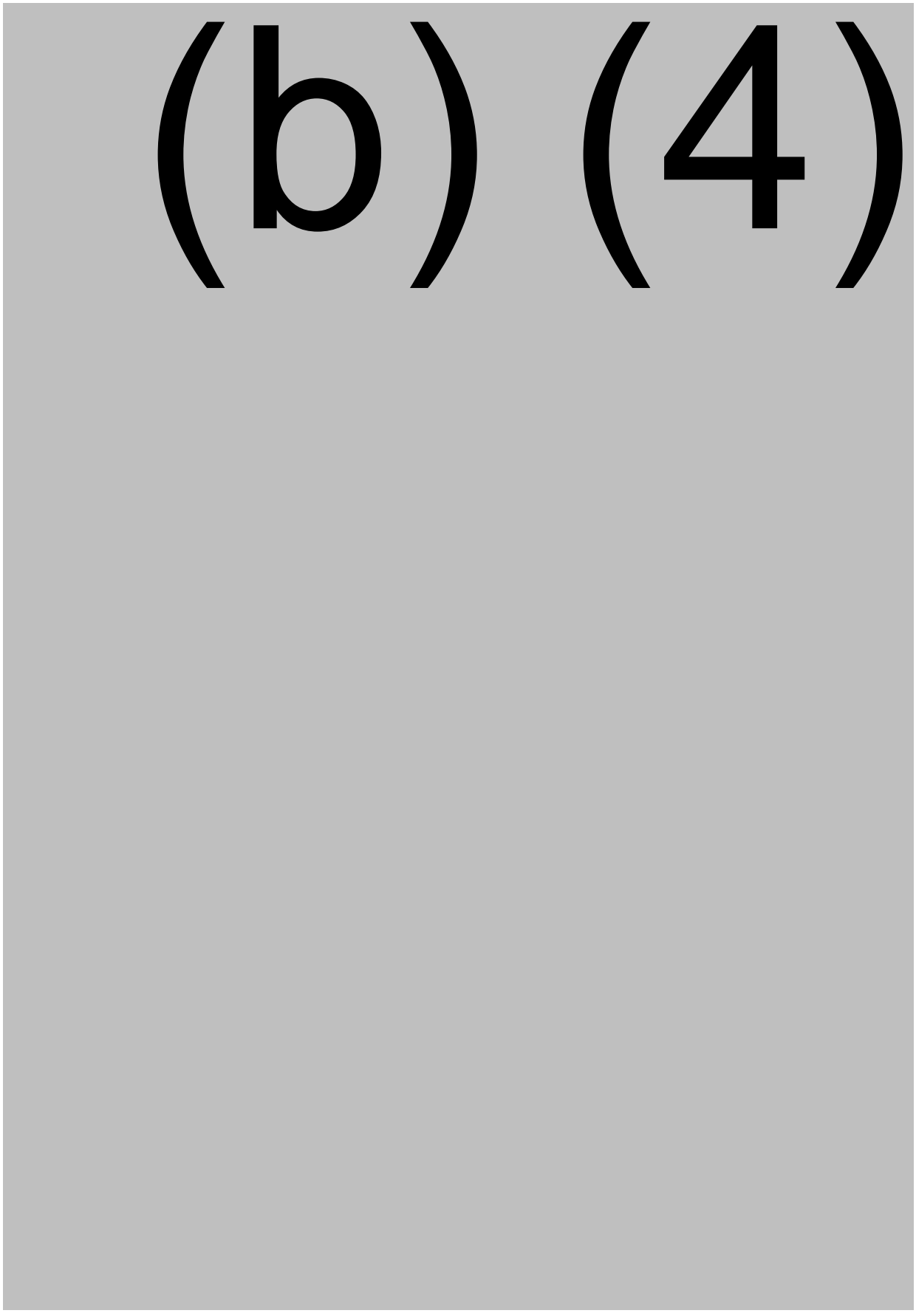
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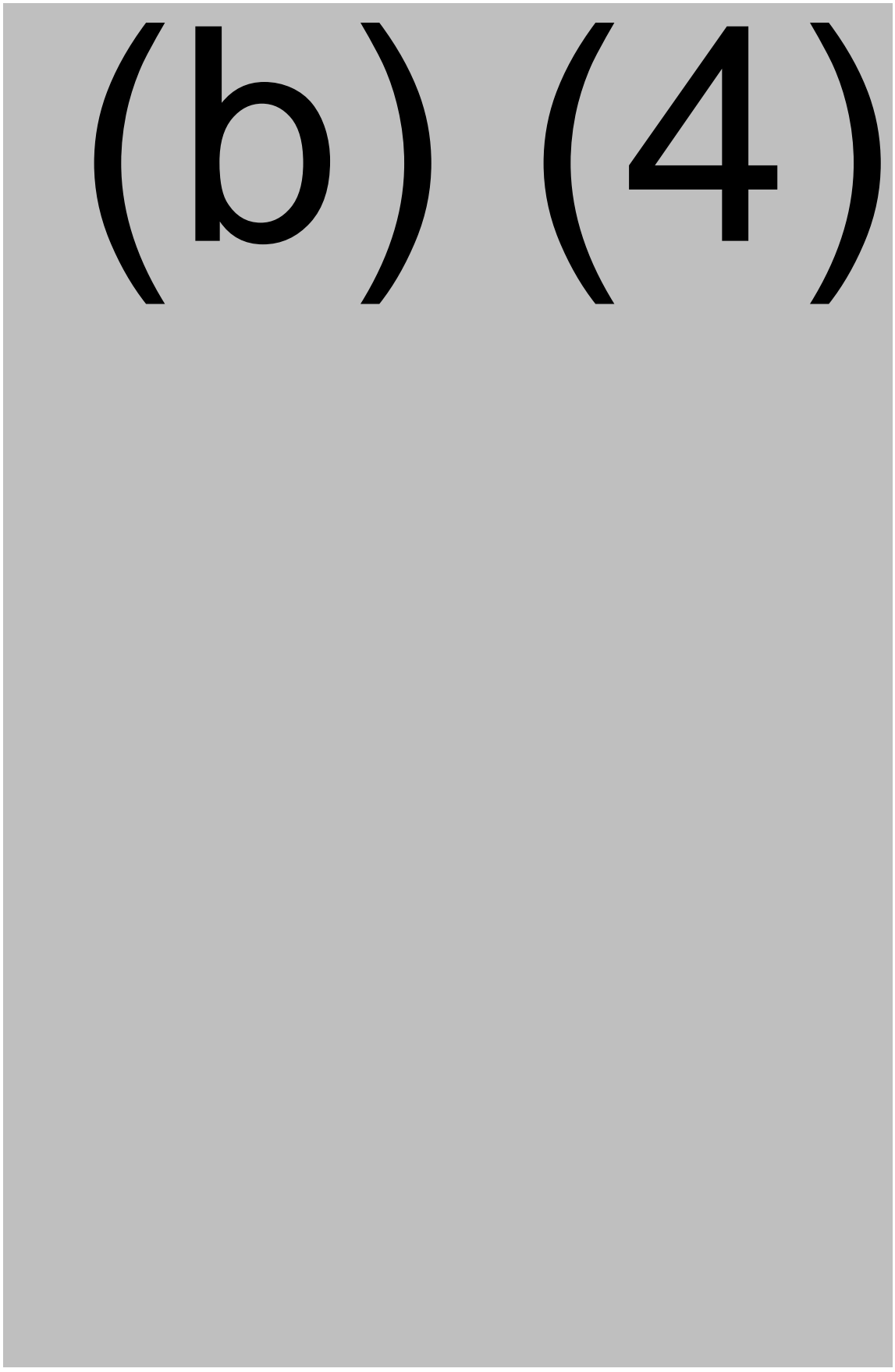
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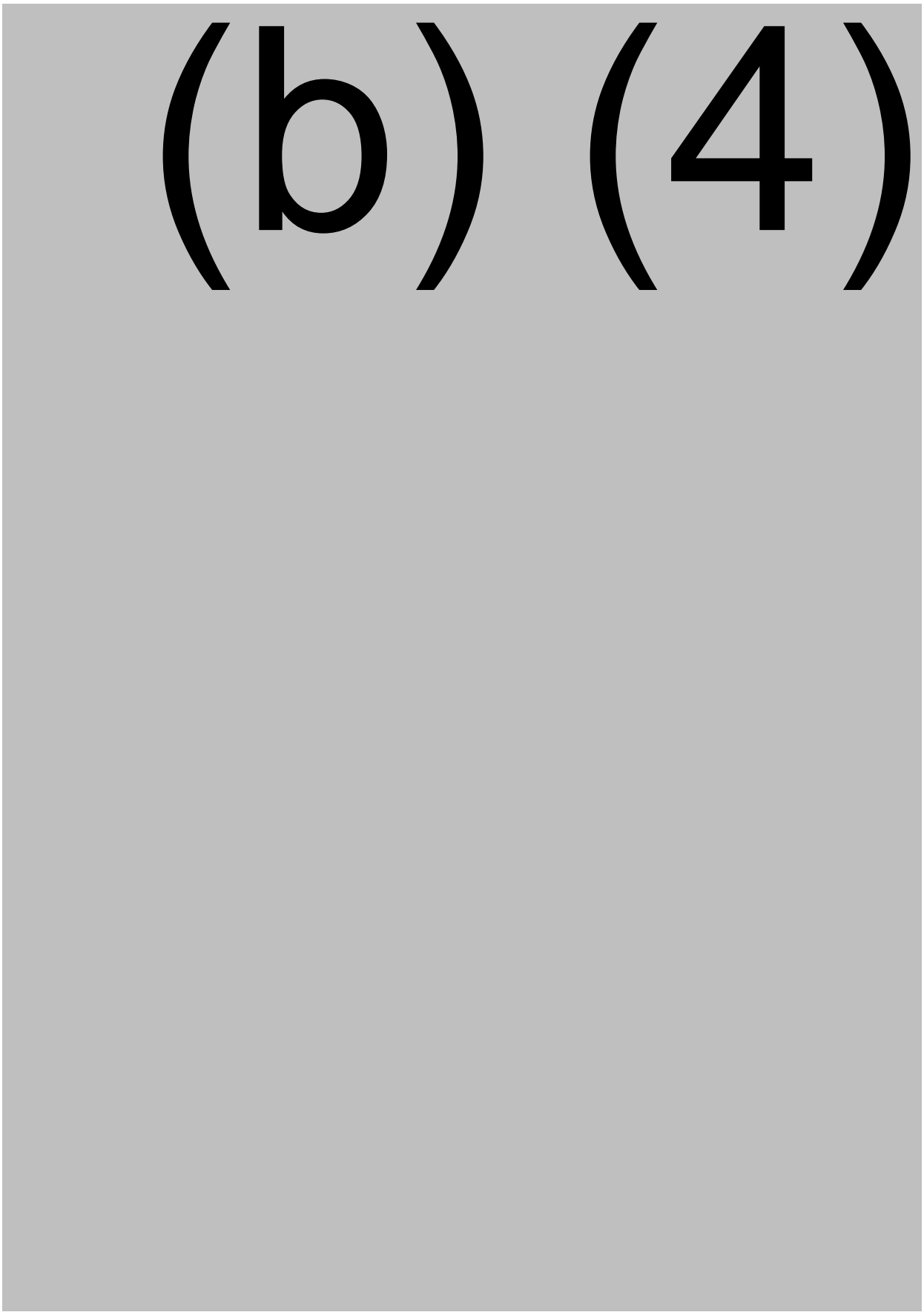
Singh, B. *Myceliophthora thermophila syn. Sporotrichum thermophile: a thermophilic mould of biotechnological potential*. Critical Reviews in Biotechnology 36 (1), pp. 59-69 (2016).

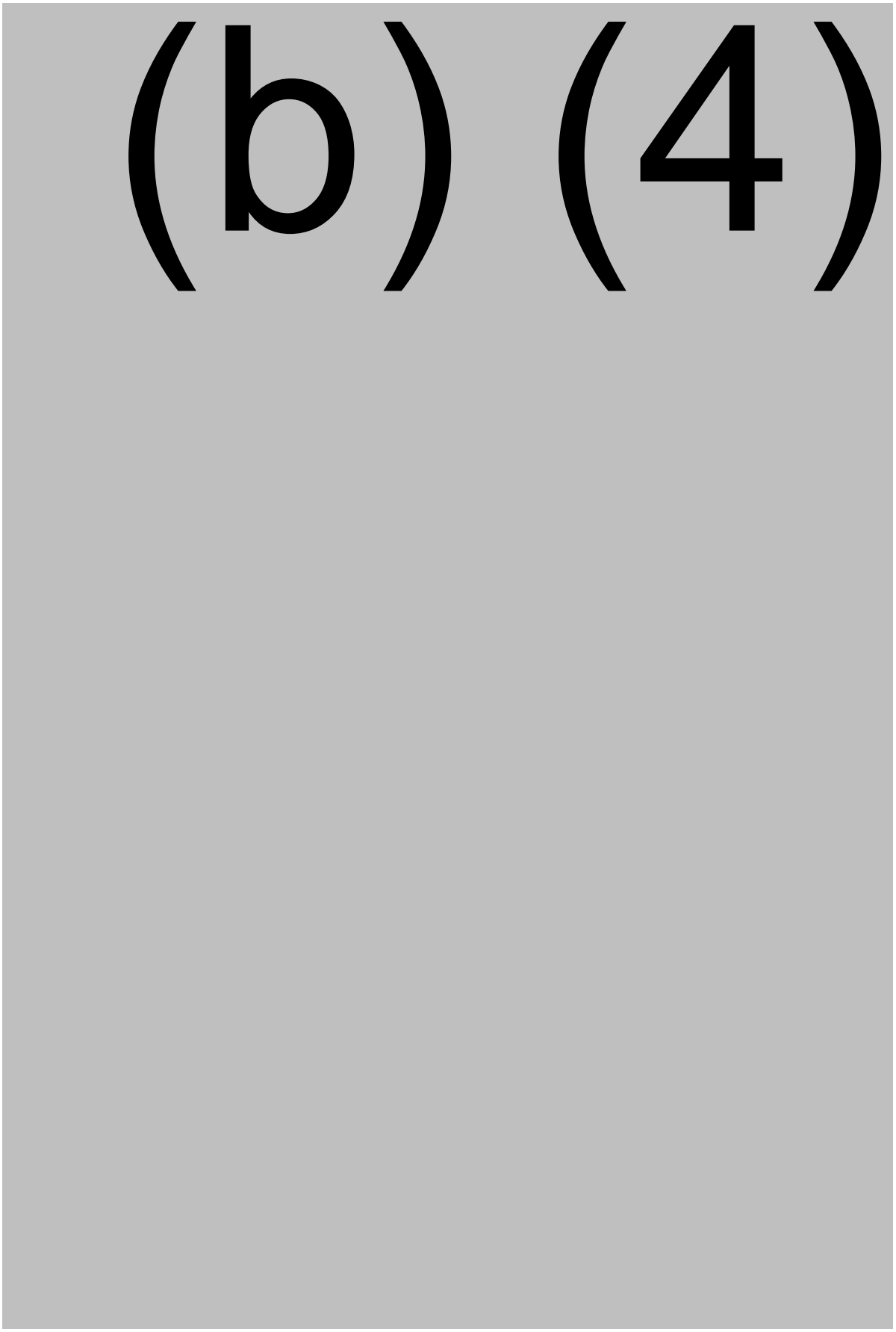
Visser, H., Joosten, V., Punt, P.J., Gusakov, A.V. et al. *Development of a mature fungal technology and production platform for industrial enzymes based on a Myceliophthora thermophila isolate, previously known as Chrysosporium lucknowense C1*. Industrial Biotechnology 7 (3), pp. 214-223 (2011).

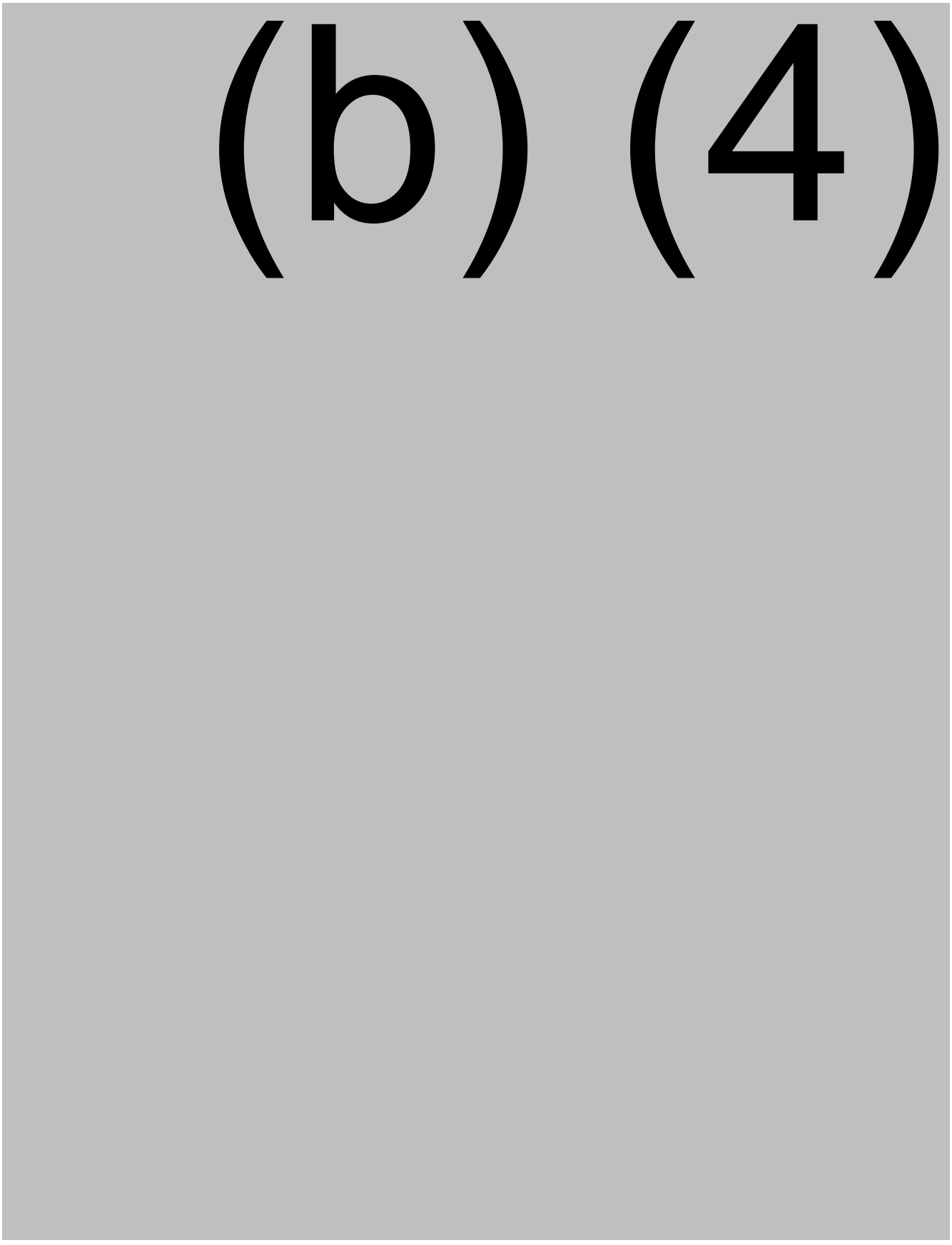

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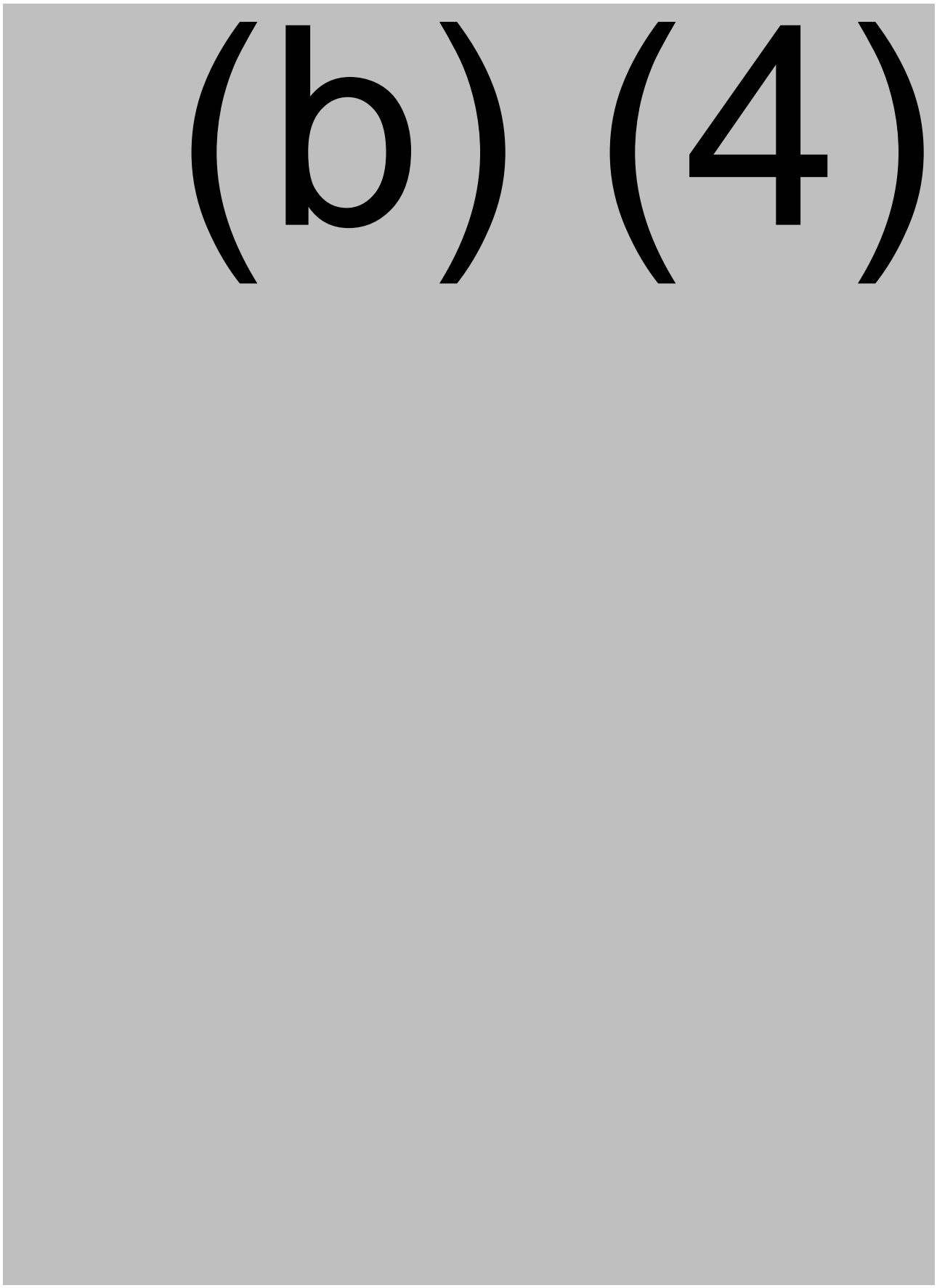

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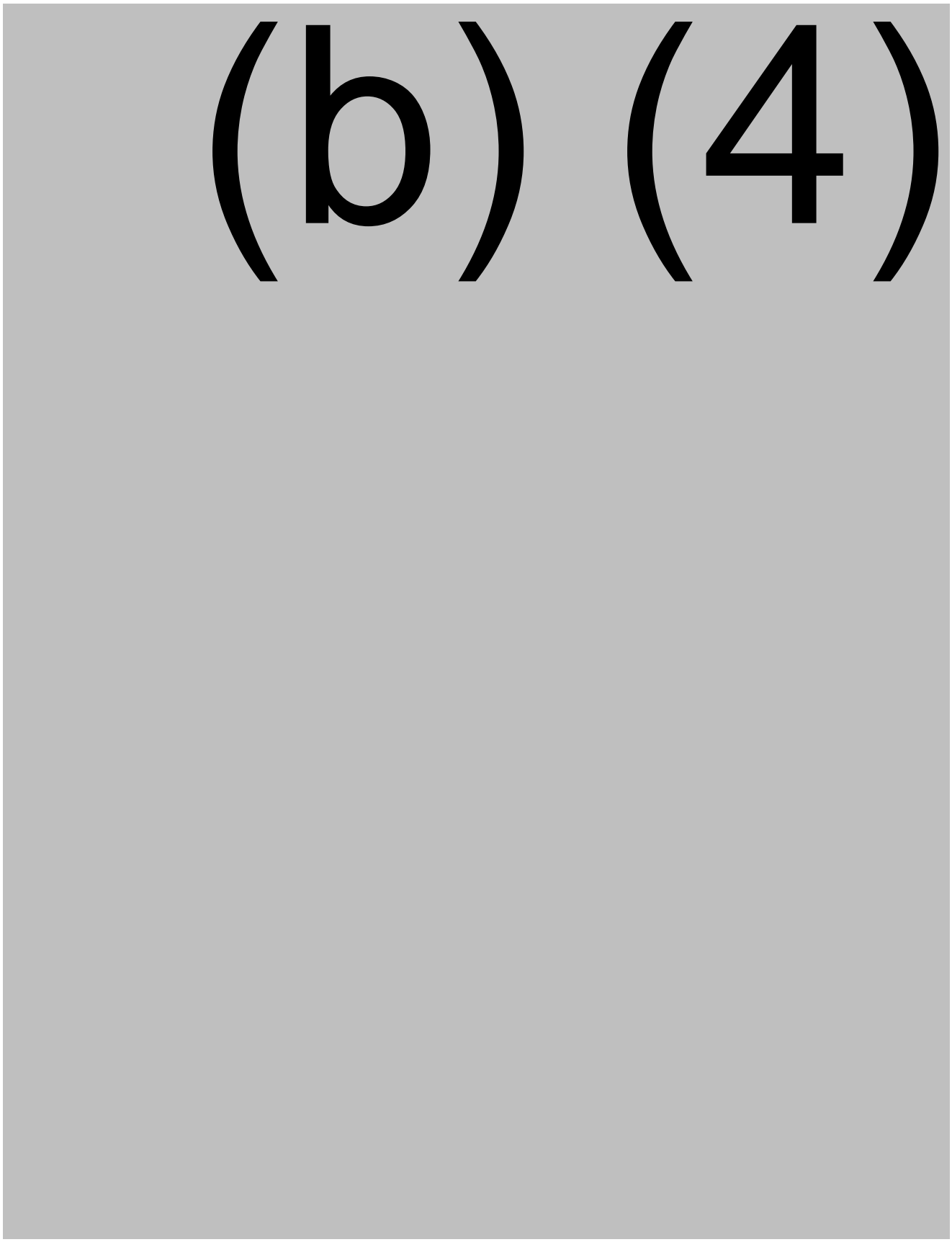

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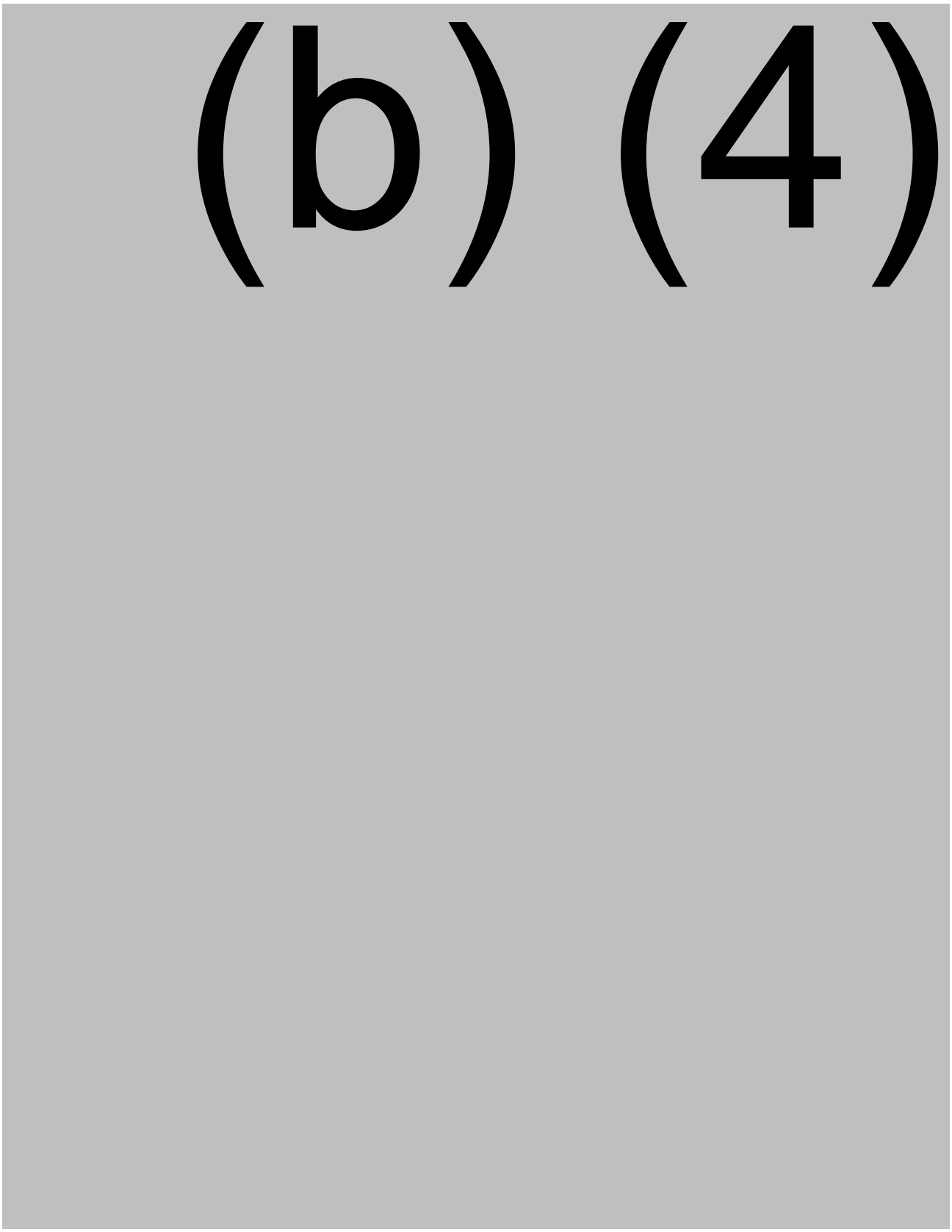

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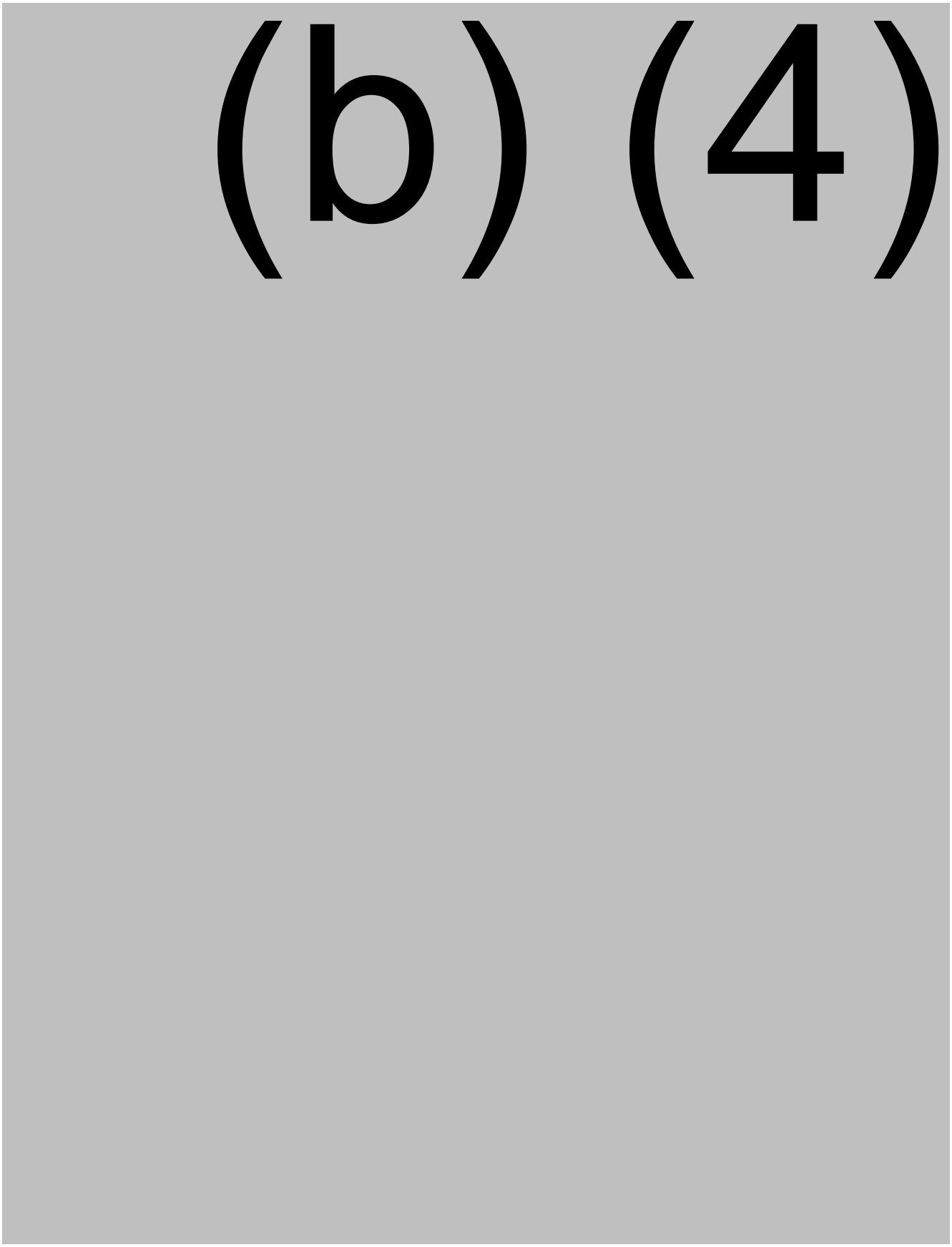

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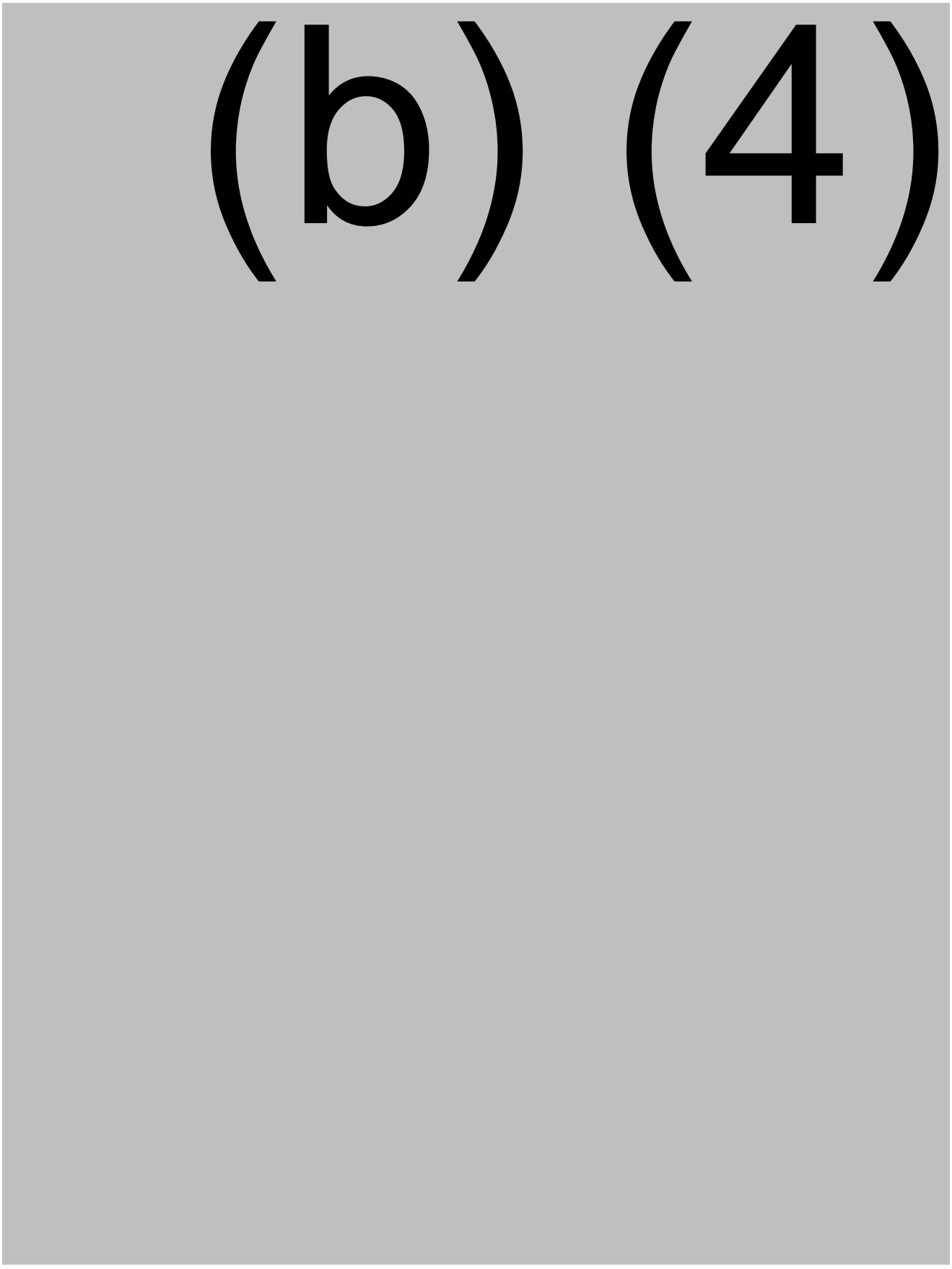

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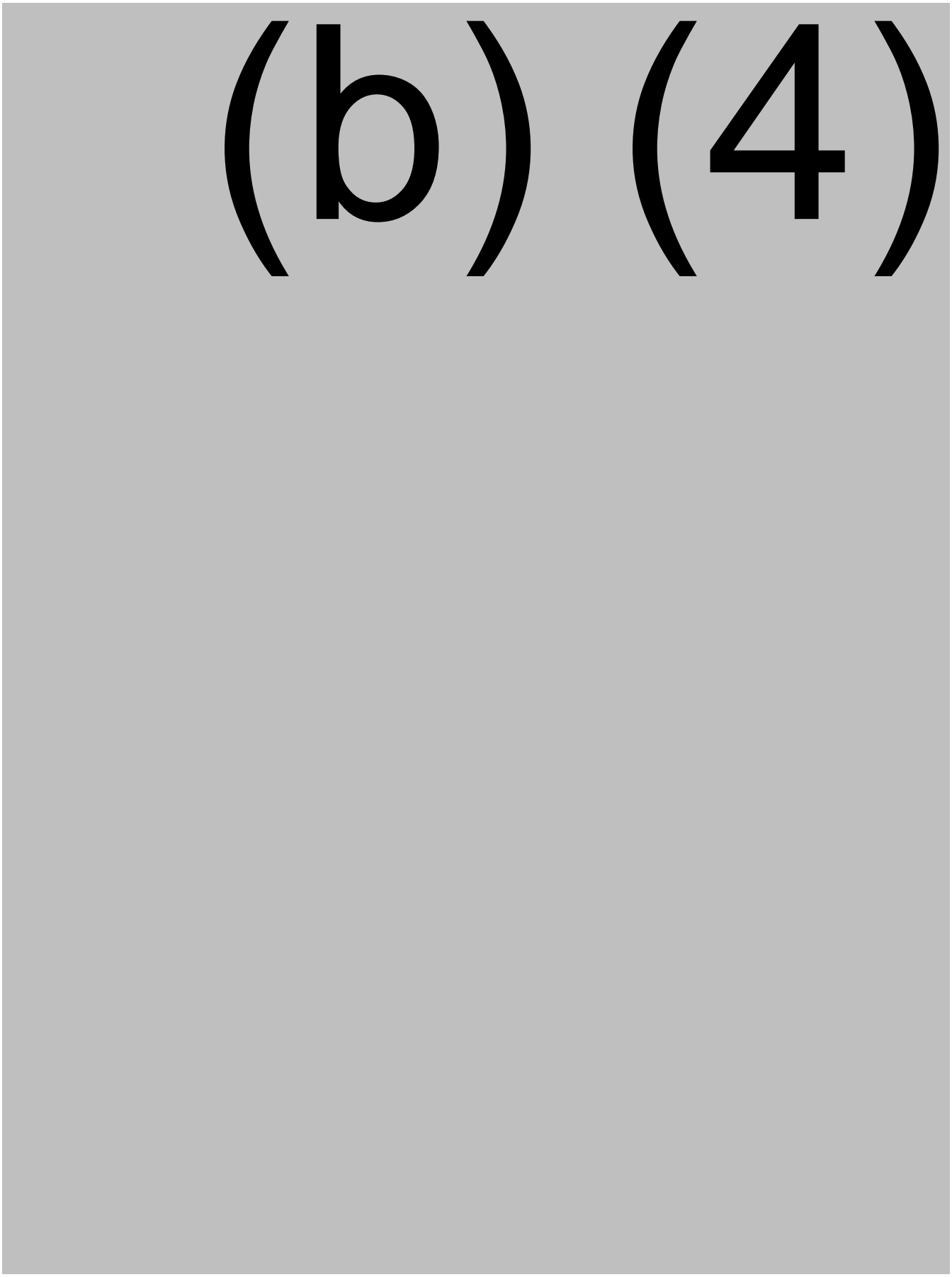

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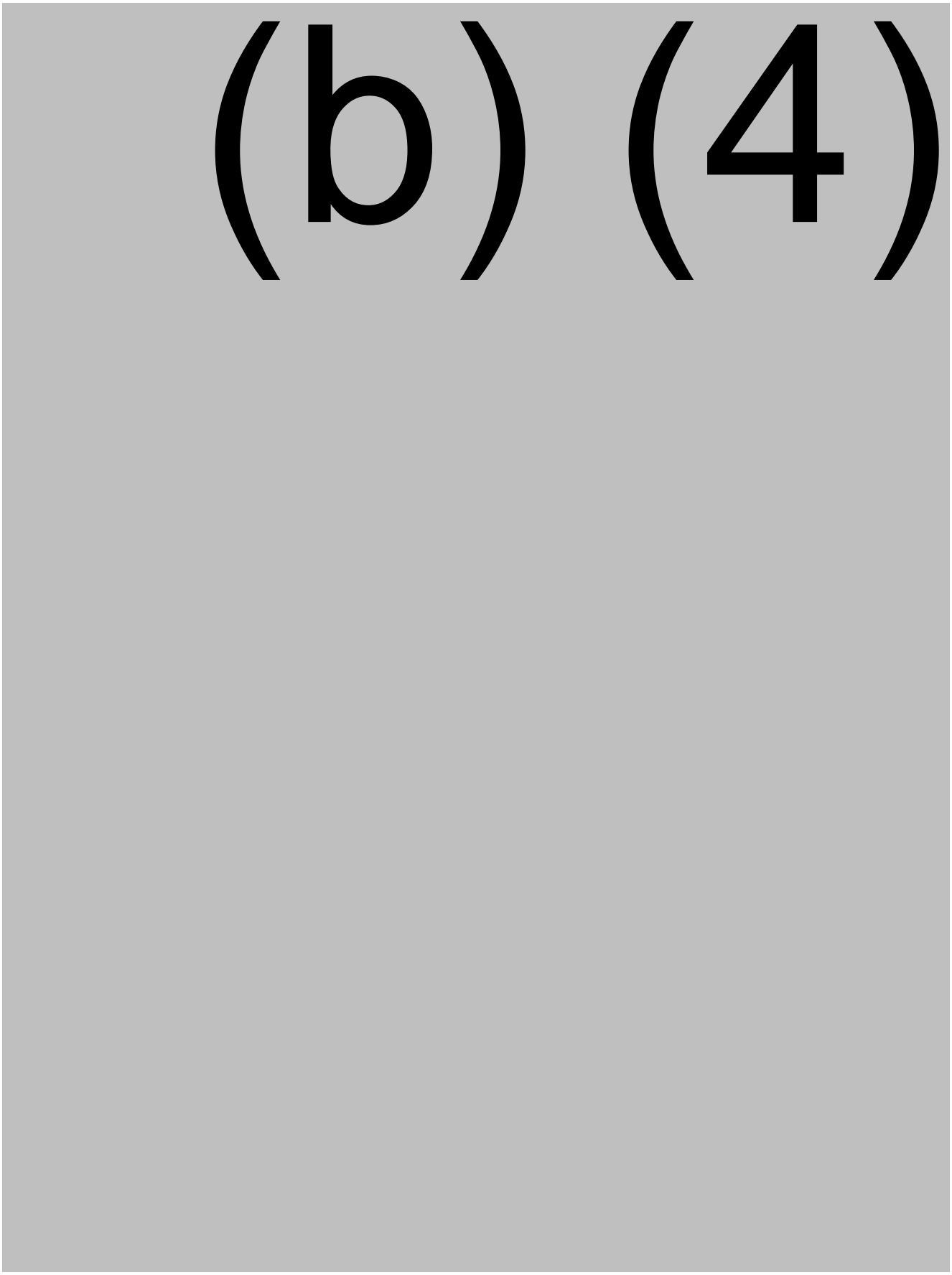

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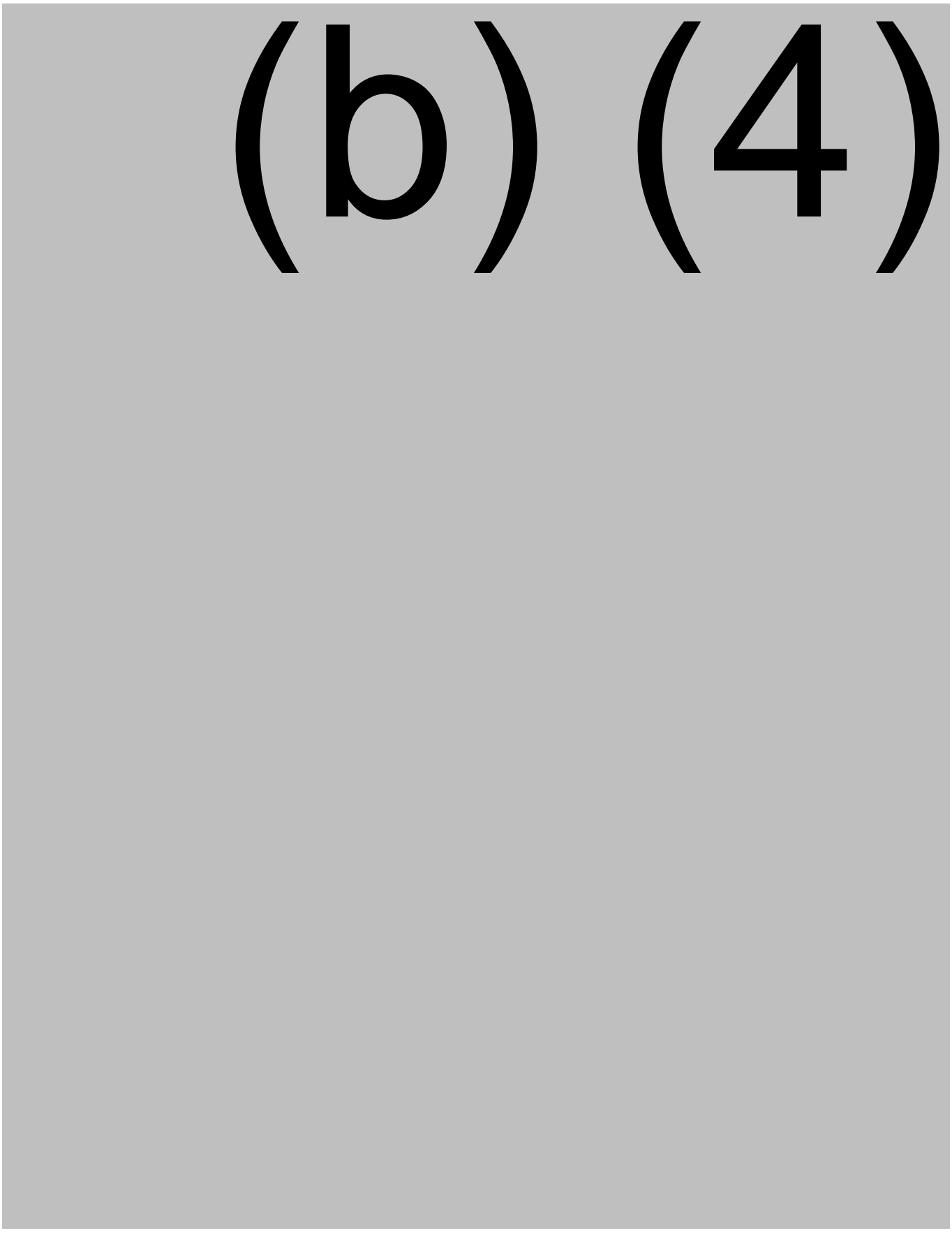

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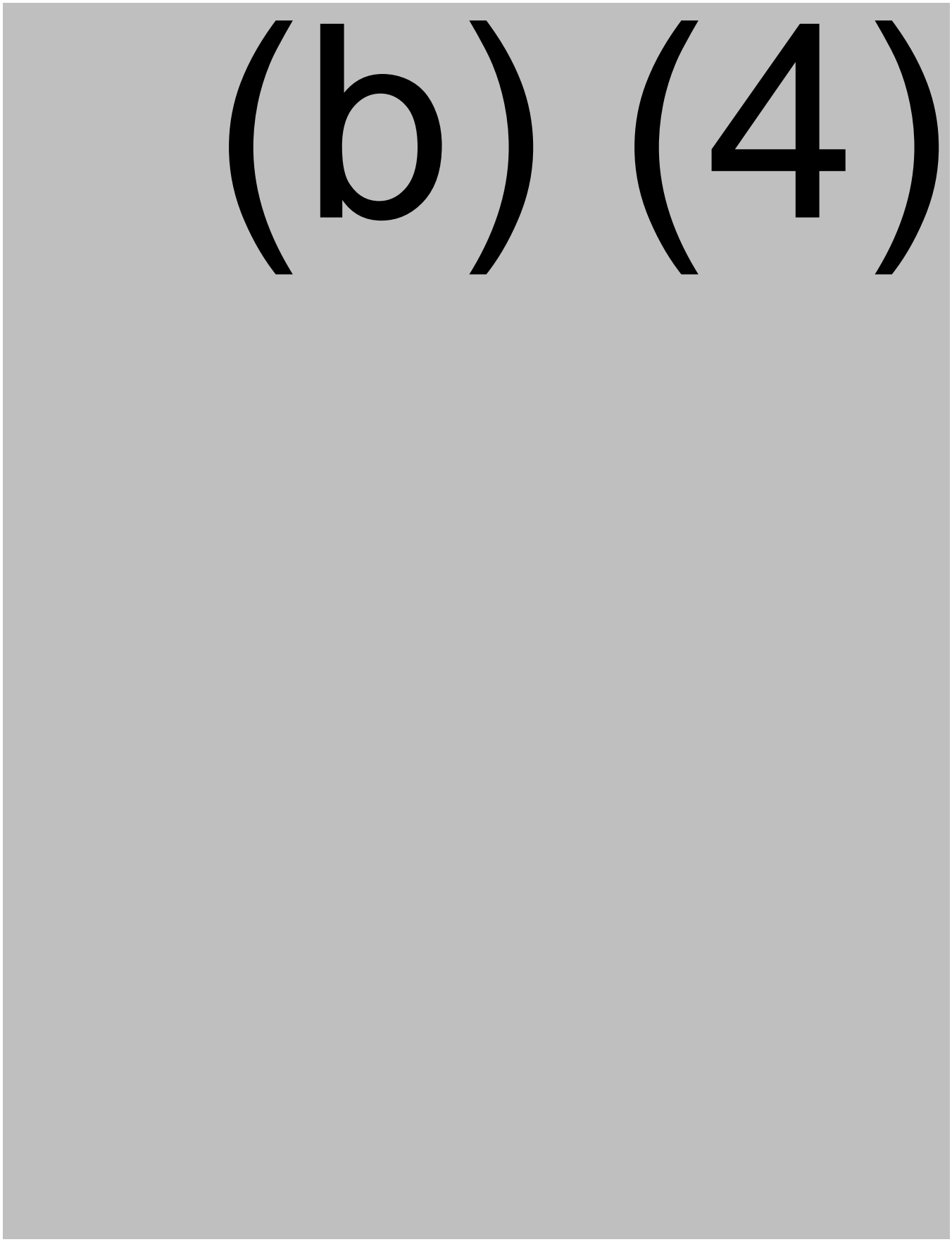

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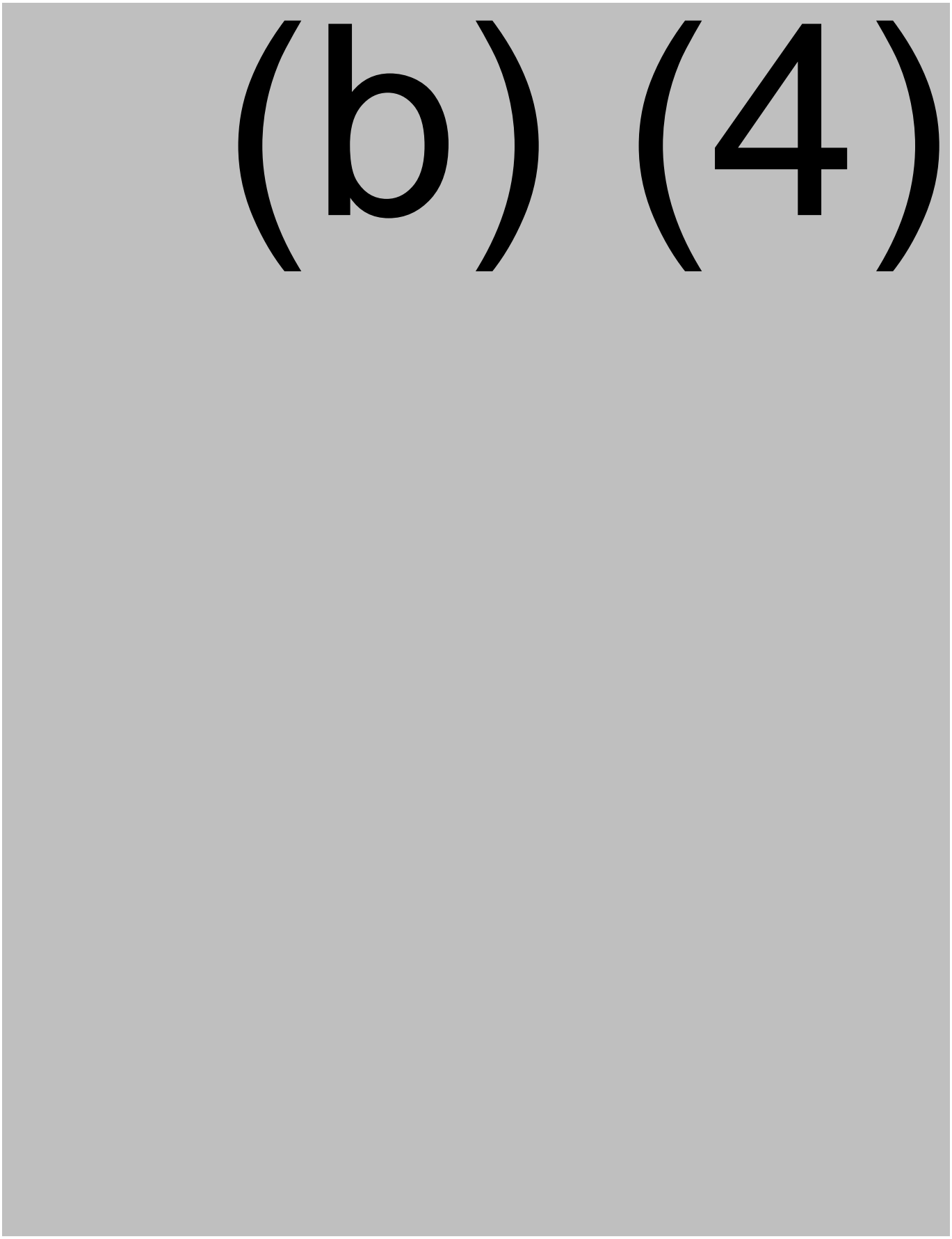

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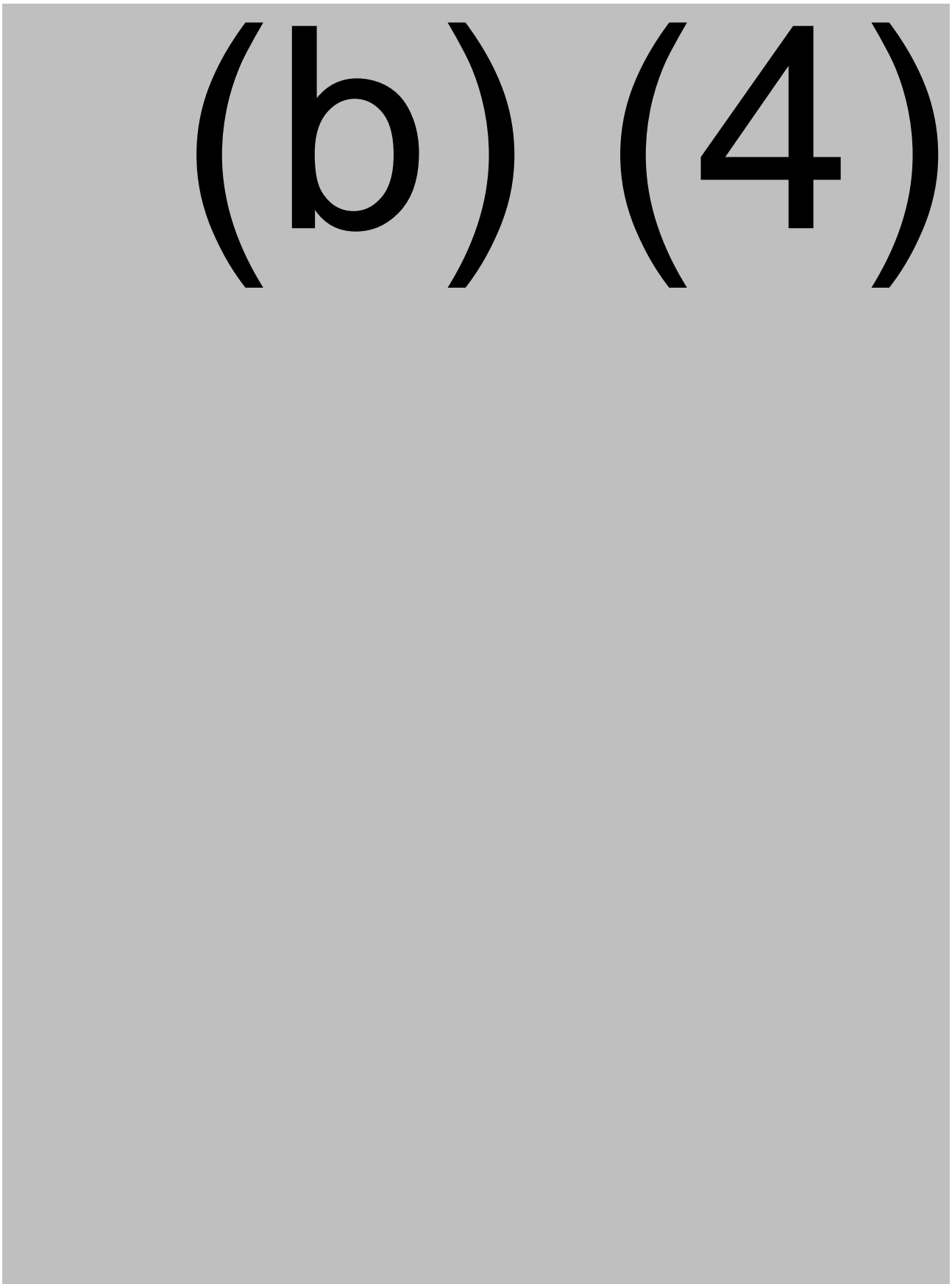

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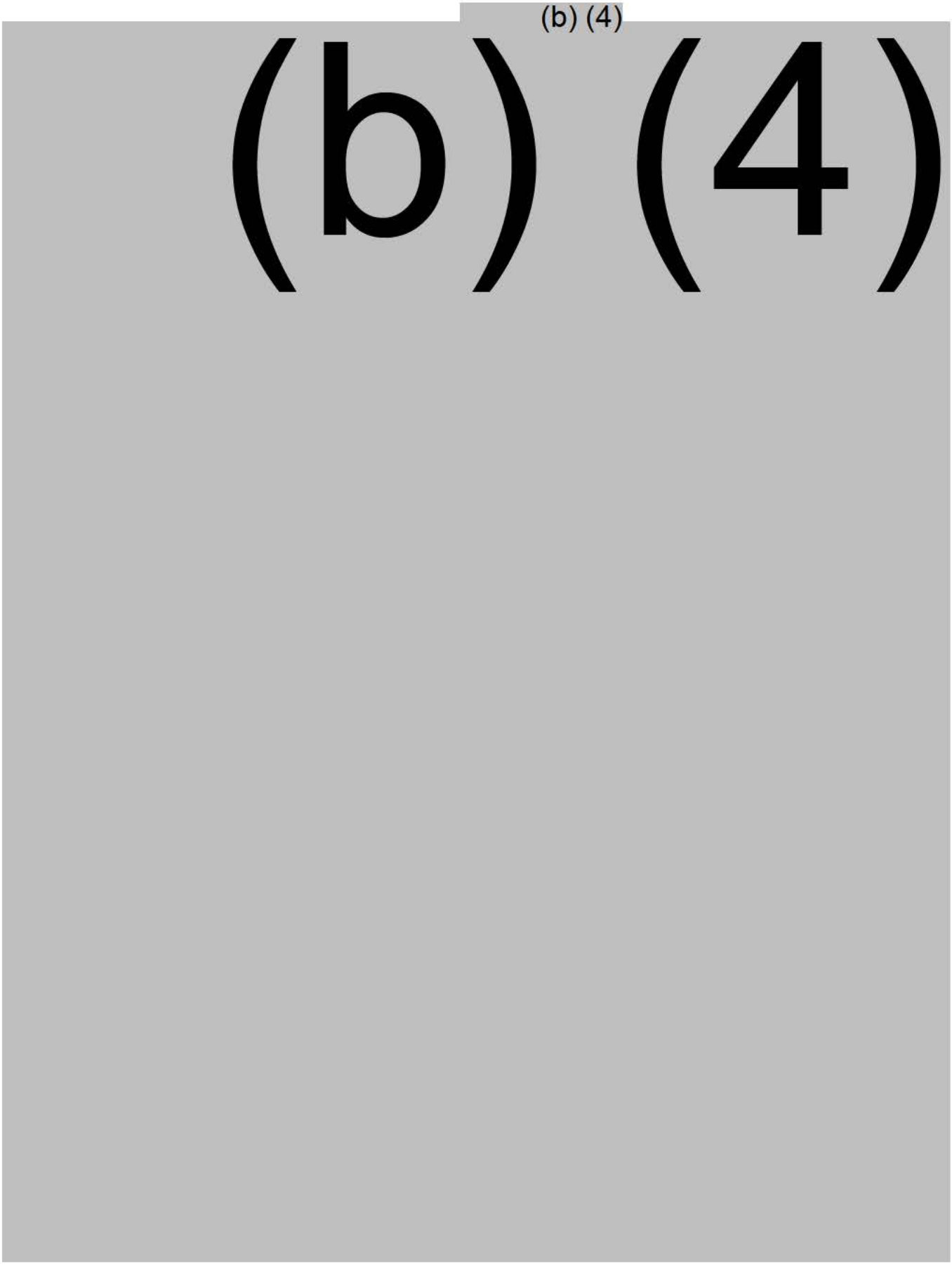

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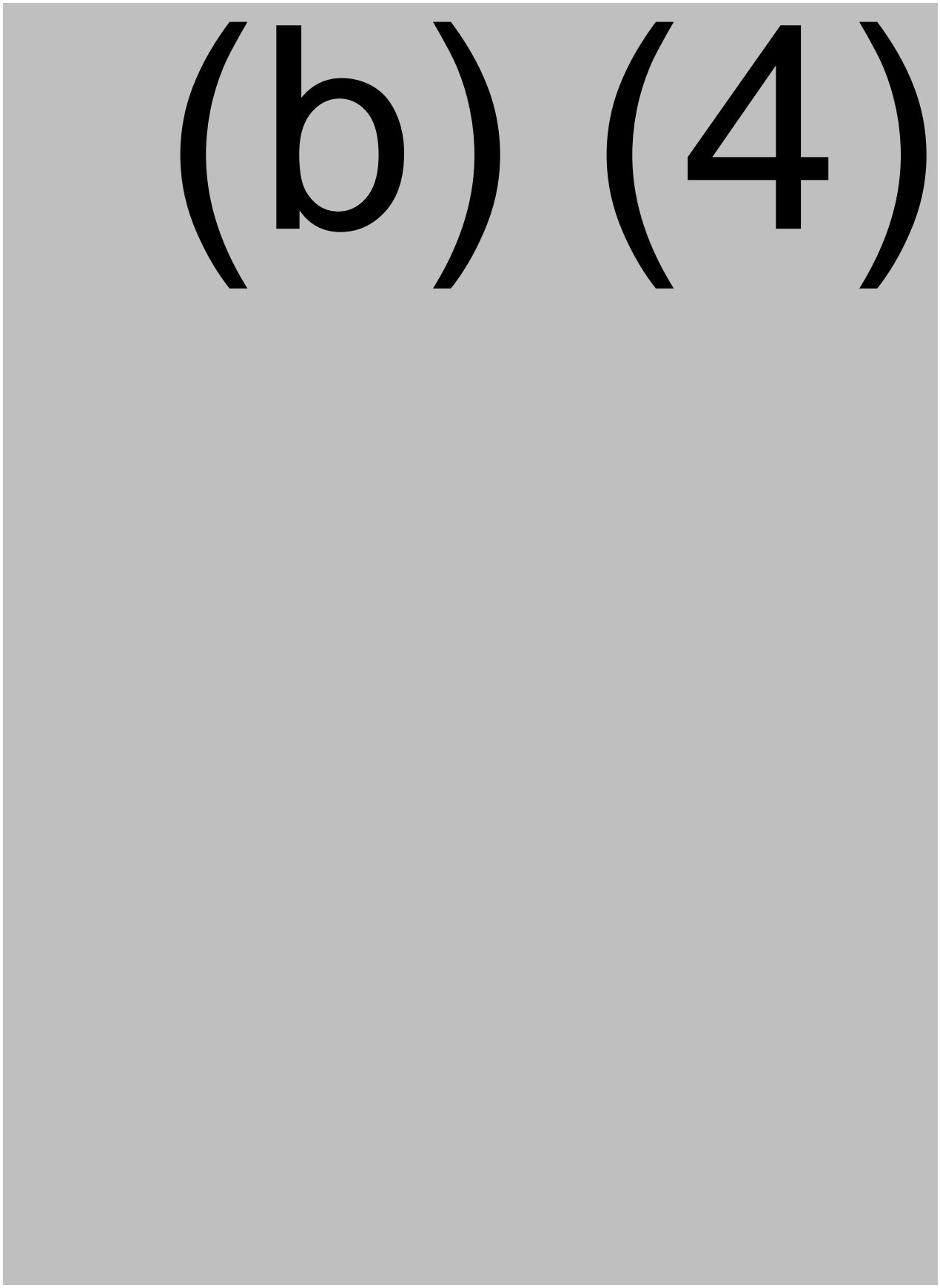

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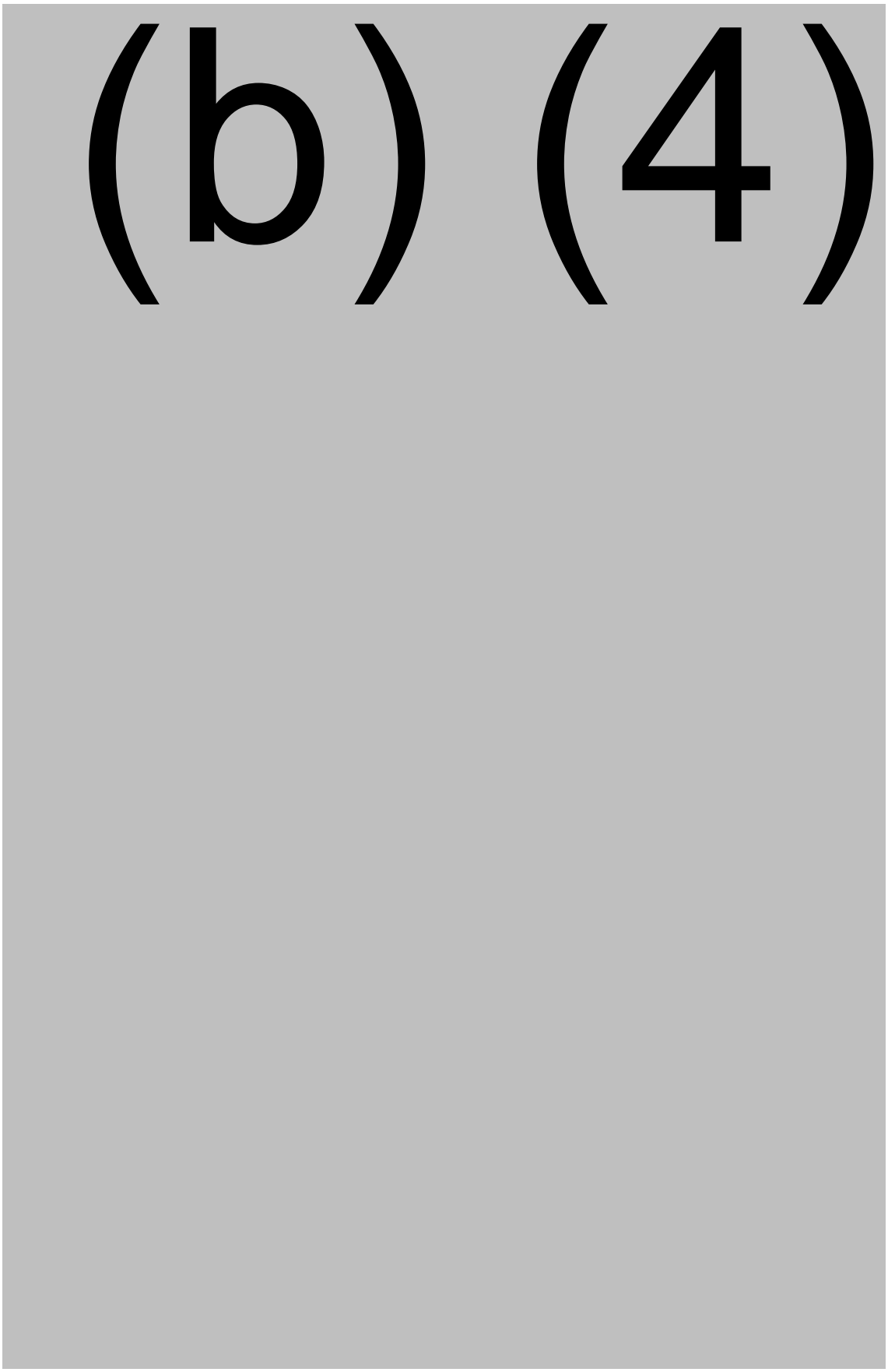

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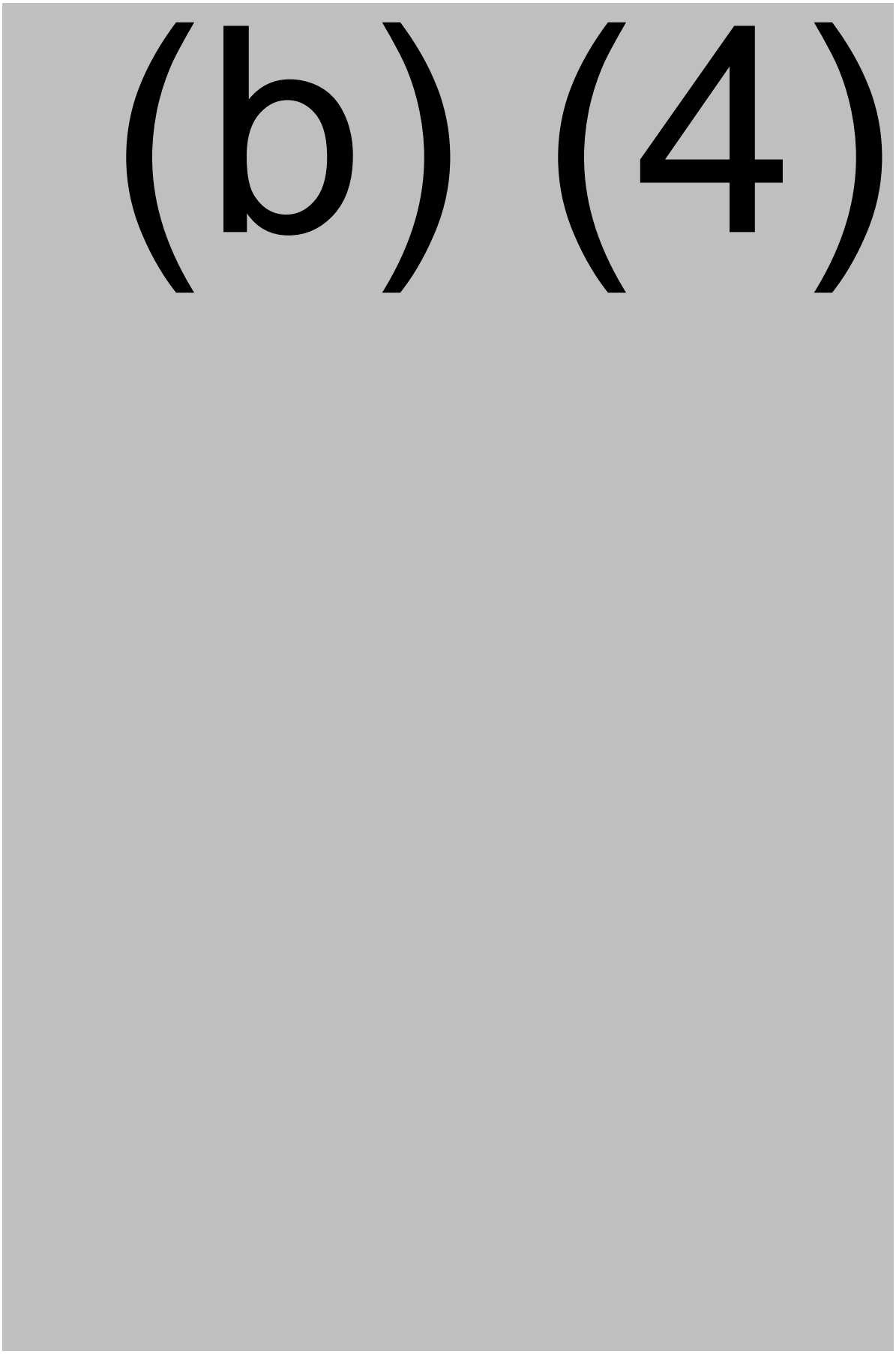

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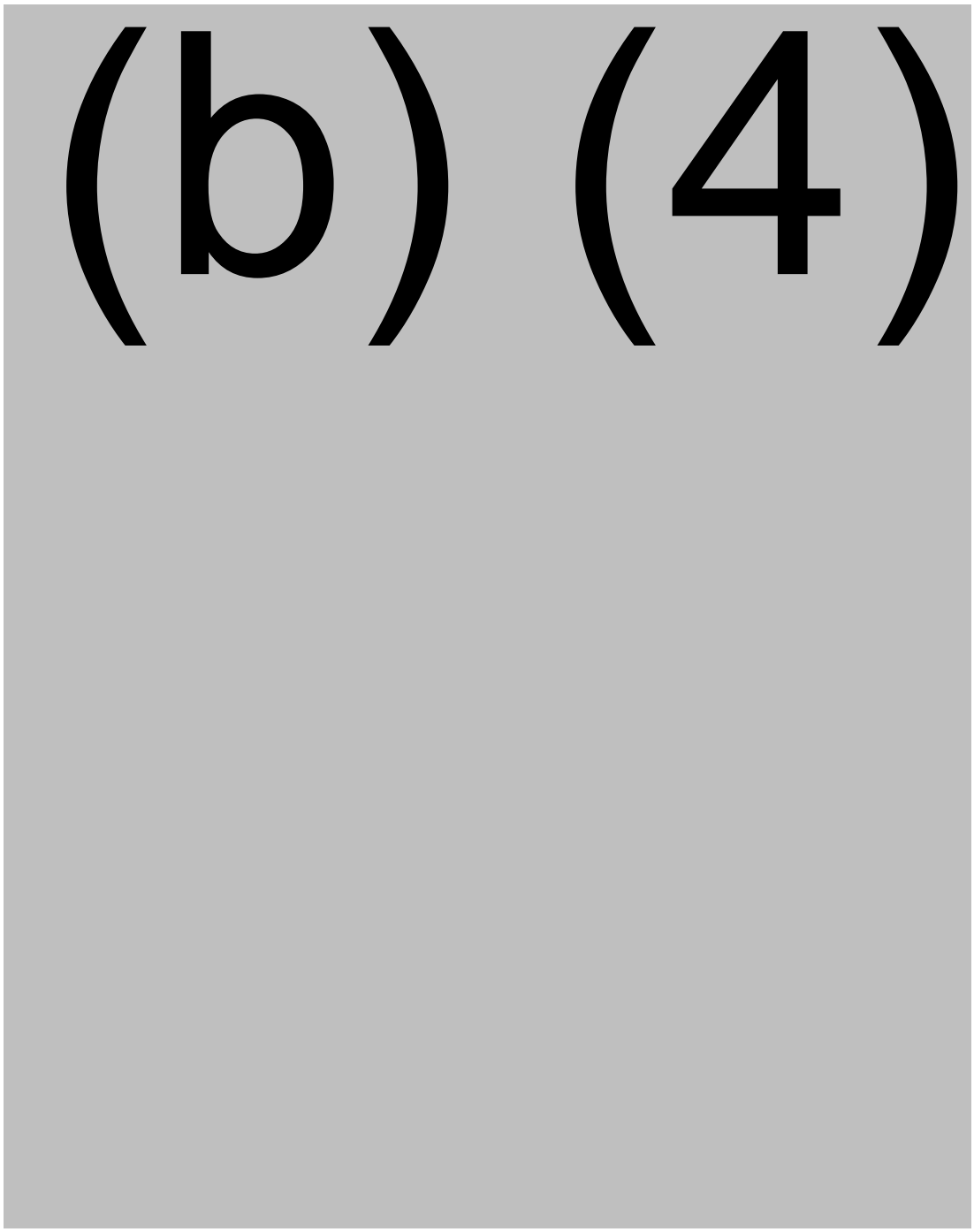
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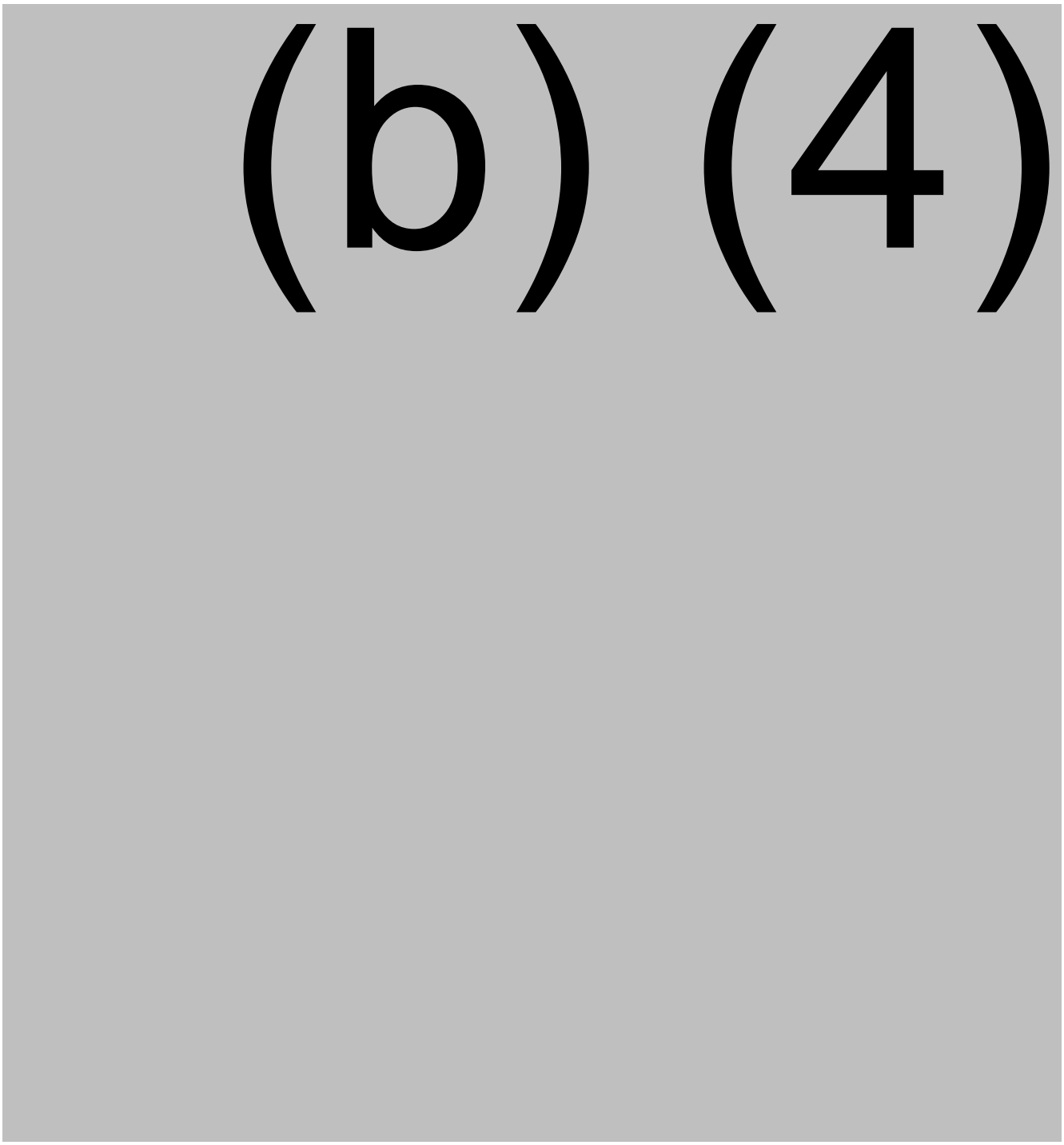

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(b) (4)

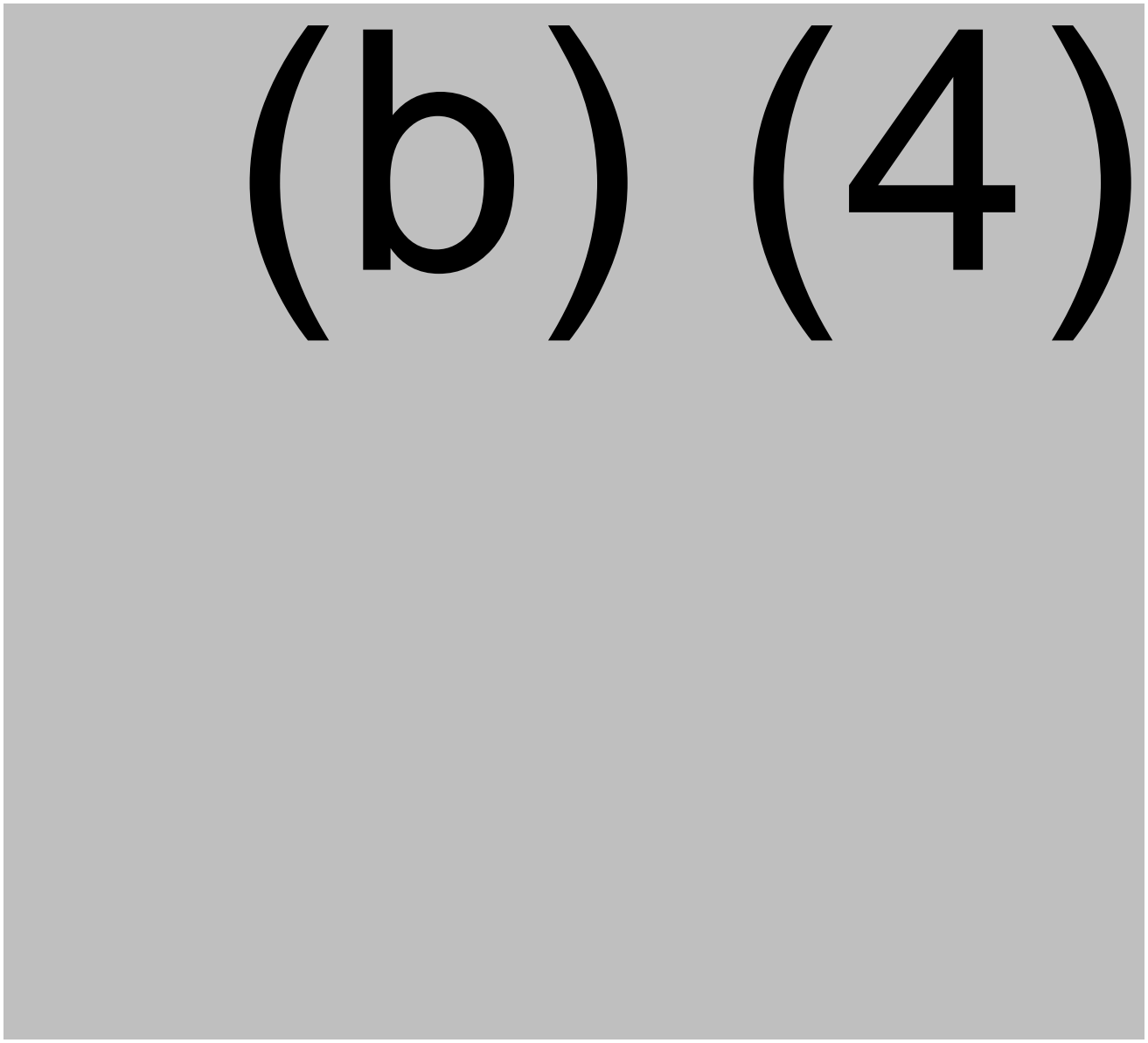

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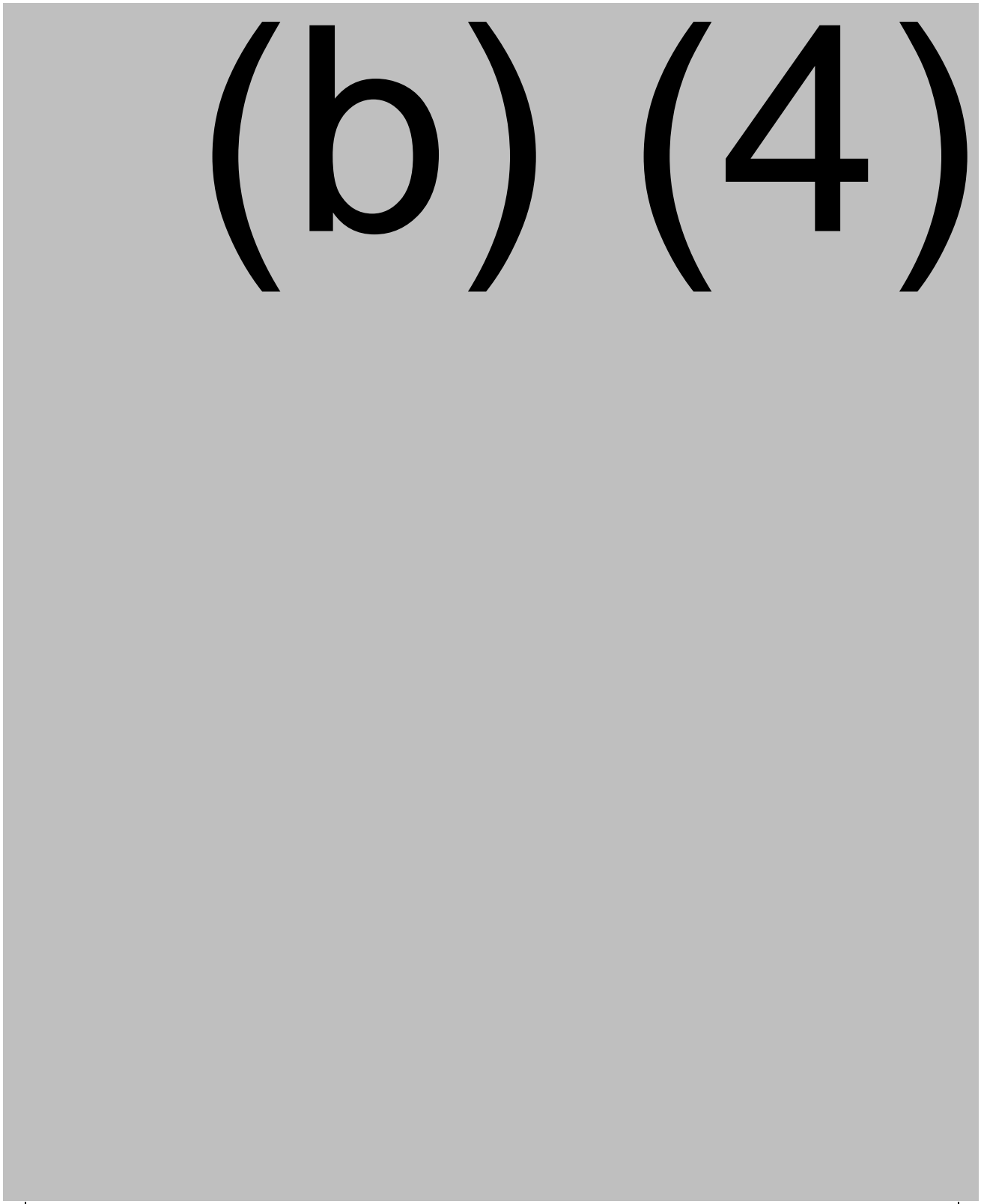
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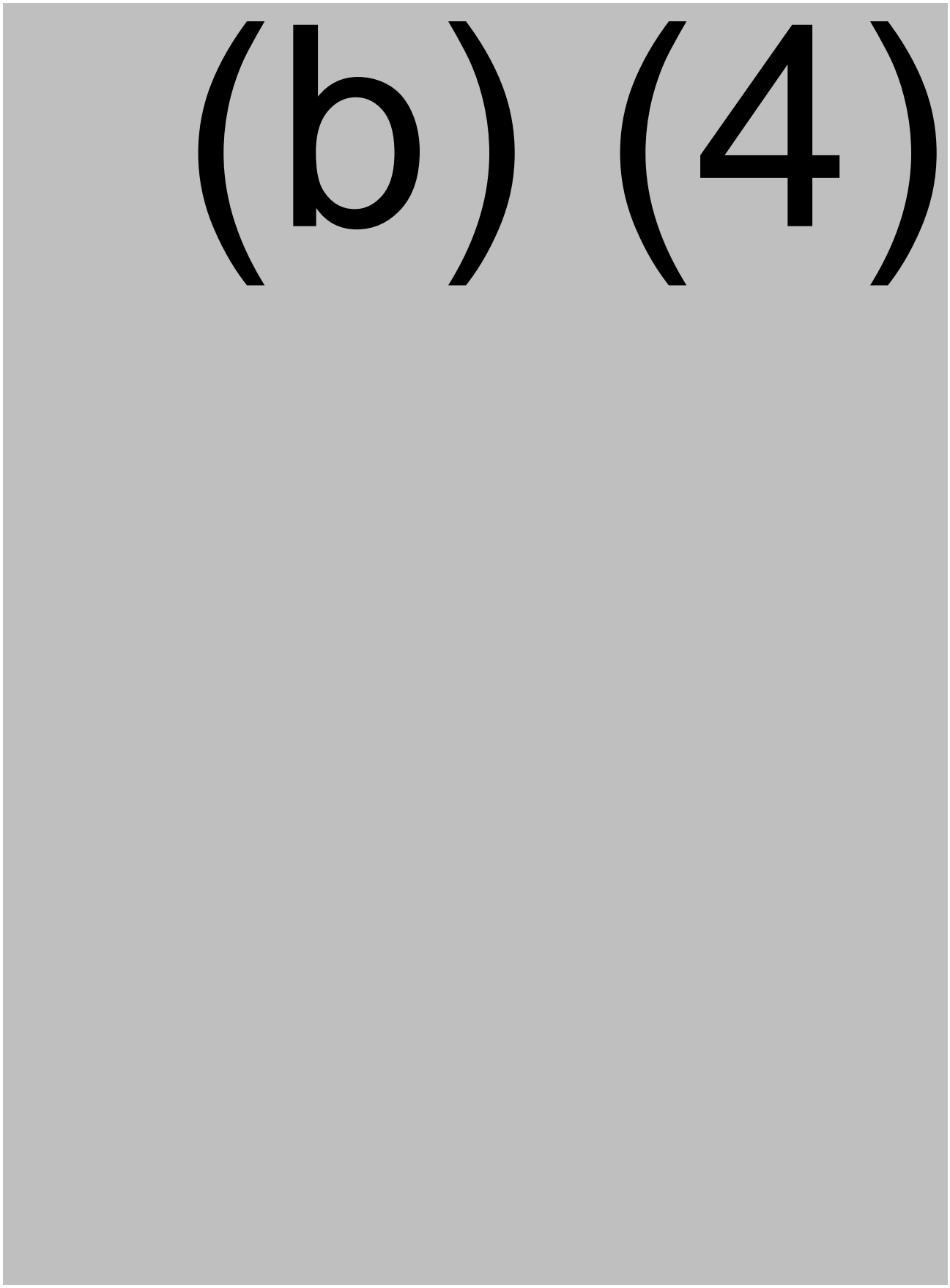


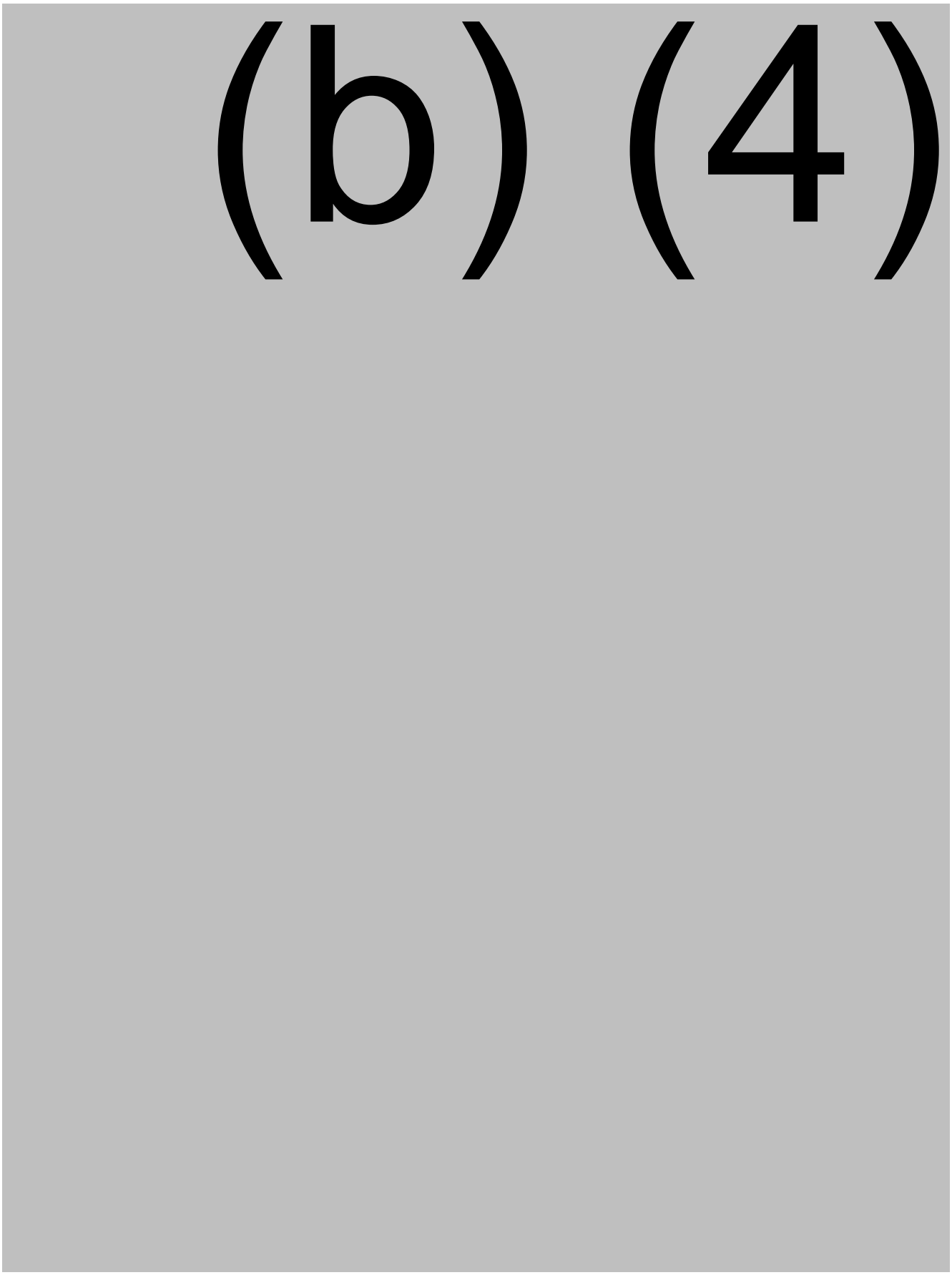
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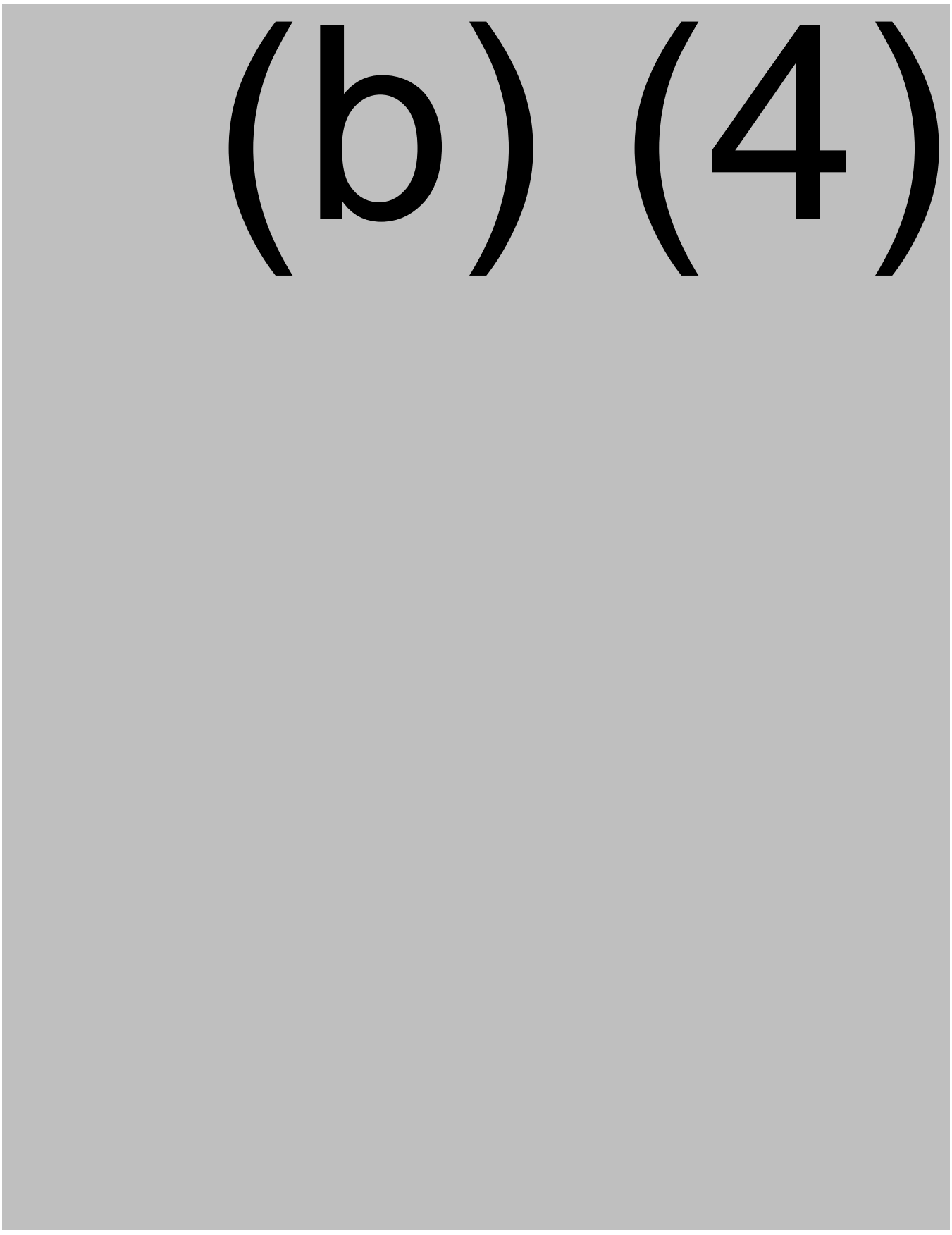


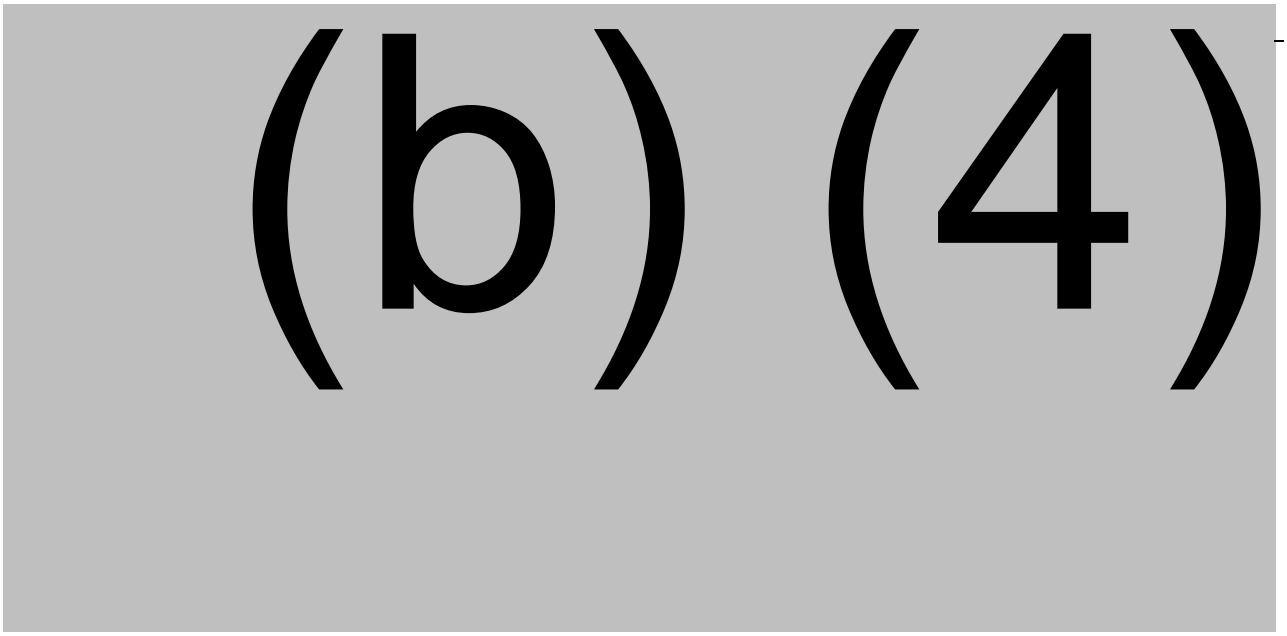
Homology Analyses of ATG-based ORFs in the (b) (4)




(b) (4)

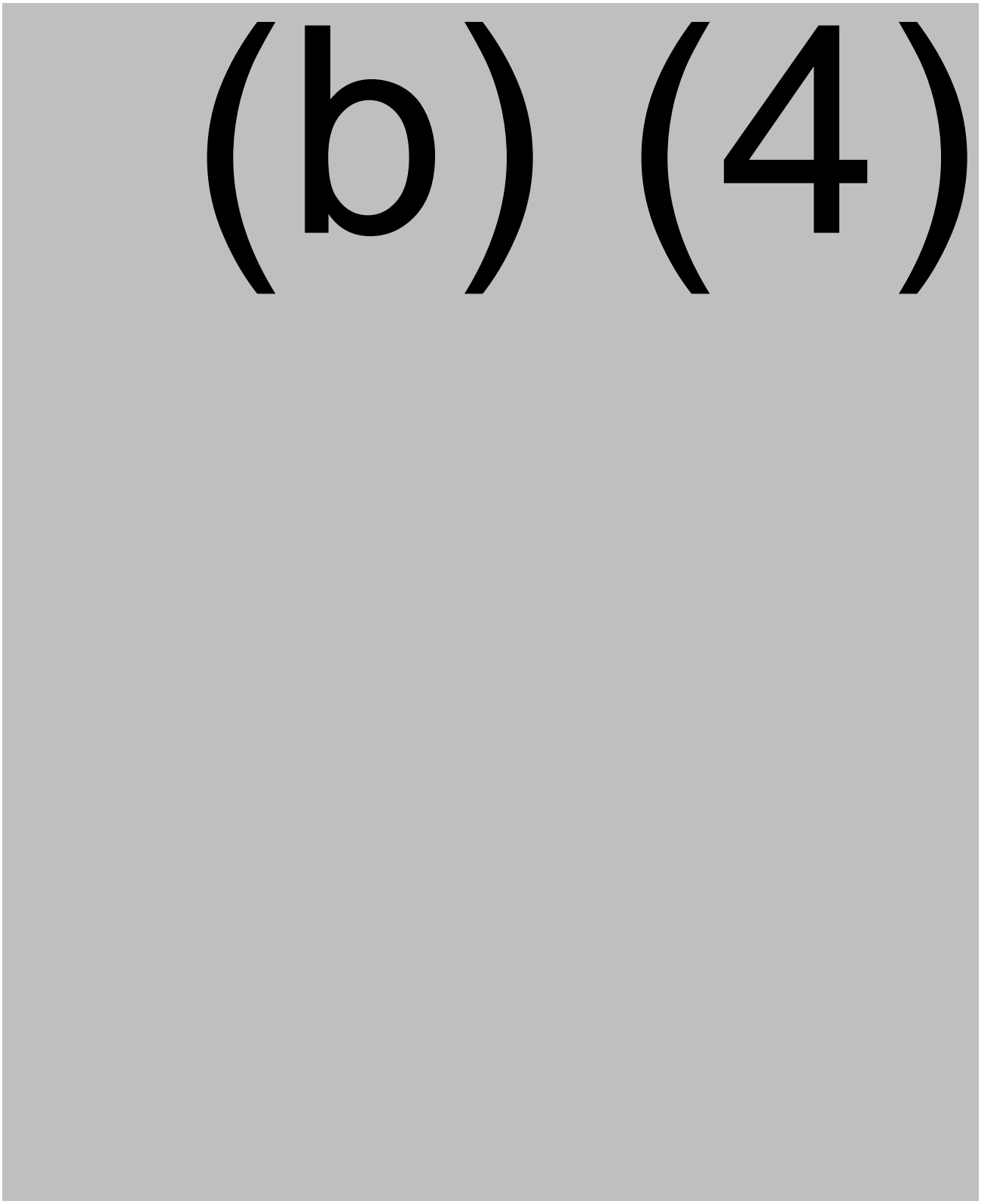

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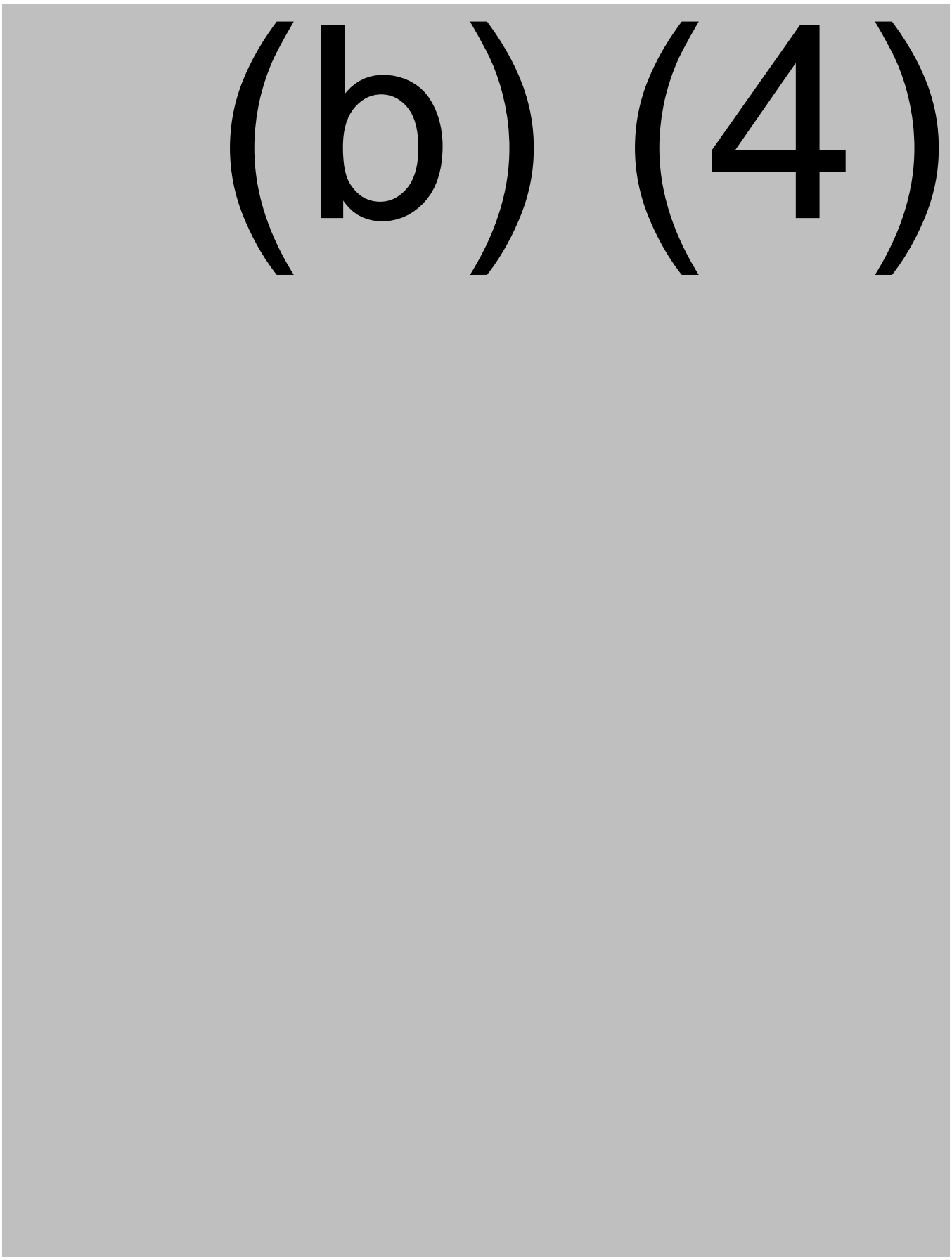

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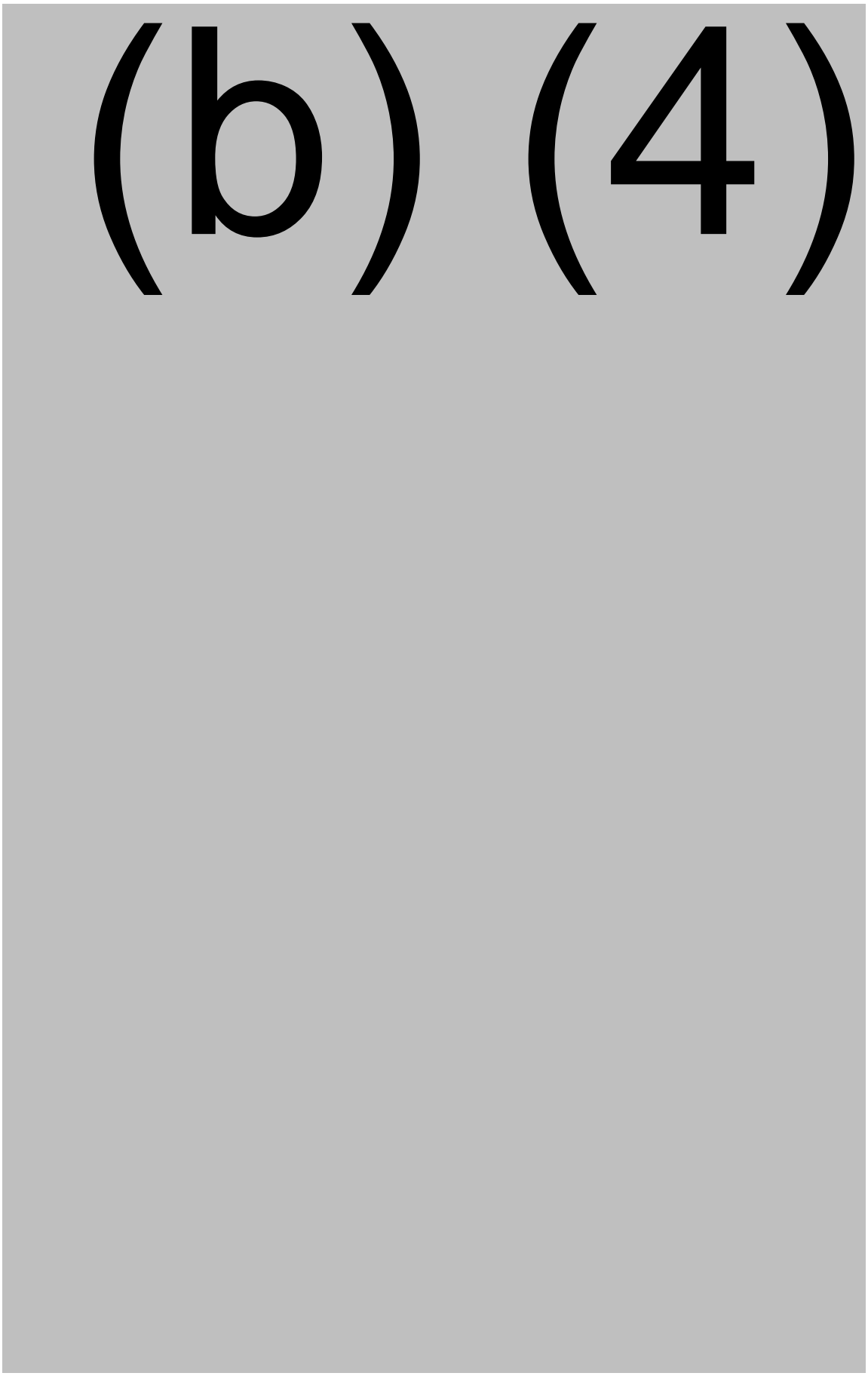

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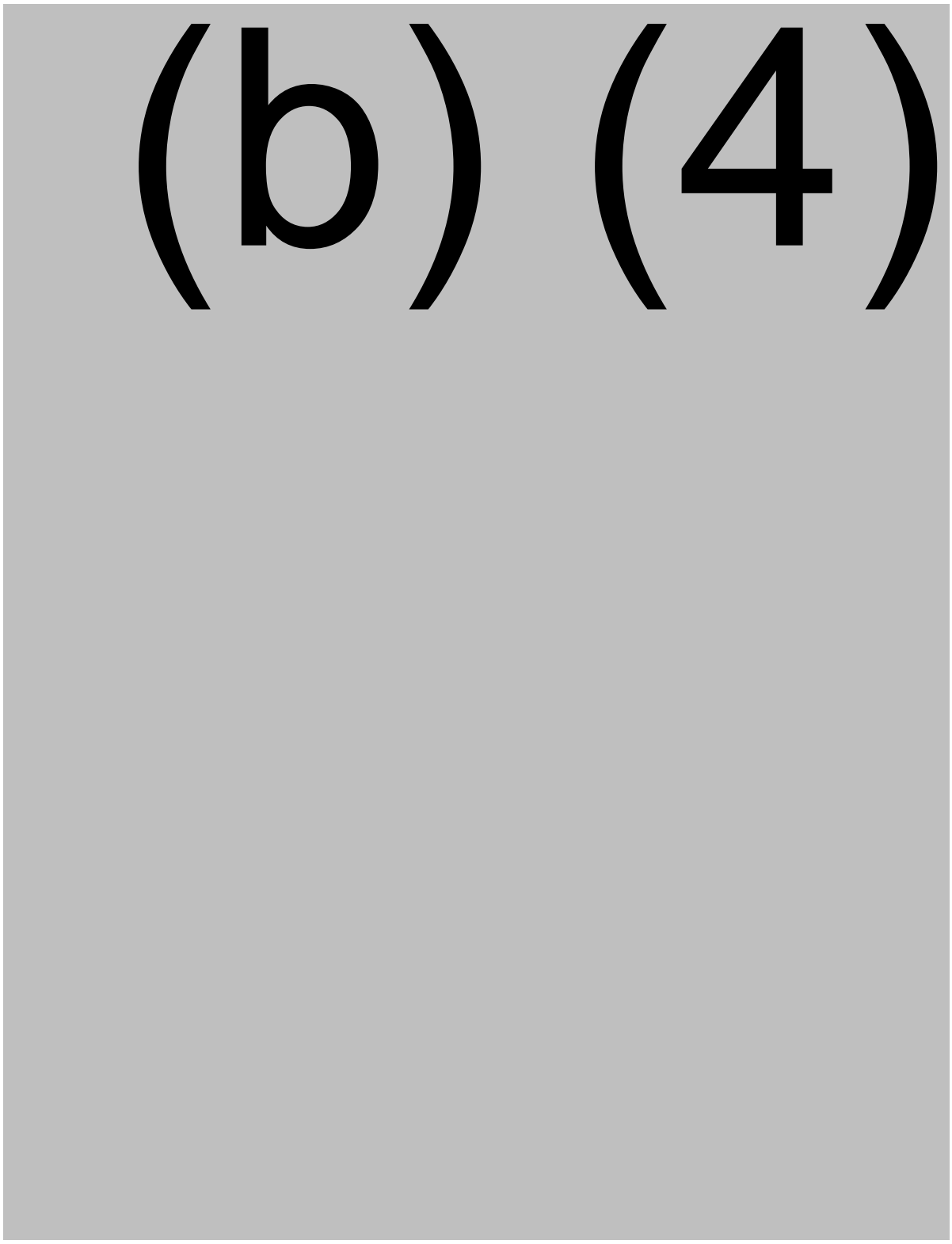
Annex 3 – (b) (4) Knock-out

Plasmid (b) (4).

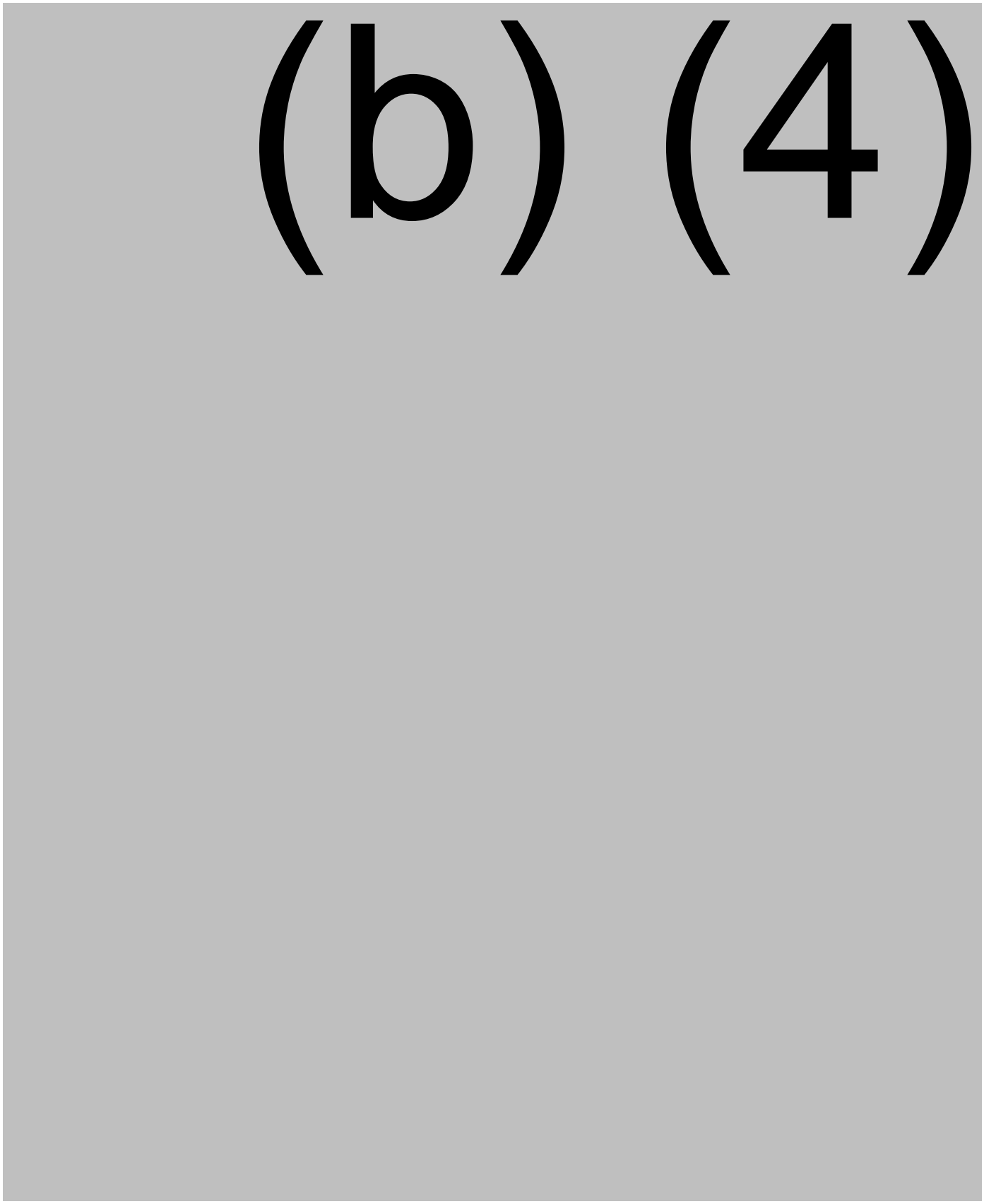


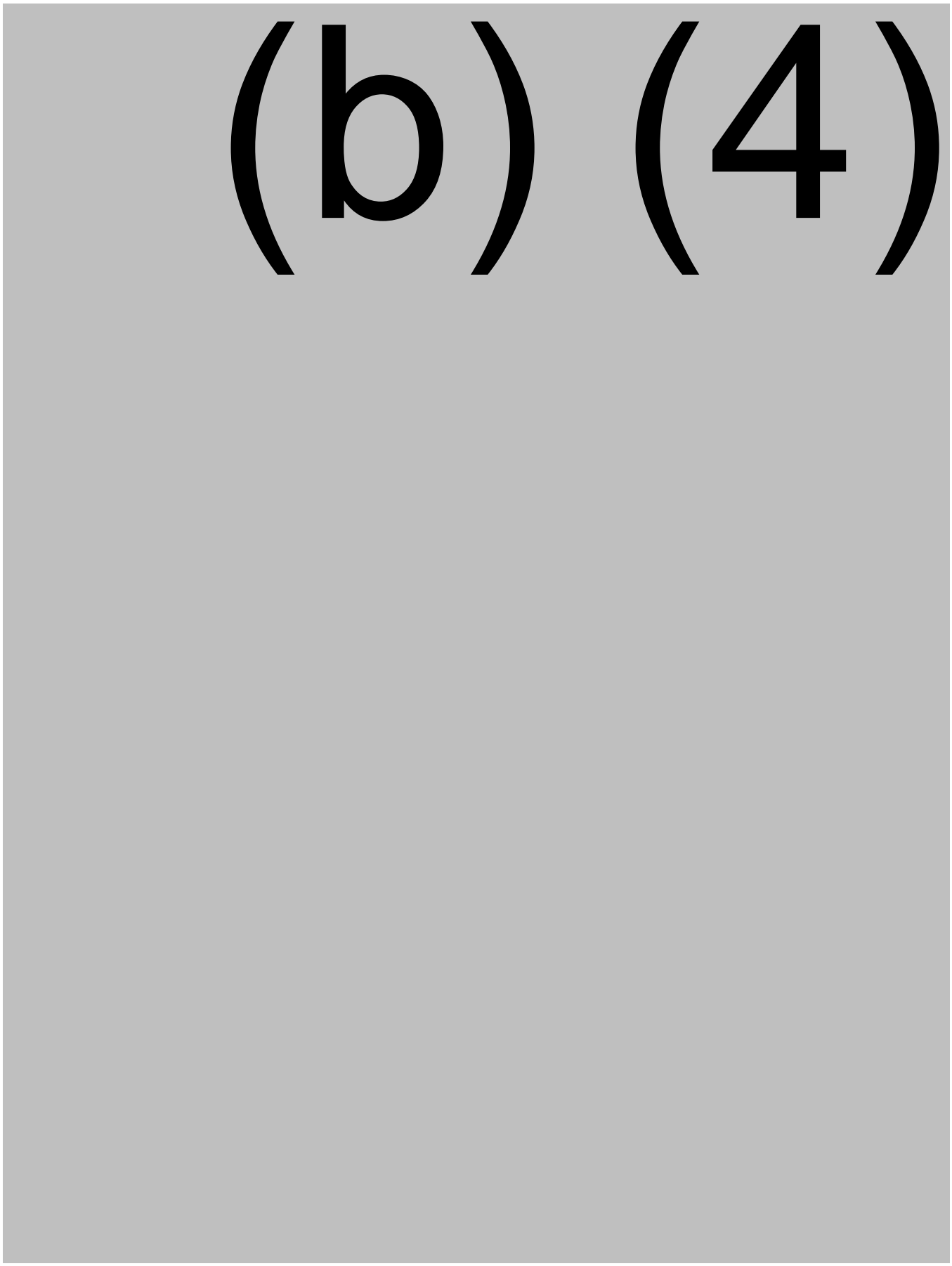

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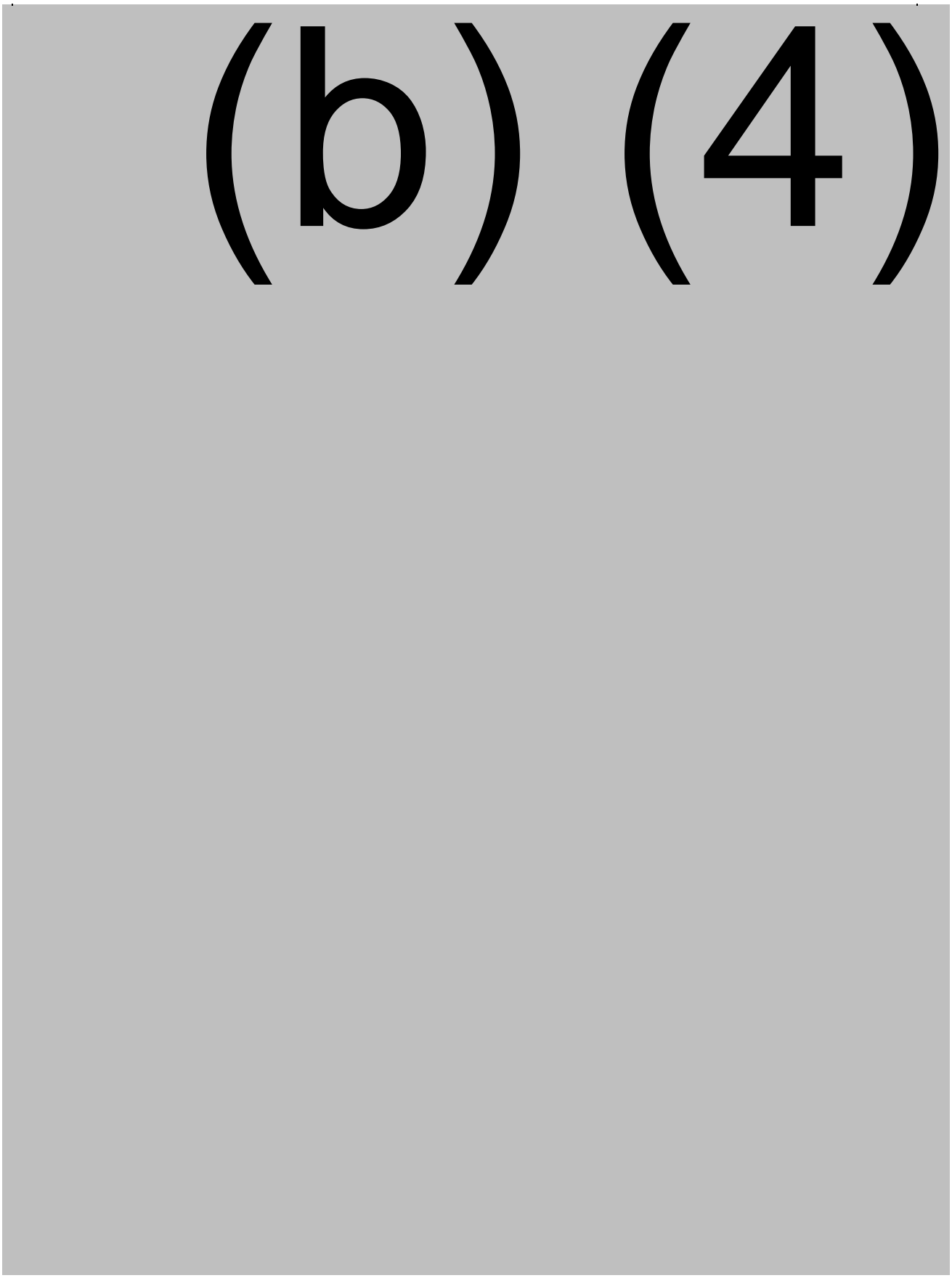

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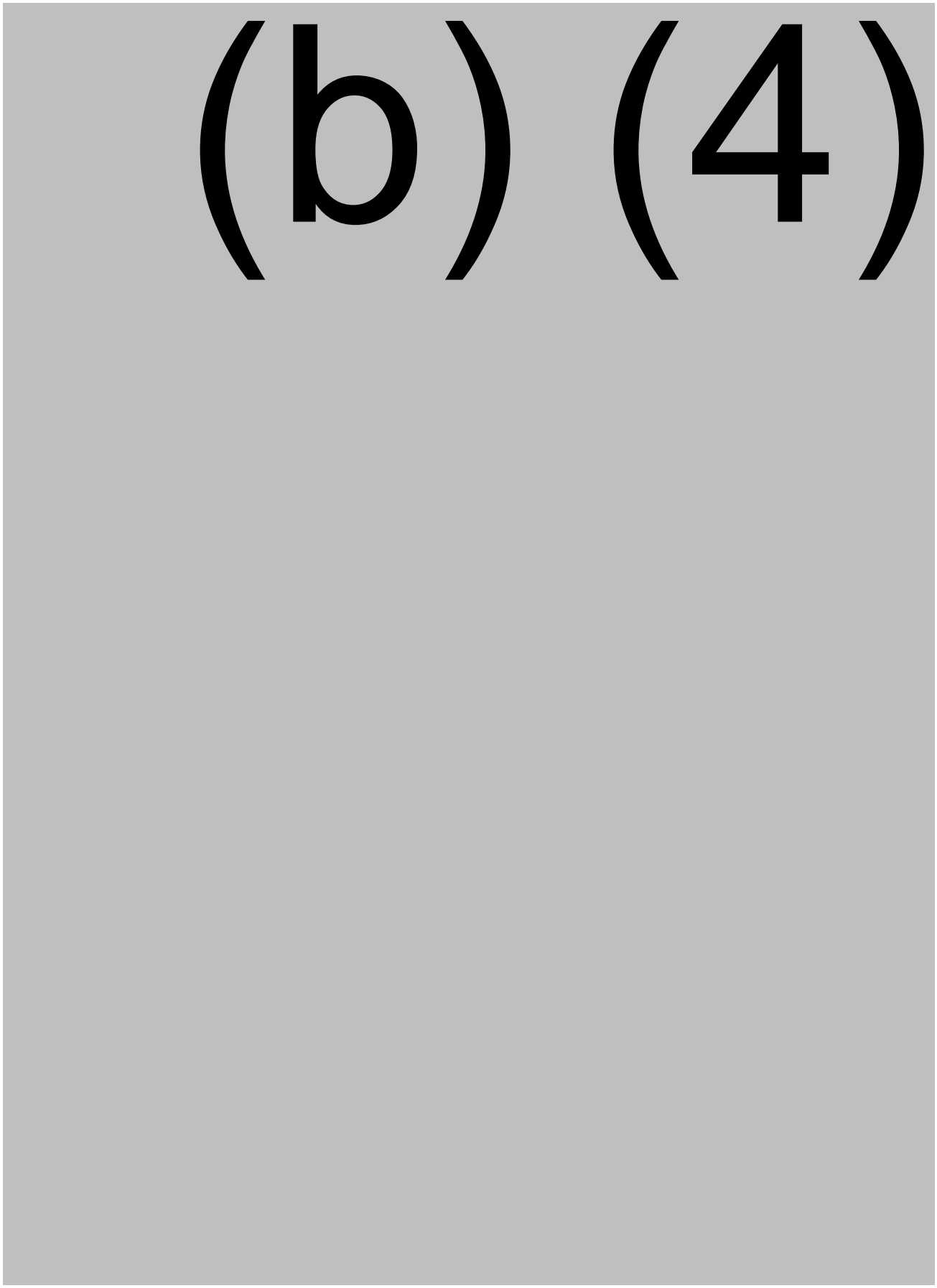

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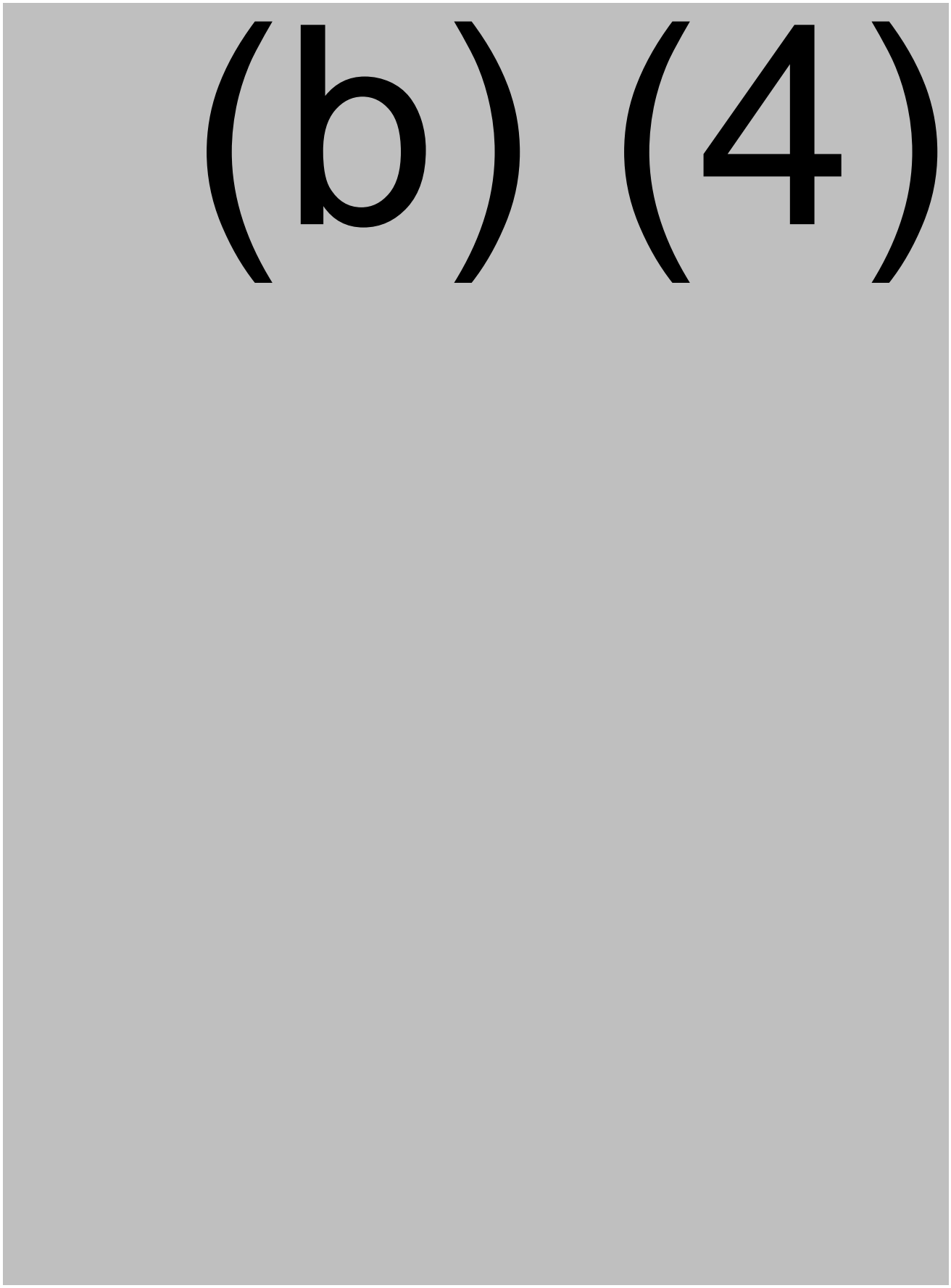
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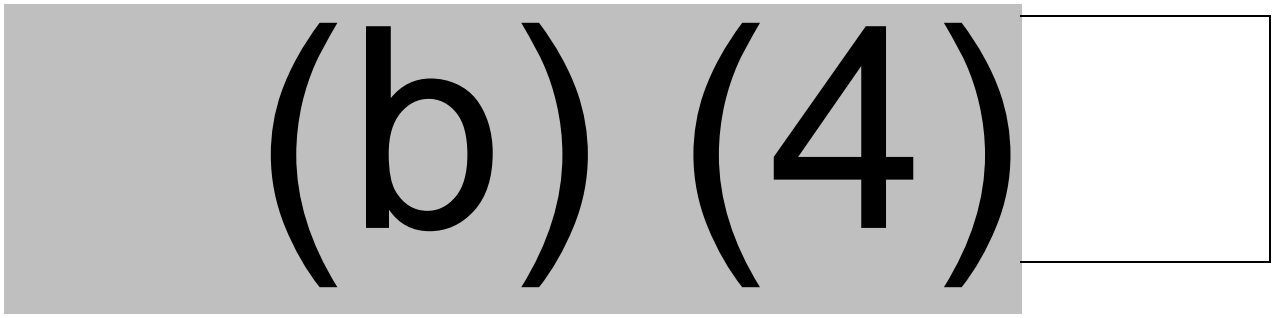



(b) (4)


(b) (4)


(b) (4)


(b) (4)


(b) (4)

Appendix 09: Absence of recombinant DNA

(b) (6), (b) (4)

(b) (6), (b) (4)

BASF SE
(b) (6)
RBW/EC
Carl-Bosch-Strasse 38
67056 Ludwigshafen

Freiburg, 2020-03-17

Certificate No.: 2063 – 01

(b) (4) Order No.: 2063 5DE0203 4210 V01 Sample received: 2020-03-11

Samples

Sample description: liquid
Sample labelling: Mannase CC
Your Sample Lot: 00001077L0
Amount of sample: 50 g
Sample Condition: No remarks

Test **Analysis for the Presence of Residual DNA of a Recombinant *Thermothelomyces thermophilus* Production Strain in an Enzyme Product (Mannanase CC) by Means of a Qualitative Gel-Based PCR Assay**

Subsample analyzed: 3 x 1 ml per batch

Method	(b) (4) Code	Start of Analysis	End of Analysis	PCR Result	Spike recovery*
Production strain-specific PCR system	GO-4529	12.03.2020	17.03.2020	**Negative**	yes

(b) (6), (b) (4)

(b) (4), (b) (6)

BASF SE

(b) (6)

RBW/EC

Carl-Bosch-Strasse 38

67056 Ludwigshafen

Freiburg, 2020-03-17

Certificate No.: 2063 – 02

(b) (4) Order No.: 2063 5DE0203 4210 V01 Sample received: 2020-03-11

Samples

Sample description: liquid

Sample labelling: Mannase CC

Your Sample Lot: 00001177L0

Amount of sample: 50 g

Sample Condition: No remarks

Test **Analysis for the Presence of Residual DNA of a Recombinant *Thermothelomyces thermophilus* Production Strain in an Enzyme Product (Mannanase CC) by Means of a Qualitative Gel-Based PCR Assay**

Subsample analyzed: 3 x 1 ml per batch

Method	(b) (4) Code	Start of Analysis	End of Analysis	PCR Result	Spike recovery*
Production strain-specific PCR system	GO-4530	12.03.2020	17.03.2020	**Negative**	**yes**

(b) (4)

BASF SE
Dr. Katja Gemperlein
RBW/EC
Carl-Bosch-Strasse 38
67056 Ludwigshafen

Freiburg, 2020-03-17

Certificate No.: 2063 – 03

(b) (4) Order No.: 2063 5DE0203 4210 V01 Sample received: 2020-03-11

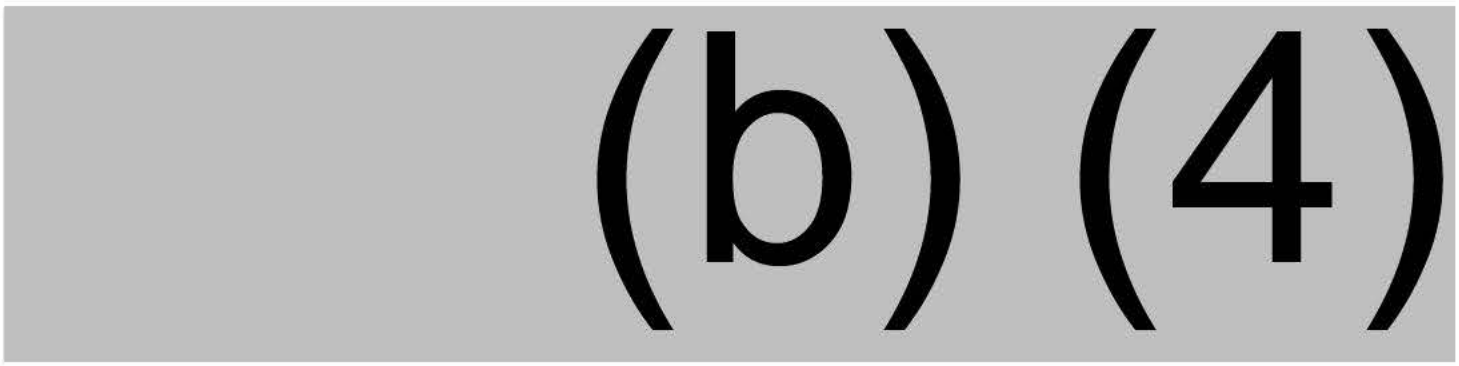
Samples

Sample description: liquid
Sample labelling: Mannase CC
Your Sample Lot: 00001277L0
Amount of sample: 50 g
Sample Condition: No remarks

Test **Analysis for the Presence of Residual DNA of a Recombinant *Thermothelomyces thermophilus* Production Strain in an Enzyme Product (Mannanase CC) by Means of a Qualitative Gel-Based PCR Assay**

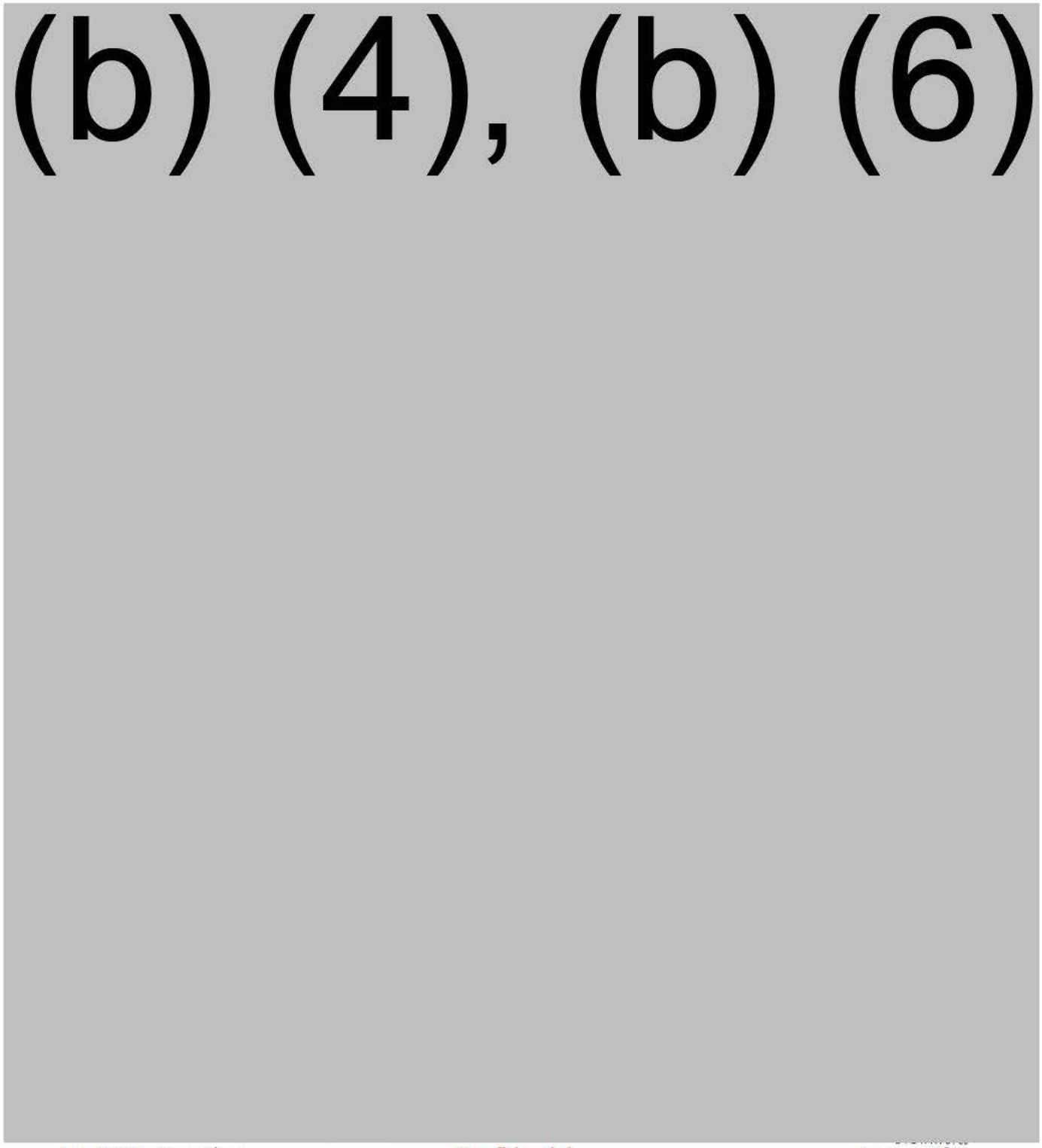
Subsample analyzed: 3 x 1 ml per batch

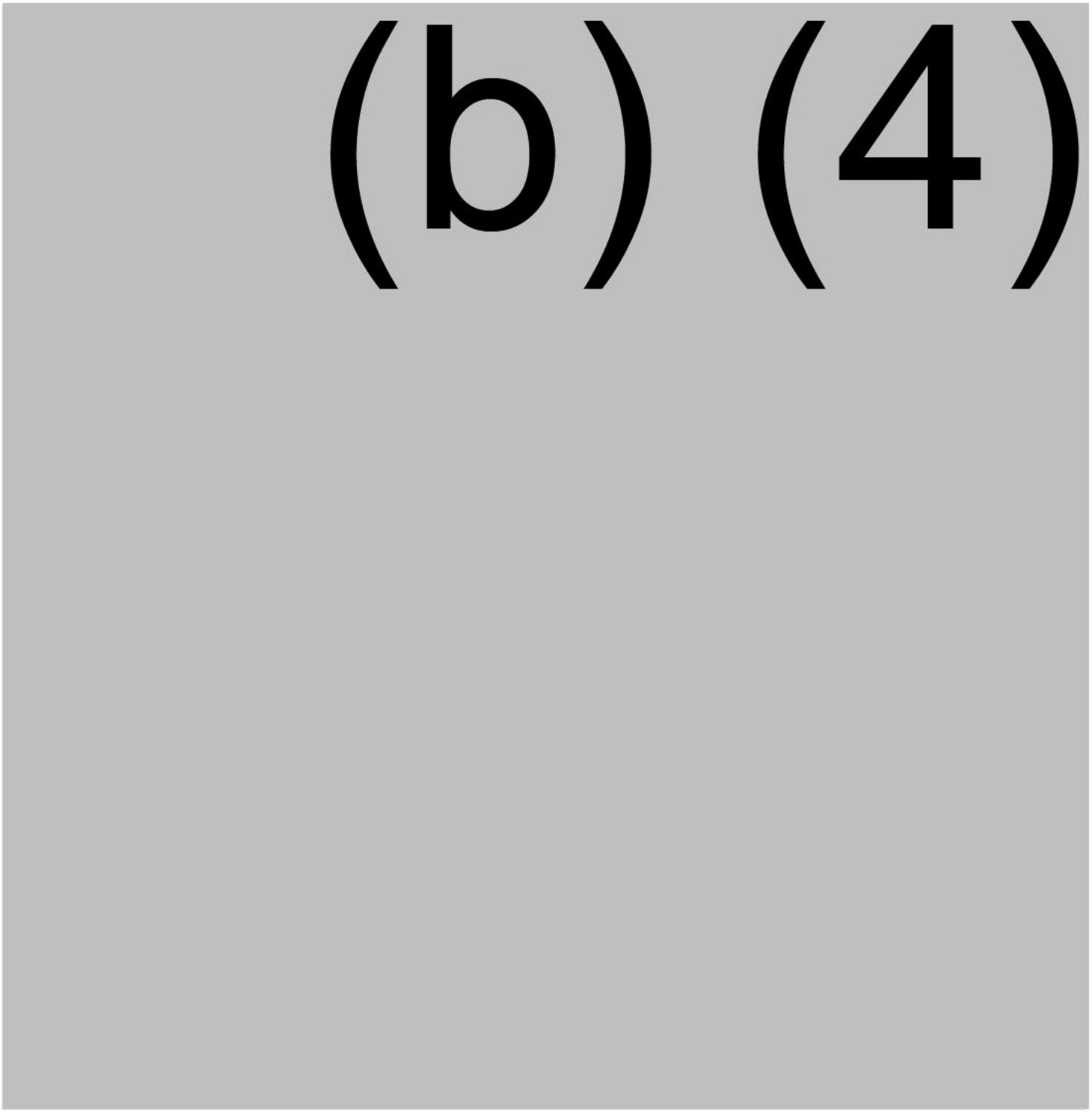
Method	(b) (4) Code	Start of Analysis	End of Analysis	PCR Result	Spike recovery*
Production strain-specific PCR system	GO-4531	12.03.2020	17.03.2020	**Negative**	**yes**



Supplementary Information to Certificate No.: 2063 - 01 to - 03

Results of the Analysis for the Presence of Residual DNA of a Recombinant *Thermothelomyces thermophilus* Production Strain in an Enzyme Product (Mannanase CC) by Means of a Qualitative Gel-Based PCR Assay




(b) (4)

Appendix 10: Absence of Production Organism SOP

Absence of *Aspergillus niger* or *Thermothelomyces thermophila* (C1) Production Organisms in Enzyme Concentrates or Formulations

Table of Contents

1 Purpose 2
2 Scope 2
3 Explanations, Terms, Abbreviations 2
4 Procedure 2
4.1 Preparation of Potato Dextrose Agar (PDA) 2
4.2 Plating of the enzyme concentrate / liquid enzyme formulation 3
4.3 Control Sample Preparation 3
4.4 Plate Culture 3
4.5 Evaluation 4
4.6 Notification of Quality Control 4
5 Responsibilities 4
6 Applicable documents 5

Disclaimer: This document is an English translation of the original procedure in German. Some minor text may still appear in German.

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1 Purpose

This working instruction describes the procedure for the detection of the absence of the production strain (*Aspergillus niger* or *Thermothelomyces thermophila*) in enzyme concentrates and liquid enzyme formulations.

2 Scope

(b) (4)

3 Explanations, Terms, Abbreviations

(b) (4)

4 Procedure

4.1 Preparation of Potato Dextrose Agar (PDA)

(b) (4)

4.2 Plating of the enzyme concentrate / liquid enzyme formulation

(b) (4)

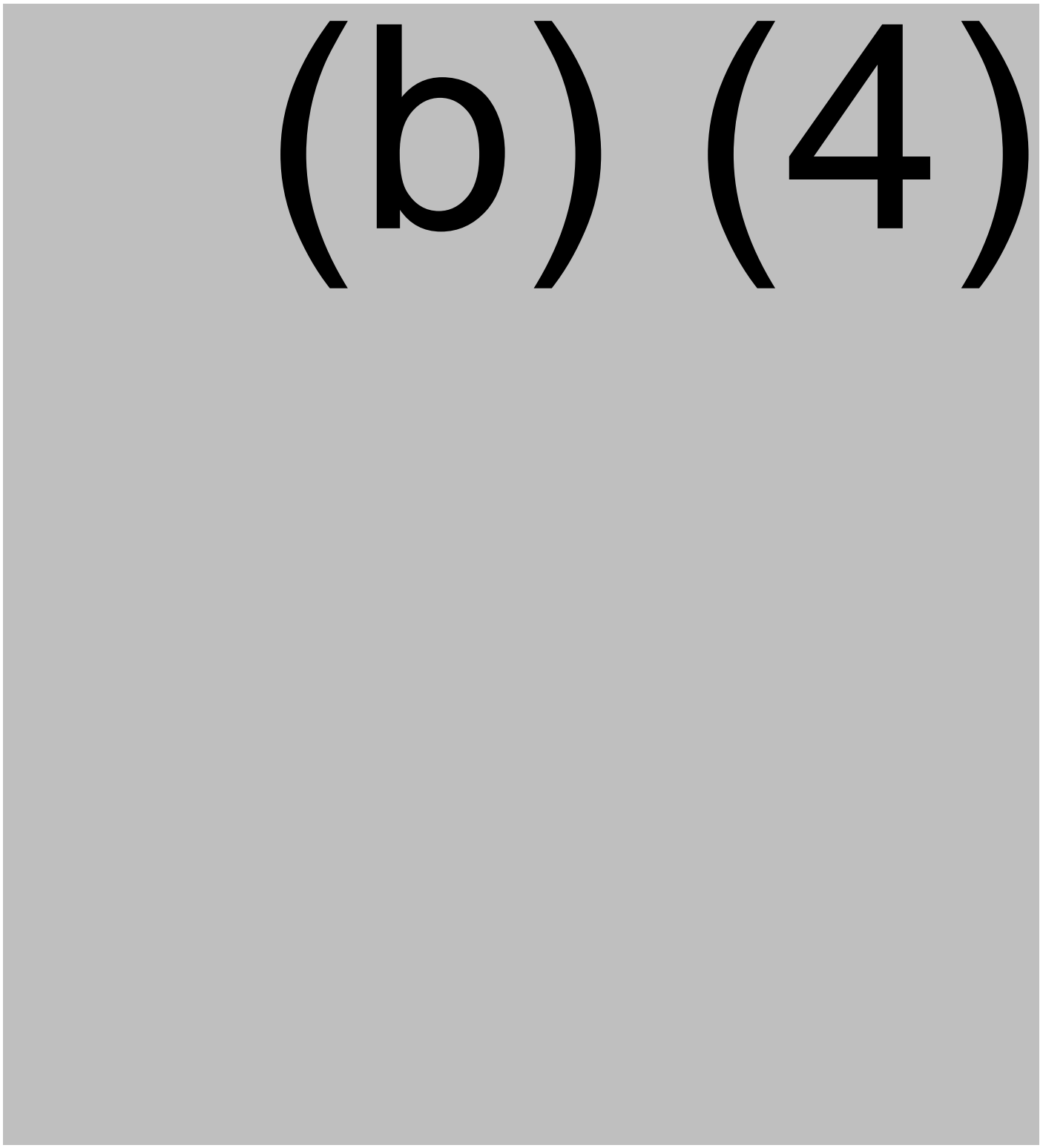
4.5 Evaluation

(b) (4)

6 Applicable documents

(b) (4)

Absence of *Aspergillus niger* or *Thermothelomyces thermophila* Production



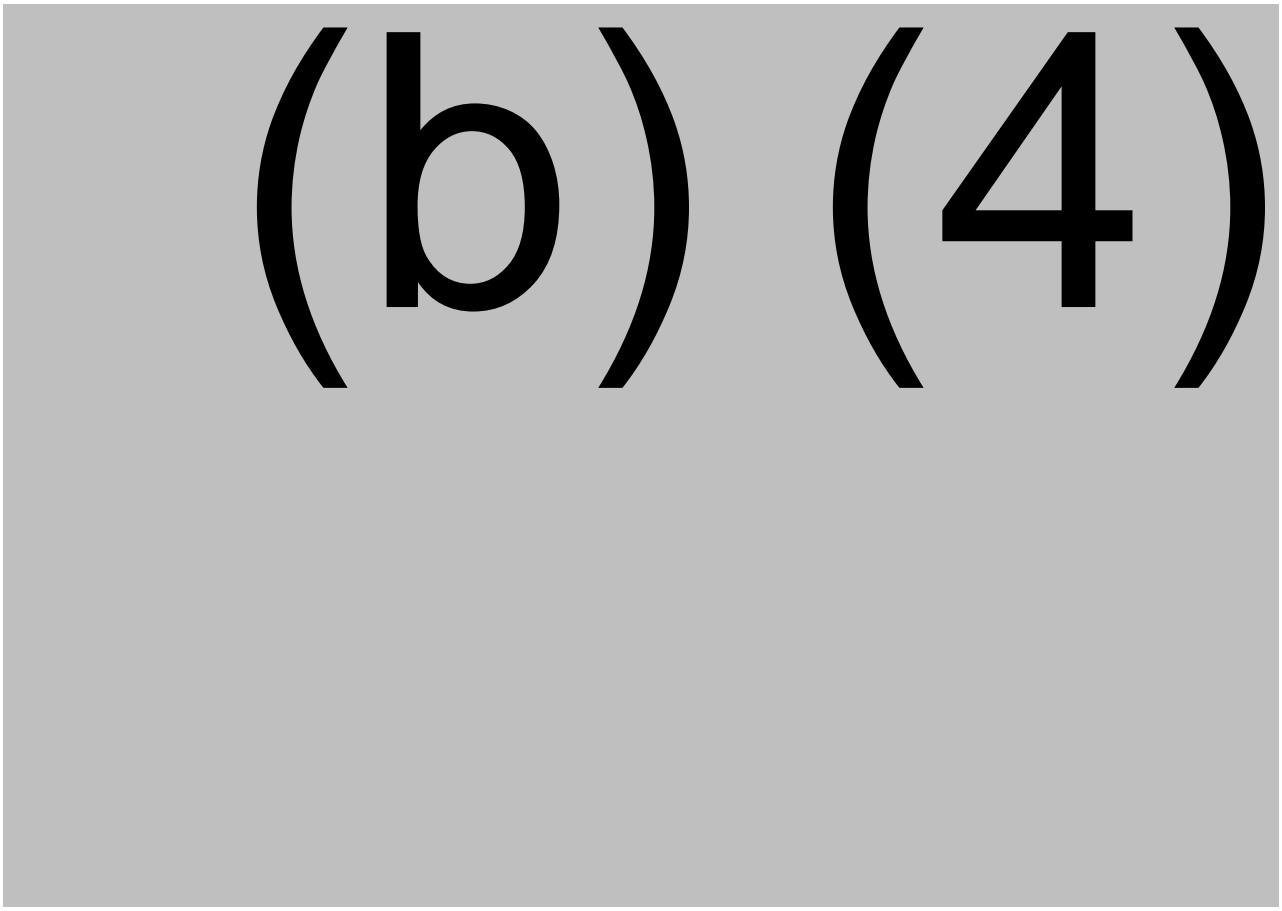
Appendix 12: Detailed Manufacturing Information: Fermentation, Recovery, and Formulation

Detailed Manufacturing Information: Fermentation, Recovery, and Formulation

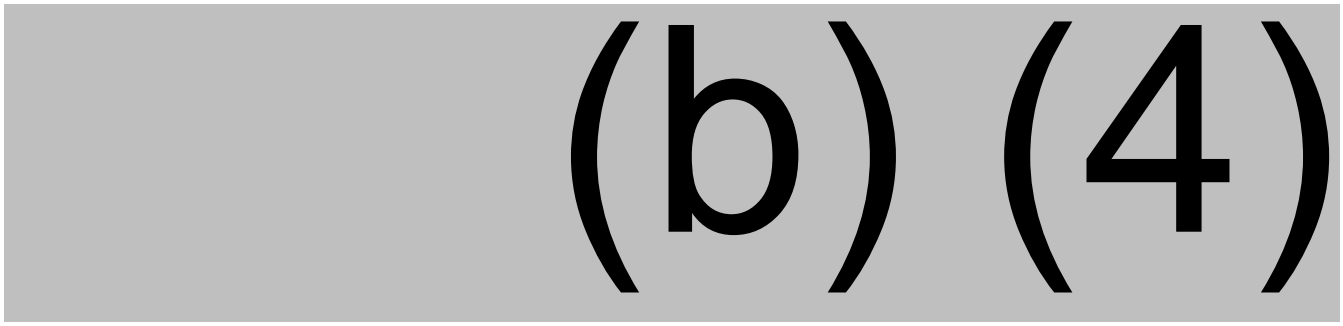
A. Fermentation

The fermentation process is described below and is illustrated in the Figure 1 below.

Figure 1. Fermentation Overview



1. Seed Build-up Fermentation



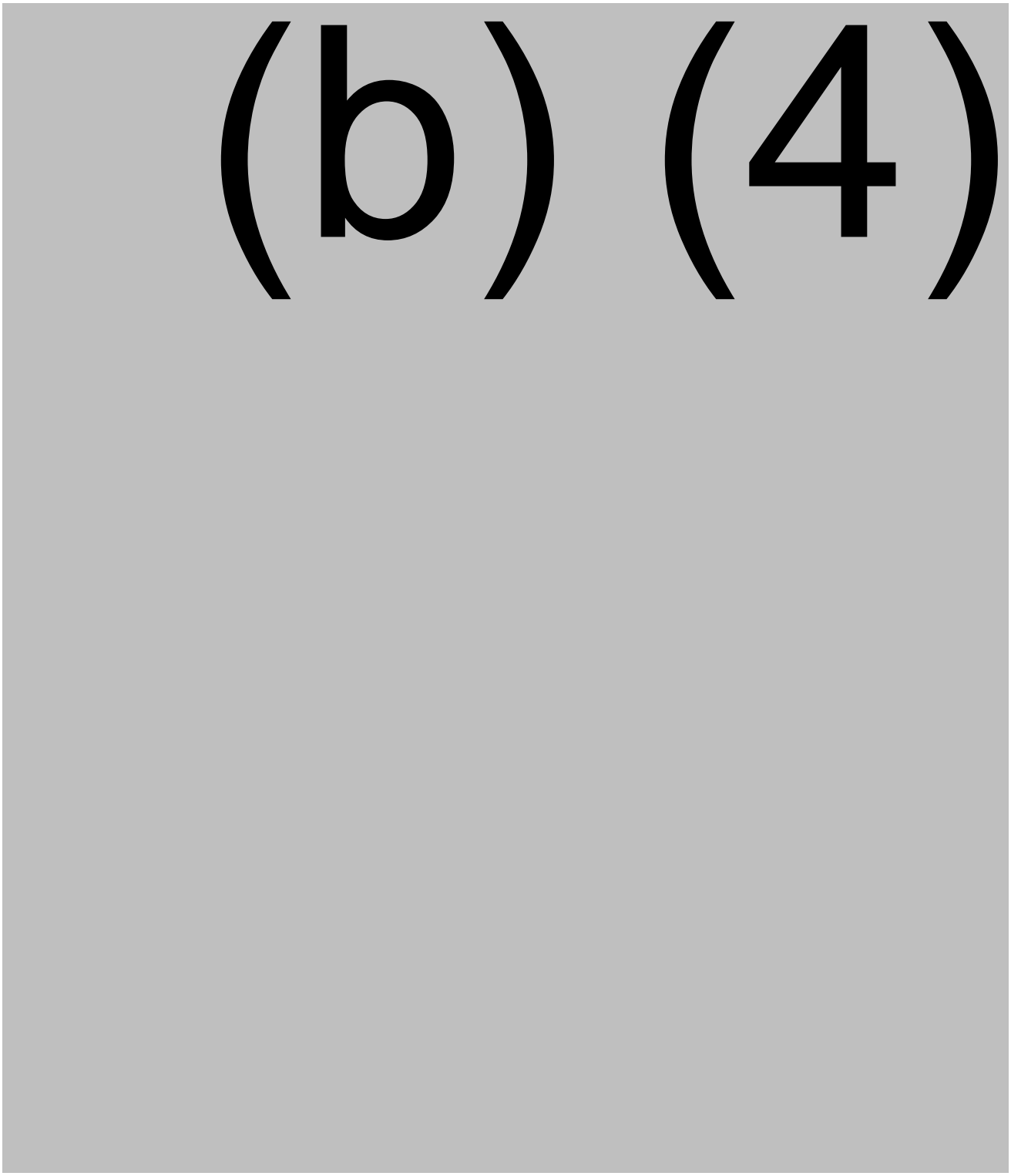
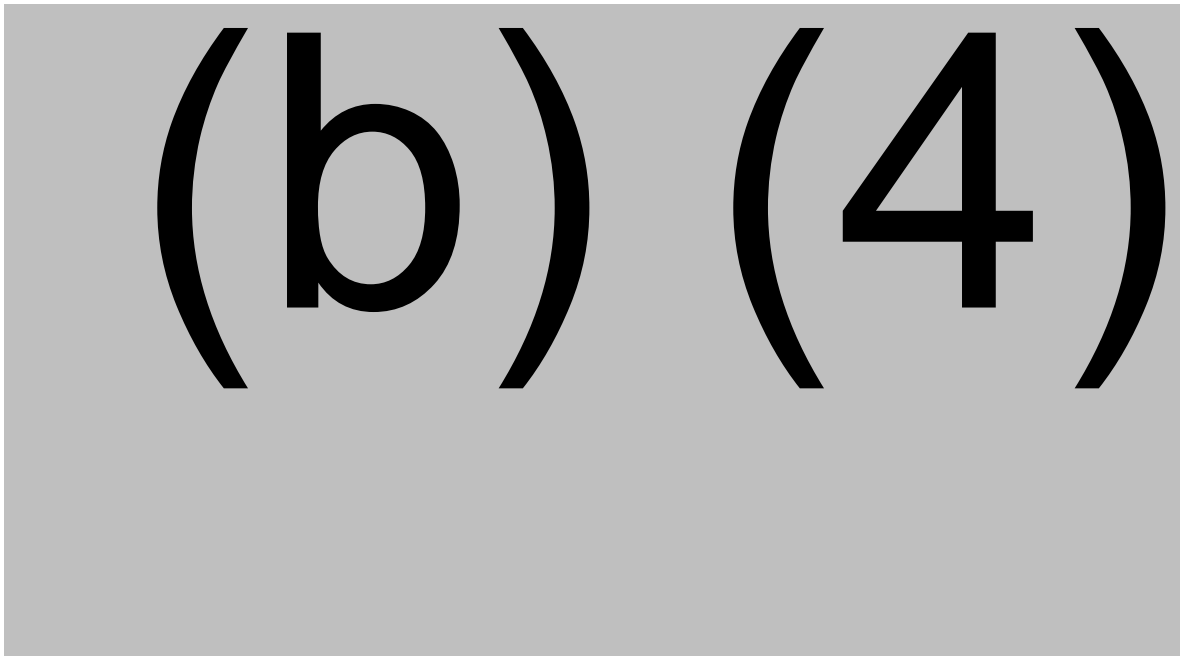

(b) (4)

Table 2. Process Control Parameters: Preculture 2 Fermentation



2. Main Fermentation

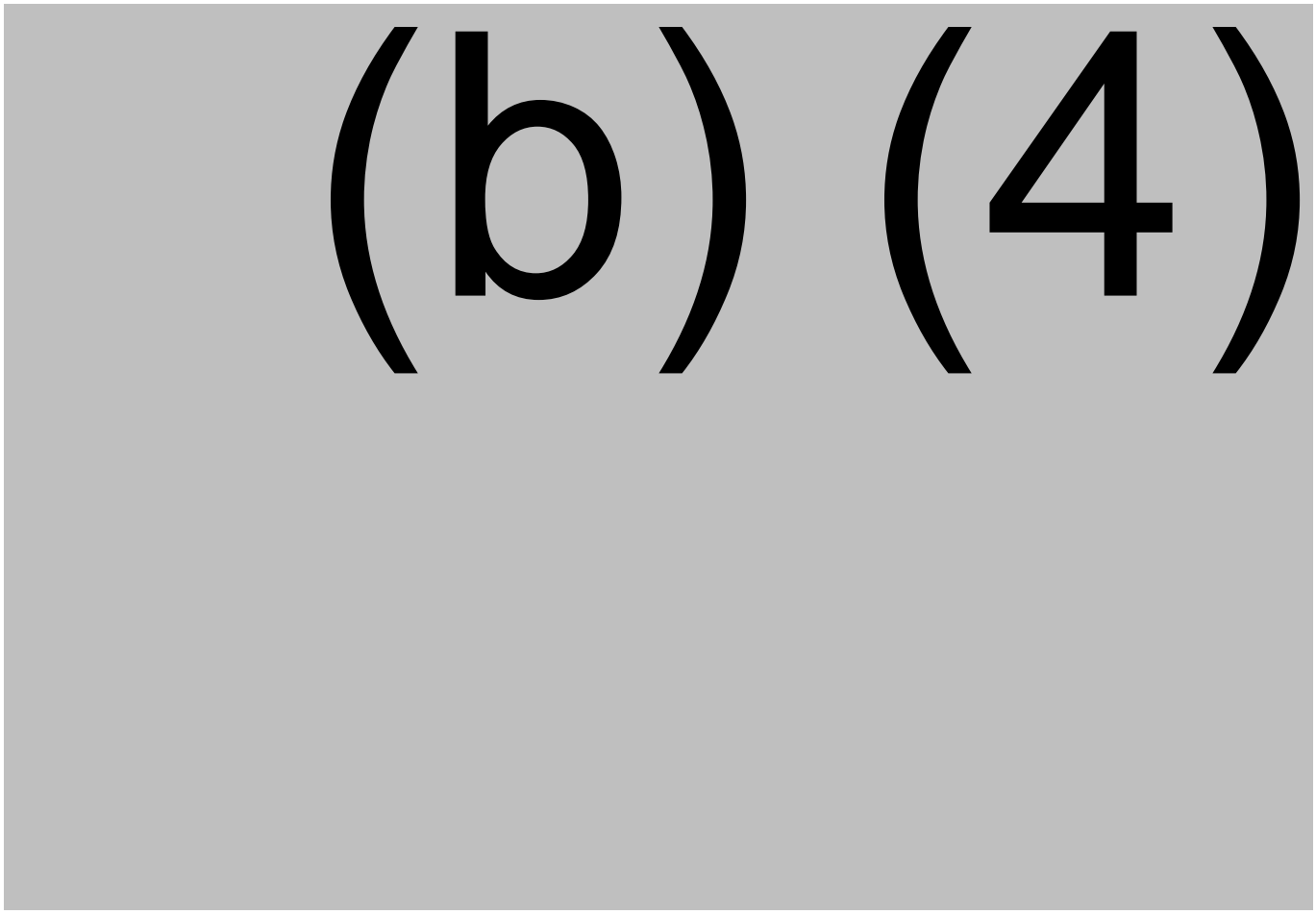


Table 3. Process Control Parameters: Main Fermentation

(b) (4)

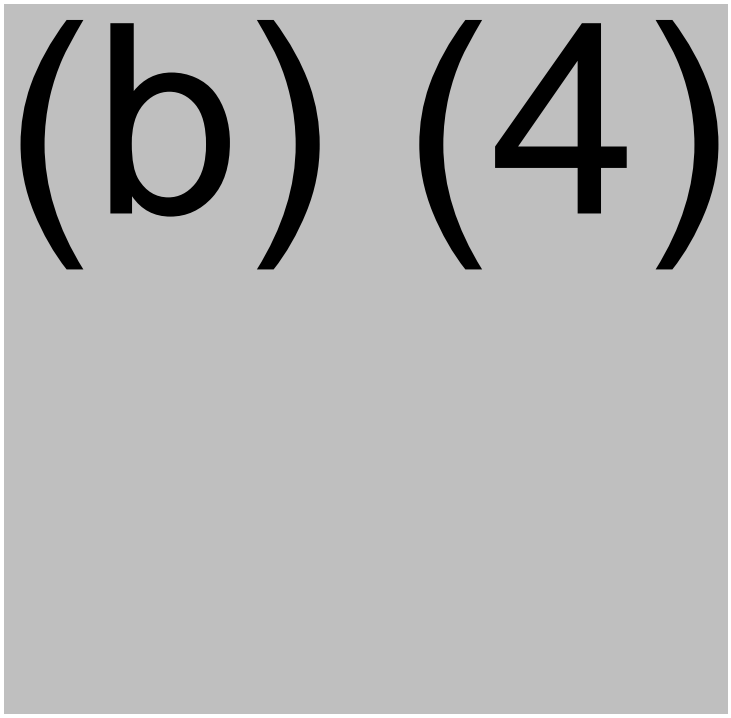
B. Cell Kill

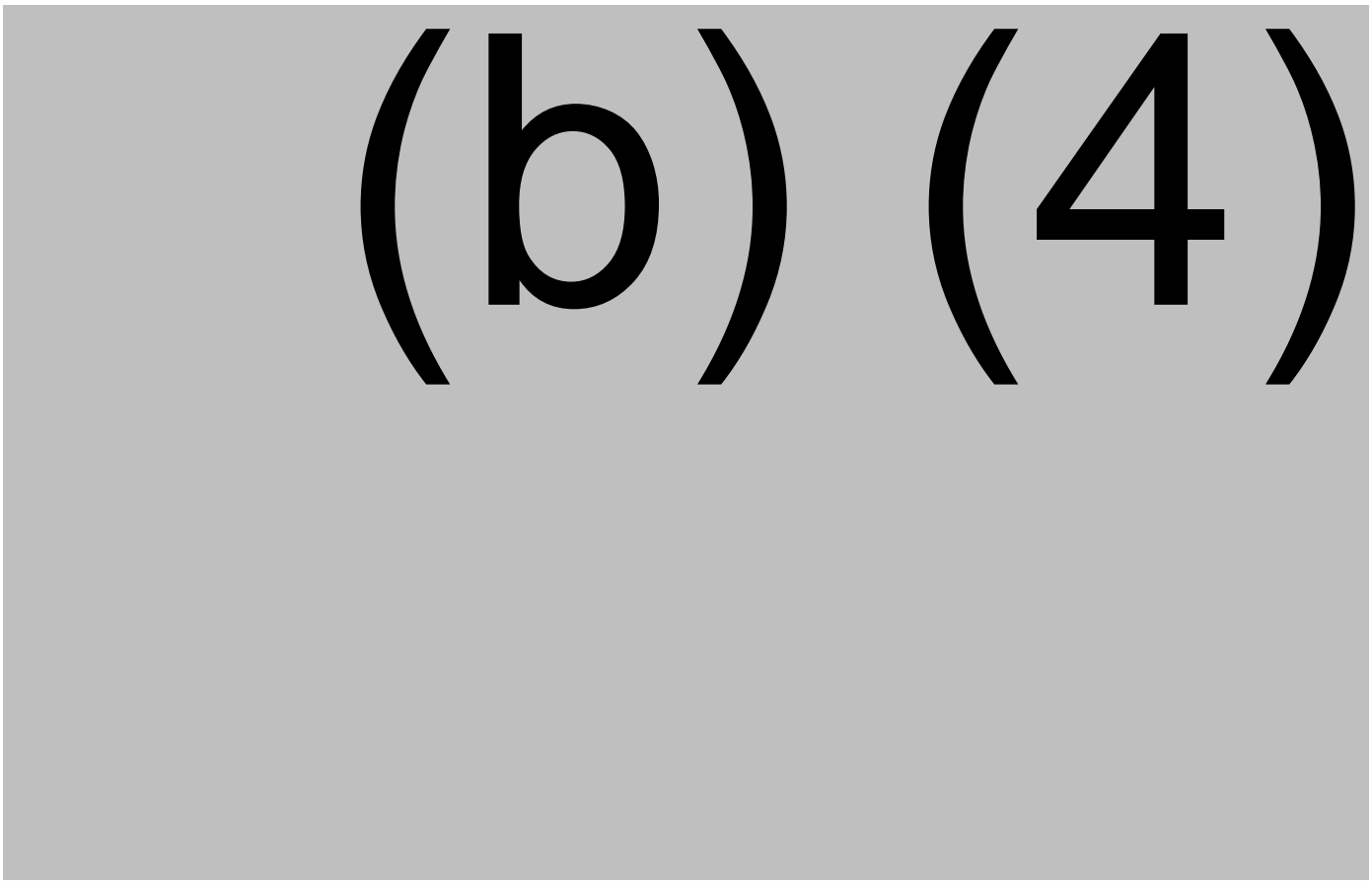
(b) (4)

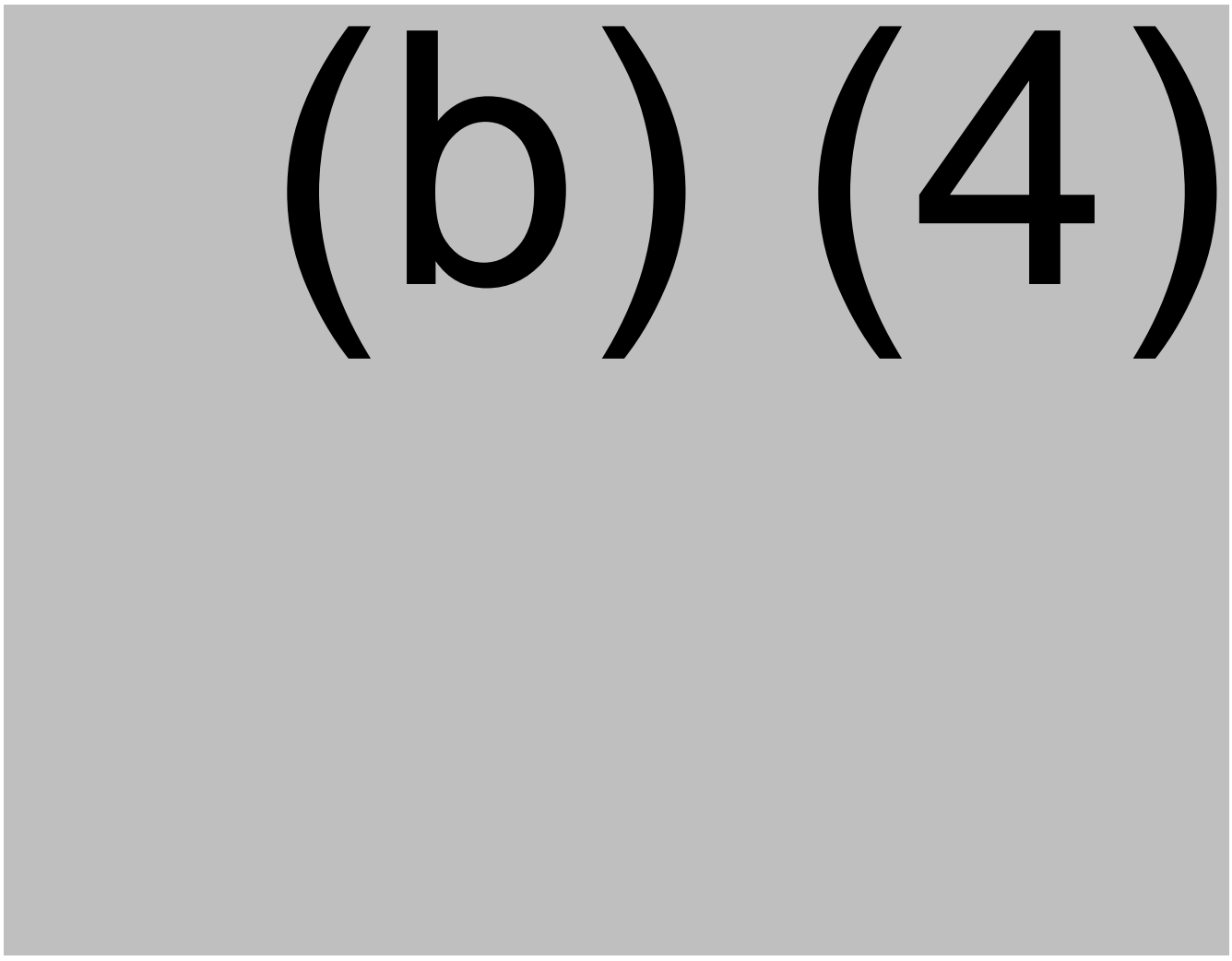
C. Recovery

(b) (4)

Figure 2. Recovery Overview

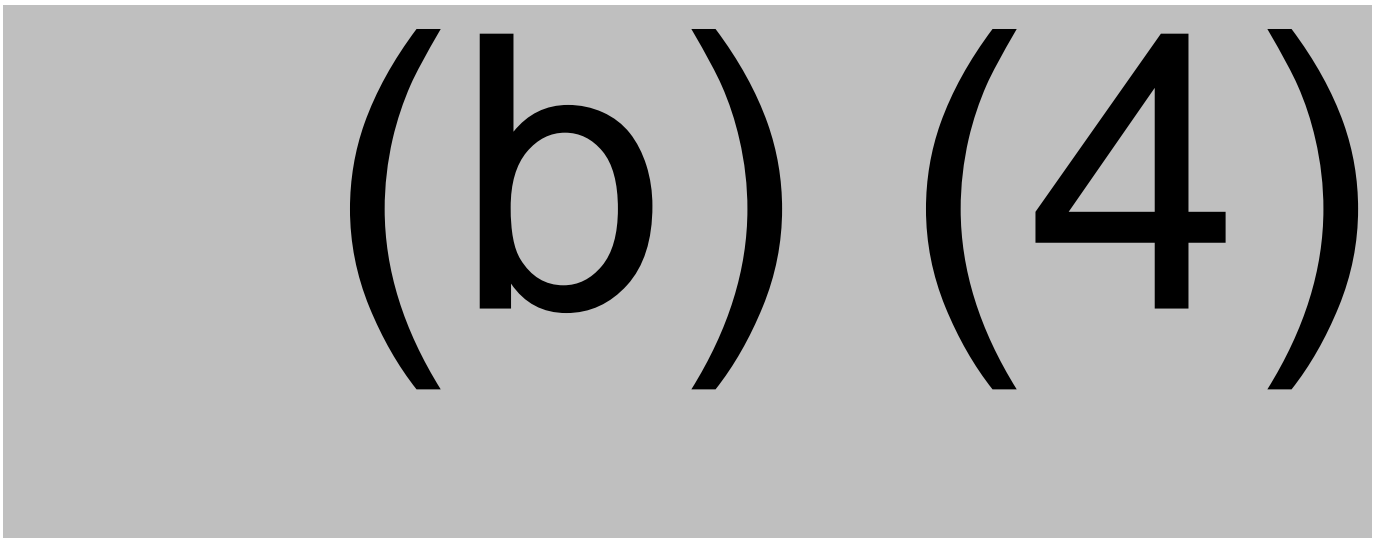


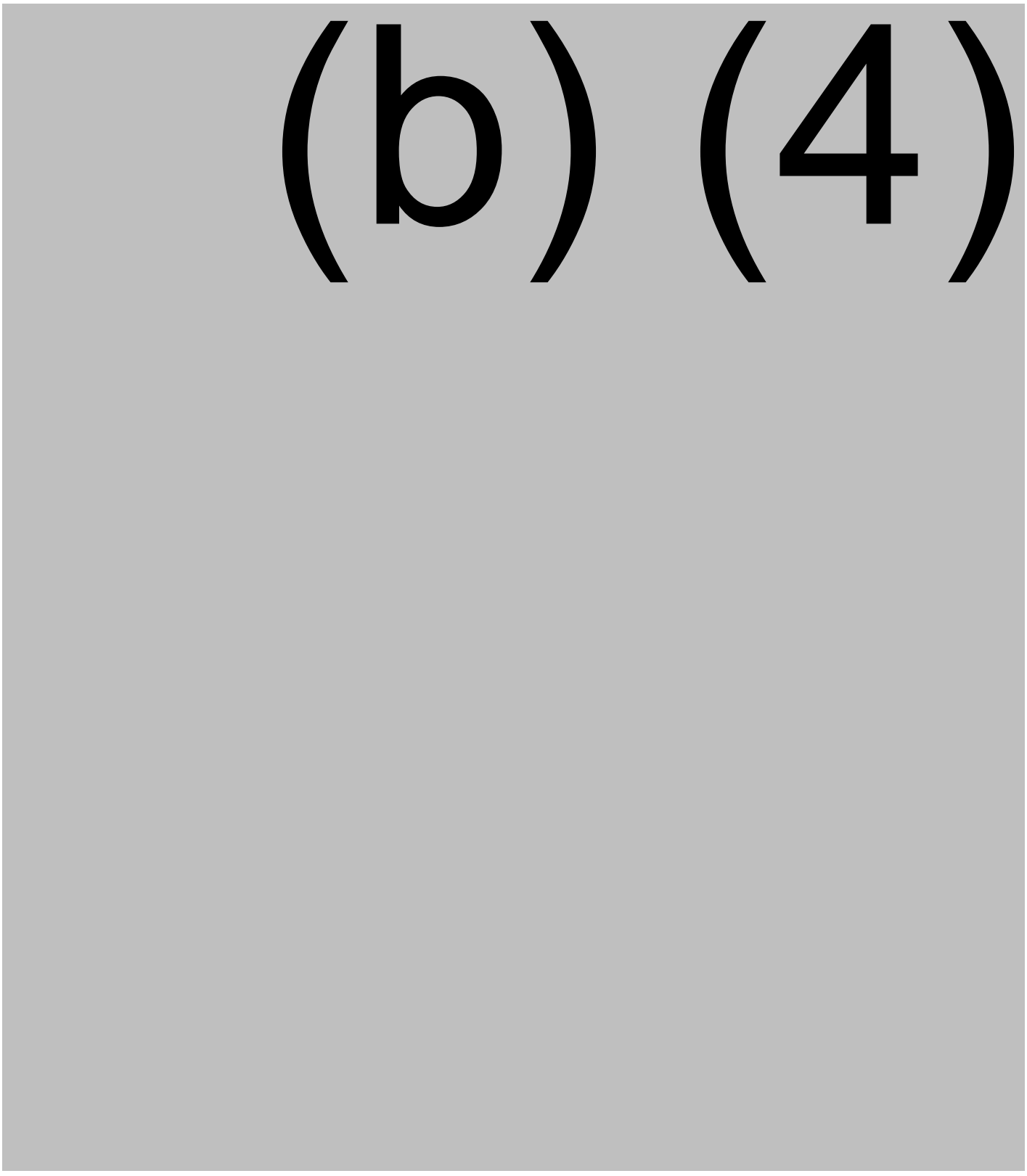




D. Formulation

1. Formulation of the Liquid Product




(b) (4)

Appendix 13: Manufacturing Raw Materials

Fermentation Media Raw Materials

NO.	INGREDIENT	FORMULA	AAFCO Listing or 21.CFR Section
1	(b) (4)		
2			
3			
4			
5			
6			
7			
8			
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10			
11			
12			
13			
14			
15			
16			
17			
18			
19			

Recovery Materials

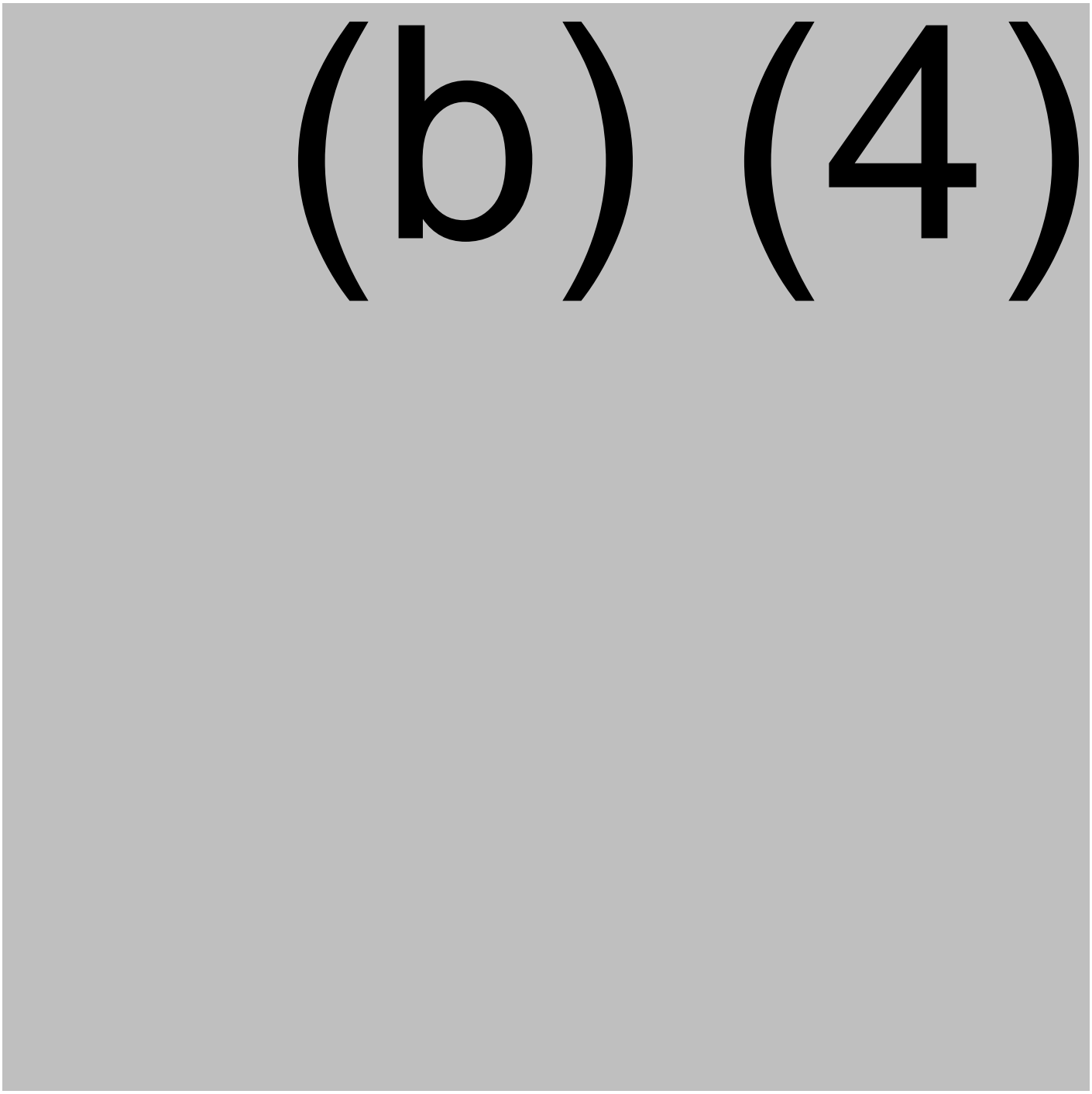
NO.	INGREDIENT	FORMULA	AAFCO Listing
17	(b) (4)		
18			
19			

Natupulse® TS Formulation

NO.	INGREDIENT	FORMULA	AAFCO Listing
20	(b) (4)		
21			
22			

Natupulse® TS L Formulation

NO.	INGREDIENT	FORMULA	AAFCO Listing or 21.CFR Section
23	(b) (4)		
24			
25			



References cited above:

Ayotte, J.D., Gronberg, J.A.M. and Apodaca, L.E. *Trace elements and radon in groundwater across the United States, 1992-2003*. 2011-5059 Survey, U. S. G. pp. i-115 (2011).

Enzyme Technical Association. Olempska-Beer, Z. *Flocculants, Antifoams, and Enzyme Use Levels.* (4/24/1998). Written Communication.

FDA Center for Food Safety and Applied Nutrition. Enzyme Technical Association. *Defoaming and Flocculating Agents Used in the Manufacture of Enzyme Preparations Used in Foods.* (2003). Written Communication.

(b) (4)

SPECIFICATIONS:

(b) (6), (b) (4)

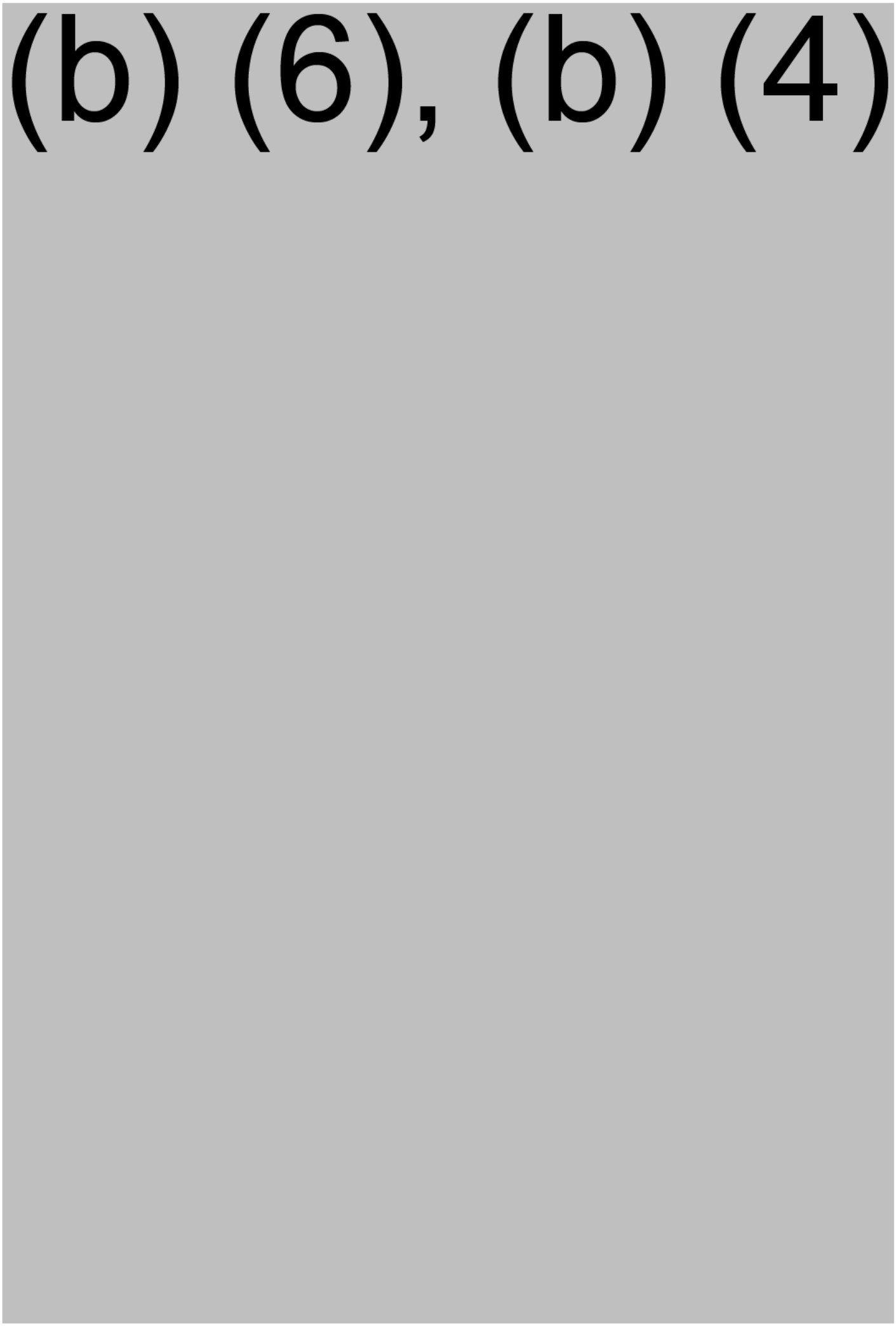
22/01

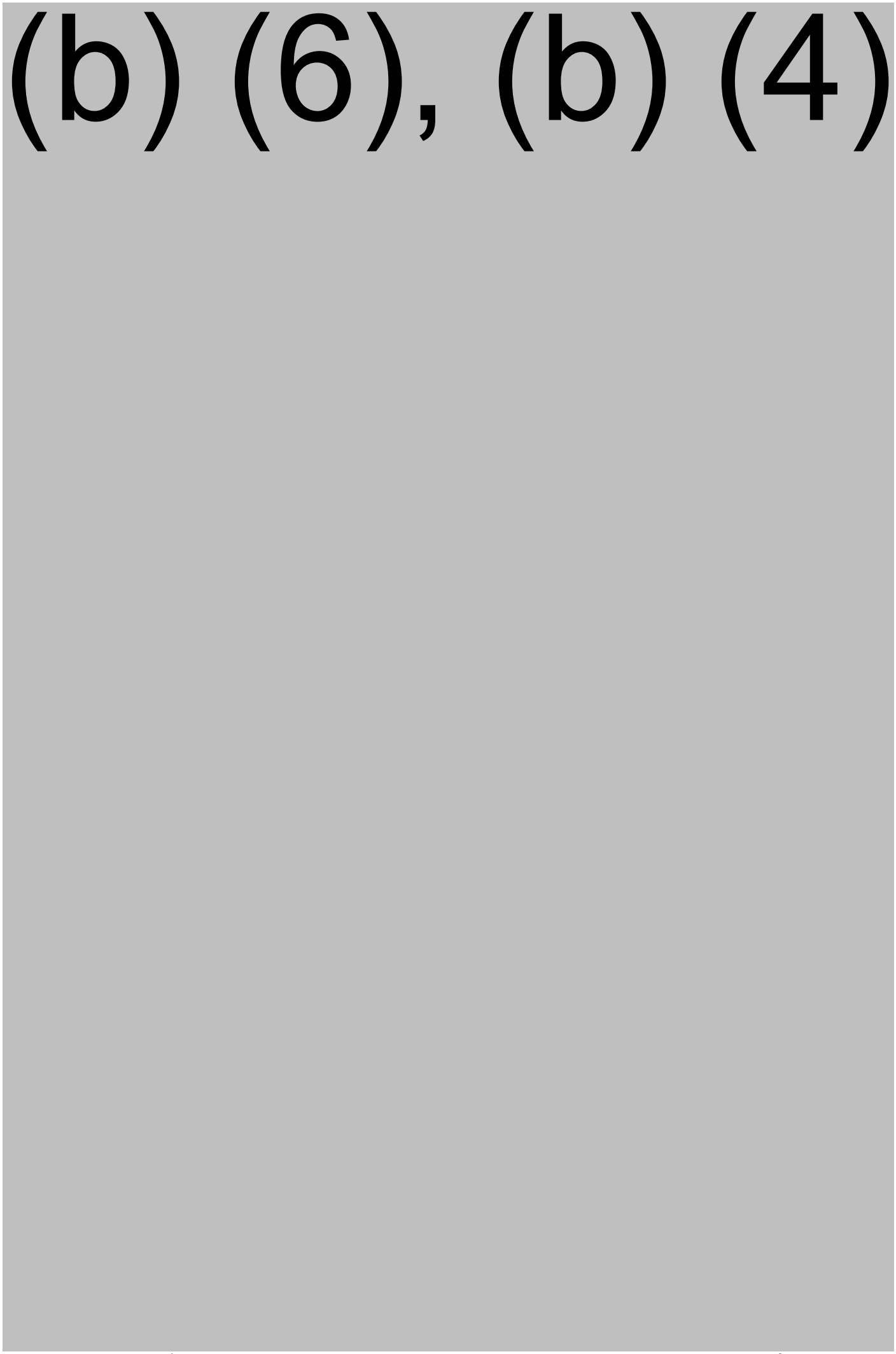
(b) (4) **Certificate of Analysis**

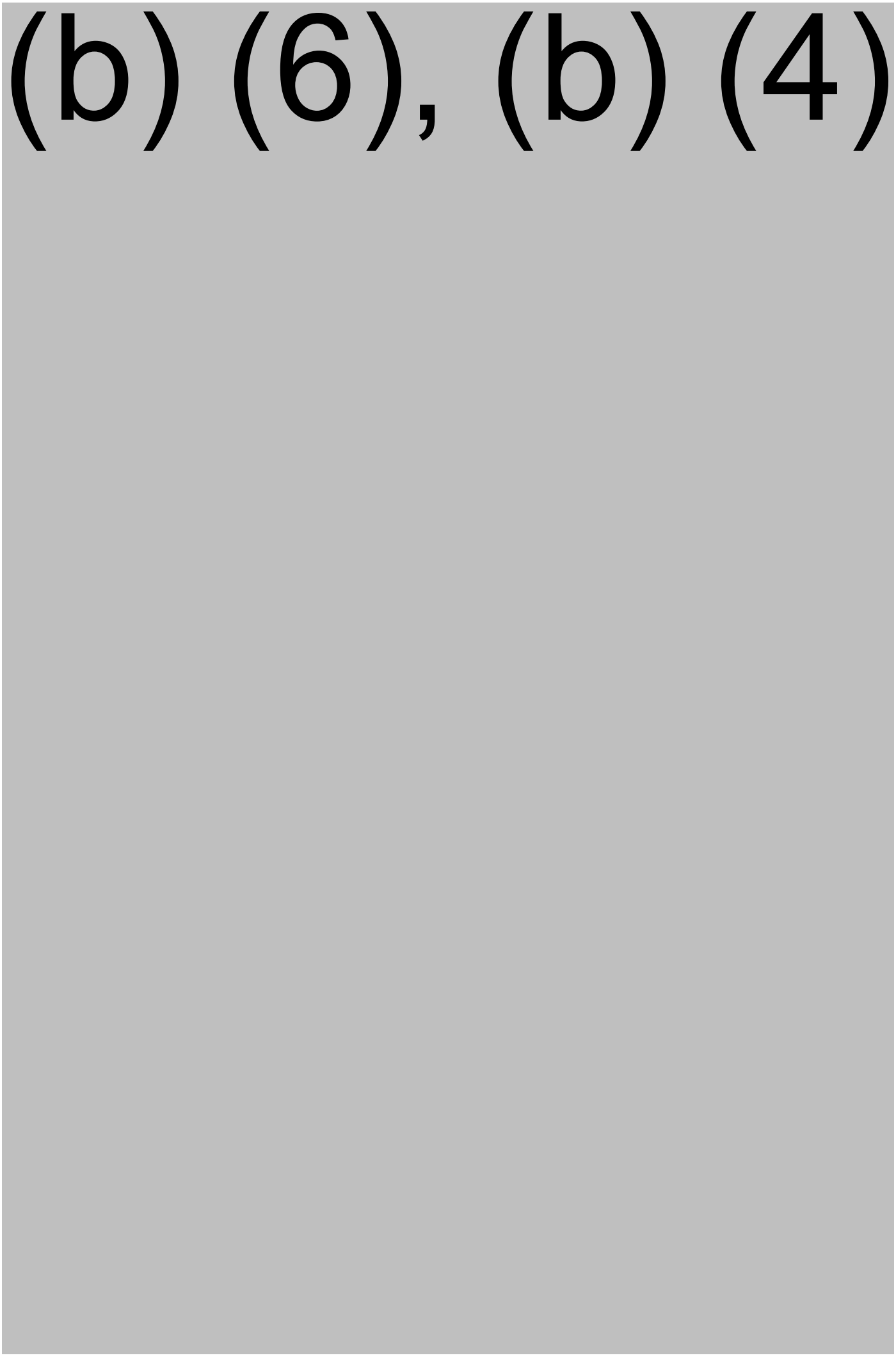
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Versie: 1
08/06/2016
1/1

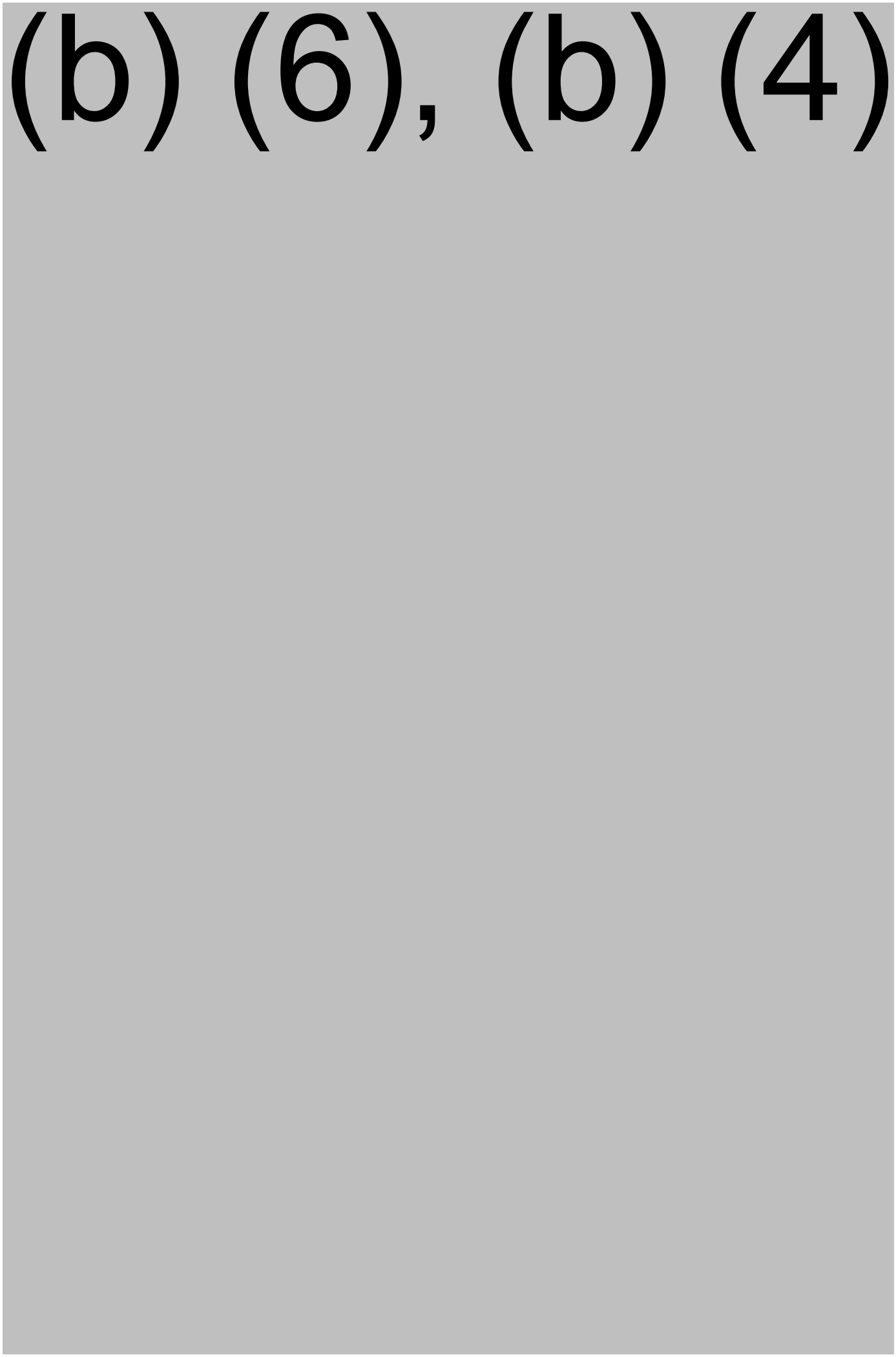
Customer: BASF SE

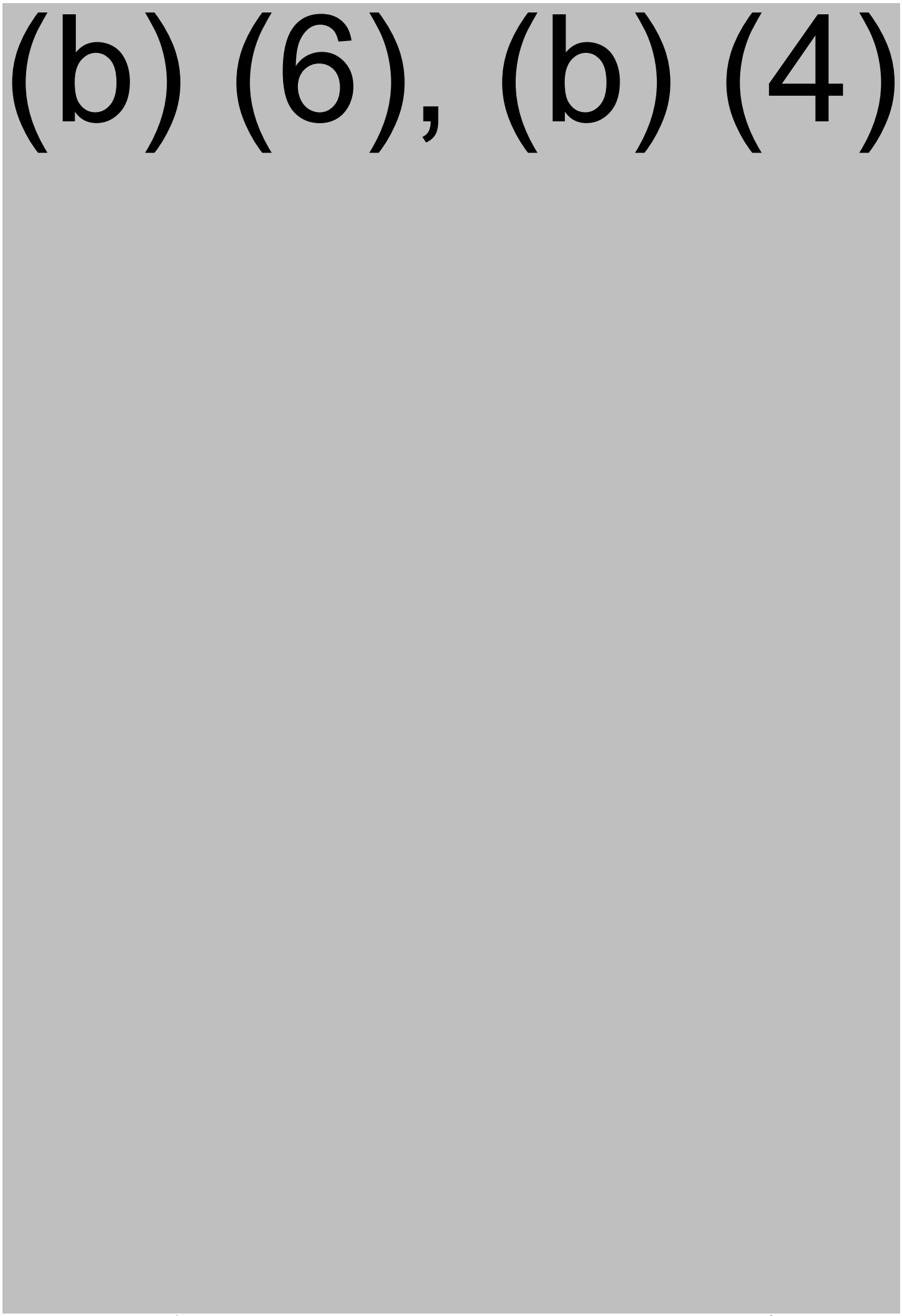
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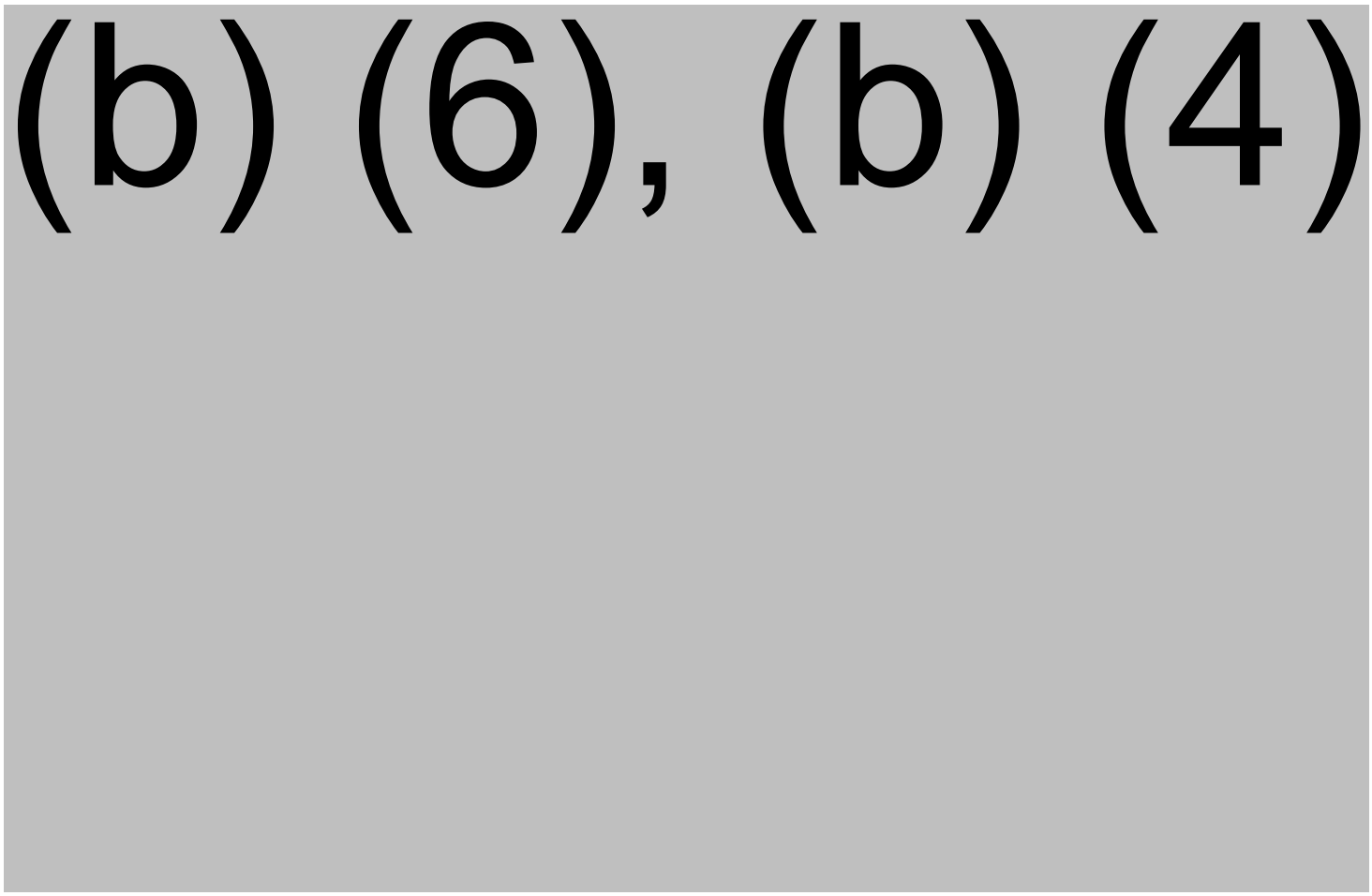

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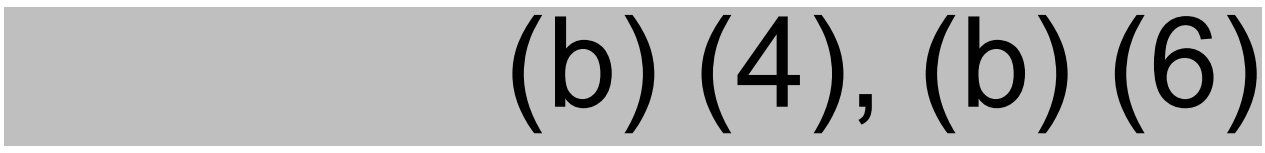

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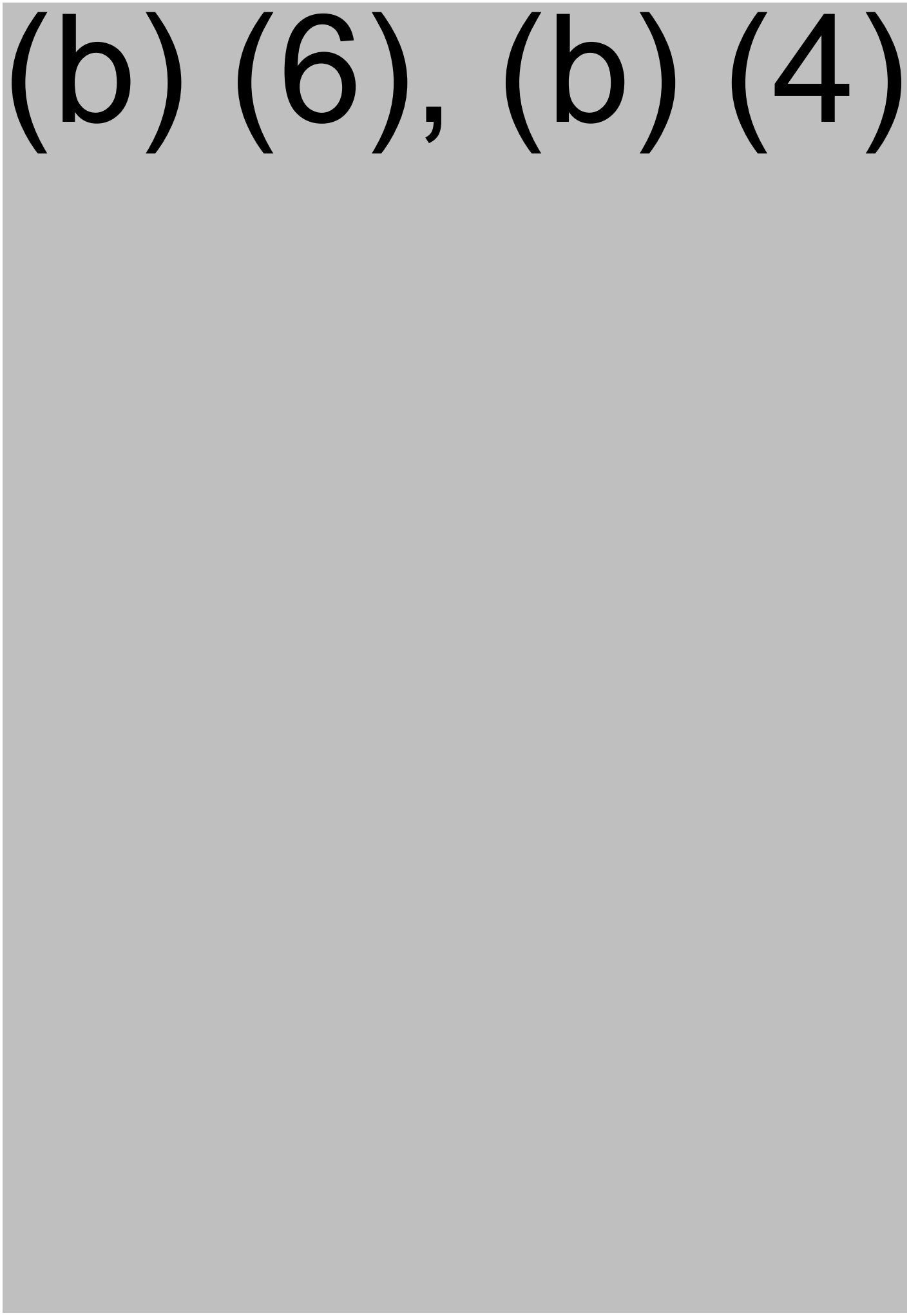

(b) (6), (b) (4)


(b) (6), (b) (4)

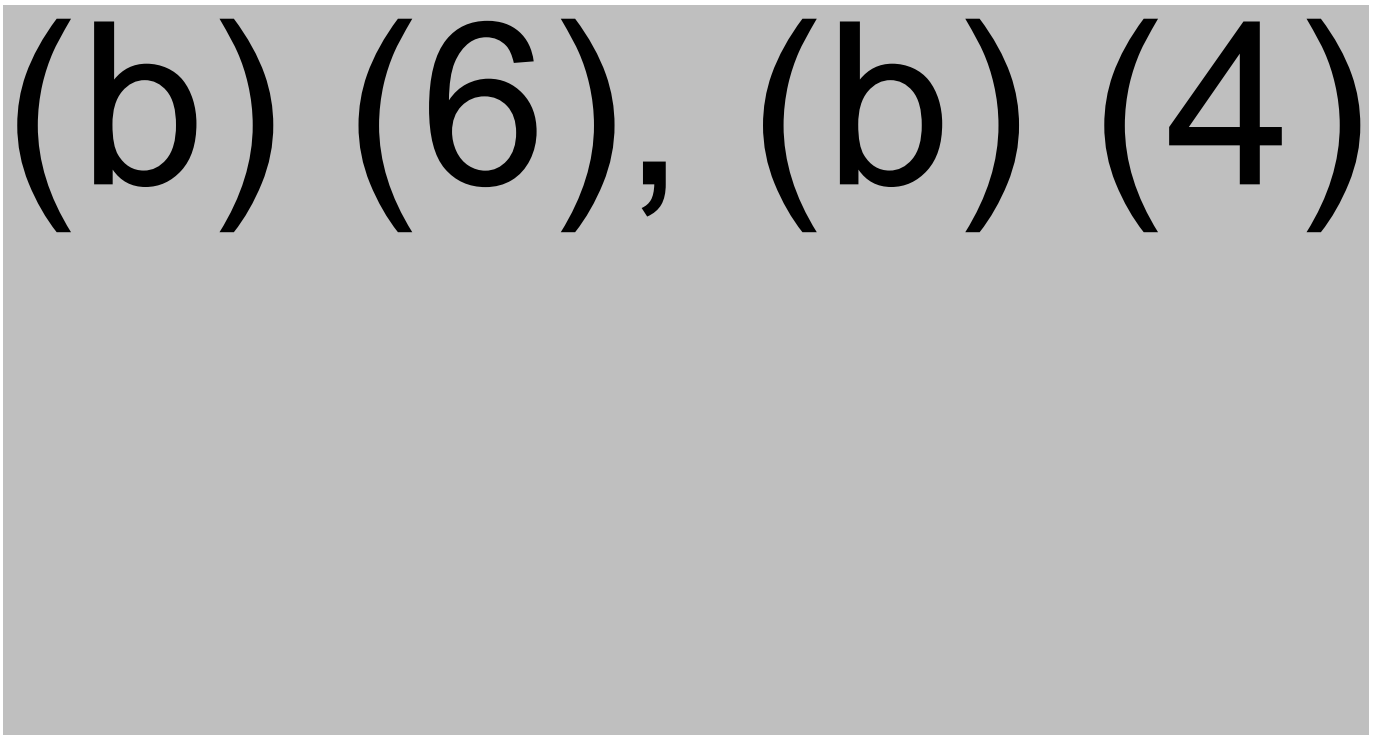

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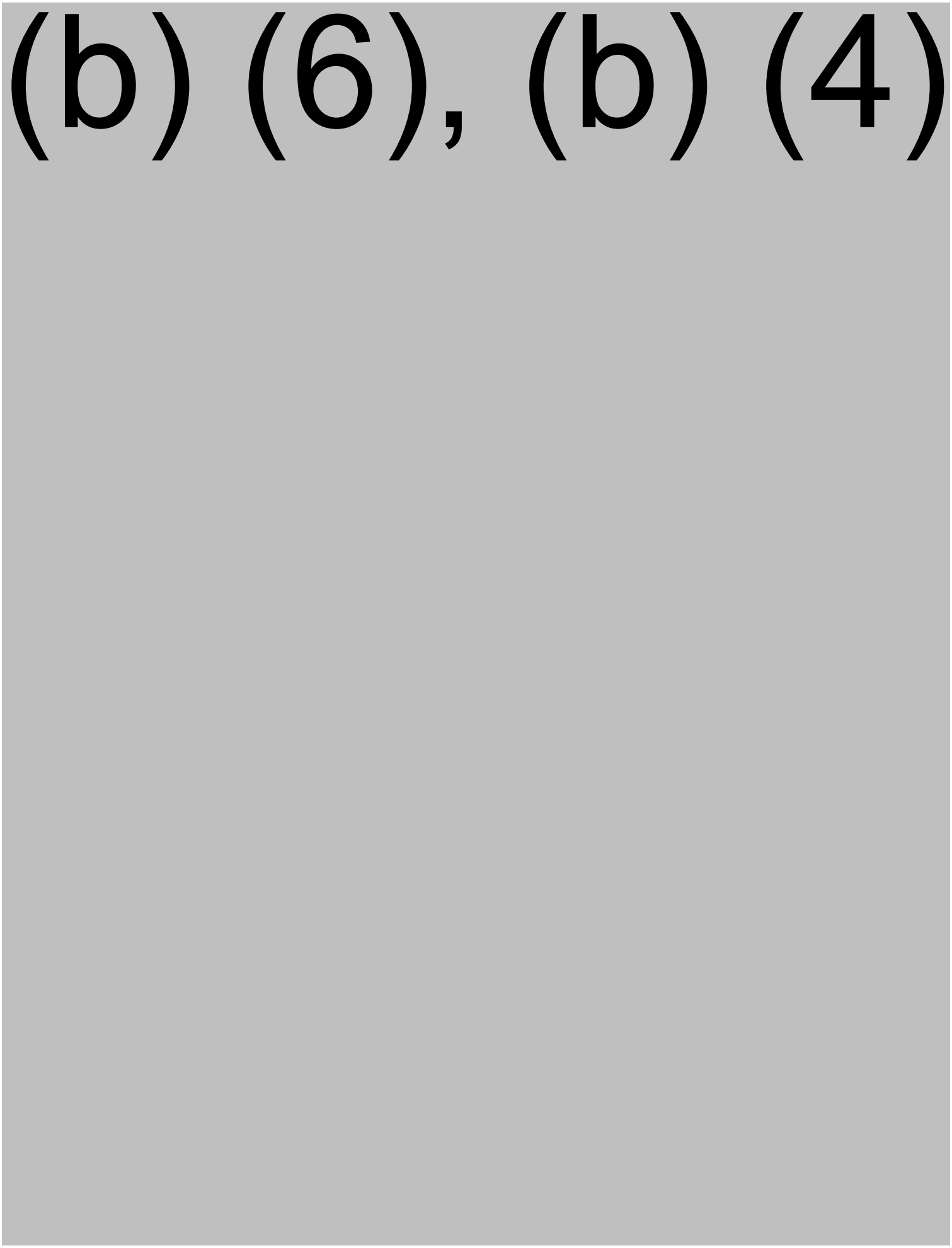

(b) (6), (b) (4)

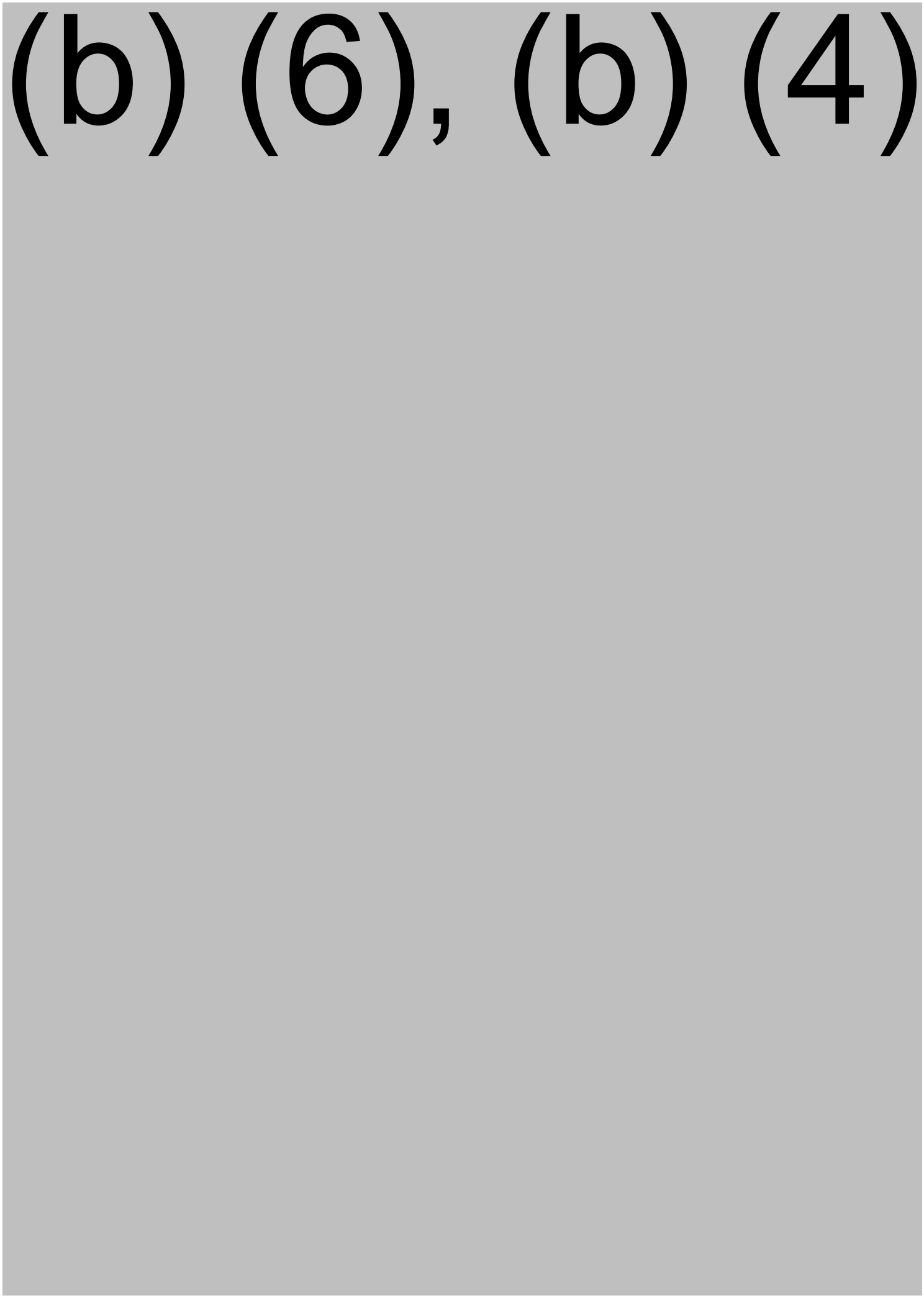

(b) (4), (b) (6)

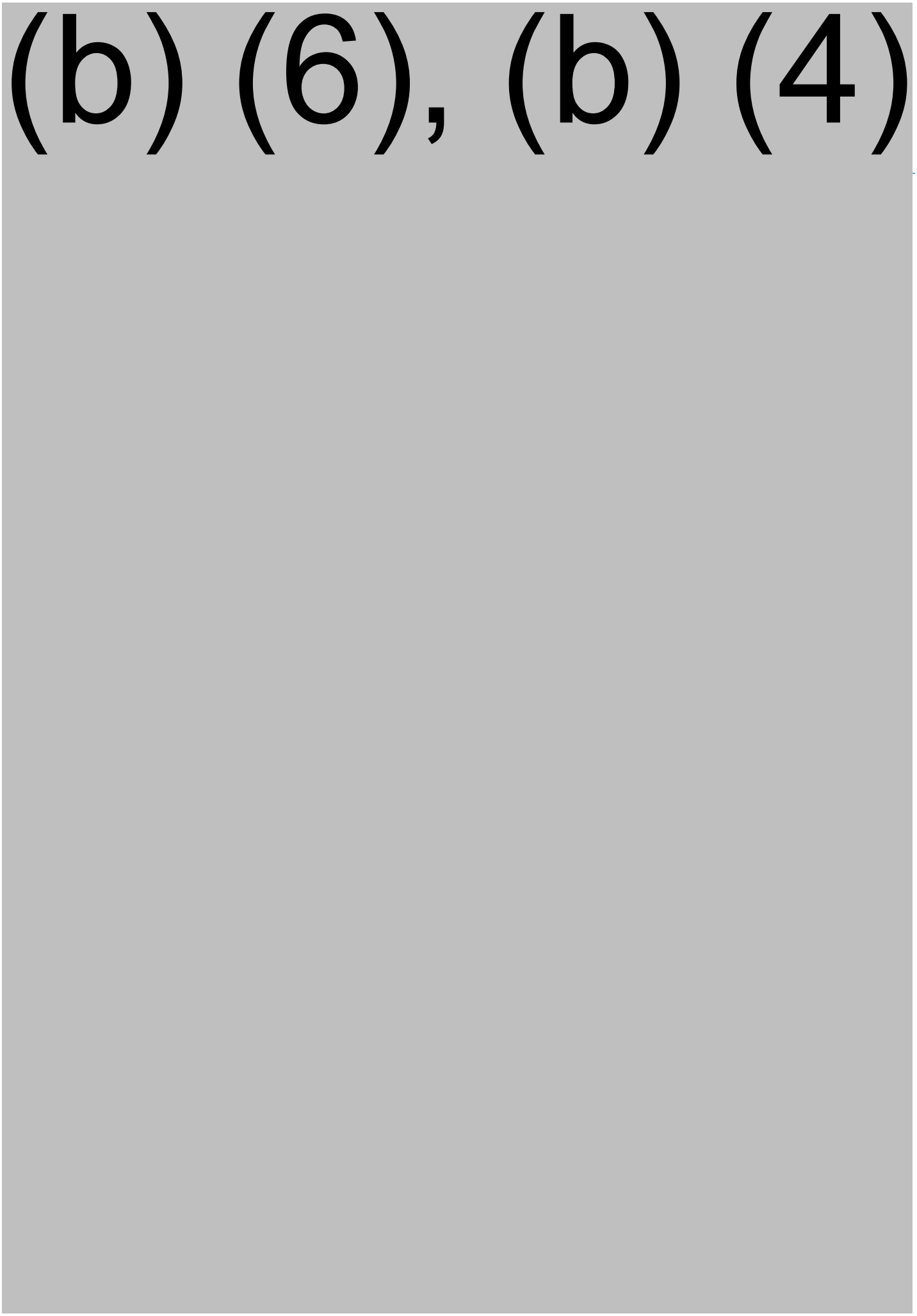


111A


(b) (6), (b) (4)


(b) (6), (b) (4)


(b) (6), (b) (4)


(b) (6), (b) (4)

Appendix 14: Product Compositions

Natupulse® TS composition

Natupulse® (b) (4) (b) (4)	(b) (4)
Mannanase activity	
Wheat bran	
Magnesium sulfate	
Vegetable oil	

Natupulse® TS L composition

Mannanase CC	(b) (4)
Mannanase activity	
Glycerol	
Sodium benzoate	
Water	

Appendix 15: Mannanase Activity Assay

Table of Contents

Clarification for the Calculation of Dilution Factors: 2
Appendix 15a: Activity Assay FEN-0003– Reference Materials 3
Appendix 15b: Activity Assay FEN-0004– Feed Additives 11
Appendix 15c: Activity Assay FEN-0005– Premix and Feeds 21
Appendix 15d: EURL Evaluation Report 30
Appendix 15e: Validation Reports 41

Clarification for the Calculation of Dilution Factors:

Regarding FEN-0004 – Photometric assay method for the determination of mannanase 19287 activity in feed additives, page 17, Table 4a:

Table 4 a
Mannanase SD powder
(b) (4)

Please note, the method calculates the dilution steps based on the dilutions (b) (4) In FEN_004 – "Photometric assay method for the determination of mannanase 19287 activity in feed additives", the calculation of the total dilution factor takes into account the extraction volume (b) (4) This is indicated below in Table 1 copied from page 16: "The extraction volume was taken into consideration when the dilution factor was calculated."

(b) (4)

Appendix 15a: Activity Assay FEN-0003– Reference Materials

Method of Analysis FEN/0003/01

BASF
We create chemistry

Title: **Determination of β-Mannanase activity in reference materials using DNS**

Effective date: April 20th, 2018

Prepared by: (b) (6)

Reviewed and approved: Dr. Benjamin Peikert

(b) (6)

Signature, date

1 Keywords

1.1 To be determined

Mannanase activity - Reference method

1.2 Matrix

Reference materials such as

- Mannanase SD powder

2 Principle

Mannanase in the sample hydrolyses the substrate, galactomannan, and the amount of released reducing carbohydrates is determined spectrophotometrically using (b) (4) .

One mannanase unit (TMU) is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one µmol mannose from mannan in one minute under the assay conditions

3 Working range

The working range of this method is (b) (4) per ml.

4 Safety note

The method described involves the handling of hazardous substances. Attention is therefore drawn to the various provisions governing the handling of potentially dangerous material.

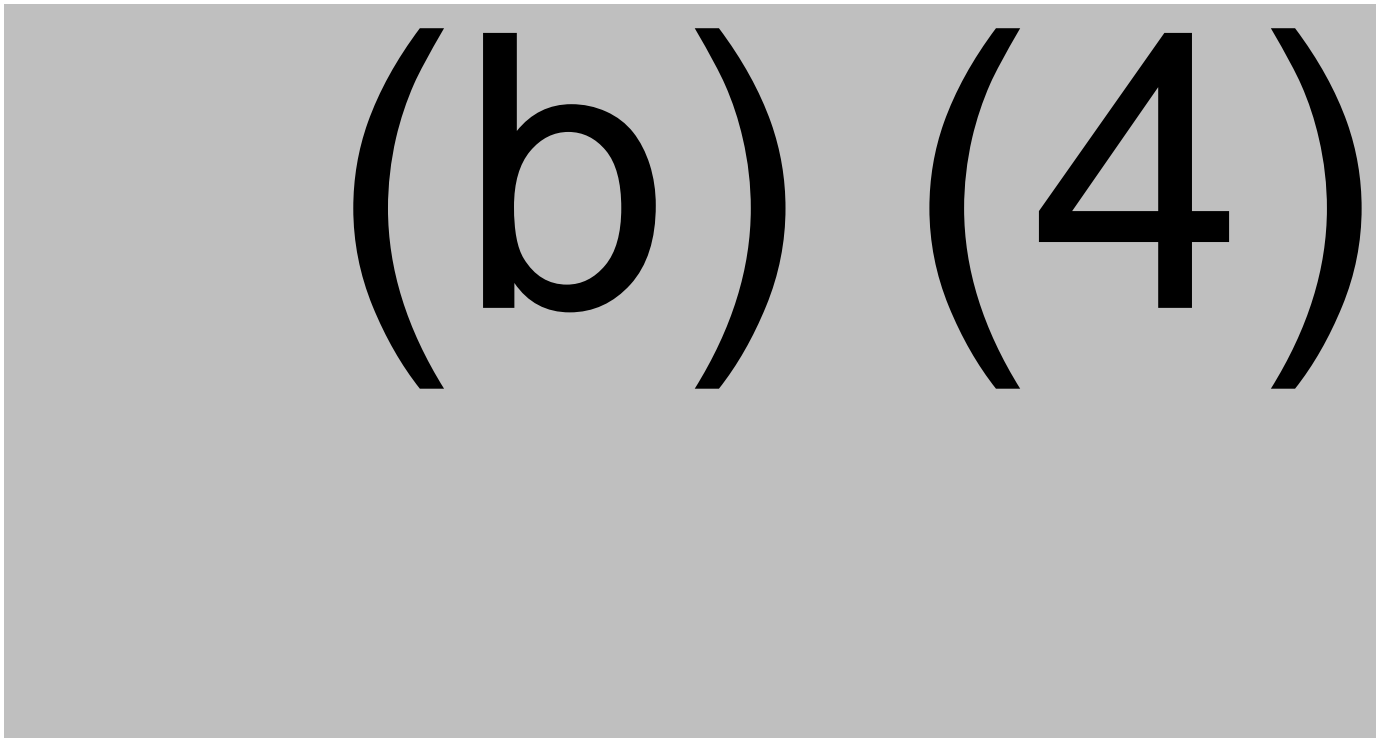
5 Reagents

(b) (4)

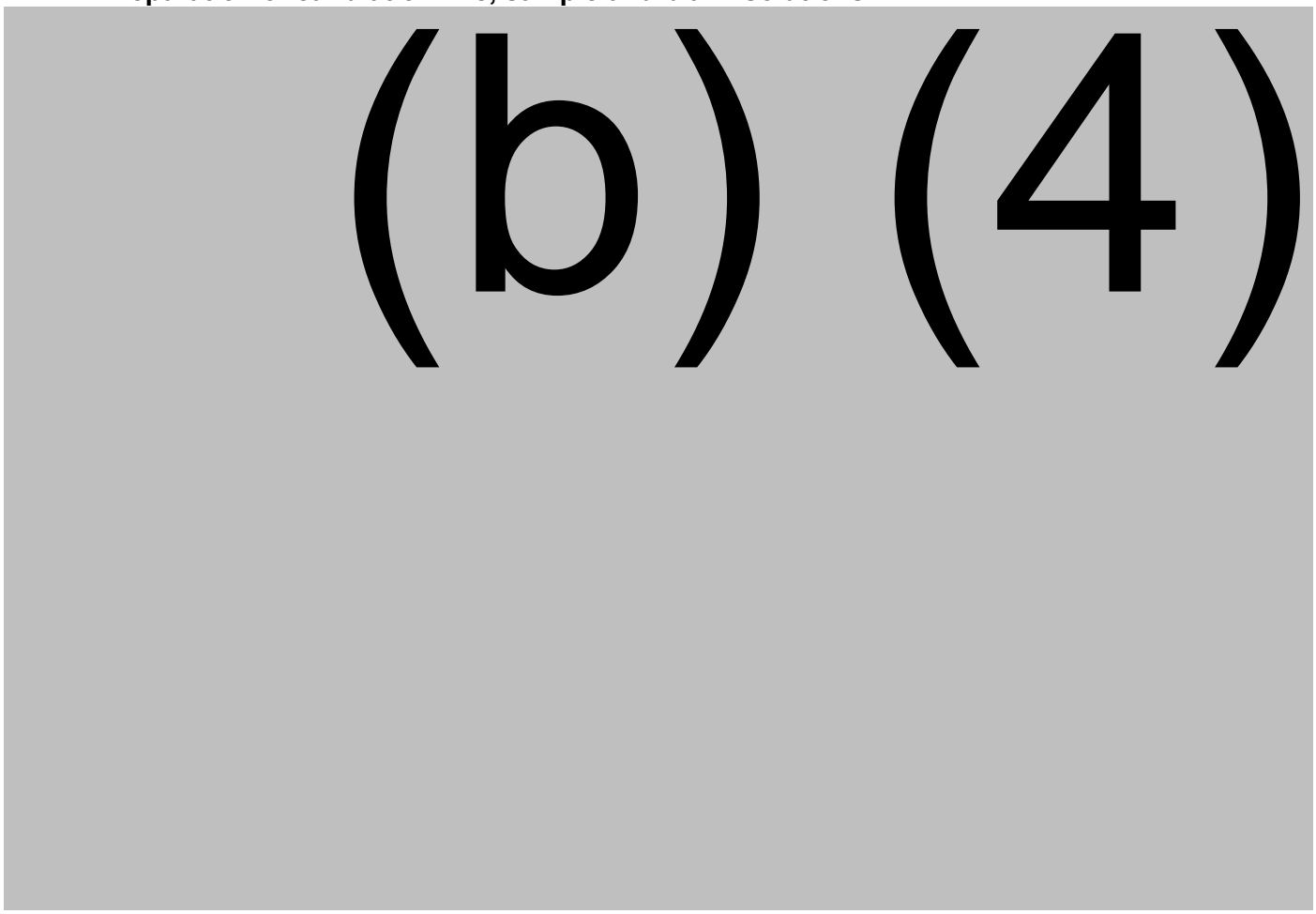
(b) (4)

6 Apparatus

(b) (4)



7 Preparation of calibration line, sample and blank solutions



(b) (4)

8 Procedure

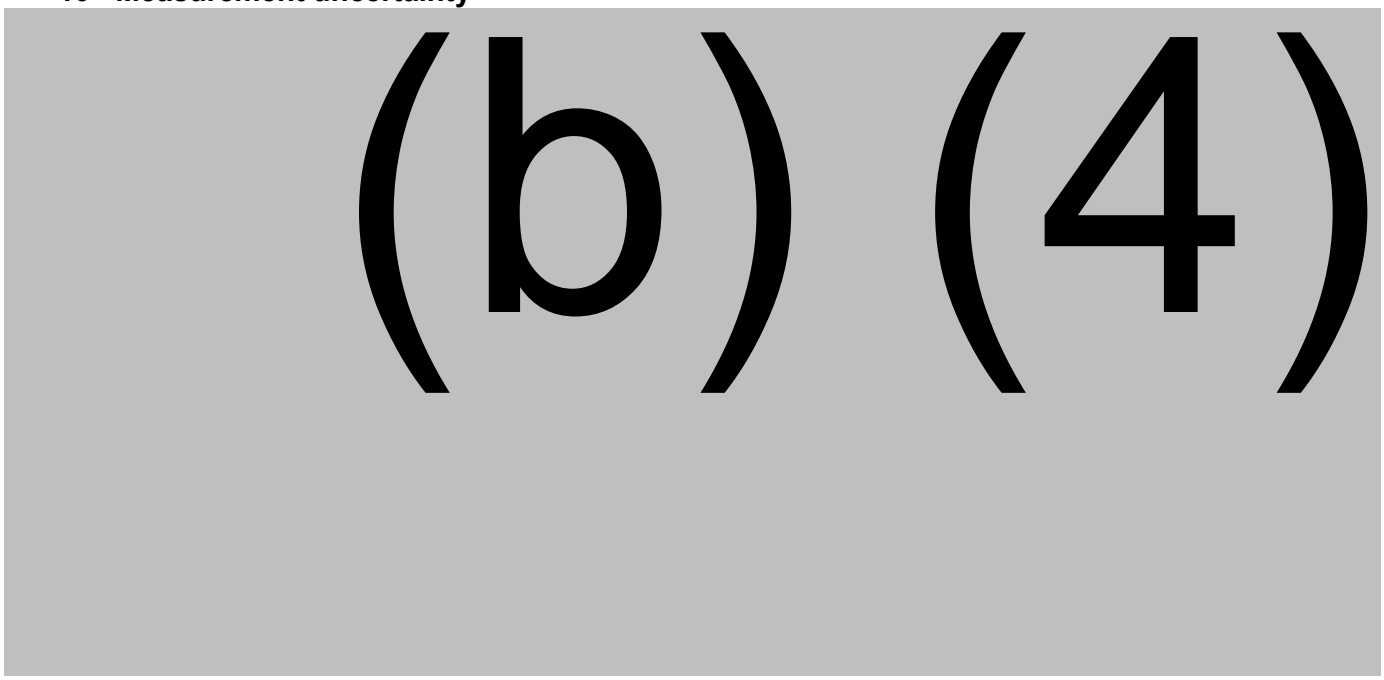
(b) (4)

(b) (4)

9 Calculation of mannanase activity

(b) (4)

10 Measurement uncertainty



Appendix 15b: Activity Assay FEN-0004– Feed Additives

Method of Analysis FEN/0004/01

BASF
We create chemistry

Title: **Photometric assay of mannanase activity in feed additives**

Effective date: April 20th, 2018

Prepared by: (b) (6)

Signature, date

Reviewed and approved: Dr. Benjamin Peikert

(b) (6)

Signature, date

1 Keywords

1.1 To be determined

Mannanase activity

1.2 Matrix

Feed additives such as

- Mannanase SD powder
- Mannanase CC
- Mannanase 8000
- Mannanase 8000 L

2 Principle

The assay procedure is specific for endo-1,4-β-D-mannanase activity. On incubation of dyed carob galactomannan with β-mannanase, the substrate is depolymerised by an endo-mechanism to produce low-molecular weight dyed fragments which remain in solution on addition of ethanol to the reaction mixture. High-molecular weight material is removed by centrifugation, and the colour of the supernatant is measured. By reference to a standard curve, endo-β-mannanase in the assay solution is determined.

3 Working range

The working range of this method is (b) (6) per ml.

4 Safety note

The method described involves the handling of hazardous substances. Attention is therefore drawn to the various provisions governing the handling of potentially dangerous material.

5 Reagents

(b) (4)

(b) (4)

(b) (4)

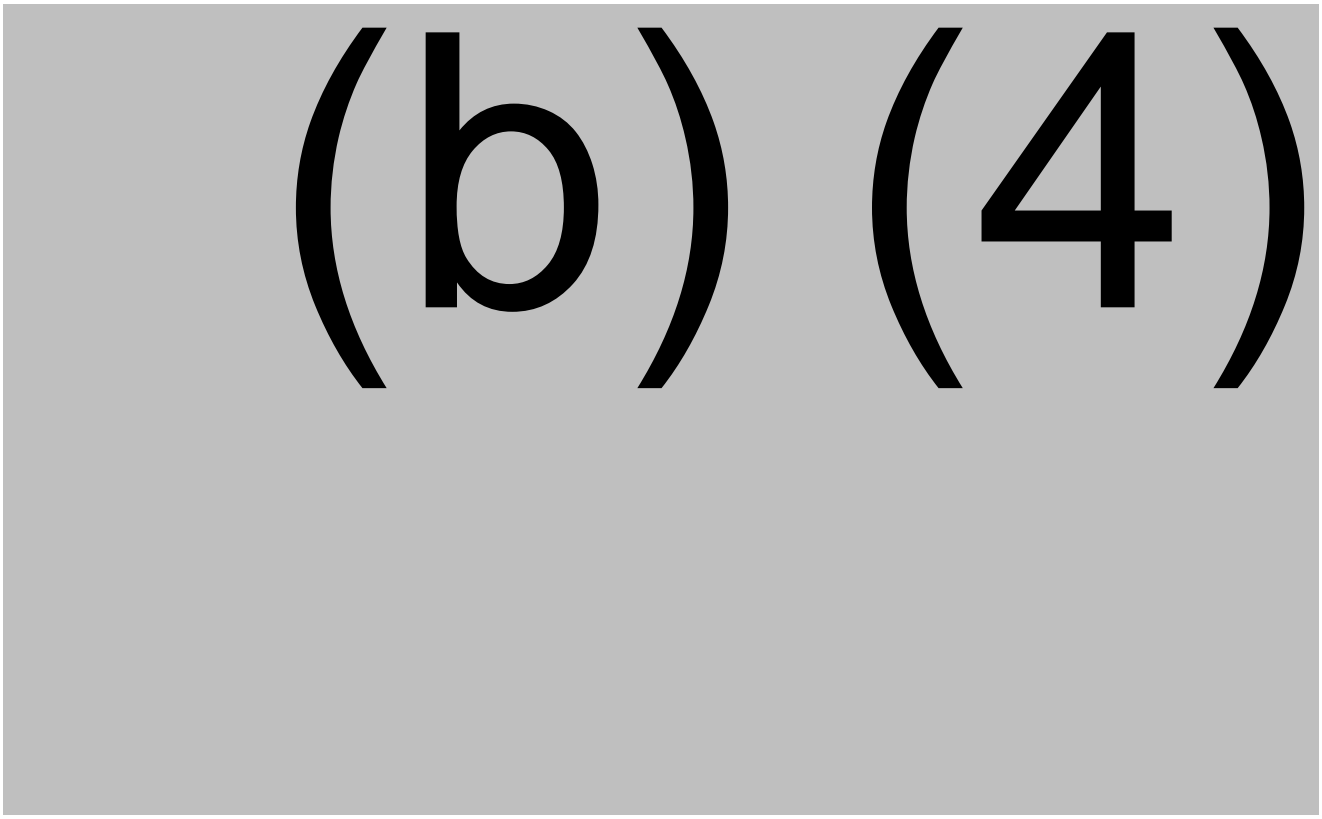
7 Preparation of standard, sample and blank solutions

(b) (4)

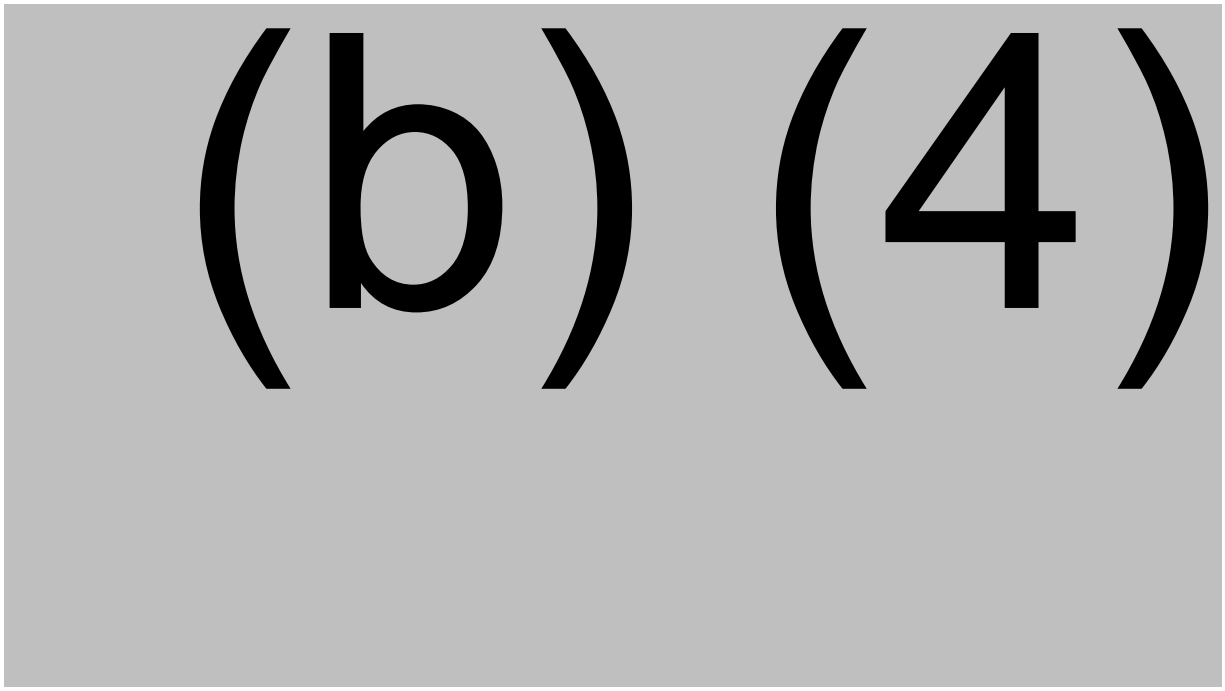
(b) (4)

(b) (4)

8 Procedure



9 Calculation of mannanase activity



10 Measurement uncertainty

(b) (4)

11 Comments

(b) (4)

Appendix 15c: Activity Assay FEN-0005– Premix and Feeds

Method of Analysis FEN/0005/01

BASF
We create chemistry

Title: **Photometric assay of mannanase activity in feed and mineral premix**

Effective date: May 02nd, 2018

Prepared by: (b) (6)

Reviewed and approved: Dr. Benjamin Peikert

(b) (6)

1 Keywords

1.1 To be determined

Mannanase activity

1.2 Matrix

Feed
Mineral premix

2 Principle

The method is suitable for measurement of endo-mannanase activity in feed and mineral premix. The substrate is azurine-crosslinked carob galactomannan. Hydrolysis by endo-1,4-β-mannanases produces water soluble dyed fragments. The rate of release of these (increase in absorbance at 590 nm) can be related directly to enzyme activity.

One mannanase unit (TMU) is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one µmol mannose from mannan in one minute as defined by the sugar release method.

3 Working range

The working range of this method is (b) (4) per ml.

4 Safety note

The method described involves the handling of hazardous substances. Attention is therefore drawn to the various provisions governing the handling of potentially dangerous material.

5 Reagents

(b) (4)

(b) (4)

(b) (4)

(b) (4)

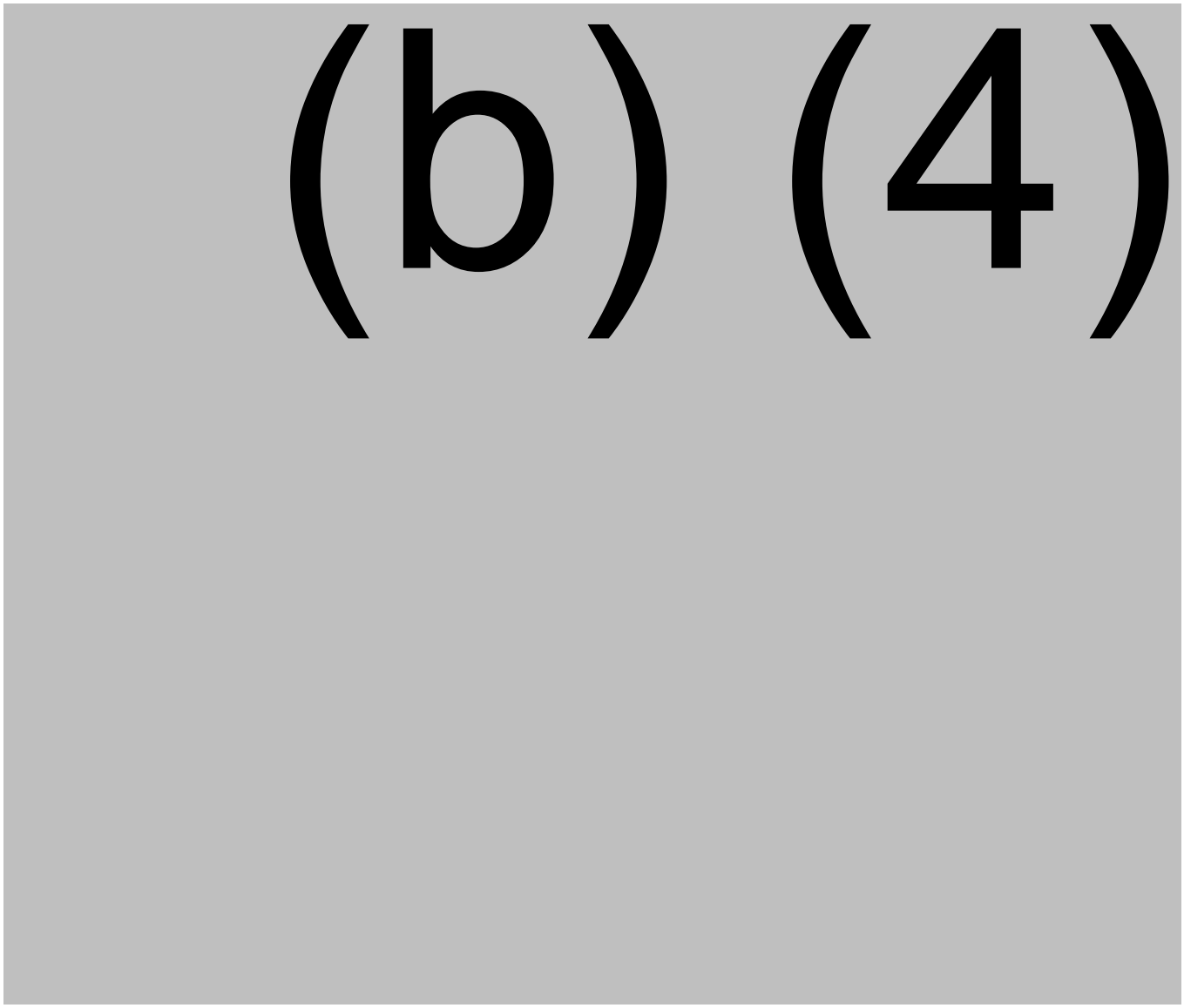
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8 Procedure

(b) (4)

9 Calculation of mannanase activity

(b) (4)


(b) (4)

Appendix 15d: EURL Evaluation Report

Ref. Ares(2021)3515639 - 27/05/2021

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate F - Health, Consumers and Reference Materials (Geel)
Food and Feed Compliance

European Union Reference Laboratory
Feed Additives

JRC F.5/CvH/ZE/AS/Ares

Evaluation Report on the Analytical Methods submitted in connection with the Application for Authorisation of a Feed Additive according to Regulation (EC) No 1831/2003

Endo-1,4-beta-D-mannanase
(FAD-2020-0107; CRL/200066)

European Union Reference Laboratory
Feed Additives

Evaluation Report on the Analytical Methods submitted in connection with the Application for Authorisation of a Feed Additive according to Regulation (EC) No 1831/2003

Dossier related to:	**FAD-2020-0107 - CRL/200066**
Name of Product:	***Endo-1,4-beta-D-mannanase***
Active Agent (s):	**Endo-1,4-beta-D-mannanase**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC Geel, Belgium**
Report prepared by:	**Zigmas Ezerskis**
Report checked by: Date:	**María José González de la Huebra** **27/05/2021**
Report approved by: Date:	**Christoph von Holst** **27/05/2021**

EXECUTIVE SUMMARY

In the current application an authorisation is sought under Article 4(1) for *endo-1,4-beta-D-mannanase* under the category / functional group 4(a) "zootechnical additives / digestibility enhancers", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, the authorisation is sought for chickens for fattening, turkeys for fattening, other poultry for fattening, minor growing poultry species and ornamental birds.

According to the Applicant, the *feed additive* contains as an active substance *endo-1,4-beta-D-mannanase* produced by the genetically modified *Thermothelomyces thermophilus* strain LU19287 (DSM 33149).

The *feed additive* is intended to be marketed under the trade name *Natupulse*® as solid (*Natupulse*®*TS*) or liquid (*Natupulse*®*TS L*) formulations with a minimum activity of *endo-1,4-beta-D-mannanase* of 8000 thermostable mannanase units (TMU) / g *feed additive*.

According to the Applicant, one TMU is defined as the amount of enzyme that produces reducing carbohydrates, having a reducing power corresponding to one µmol of mannose, from locust bean gum (0.3 g/100 ml buffer solution) in one minute at 50 °C and pH 3.5.

The *feed additive* is intended to be used directly into *feedingstuffs* or through *premixtures* at a proposed minimum *endo-1,4-beta-D-mannanase* activity of 800 TMU/kg complete *feedingstuffs*.

For the quantification of the *endo-1,4-beta-D-mannanase* activity in the *feed additive*, *premixtures* and *feedingstuffs*, the Applicant submitted two single-laboratory validated and further verified methods based on the enzymatic hydrolysis of dyed carob galactomannan with the *endo-1,4-beta-D-mannanase*, where the amount of released low-molecular weight dyed fragments are measured by photometry. Based on this measurement, the enzyme activity of the sample is then quantified against a standard enzyme with known activity.

Based on the overall available performance data, the EURL recommends for official control the two above mentioned single-laboratory validated and further verified methods based on the enzymatic hydrolysis of dyed carob galactomannan with the *endo-1,4-beta-D-mannanase*, followed by photometric determination of dyed fragments, for official control for the determination of *endo-1,4-beta-D-mannanase* activity in the *feed additive*, *premixtures* and *feedingstuffs*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

KEYWORDS

Endo-1,4-beta-D-mannanase, *Natupulse*®, zootechnical additives, digestibility enhancers, chickens for fattening, turkeys for fattening, other poultry for fattening, minor growing poultry species, ornamental birds.

1. BACKGROUND

In the current application an authorisation is sought under Article 4(1) (new feed additive) for *endo-1,4-beta-D-mannanase* under the category / functional group 4(a) "zootechnical additives / digestibility enhancers", according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1,2]. Specifically, the authorisation is sought for chickens for fattening, turkeys for fattening, other poultry for fattening, minor growing poultry species and ornamental birds [2].

According to the Applicant, the *feed additive* contains as an active substance *endo-1,4-beta-D-mannanase* produced by the genetically modified *Thermothelomyces thermophilus* strain LU19287 (DSM 33149) [3].

The *feed additive* is intended to be marketed under the trade name *Natupulse*® as solid (*Natupulse*®*TS*) or liquid (*Natupulse*®*TS L*) formulations [3] with a minimum activity of *endo-1,4-beta-D-mannanase* of 8000 thermostable mannanase units (TMU) / g *feed additive* [4].

According to the Applicant, one TMU is defined as the amount of enzyme that produces reducing carbohydrates, having a reducing power corresponding to one µmol of mannose, from locust bean gum (0.3 g/100 ml buffer solution) in one minute at 50 °C and pH 3.5 [4].

The *feed additive* is intended to be used directly into *feedingstuffs* or through *premixtures* at a proposed minimum *endo-1,4-beta-D-mannanase* activity of 800 TMU/kg complete *feedingstuffs* [2,5].

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761, on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the duties and the tasks of the European Union Reference Laboratory concerning applications for authorisations of feed additives, the EURL is requested to submit a full evaluation report to the European Food Safety Authority for each application or group of applications. For this particular dossier, the methods of analysis submitted in connection with *endo-1,4-beta-D-mannanase* and their suitability to be used for official controls in the frame of the authorisation were evaluated.

3. EVALUATION

Description of the analytical methods for the determination of the active substance in the feed additive, premixtures, feedingstuffs and when appropriate water (section 2.6.1 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

For the quantification of the *endo-1,4-beta-D-mannanase* activity in the *feed additive*, *premixtures* and *feedingstuffs*, the Applicant submitted two single-laboratory validated and further verified methods [6,7] based on the enzymatic hydrolysis of dyed carob galactomannan with the *endo-1,4-beta-D-mannanase*, where the amount of released low-molecular weight dyed fragments are measured by photometry. Based on this measurement, the enzyme activity of the sample is then quantified against a standard enzyme with known activity.

For the quantification of the *endo-1,4-beta-D-mannanase* activity in the *feed additive*, the samples (0.5 to 2 g), for which the carrier is soluble in water, are diluted with citrate buffer containing Tween® 20 (pH 3.5). The samples, for which the carrier of the *feed additive* preparation is not soluble in water, are suspended by mixing them in the above mentioned buffer solution for 30 min followed by filtration. Aliquots of the prepared samples, after their pre-incubation at 40 °C for 5 min, are mixed with the Azo-Carob-Galactomannan substrate solution in the citrate buffer (2 g/ml) and additionally incubated at 40 °C for 20 min. The enzymatic reaction is stopped by adding the precipitation solution composed of industrial methylated spirits (95 %, v/v) or pure ethanol (95 %, v/v) into the reaction mixture followed by mixing. The resulting solutions are centrifuged after equilibration at room temperature for 10 min at 3000 *g* for 15 min. The supernatants, containing low-molecular weight dyed fragments formed from the reaction of the *endo-1,4-beta-D-mannanase* on the substrate, are analysed by photometry at 590 nm [6].

The blank samples are prepared from the above mentioned citrate buffer, the substrate and the precipitation solution. The absorbance of the blank samples is subtracted from the absorbance values of the sample and the standard solutions, respectively. In addition, for quality control purposes, control samples containing a known activity of the enzyme are prepared. The quantification of the *endo-1,4-beta-D-mannanase* activity is performed using an external calibration curve prepared from the standard samples of the *endo-1,4-beta-D-mannanase* [6] with known activity, which were subjected to identical experimental conditions as the blank, the control and target samples.

For the quantification of the *endo-1,4-beta-D-mannanase* activity in *premixtures* and *feedingstuffs*, the ground samples of the *premixtures* (0.5 g) or *feedingstuffs* (50 g) are stirred with added citrate buffer containing Tween® 20 (pH 3.5) for 30 min. Aliquots of the extracts are centrifuged at 3000 *g* for 15 min and the supernatants are filtered. The prepared samples

are mixed, after their pre-incubation at 50 °C for 5 min, with a Beta-Mannazyme tablet (T-MNZ, Megazyme) and left for an enzymatic reaction at 50 °C for 30 min without stirring. The reaction is stopped by adding the above mentioned precipitation solution followed by vigorous mixing. Afterwards, the mixtures are filtered for further analysis by photometry at 590 nm. The blank and control samples are prepared similarly as in case of the *feed additive*. For the quantification of the *endo-1,4-beta-D-mannanase* activity, the absorbance of the blank samples is subtracted from the absorbance values of the target and standard samples, respectively. The quantification is performed using an external calibration curve prepared from the standard samples of the *endo-1,4-beta-D-mannanase* with known activity, which were subjected to identical experimental conditions as the blank, the control and target samples [7].

The performance characteristics derived from the validation and verification studies [8-15] for the quantification of the *endo-1,4-beta-D-mannanase* activity in the *feed additive*, *premixtures* and *feedingstuffs* are presented in Table 1. In addition, the Applicant reported a limit of quantification (LOQ) of 33 TMU/kg *feedingstuffs* [12], which is below the minimum activity recommended by the Applicant in the conditions of use [2,5].

In addition, the Applicant presented stability data for the different batches of the *feed additive* at different storage conditions [16,17]. An RSD_r ranging from 0.1 to 2.2 % and 0.4 to 1.3 %, respectively, was derived for the *feed additive* samples with the enzymatic activity ranging from 8690 to 9631 TMU/g and 167371 to 247971 TMU/g [16,17]. Furthermore, the Applicant presented data from homogeneity studies for *premixtures* [18], mash and pelleted *feedingstuffs* [19].

Table 1. Performance characteristics of the methods for the quantification of *endo-1,4-beta-D-mannanase* activity in the *feed additive*, *premixtures* and *feedingstuffs*

	Feed additive		***Premixtures***		***Feedingstuffs***	
	Validation	**Verification**	**Validation**	**Verification**	**Validation**	**Verification**
Activity, TMU/g (or kg)	8144 – 241534(*)	224750(*)	22000(**)	23487(**)	461 – 1950000(**)	1950000(**)
RSD_r, %	1.7 – 4.8	3.4	11.3	8.8	3.3 – 6.1	1.8
RSD_{ip}, %	2.3 – 5.0	8.8	17.1	11.6	3.8 – 6.5	2.8
R_{Rec}, %	94 – 111	95	107	100	100 – 102	107
Reference	[8-11,13]	[13]	[15]	[15]	[12,14]	[14]

RSD_r and RSD_{ip}: relative standard deviations for *repeatability* and *intermediate precision*, respectively; R_{Rec}: a recovery rate; (*) TMU/g; (**) TMU/kg.

The following performance characteristics were obtained in the frame of the homogeneity studies: (i) an RSD_r ranging from 9.6 to 13.7 % and an R_{Rec} ranging from 94 to 104 % for *premixtures* with the enzymatic activity ranging from 78386 to 84489 TMU/kg [18]; and (ii) an RSD_r ranging from 3.6 to 10.4 % and an R_{Rec} ranging from 81 to 111 % for *feedingstuffs* with the enzymatic activity ranging from 728 to 1006 TMU/kg [19].

The obtained performance characteristics from the stability and homogeneity studies were additionally used by the EURL when evaluating the suitability of the proposed methods for official control.

Based on the overall available performance data, the EURL recommends for official control the two above mentioned single-laboratory validated and further verified methods for official control for the determination of the *endo-1,4-beta-D-mannanase* activity in the *feed additive*, *premixtures* and *feedingstuffs*.

Methods of analysis for the determination of the residues of the additive in food (section 2.6.2 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

An evaluation of corresponding methods of analysis is not relevant for the present application.

A further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control two single-laboratory validated and further verified methods based on the enzymatic hydrolysis of dyed carob galactomannan with the *endo-1,4-beta-D-mannanase*, followed by a photometric determination of dyed fragments, for official control for the determination of the *endo-1,4-beta-D-mannanase* activity in the *feed additive*, *premixtures* and *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the determination of the *endo-1,4-β-mannanase* activity in the *feed additive, premixtures* and *feedingstuffs:*

- Enzymatic hydrolysis of dyed carob galactomannan with the *endo-1,4-beta-D-mannanase* followed by photometry

One TMU is defined as the amount of enzyme that produces reducing carbohydrates, having a reducing power corresponding to one µmol of mannose, from locust bean gum (0.3 g/100 ml buffer solution) in one minute at 50 °C and pH 3.5.

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *endo-1,4-β-mannanase* have been sent to the European Union Reference Laboratory for Feed Additives. The dossier has been made available to the EURL by EFSA.

6. REFERENCES

[1] *Application, Reference SANTE_E5_FWD. APPL. 1831-0002-2021

[2] *Application, Annex 1 – submission number 1607680358379-2752

[3] *Technical dossier, Section II: 2.1.1. Name of the additive

[4] *Technical dossier, Section II: 2.1.3. Qualitative and Quantitative composition of the additive

[5] *Technical dossier, Section II: 2.5.1. Proposed mode of use in animal nutrition

[6] *Technical dossier, Section II – Annex_II_19

[7] *Technical dossier, Section II – Annex_II_165

[8] *Technical dossier, Section II – Annex_II_167

[9] *Technical dossier, Section II – Annex_II_168

[10] *Technical dossier, Section II – Annex_II_169

[11] *Technical dossier, Section II – Annex_II_170

[12] *Technical dossier, Section II – Annex_II_172

[13] *Technical dossier, Section II – Annex_II_173

[14] *Technical dossier, Section II – Annex_II_174

[15] Supplementary information – Annex I_Verification Report_Mannanase-Natupulse Premix

[16] *Technical dossier, Section II – Annex_II_153

[17] *Technical dossier, Section II – Annex_II_154

[18] *Technical dossier, Section II – Annex_II_159

[19] *Technical dossier, Section II – Annex_II_160

*Refers to Dossier no: FAD-2020-0107

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation is the European Union Reference Laboratory for Feed Additives, JRC, Geel, Belgium. This report is in accordance with the opinion of the consortium of National Reference Laboratories as referred to in Article 6(2) of Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761.

8. ACKNOWLEDGEMENTS

The following National Reference Laboratories contributed to this report:

- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Instytut Zootechniki — Państwowy Instytut Badawczy, Krajowe Laboratorium Pasz, Lublin (PL)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, PESCA, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)

Appendix 15e: Validation Reports

(b) (6), (b) (4)

Validation of analytical method for measuring β-mannanase activity in reference materials

Experiment number: L-251a

FINAL REPORT

Revision number: 0

Date: 21st August 2019

RESEARCH ACTIVITY CONTRACTED WITH:

BASF SE
Analyt. & Appl. Technology Feed Enzymes
G-ENA/MD – A110/R0316
67056 Ludwigshafen am Rhein (Germany)

Study monitors: Benjamin Georg Peikert and (b) (6)

Study director: (b) (6)

(b) (4)

(b) (4)

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SUMMARY

The objective of the present study was the validation of the analytical method FEN/0003/01 for measurement of ß-mannanase activity present in reference materials.

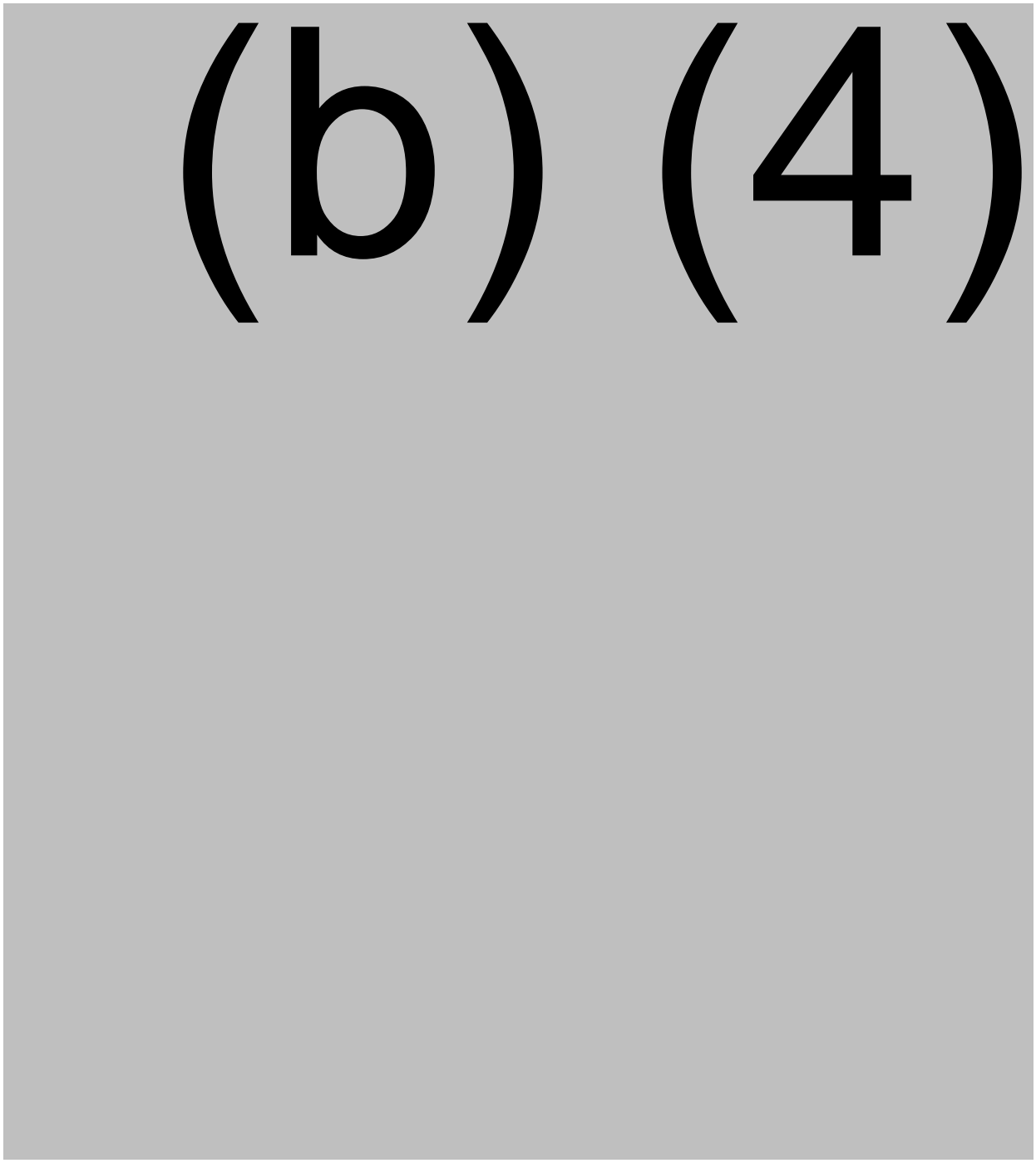
The study was performed using 3 types of ß-mannanase Reference Materials (blank, known, unknown) and mannose was used for preparation of calibration curves.

The determination of ß-mannanase activity in ß-mannanase Reference Materials (BASF method FEN/0003/01) is based on the measurement of reducing sugars, which have the property to reduce the 3,5-dinitrosalicylic acid (DNS) reagent to 3-amino-5-nitrosalicylic acid, producing a change in the amount of light absorbed, at wavelength 540 nm.

The study consisted on the determination of the following quality parameters of a method of analysis: range and linearity, limits of detection (LOD) and quantification (LOQ), and accuracy and precision parameters, which included recovery, repeatability, intermediate precision and inter-laboratory reproducibility. Robustness and Uncertainty were also evaluated.

(b) (4)

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(b) (4)

(b) (4)

RESPONSIBILITIES

R&D

STUDY DIRECTOR

(b) (6), (b) (4)

STUDY MONITORS

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ANALYTICAL LABORATORY

(b) (6), (b) (4)

OBJECTIVE

The objective of the present study was the validation of the analytical method FEN/0003/01 for measurement of ß-mannanase activity present in Reference Materials.

QUALITY ASSURANCE

IRTA has an Integrated Quality Policy and the Institute is **ISO 9001:2015** certified for their R+D+T projects, contracts and services.

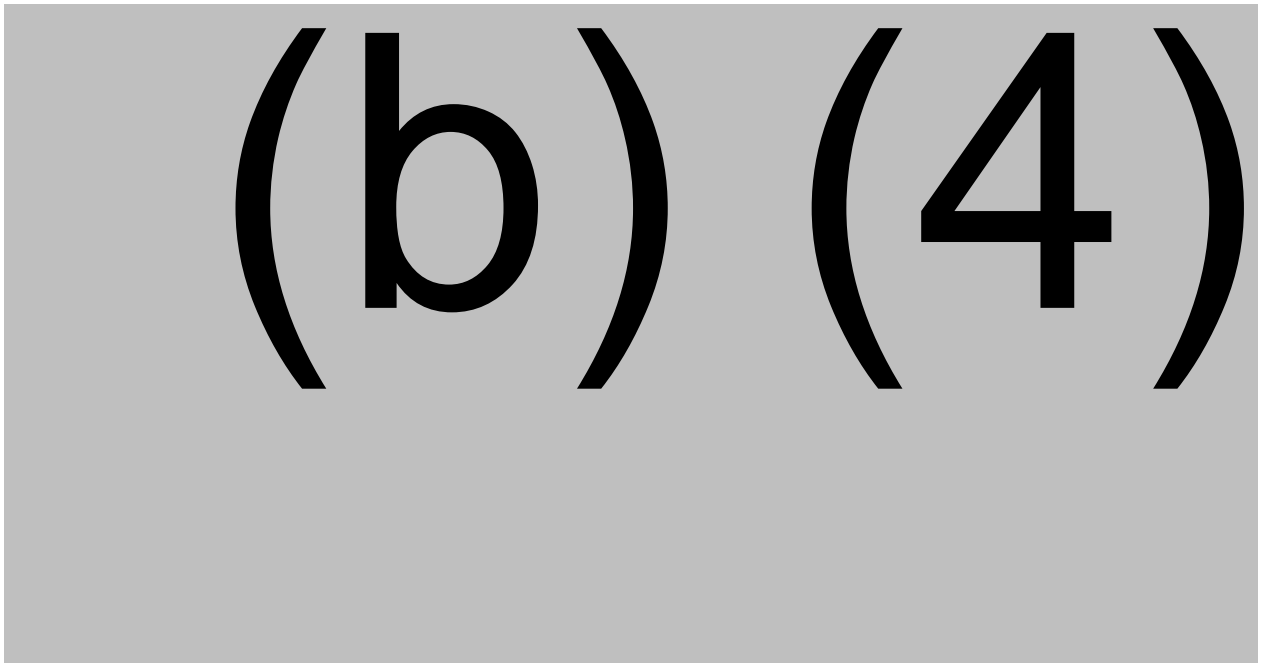
This study has been conducted in accordance with ISO 9001:2015 quality criteria, and under the global SOPs **PG-75** (Conducting Research studies).

All study staff have appropriate training and qualifications to perform their study tasks. They had been trained in Quality Systems including data recording requirements and forms for completion of the study, QC techniques, relevant SOPs and specific protocol requirements prior to study start.

SCOPE AND TEST METHOD DESCRIPTION

This procedure is for the determination of ß-mannanase activity (EC 3.2.1.78) expressed as TMU/g in ß-mannanase Reference Materials by in-house analysts.

The determination of ß-mannanase activity in ß-mannanase Reference Materials (BASF method FEN/0003/01) is based on the measurement of reducing sugars, which have the property to reduce the 3,5-dinitrosalicylic acid (DNS) reagent to 3-amino-5-nitrosalicylic acid, producing a change in the amount of light absorbed, at wavelength 540 nm.



(b) (4)

DEFINITIONS

RANGE AND LINEARITY

The linearity is the ability of a method (within a given range) to obtain test results that are directly proportional to the concentration of analyte in the sample (Eurachem, 1998). The range is the interval between upper and lower concentration (amounts) of analyte in the sample (including these concentrations for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity).

LIMIT OF DETECTION (LOD)

The limit of detection was defined as the smallest measured content from which it is possible to deduce the presence of the analyte with reasonable statistical certainty. The LOD is numerically equal to 3 times the standard deviation of the mean of blank determinations (n≥10, where n=nº of measures) performed under repeatability conditions. It can also be calculated from the slope of the calibration line as y(LOD) = a+ 3 Sy/x.

LIMIT OF QUANTIFICATION (LOQ)

The limit of quantification was defined as the lowest content of analyte that can be measured with reasonable statistical certainty. The LOQ is numerically equal to 10 times the standard deviation of the mean of blank determinations (n≥10, where n=nº of measures) performed under repeatability conditions. It can also be calculated from the slope of the calibration line as y(LOQ) = a+ 10 Sy/x.

PRECISION

The precision expresses the closeness of agreement between a series of independent measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions.

ACCURACY

The accuracy expresses the closeness of agreement between the value that is accepted either as a conventional true value or an accepted reference value and the value determined by analysis.

RECOVERY

The Relative Percent between determined and expected values of ß-mannanase samples was calculated as an indicator of recovery.

(b) (4)

REPEATABILITY

The repeatability measures precision under the same operating conditions over a short interval of time. Independent test results are obtained with the same method on identical test items in the same laboratory with the same operator using the same equipment within short intervals of time. Repeatability is expressed mathematically by the coefficient of variation (relative standard deviation) of a series of different measures.

INTERMEDIATE PRECISION

Intermediate precision expresses within-laboratory variations: e.g. different days, different analysts, different equipment, etc.

REPRODUCIBILITY

The reproducibility measures precision under different operating conditions. Reproducibility relates to the agreement of test results with different operators, test apparatus, and laboratory locations. In this case it was obtained analysing samples containing unknown concentrations of the test product.

ROBUSTNESS

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. In this case, the parameters changed have been pH, time and temperature of the enzymatic reaction.

UNCERTAINTY

The uncertainty is the expression of the statistical dispersion of the values attributed to a measured quantity. All measurements are subject to uncertainty and a measurement result is complete only when it is accompanied by a statement of the associated uncertainty. By international agreement, this uncertainty has a probabilistic basis and reflects incomplete knowledge of the quantity value.

METHODOLOGY

MATERIALS

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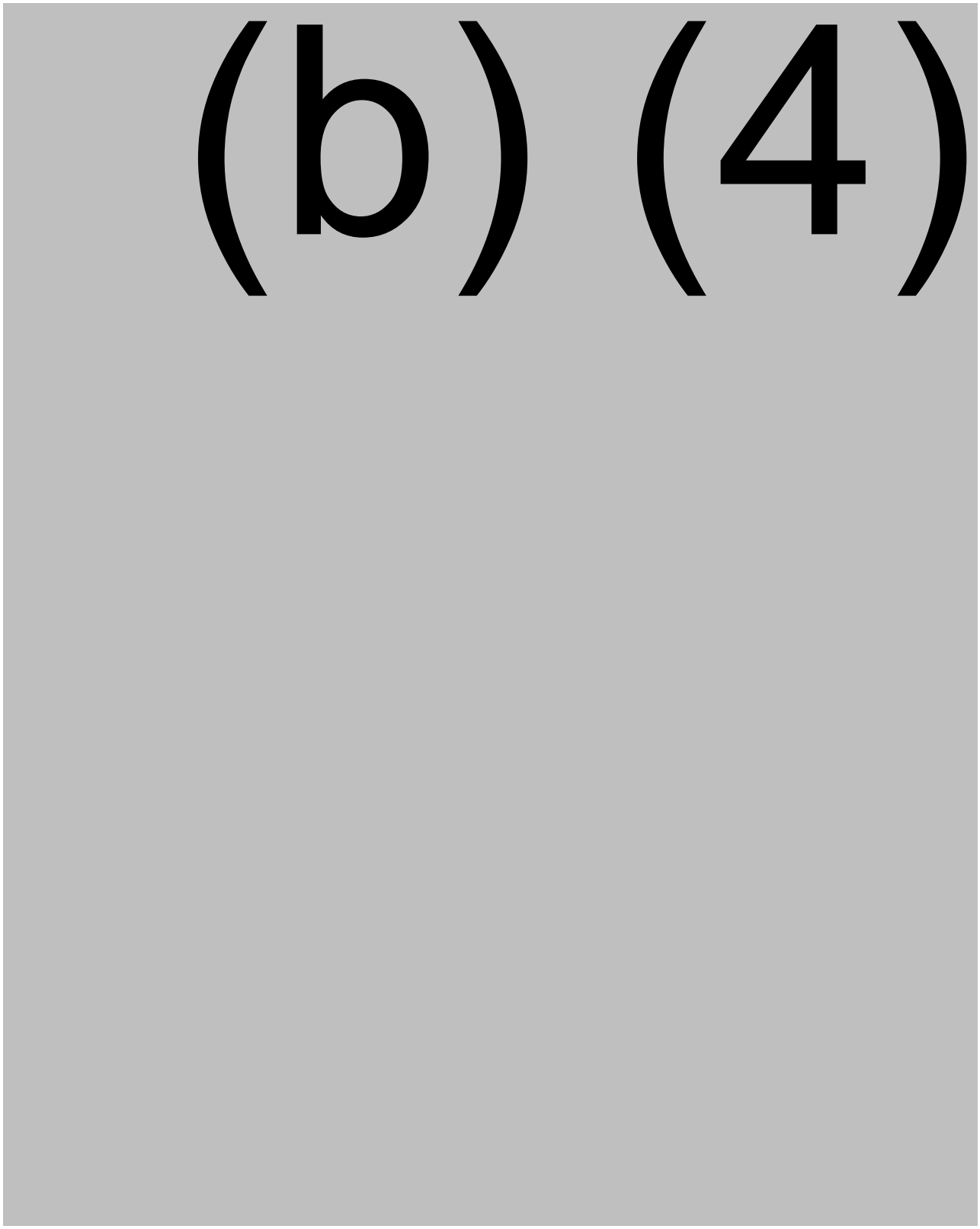
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REAGENTS

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CONFIDENTIAL

ANALYTICAL PROCEDURE



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SITE OF THE STUDY

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RESULTS AND DISCUSSION

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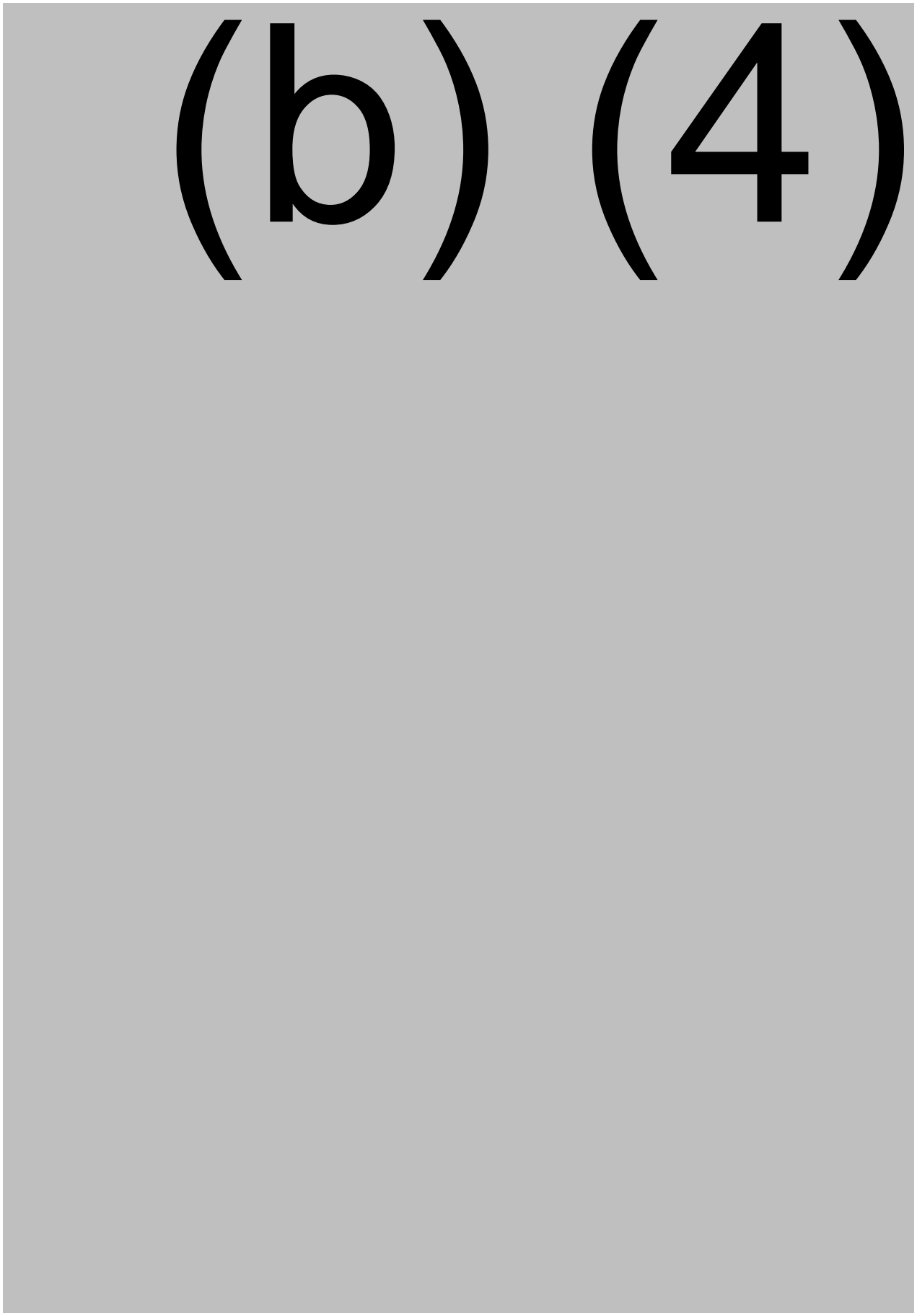
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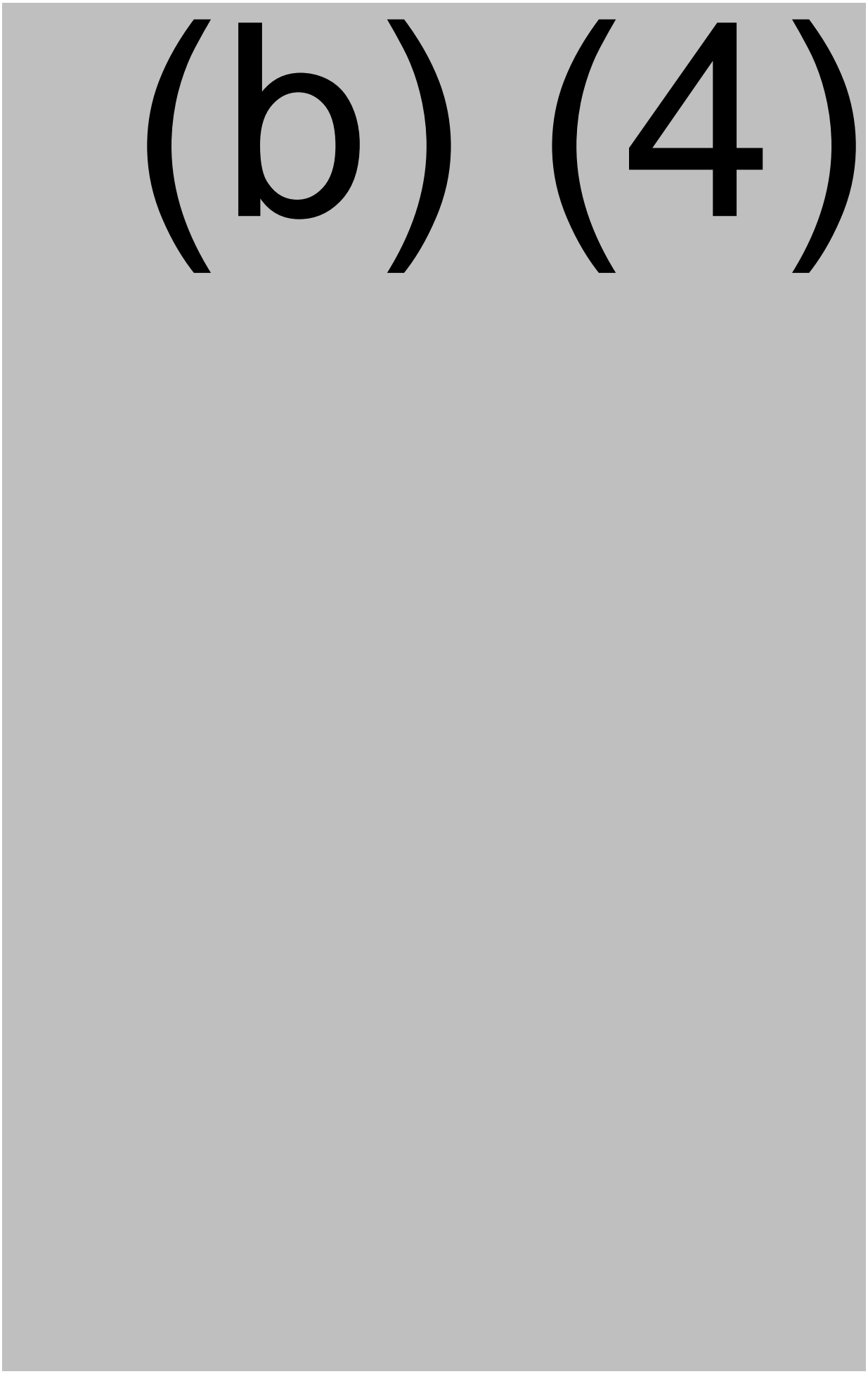
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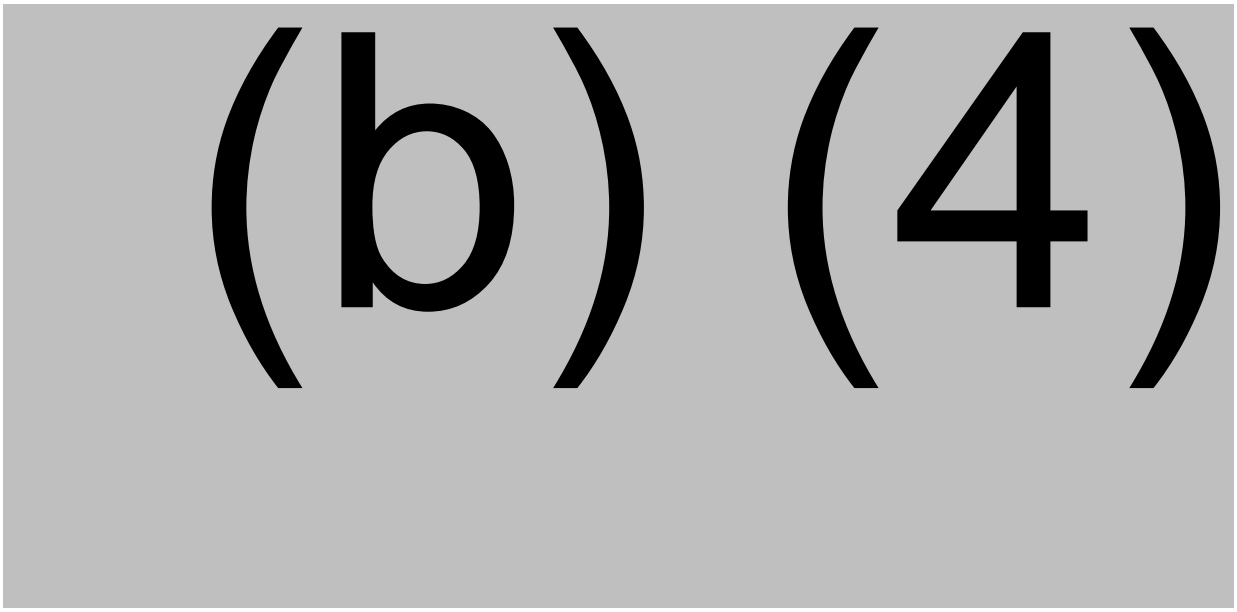
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RANGE, LINEARITY AND RECOVERY

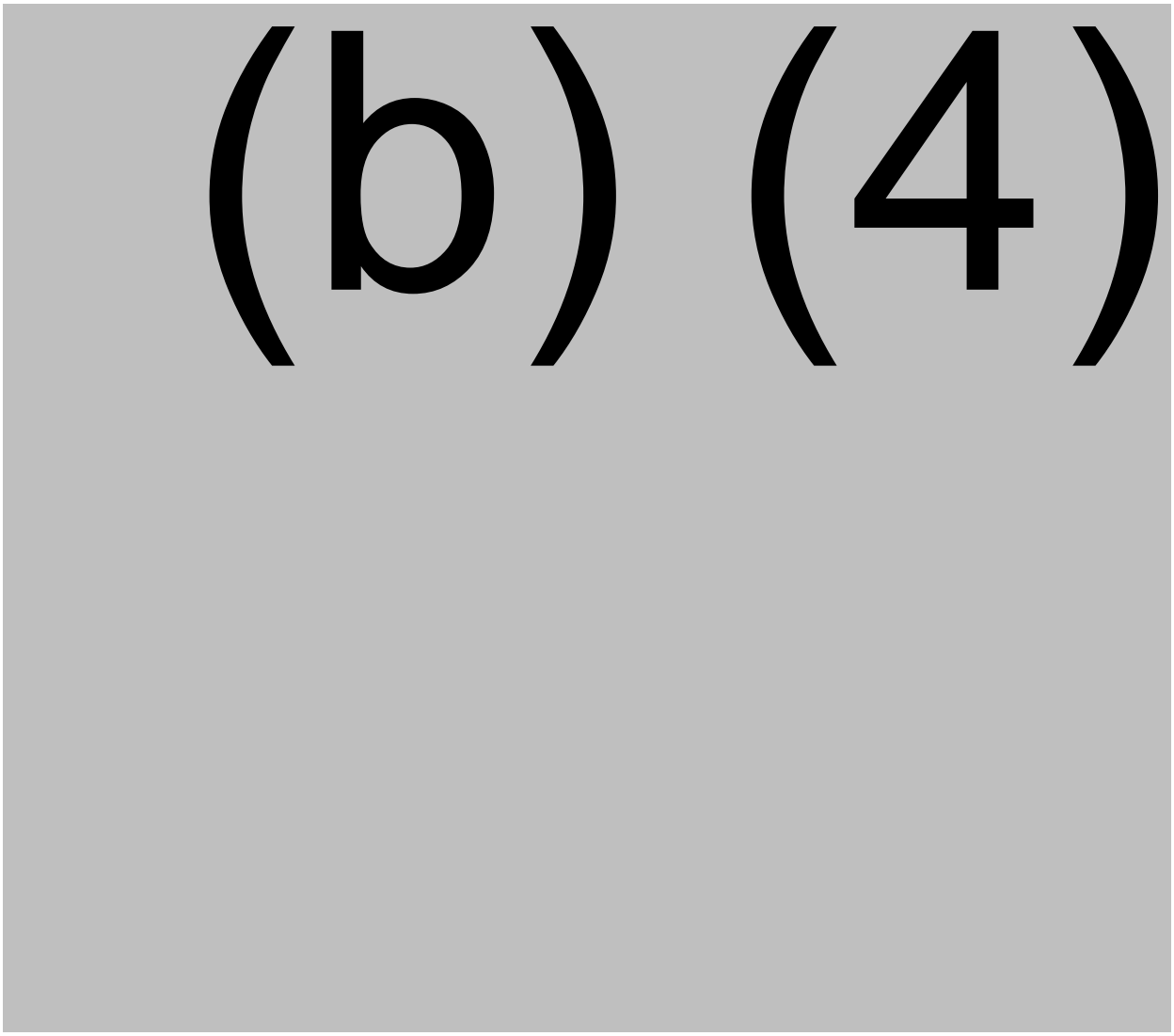
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ROBUSTNESS



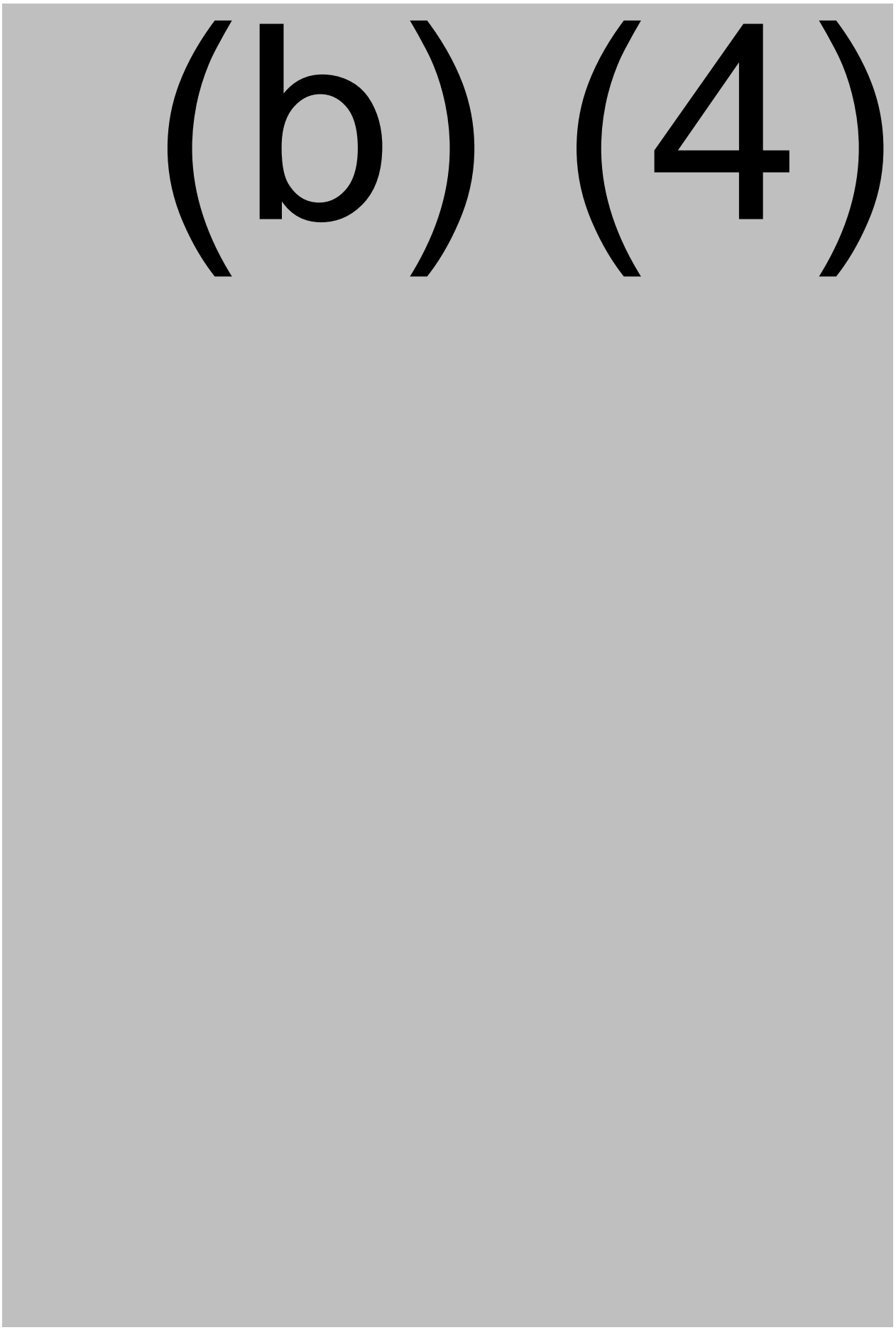
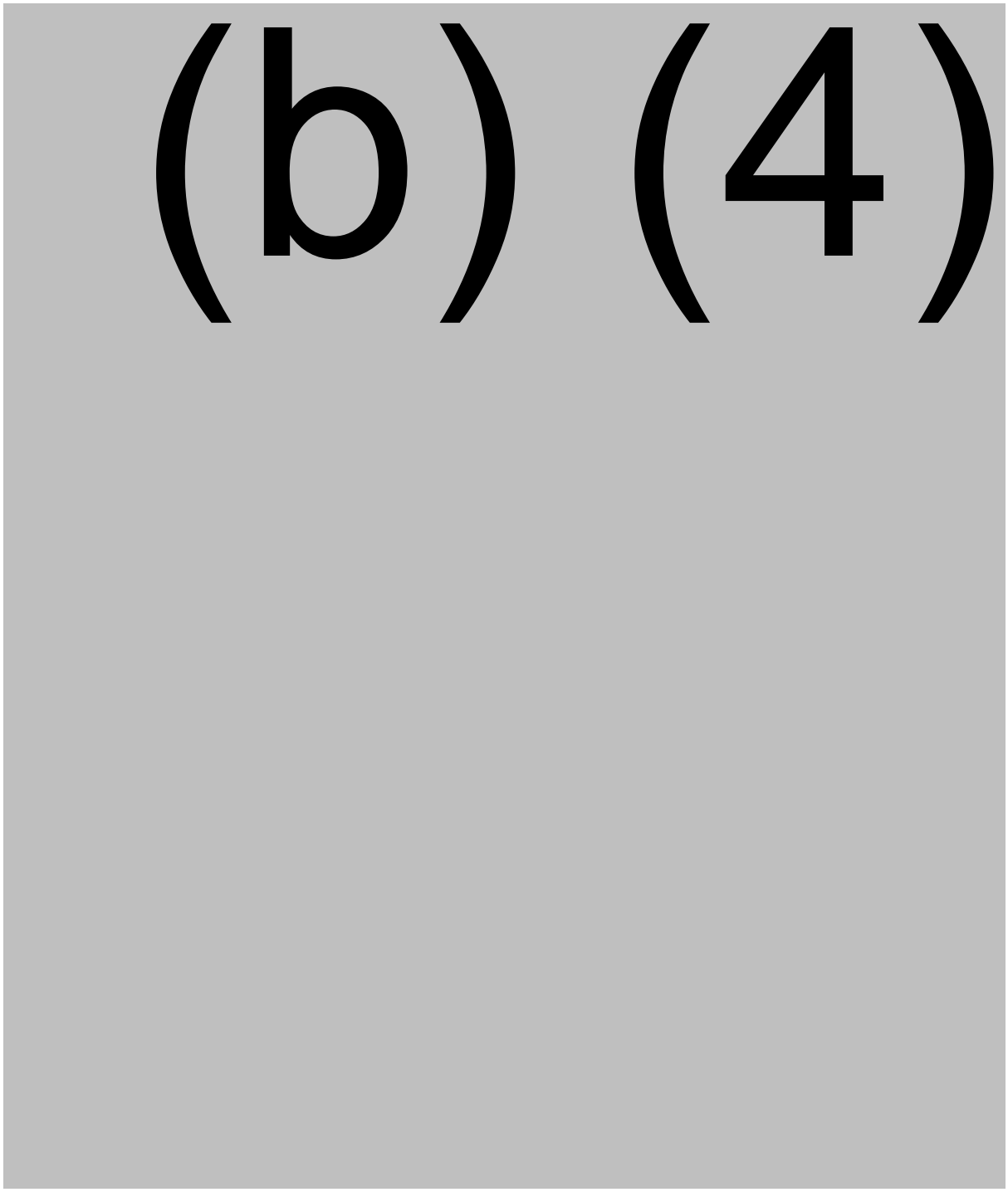

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Table 9. Evaluation of criteria fitness for robustness.

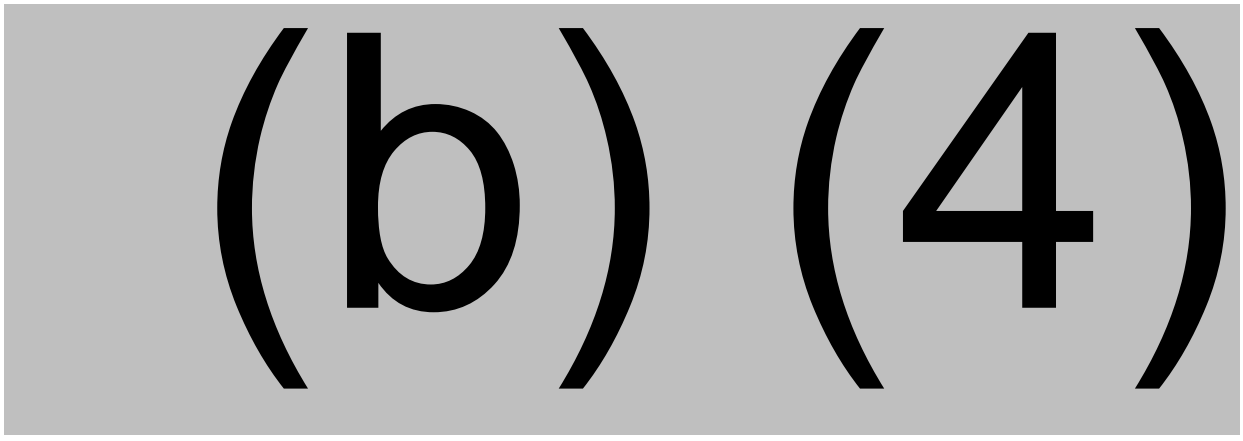
Conditions	Changes in calibration curve (mannose) and samples
	ß-mannanase (TMU/g)
Time -30 seconds	194178
Time + 30 seconds	205775
Temperature - 0.5ºC	201209
Temperature + 0.5ºC	236612
pH - 0.05 U	223709
pH + 0.05 U	201079
average	210427
St.dev.	16249
$RSD_{ROBUSTNESS}$ (%)	7.7

The determined $RSD_{ROBUSTNESS}$ (%) was 7.7% < 20% (as proposed as quality criterion). The average ß-mannanase activity determined changing the time, temperature or pH of the reaction in the ß-mannanase Reference Material known product was 210427 TMU/g, while the value determined in this sample under the optimal conditions defined in the proposed method was 226864 TMU/g. The average enzyme activity measured at all 6 different conditions was comparable to average value of repeatability. The RSD% between both means values was 7.25% (< ±15%) proposed as acceptance criterion and the calculated z score value was 2.05.

The data of ß-mannanase activity obtained during the evaluation of robustness were also statistically evaluated by ANOVA and linear contrasts. The analysis of results showed that the method proved to be robust against slight modifications of reaction time (P>0.05) while changes in pH and temperature of enzymatic reaction affect the determination of ß-mannanase (Table 10).



CONCLUSION



(b) (4)

REFERENCES

ICH HARMONISED TRIPARTITE GUIDELINE (2005). Validation of Analytical Procedures: text and methodology Q2(R1). International Conference on harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use.

Thompson, M., Ellison, S.L.R. and Wood, R. (2002). Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis (IUPAC Technical report). Pure Appl. Chem. 74 (5), 835-855.

Magnusson, B. and Örnemark, U. (2nd ed. 2014) Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to method Validation and Related Topics. ISBN 978-91-87461-59-0. Available from www.eurachem.org.

Signatures:

(b) (6)

(b) (4)

ANNEXES

(b) (4)

Annex 1. ***Validation plan for ß-mannanase activity using DNS method***

(b) (4)

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(b) (4)

Annex 2. Method of analysis fen/0003/01 provided by BASF: Determination of β-mannanase activity in reference materials using DNS.

Method of Analysis FEN/0003/01

BASF
We create chemistry

Title: **Determination of β-Mannanase activity in reference materials using DNS**

Effective date: April 20th, 2018

Prepared by: (b) (6)

Reviewed and approved: Dr. Benjamin Peikert

(b) (6)

Signature, date

1 Keywords

1.1 To be determined

Mannanase activity - Reference method

1.2 Matrix

Reference materials such as

- Mannanase SD powder

2 Principle

Mannanase in the sample hydrolyses the substrate, galactomannan, and the amount of released reducing carbohydrates is determined spectrophotometrically using dinitro-salicylic acid.

One mannanase unit (TMU) is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one µmol mannose from mannan in one minute under the assay conditions

3 Working range

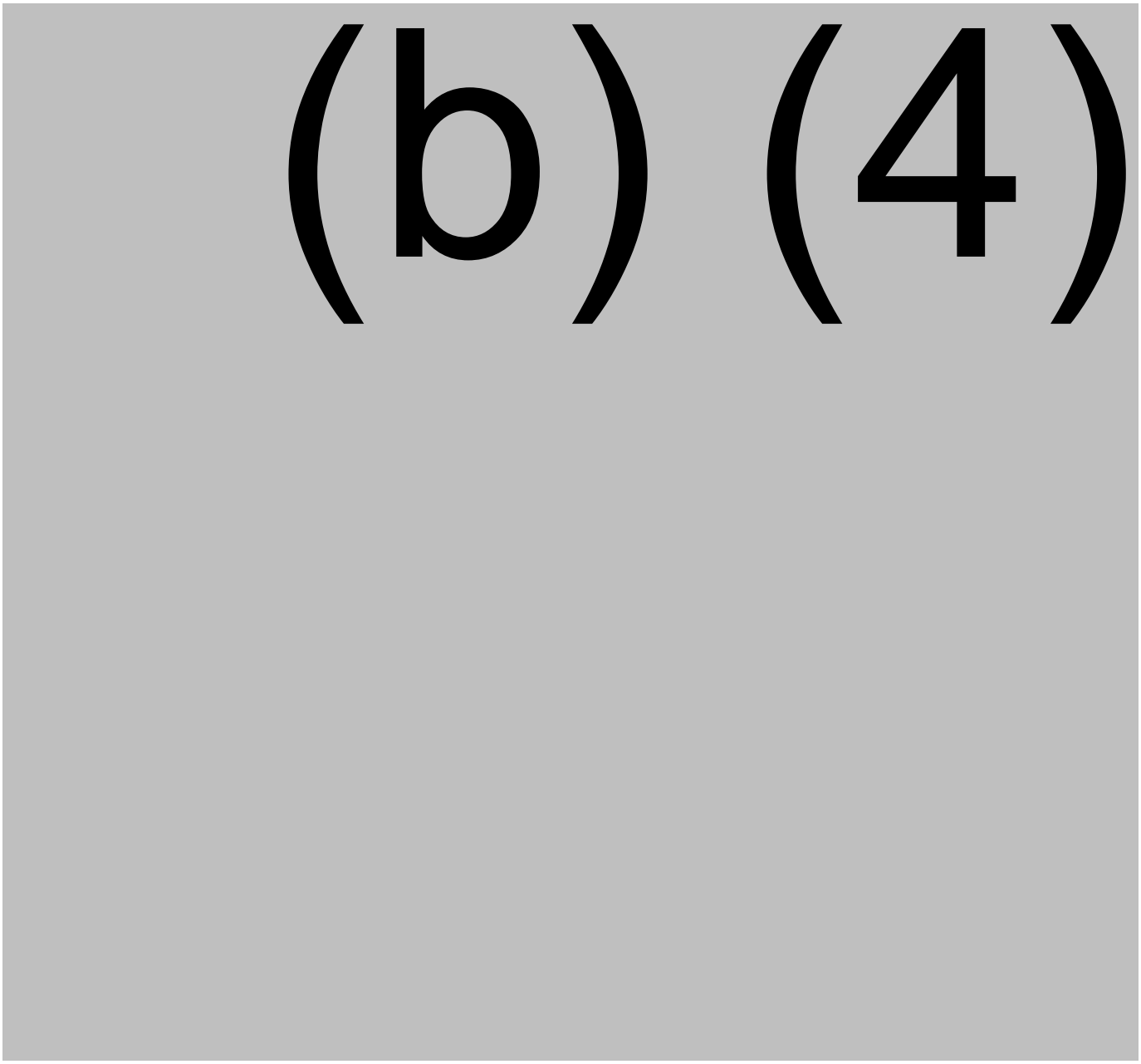
The working range of this method is (b) (4).

4 Safety note

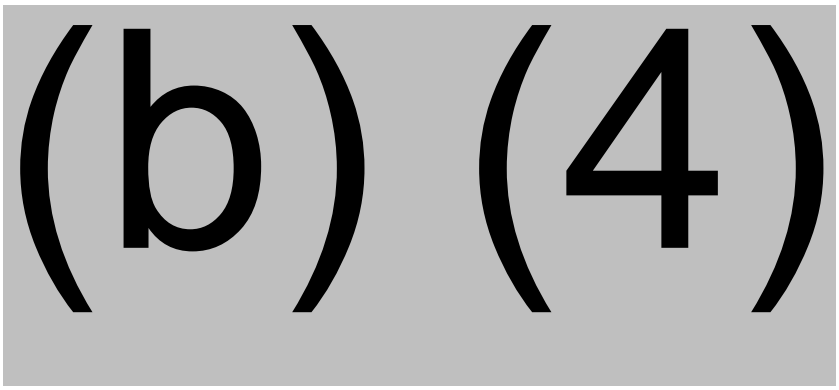
The method described involves the handling of hazardous substances. Attention is therefore drawn to the various provisions governing the handling of potentially dangerous material.

5 Reagents

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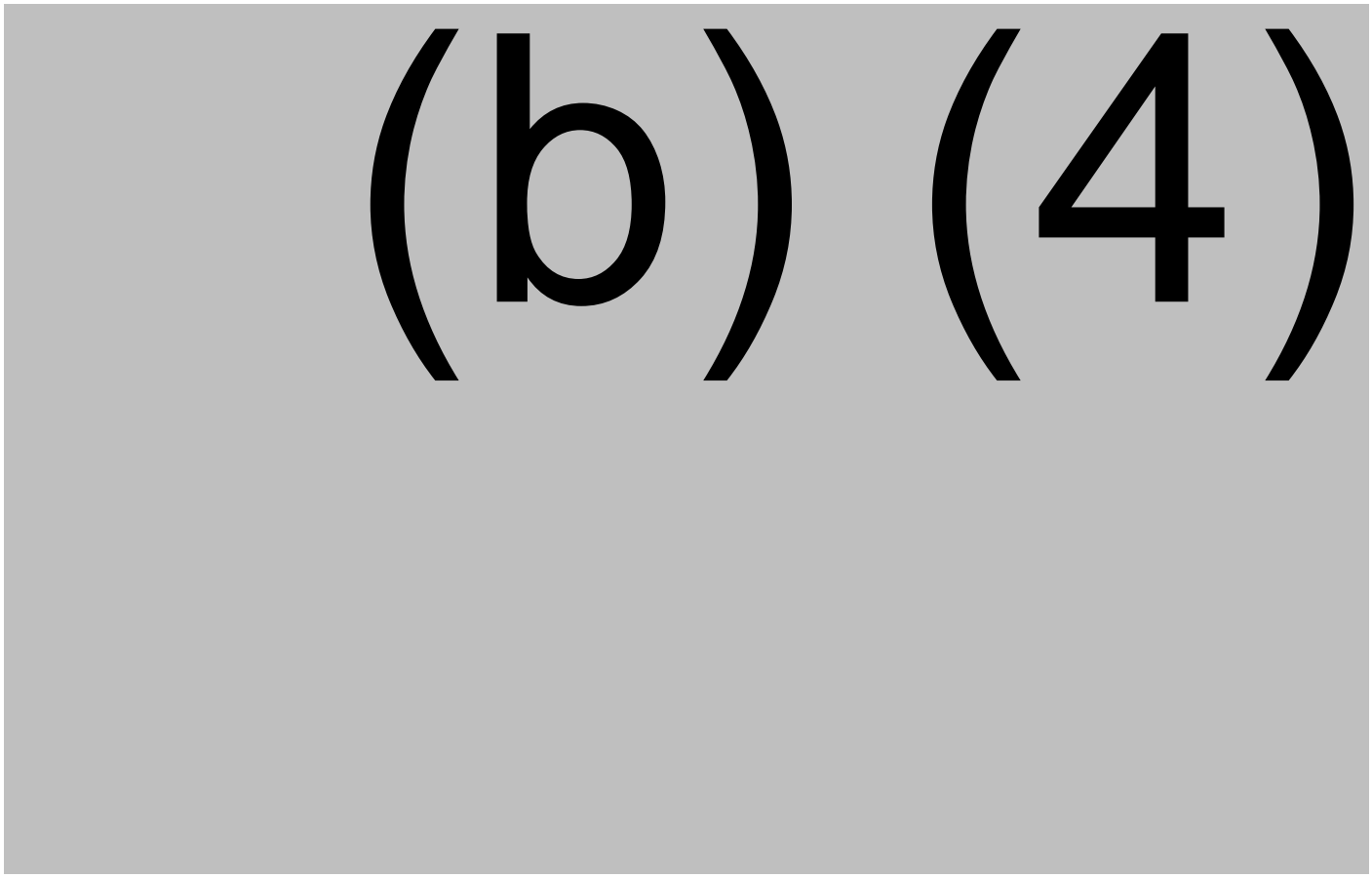
6 Apparatus



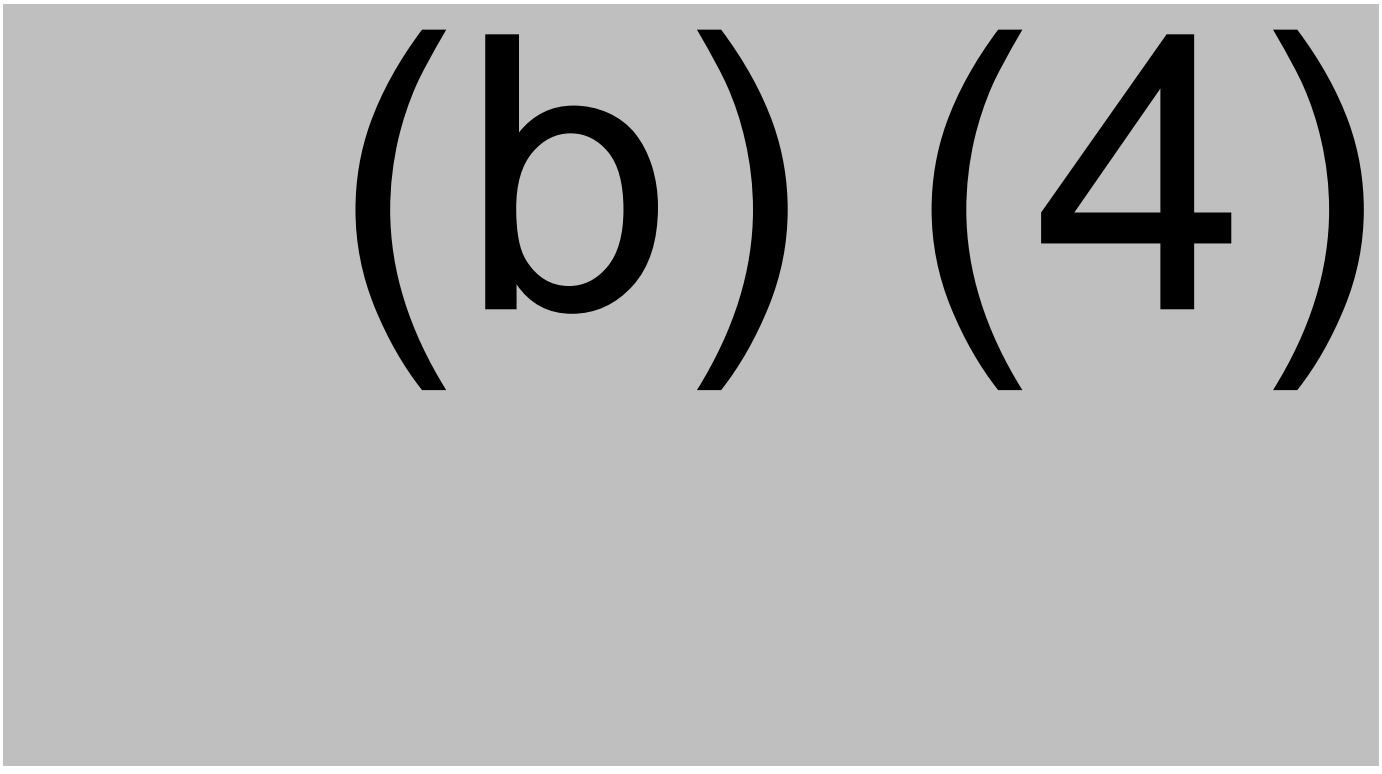
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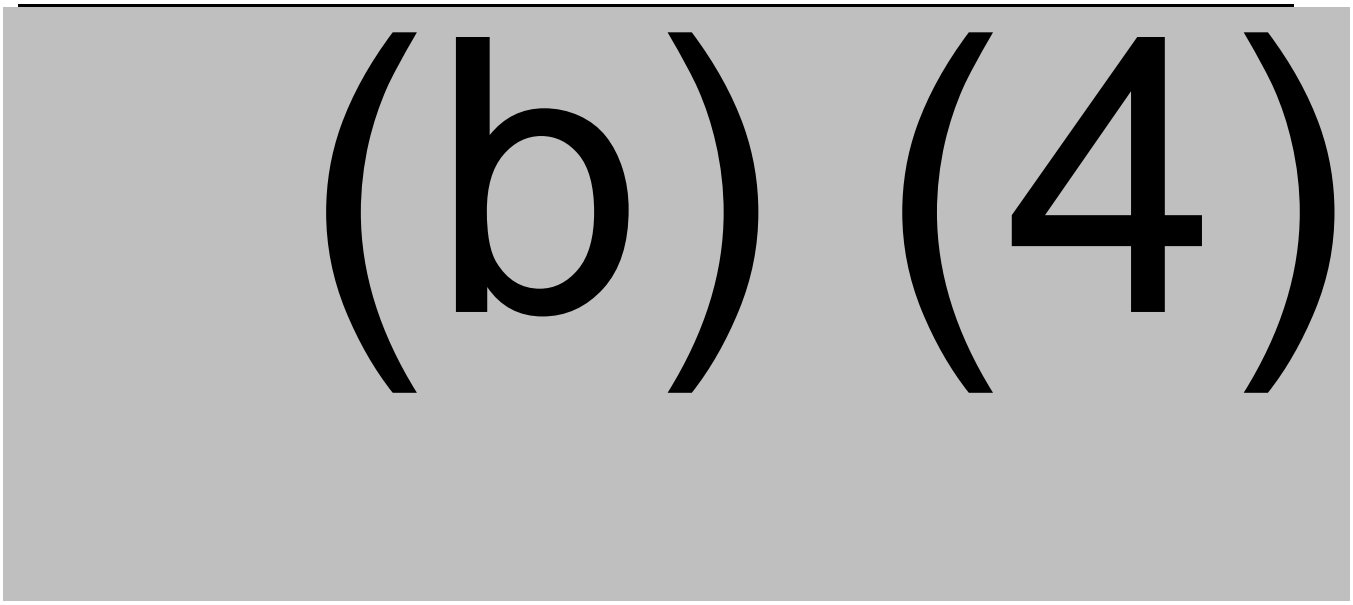
7 Preparation of calibration line, sample and blank solutions

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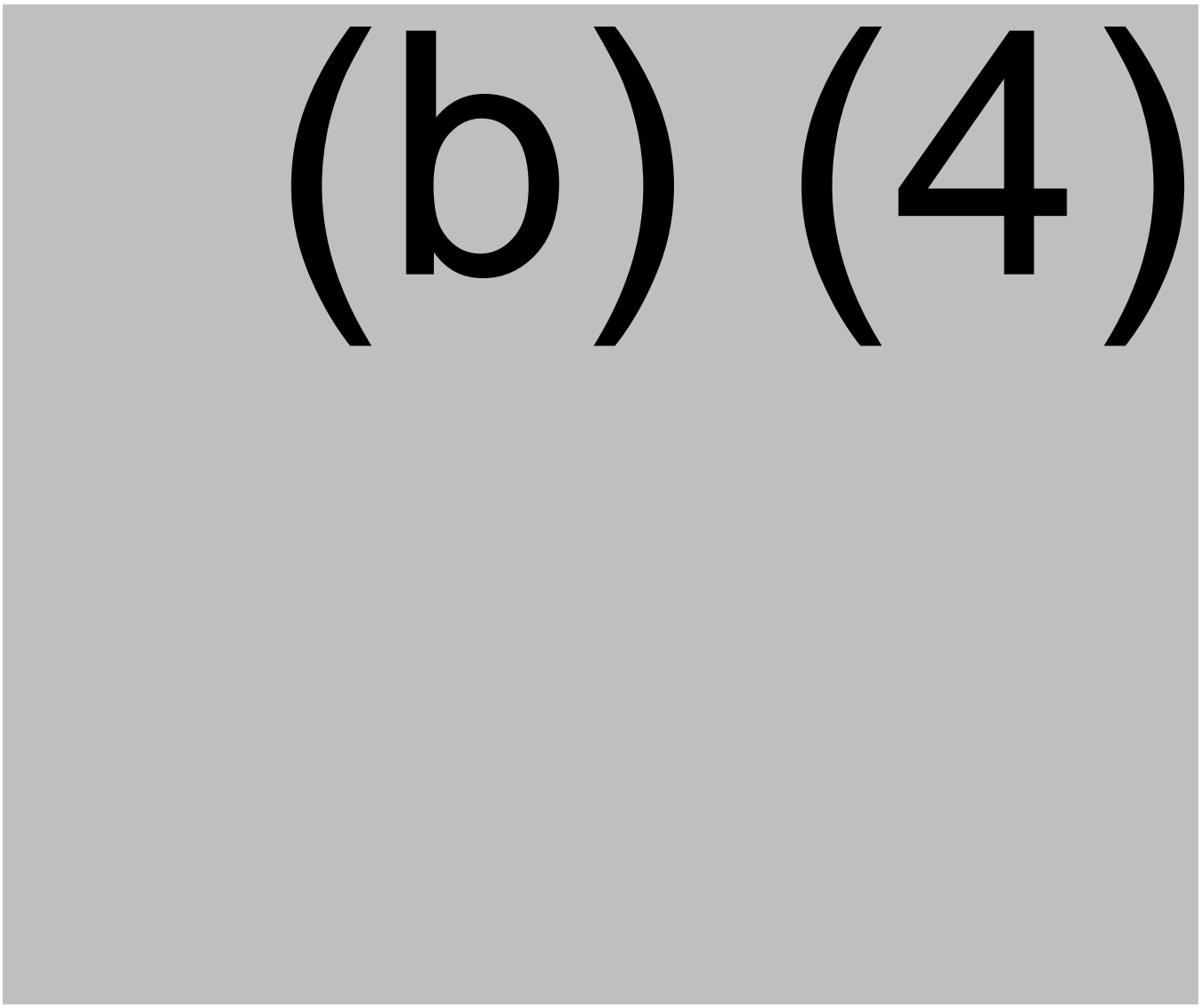


8 Procedure





9 Calculation of mannanase activity



10 Measurement uncertainty

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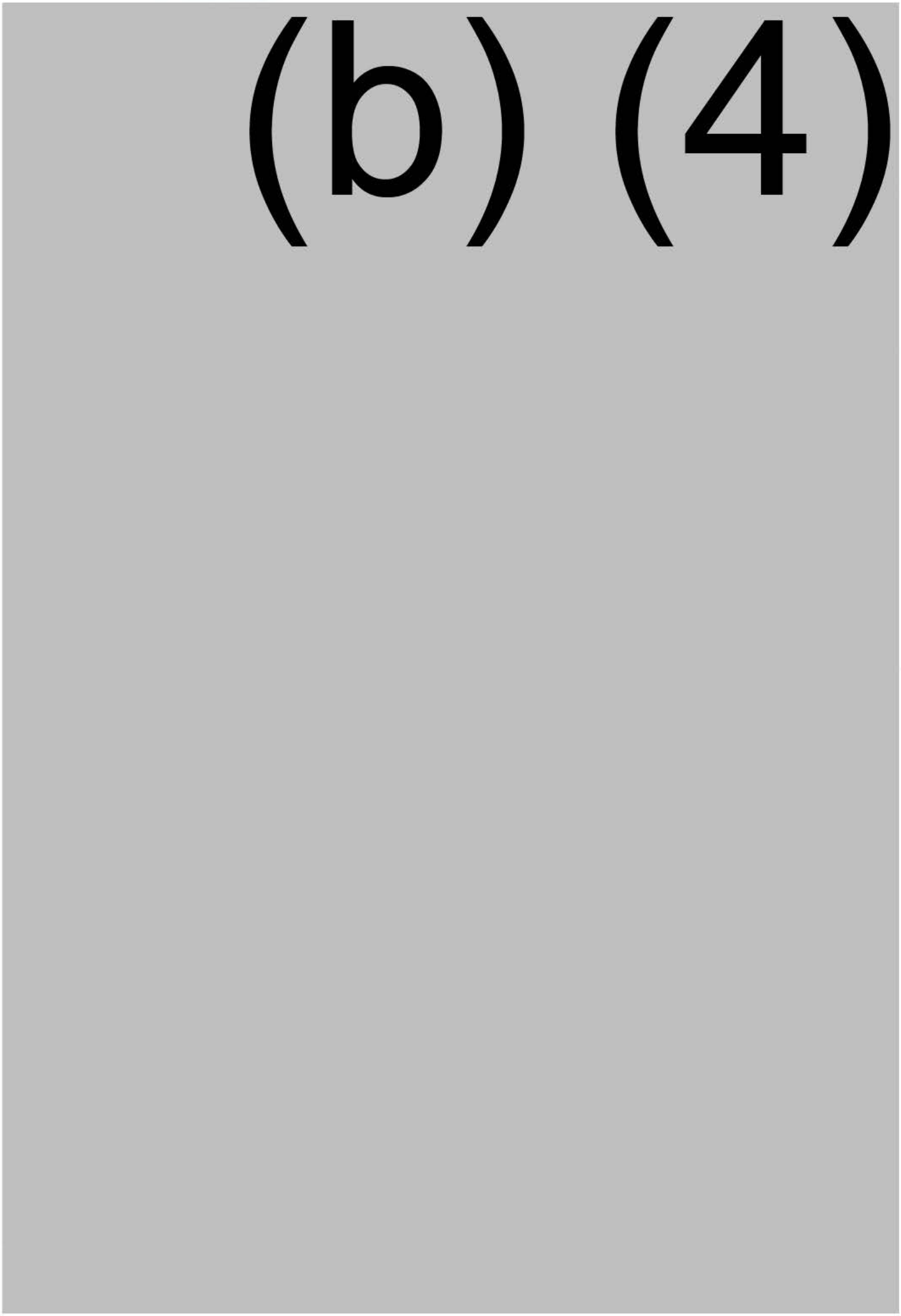
11 Comments

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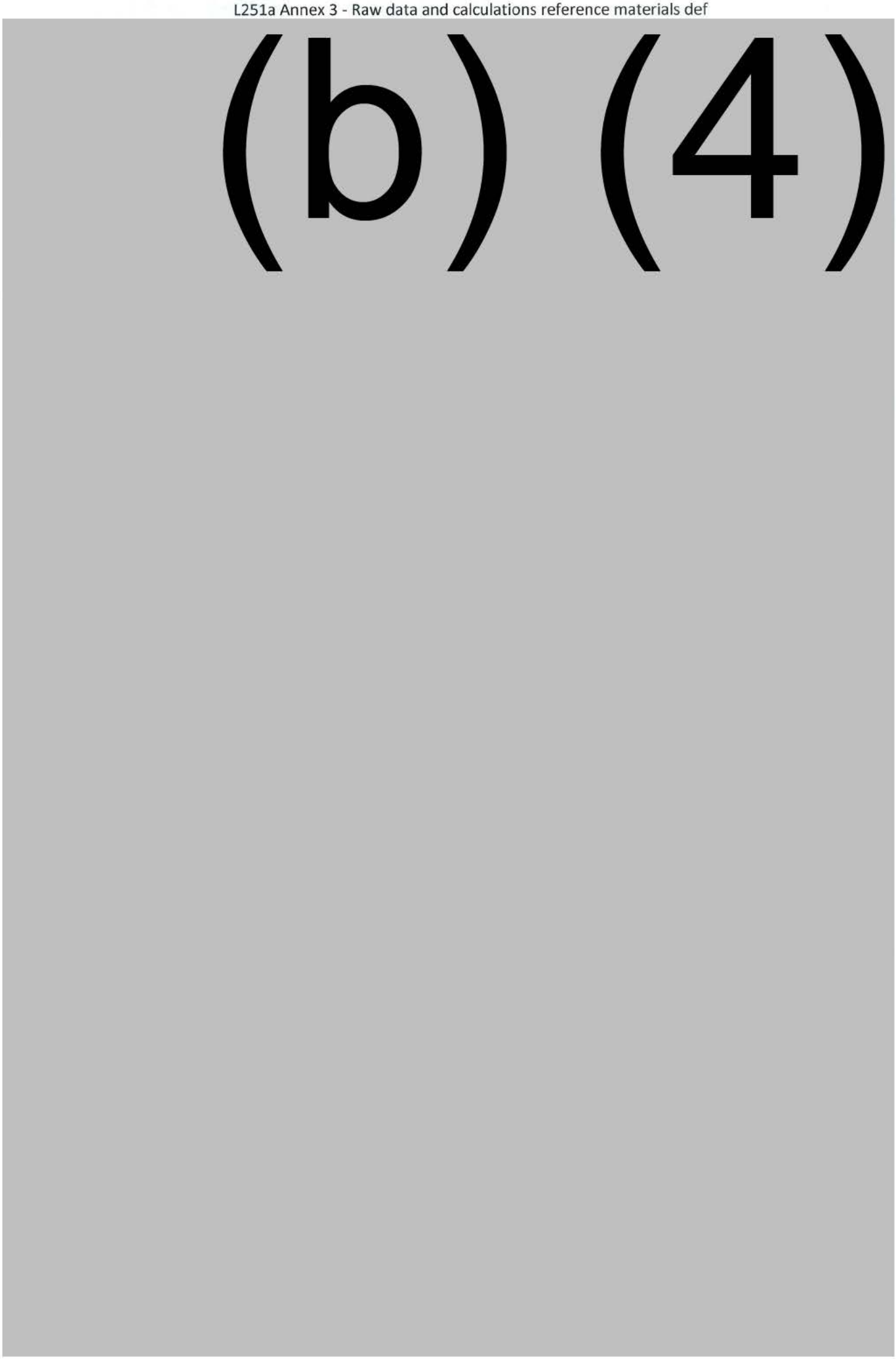
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Annex 3. Raw data and calculation for validation of ß-mannanase activity determination in reference materials.

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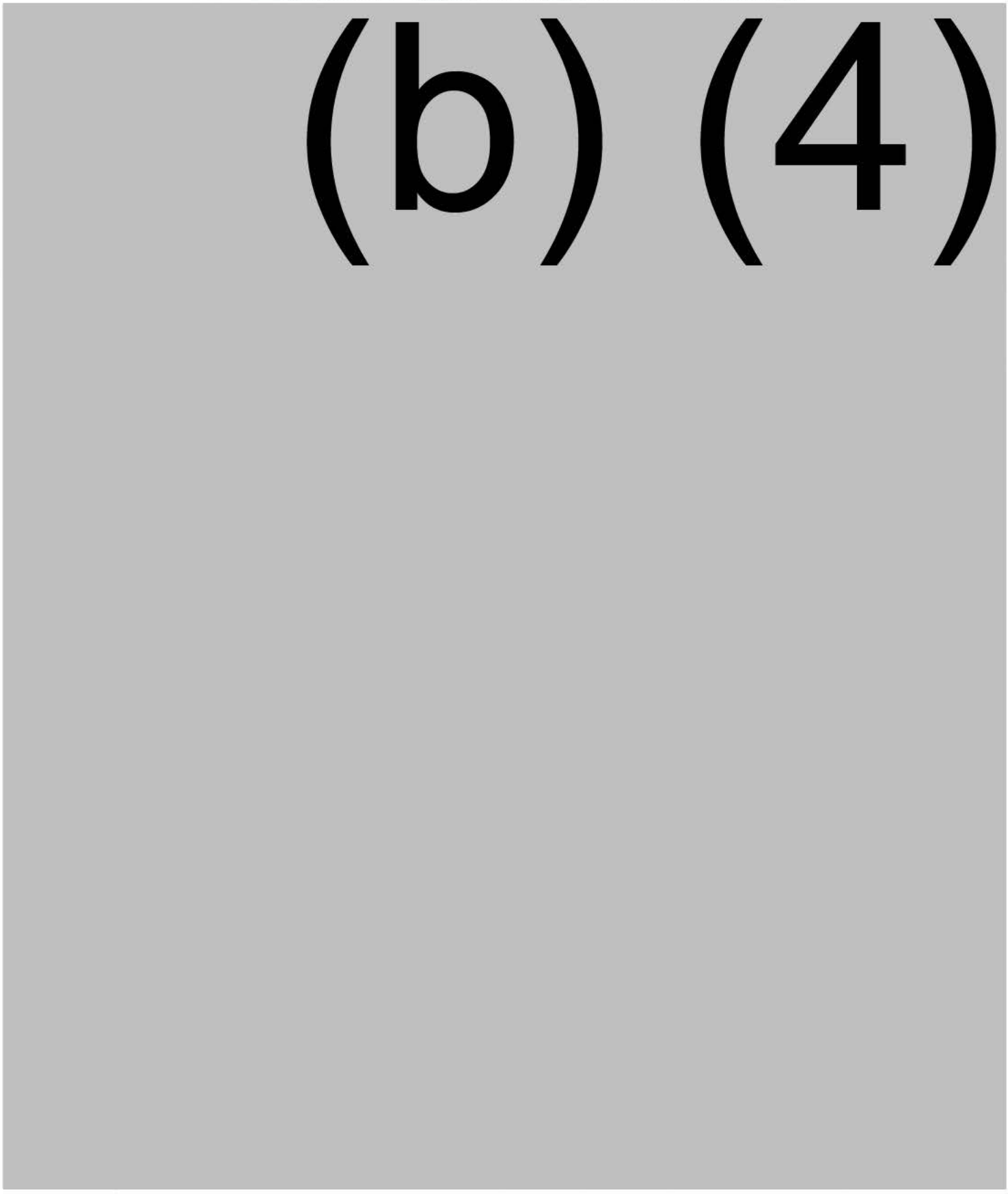


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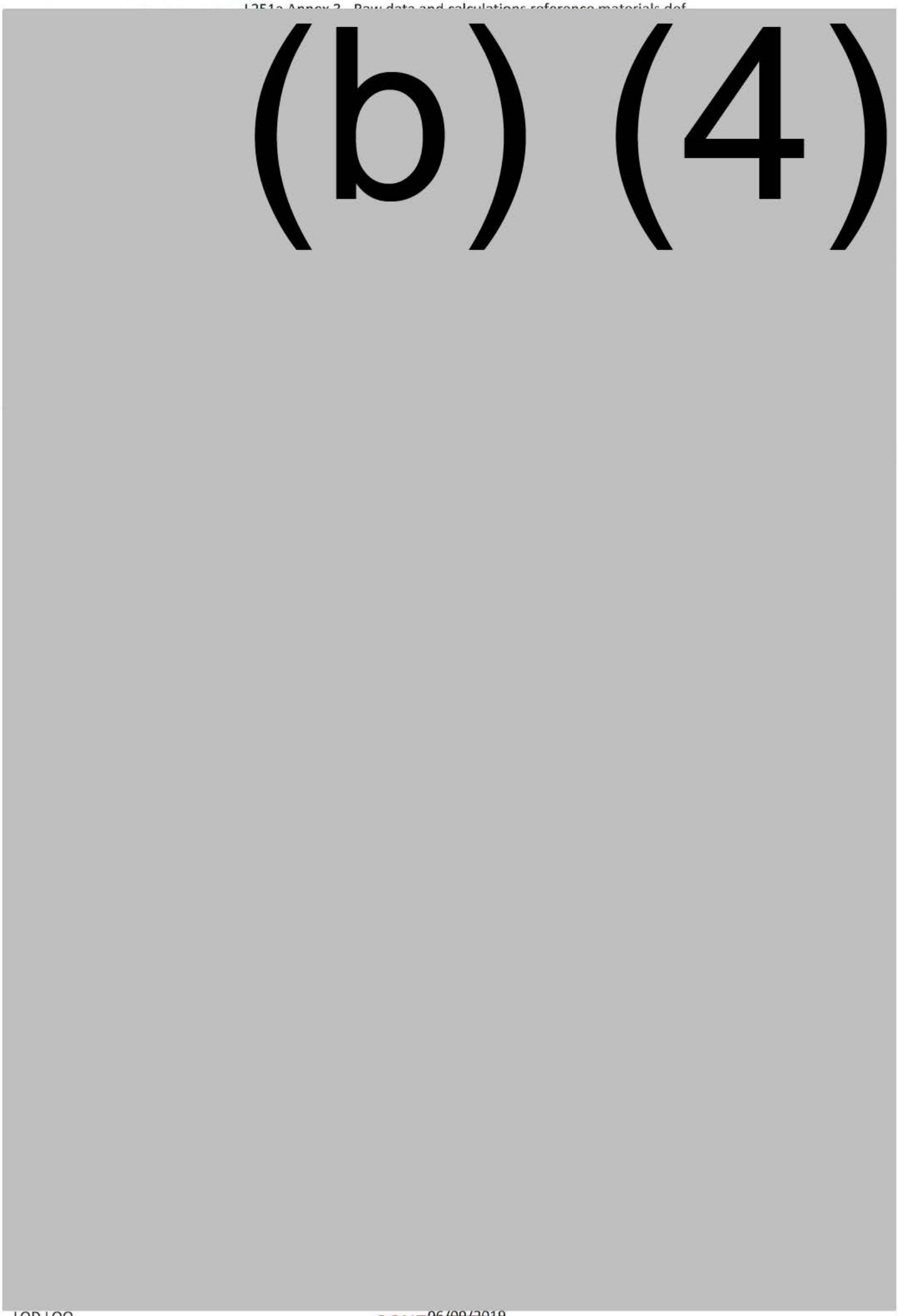


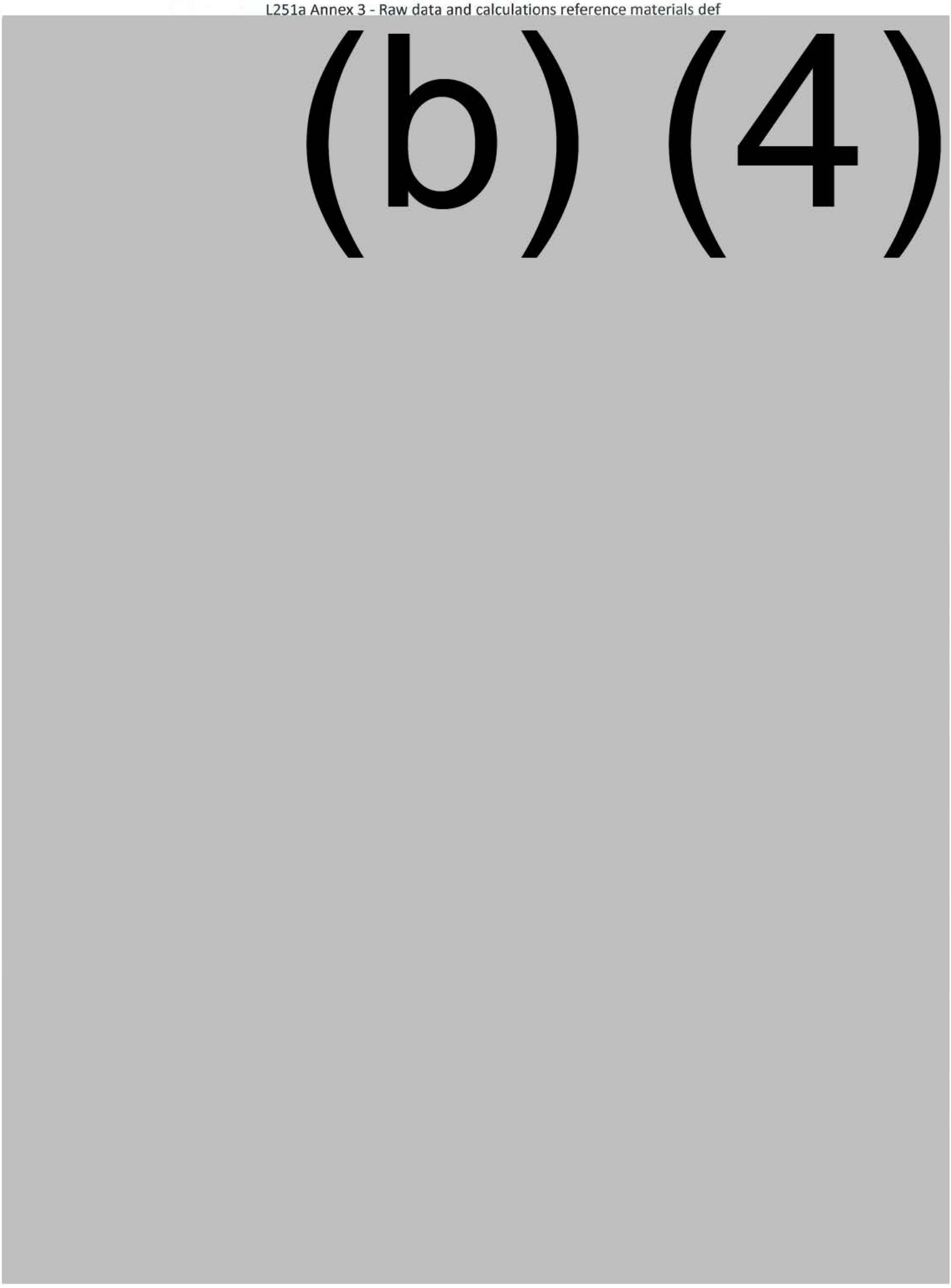
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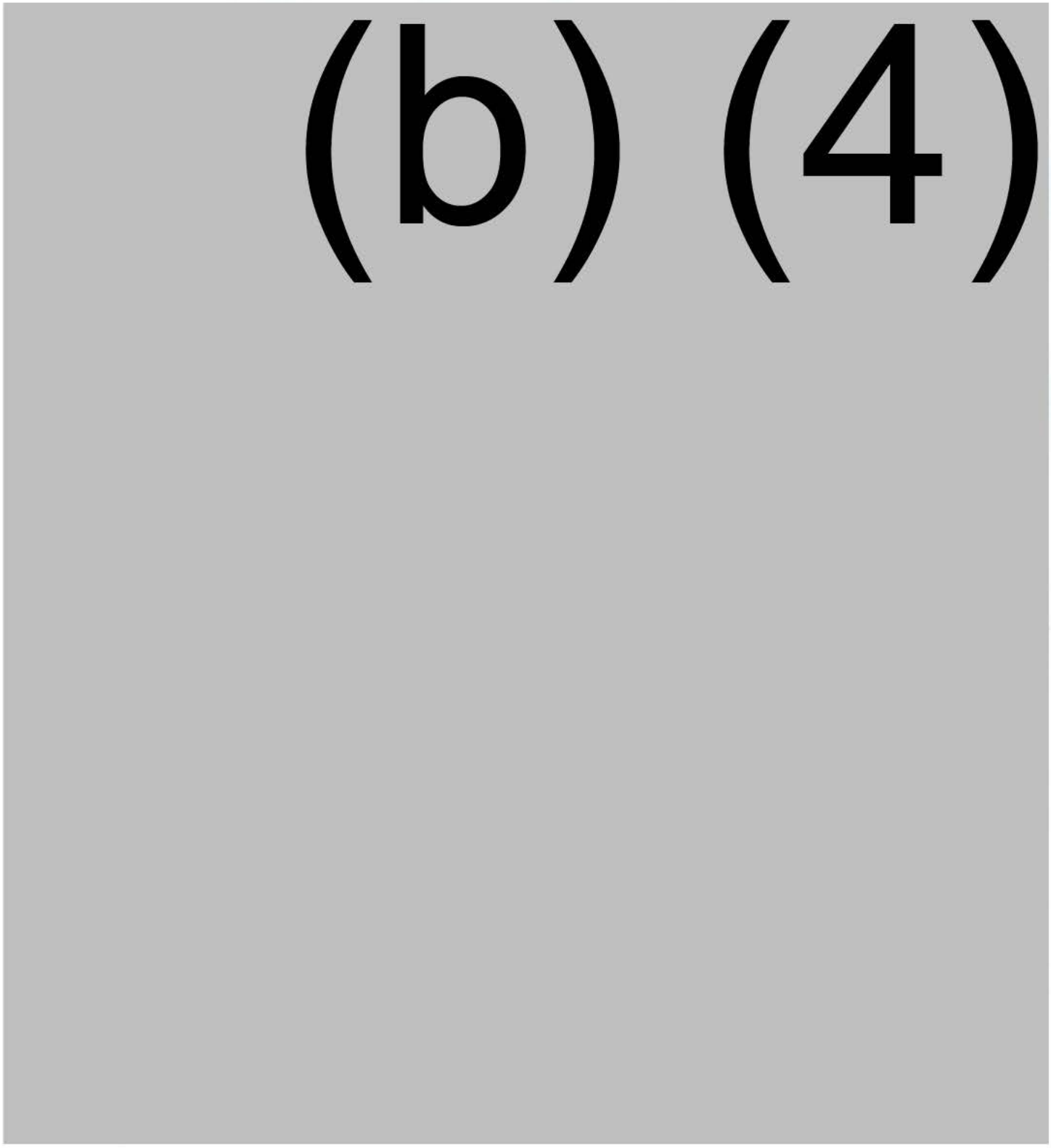


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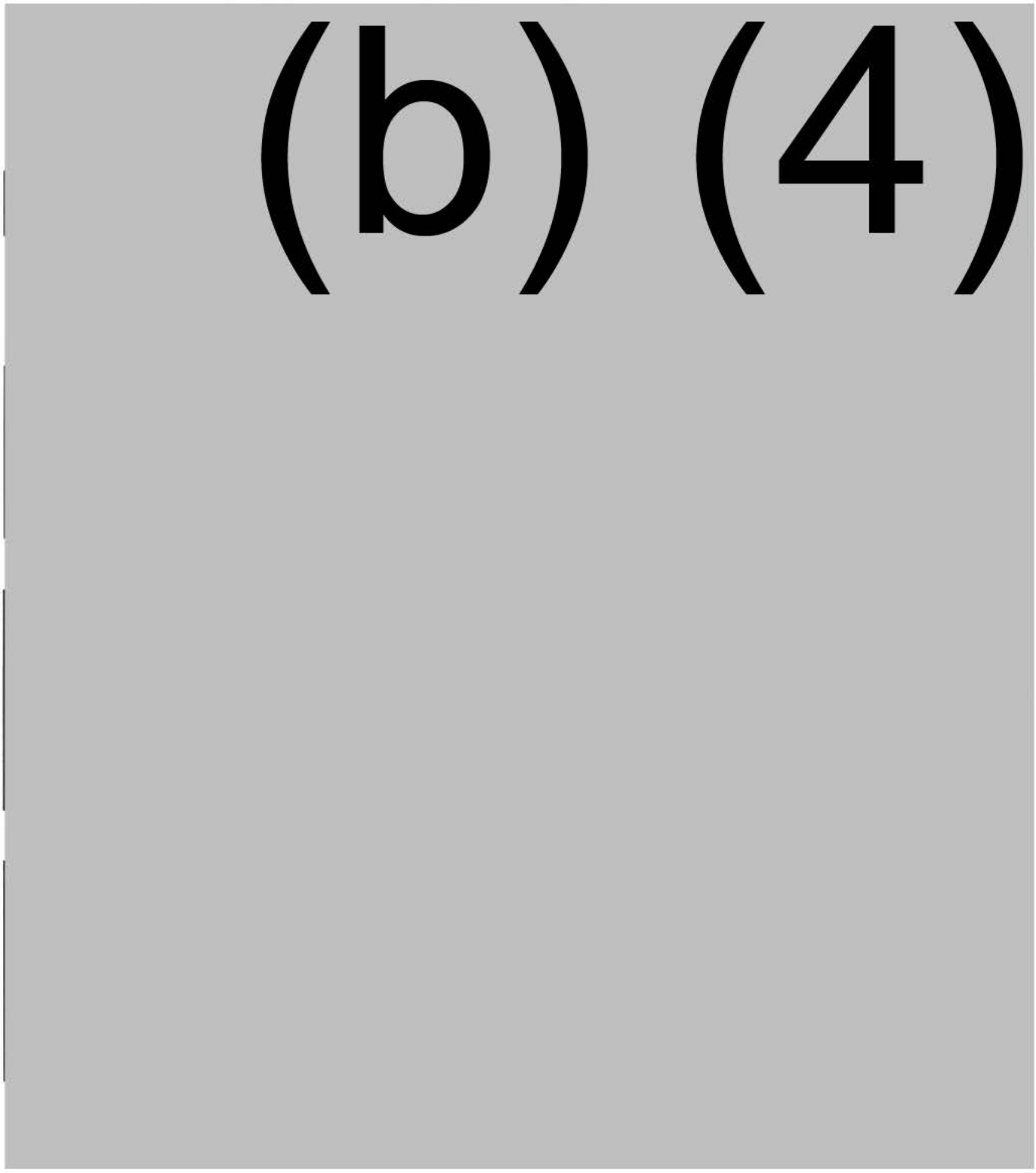

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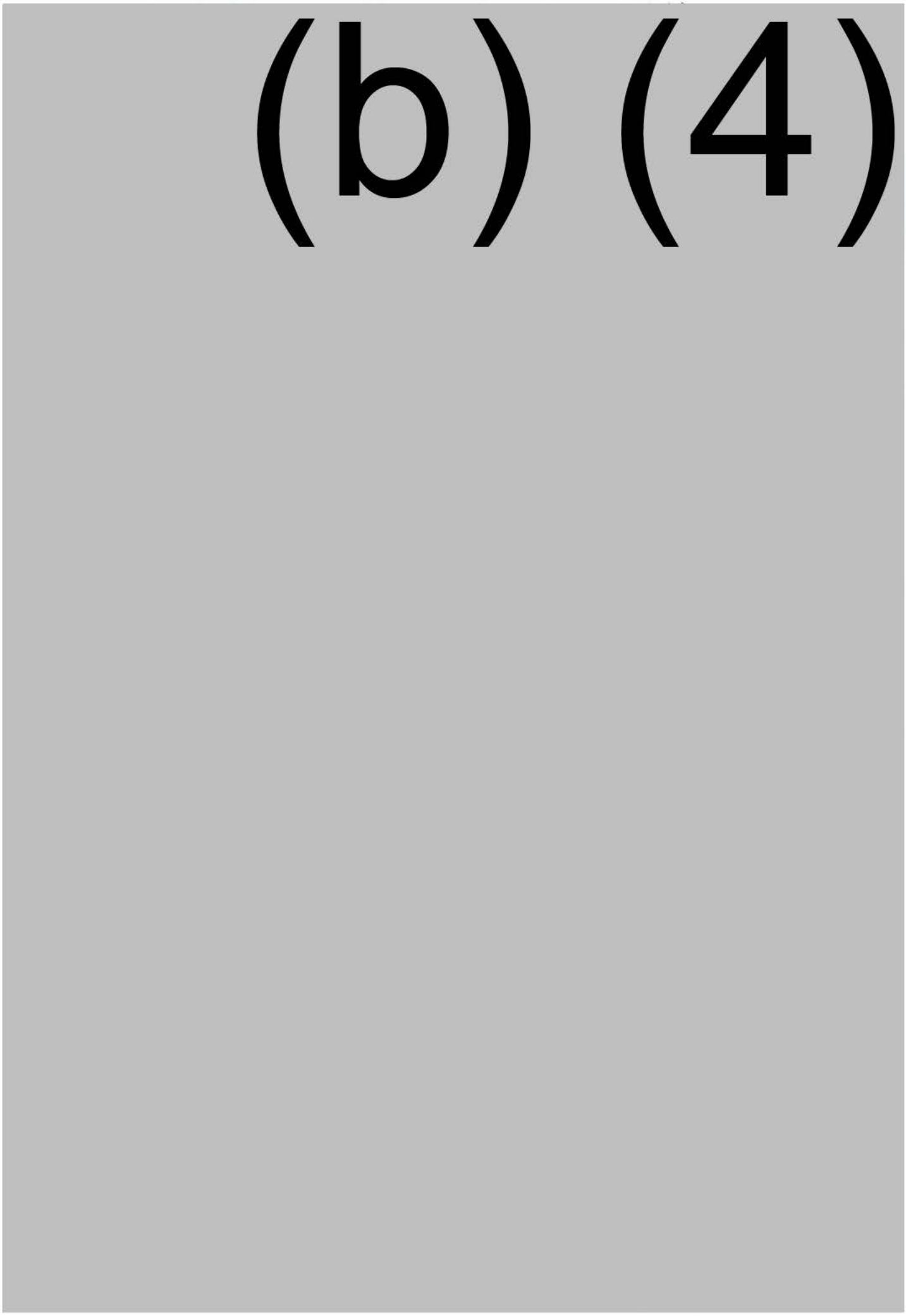


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VALIDATION OF ANALYTICAL METHOD FOR MEASURING β-MANNANASE ACTIVITY IN FEED ADDITIVE β-MANNANASE CC

xperiment number: L-251 b1

FINAL REPORT

Revision number: 0

Date: 30th April 2019

RESEARCH ACTIVITY CONTRACTED WITH:

BASF SE
Analyt. & Appl. Technology Feed Enzymes
G-ENA/MD – A110/R0316
67056 Ludwigshafen am Rhein (Germany)

Study monitors: Benjamin Georg Peikert and (b) (6)

Study director: (b) (6)

(b) (4)

(b) (4)

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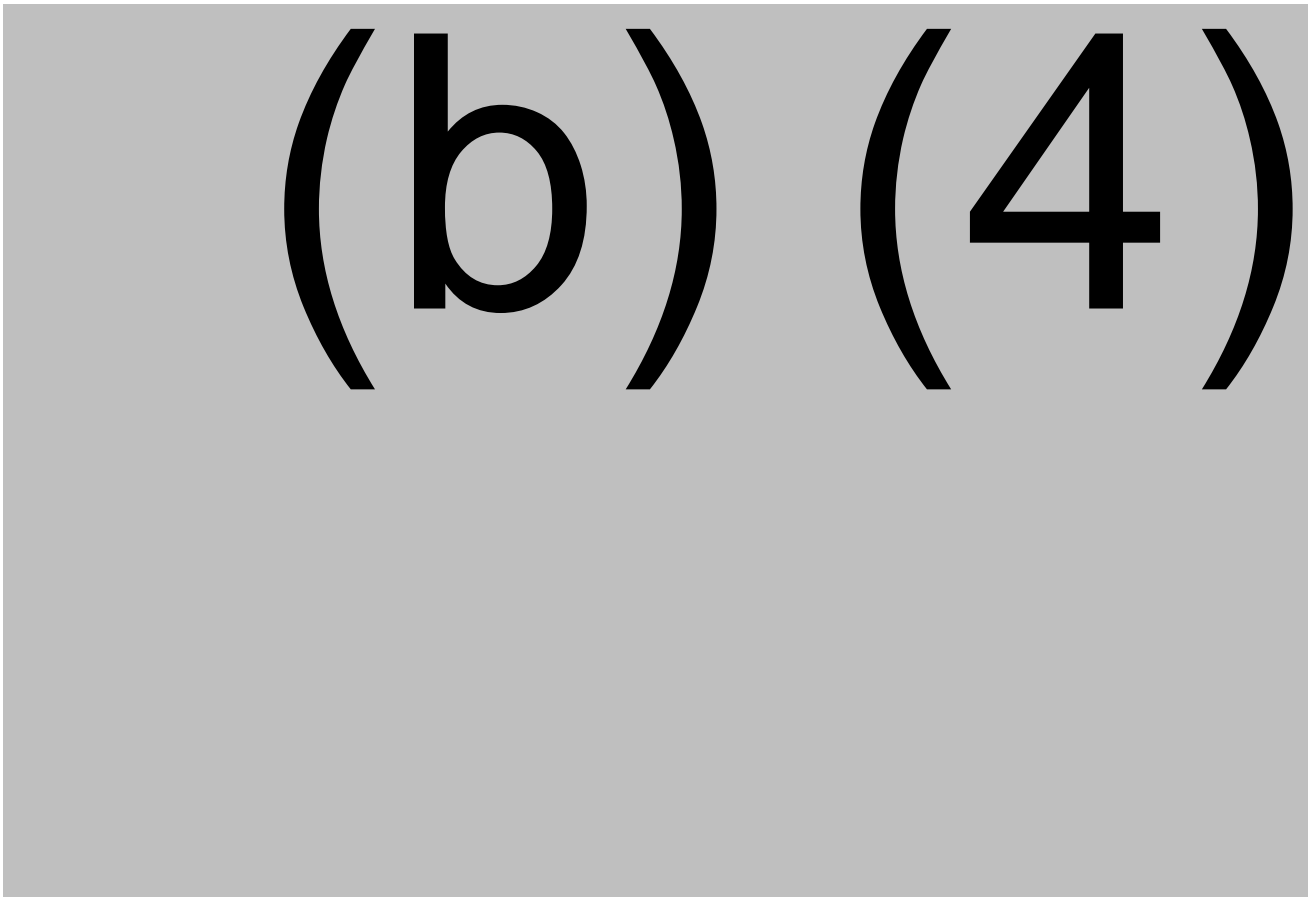
SUMMARY

The objective of the present study was the validation of the analytical method FEN/0004/01 for measurement of ß-mannanase activity present in feed additives.

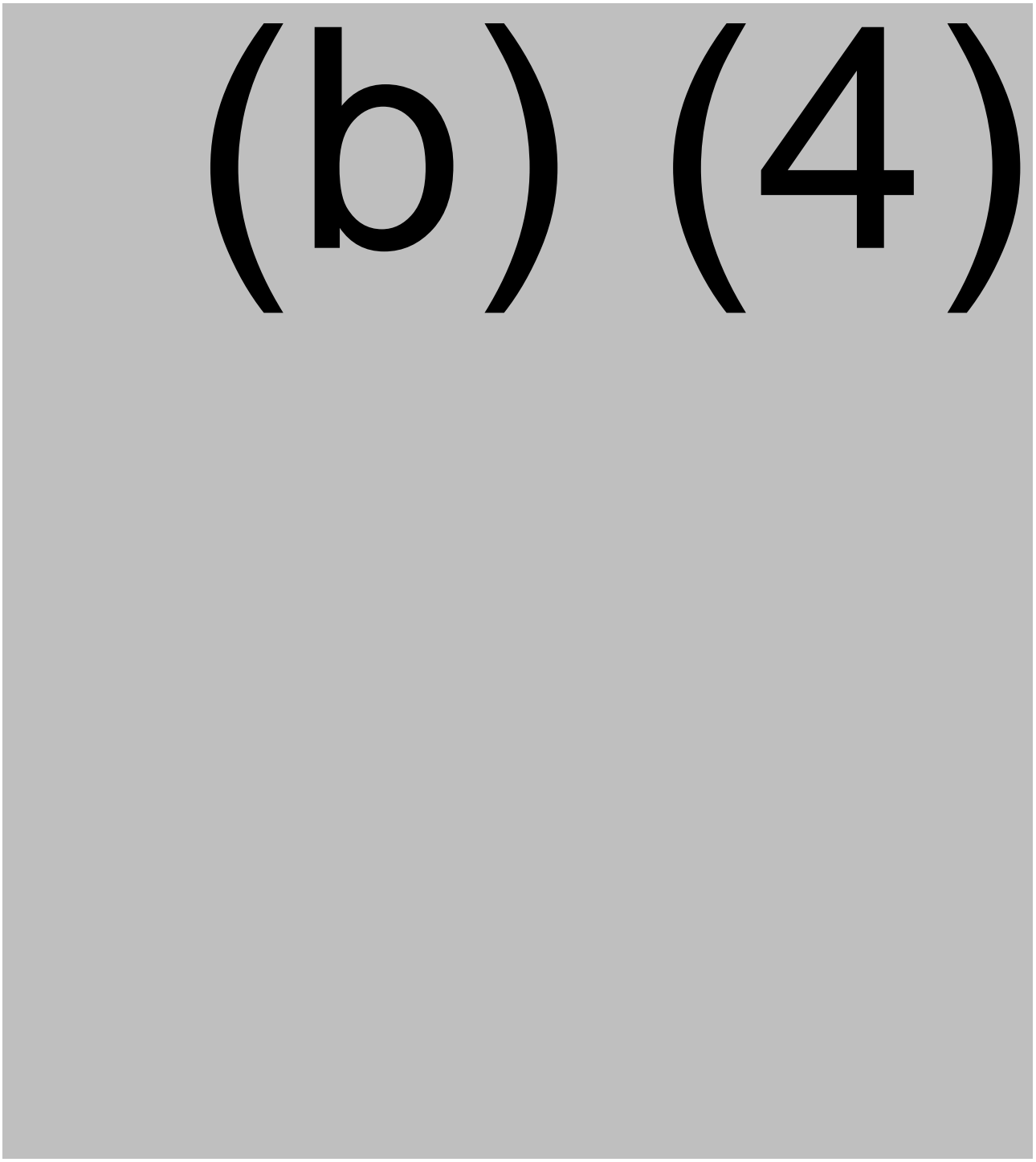
The study was performed using 3 types of Mannanase CC feed additives (blank, known, unknown) and two thermostable endo-mannanase enzymatic products: standard A 0009/001/FEN_STD (used for preparation of calibration curves) and control 0005/001/FEN_KON.

The validation of the method for the determination of ß-mannanase activity in feed additive Mannanase CC (BASF method FEN/0004/01 provided by the company) is based on the methodology using a coloured azo-substrate (azo-carob galactomannan, dyed with Remazol Brilliant Blue R). The hydrolysis of this substrate by the endo-1,4,-ß-mannanase produces water soluble dyed fragments. The rate of release of these fragments is related to the enzyme activity.

The study consisted on the determination of the following quality parameters of a method of analysis: range and linearity, limits of detection (LOD) and quantification (LOQ), and accuracy and precision parameters, which included recovery, repeatability, intermediate precision and inter-laboratory reproducibility. Robustness and Uncertainty were also evaluated.



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RESPONSIBILITIES

R&D

Study Director

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Study monitors

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Analytical Laboratory

(b) (6), (b) (4)

OBJECTIVE

The objective of the present study was the validation of the analytical method FEN/0004/01 for measurement of β-mannanase activity present in feed additives.

QUALITY ASSURANCE

IRTA has an Integrated Quality Policy and the Institute is **ISO 9001:2015** certified for their R+D+T projects, contracts and services.

This study has been conducted in accordance with ISO 9001:2015 quality criteria, and under the global SOPs **PG-75** (Conducting Research studies).

All study staff have appropriate training and qualifications to perform their study tasks. They had been trained in Quality Systems including data recording requirements and forms for completion of the study, QC techniques, relevant SOPs and specific protocol requirements prior to study start.

SCOPE AND TEST METHOD DESCRIPTION

This procedure is for the determination of β-mannanase activity (EC 3.2.1.78) expressed as TMU/g in β-mannanase CC feed additive by in-house analysts.

The determination of β-mannanase activity in feed additives (BASF method FEN/0004/01) is based on the methodology using a coloured azo-substrate (Remazol Brilliant Blue R azo-carob galactomannan). The hydrolysis of this substrate by the endo-1,4,-β-mannanase produces water soluble dyed fragments. The rate of release of these fragments is related to the enzyme activity.

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SITE OF THE STUDY

All the ß-mannanase determinations included in this study were performed in the laboratory of IRTA-Animal Nutrition, in the Centre Mas Bové (Constantí, Tarragona).

DATES AND DURATION OF THE STUDY

The duration of the study was 31 days, starting on the 22nd February 2019 and finishing on the 25th March 2019.

DEFINITIONS

Range and Linearity

The linearity is the ability of a method (within a given range) to obtain test results that are directly proportional to the concentration of analyte in the sample (Eurachem, 1998). The range is the interval between upper and lower concentration (amounts) of analyte in the sample (including these concentrations for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity).

Limit of detection (LOD)

The limit of detection was defined as the smallest measured content from which it is possible to deduce the presence of the analyte with reasonable statistical certainty. The LOD is numerically equal to 3 times the standard deviation of the mean of blank determinations (n≥10, where n=nº of measures) performed under repeatability conditions. It can also be calculated from the slope of the calibration line as y(LOD) = a+ 3 Sy/x.

Limit of Quantification (LOQ)

The limit of quantification was defined as the lowest content of analyte that can be measured with reasonable statistical certainty. The LOQ is numerically equal to 10 times the standard deviation of the mean of blank determinations (n≥10, where n=nº of measures) performed under repeatability conditions. It can also be calculated from the slope of the calibration line as y(LOQ) = a+ 10 Sy/x.

Precision

The precision expresses the closeness of agreement between a series of independent measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions.

Accuracy

The accuracy expresses the closeness of agreement between the value that is accepted either as a conventional true value or an accepted reference value and the value determined by analysis.

Recovery

The Relative Percent between determined and expected values of xylanase fortified samples was calculated as an indicator of recovery.

Repeatability

The repeatability measures precision under the same operating conditions over a short interval of time. Independent test results are obtained with the same method on identical test items in the same laboratory with the same operator using the same equipment within short intervals of time. Repeatability is expressed mathematically by the coefficient of variation (relative standard deviation) of a series of different measures.

Intermediate precision

Intermediate precision expresses within-laboratory variations: e.g. different days, different analysts, different equipment, etc.

Reproducibility

The reproducibility measures precision under different operating conditions. Reproducibility relates to the agreement of test results with different operators, test apparatus, and laboratory locations. In this case it was obtained analysing samples containing unknown concentrations of the test product.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. In this case, the parameters changed have been pH, time and temperature of the enzymatic reaction.

Uncertainty

The uncertainty is the expression of the statistical dispersion of the values attributed to a measured quantity. All measurements are subject to uncertainty and a measurement result is complete only when it is accompanied by a statement of the associated uncertainty. By international agreement, this uncertainty has a probabilistic basis and reflects incomplete knowledge of the quantity value.

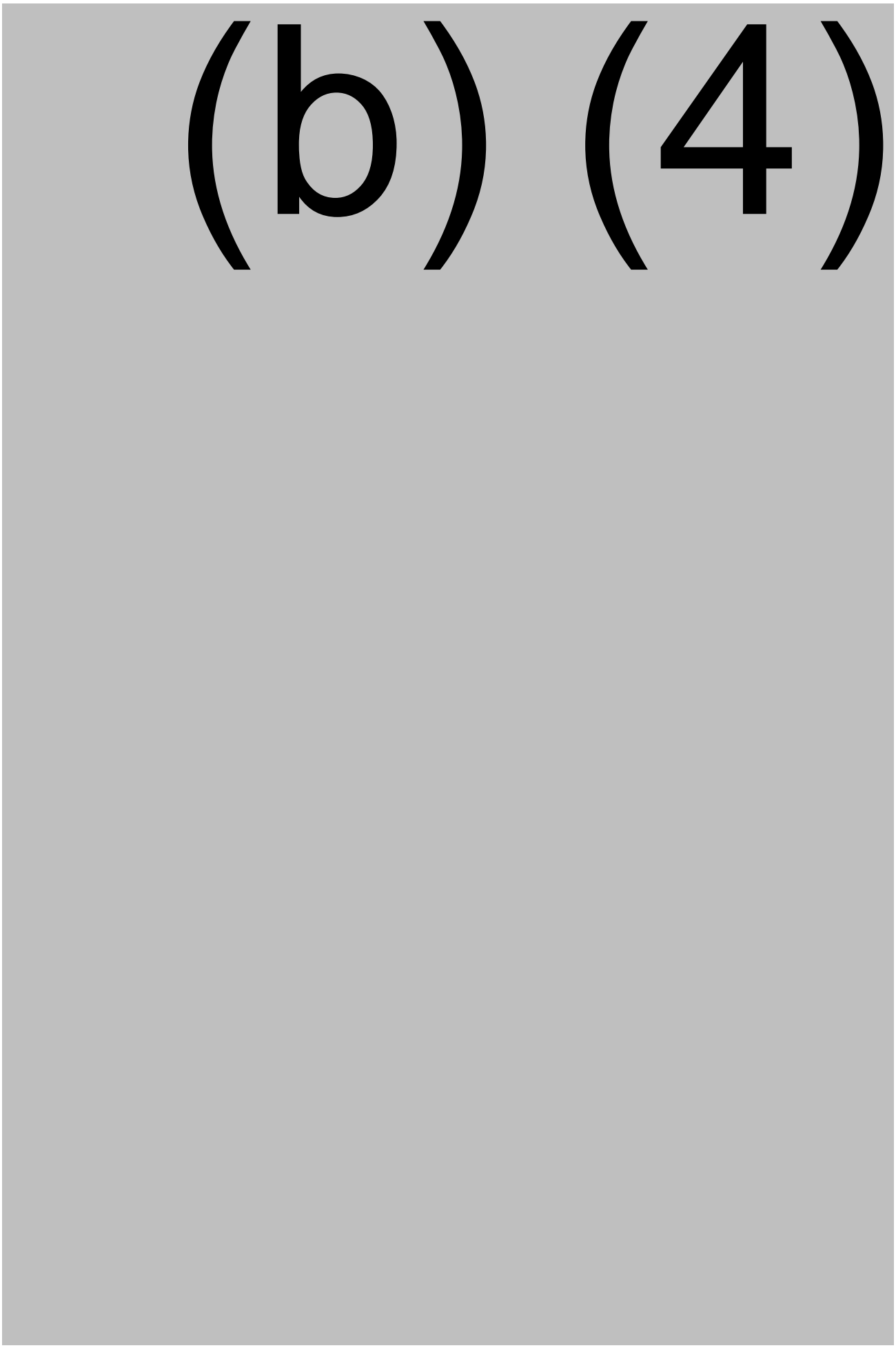
METHODOLOGY

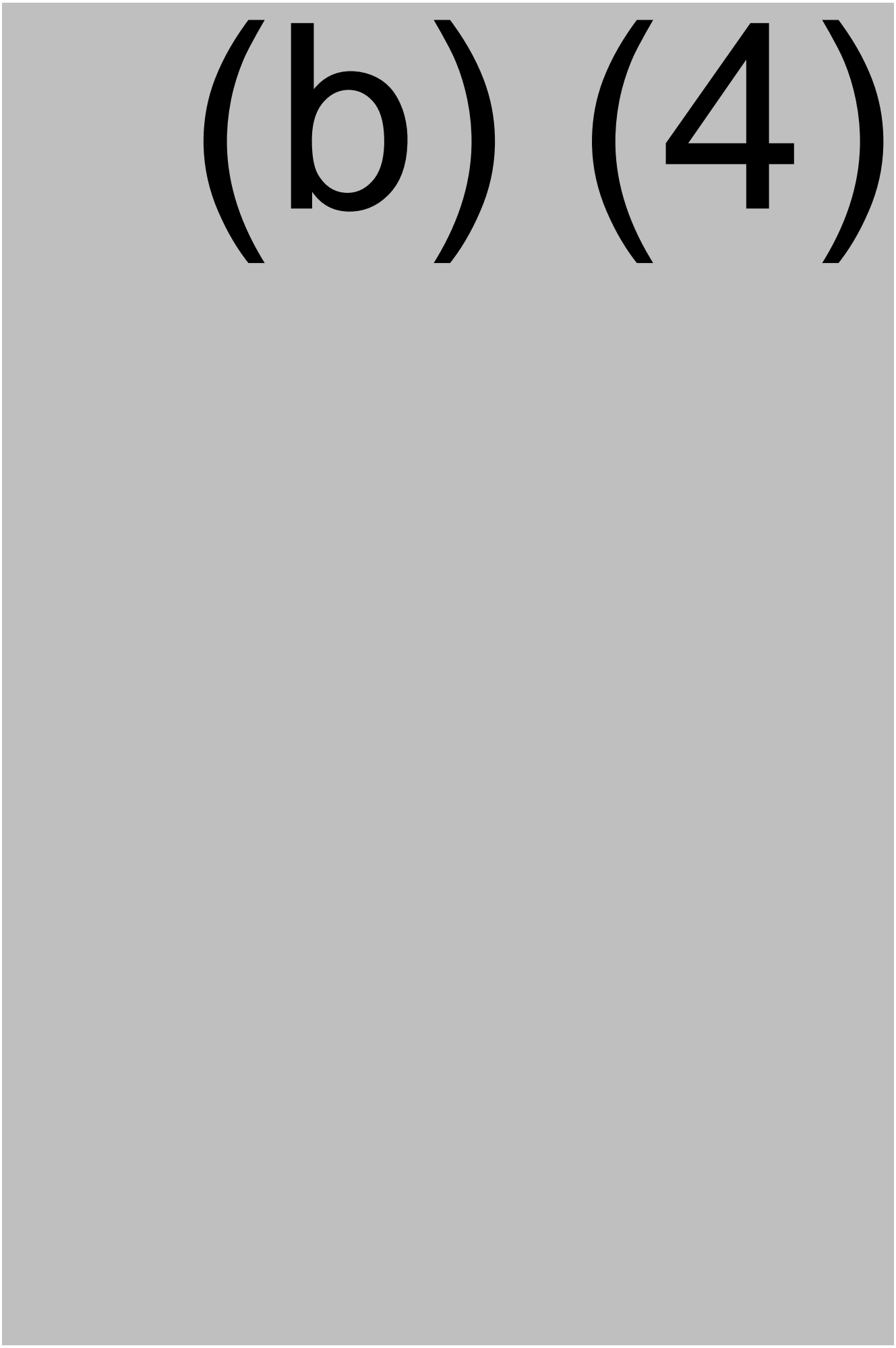
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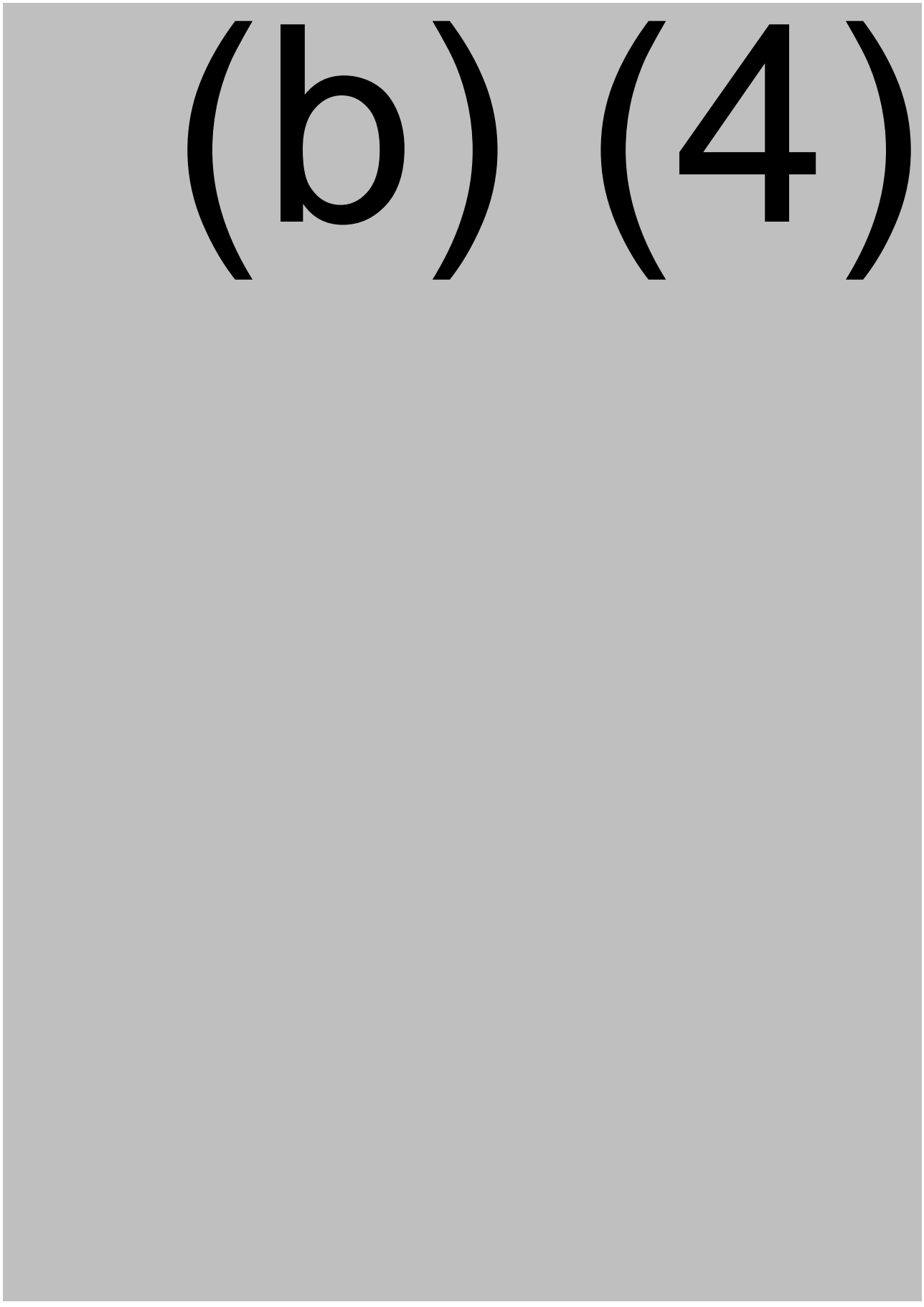
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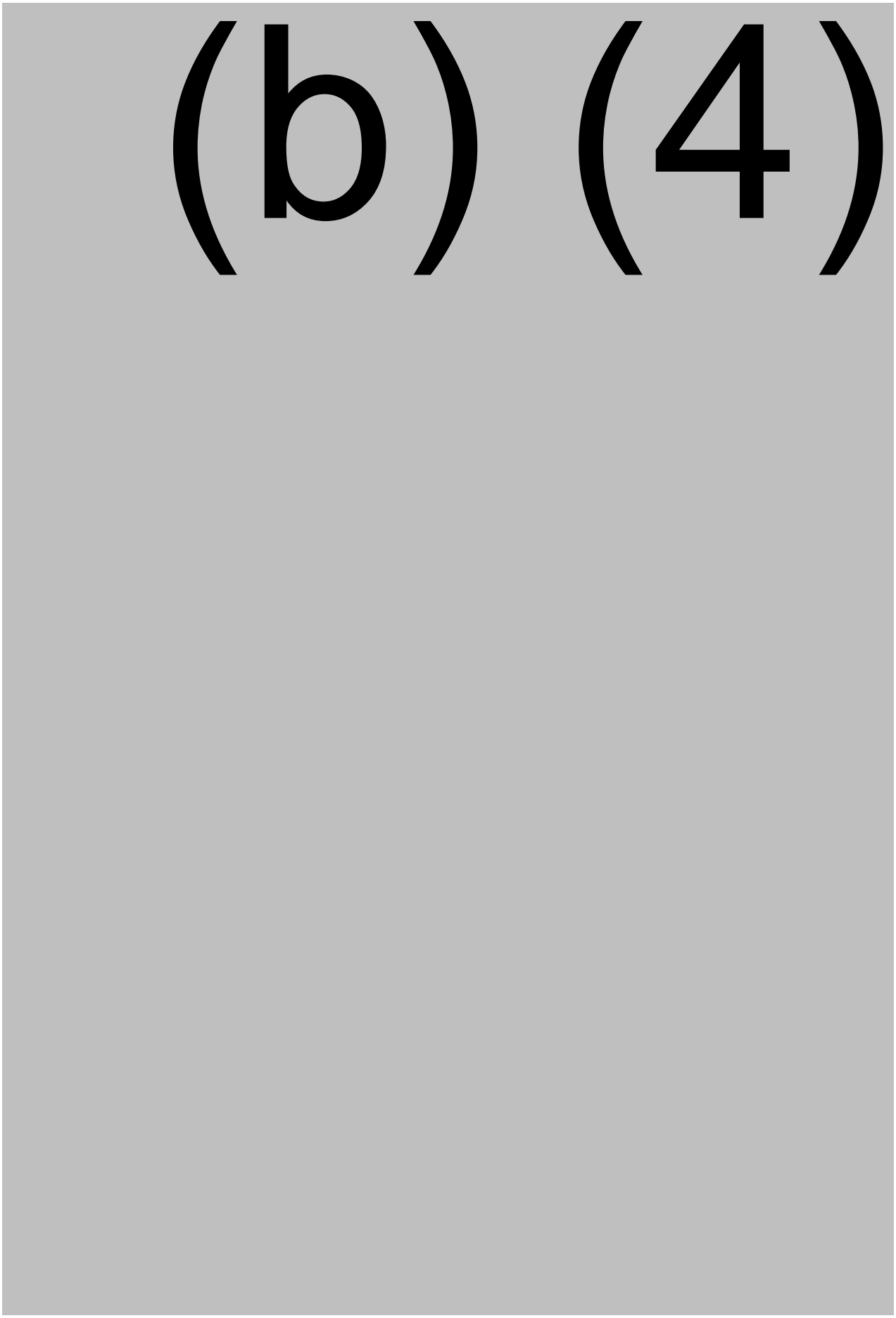
Reagents

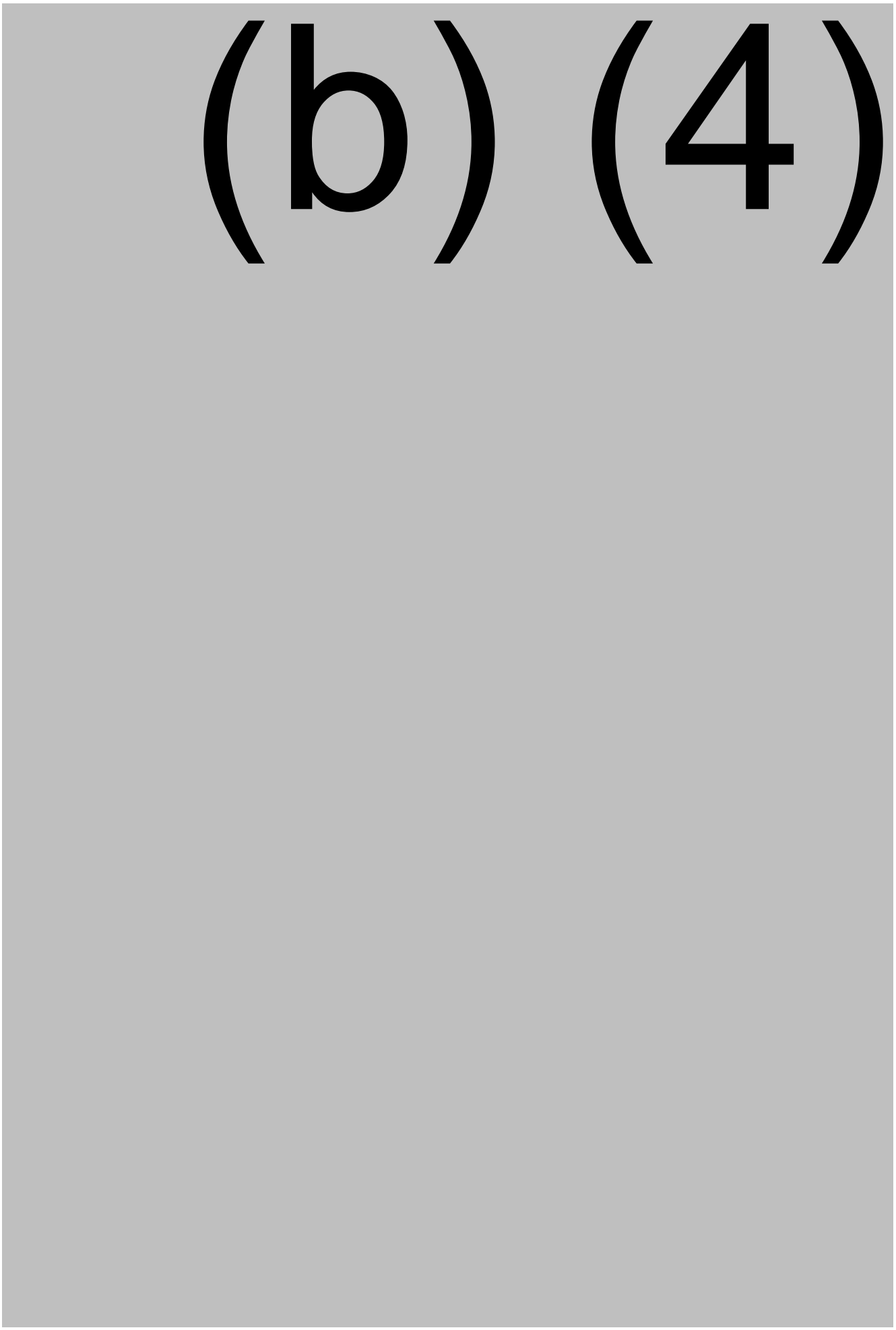
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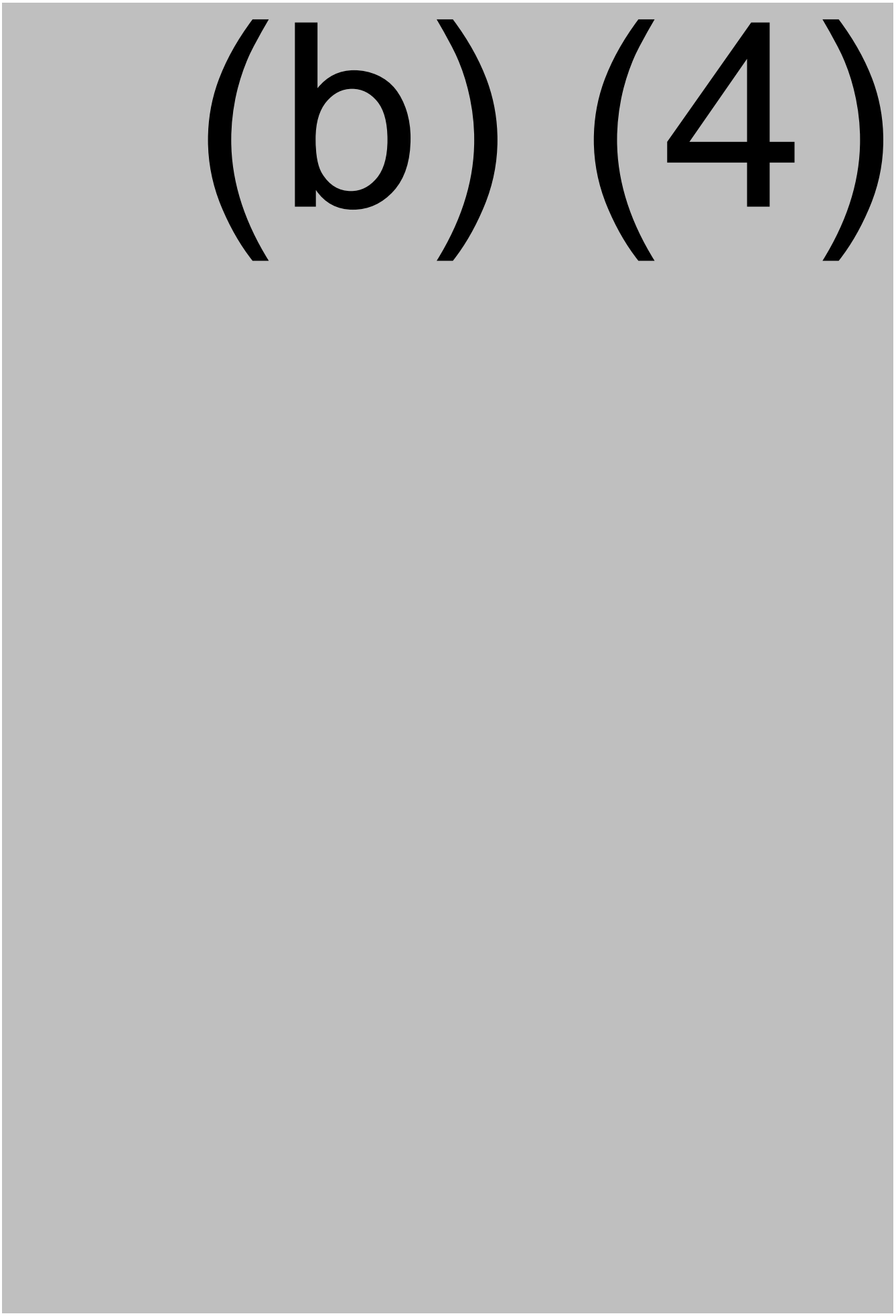

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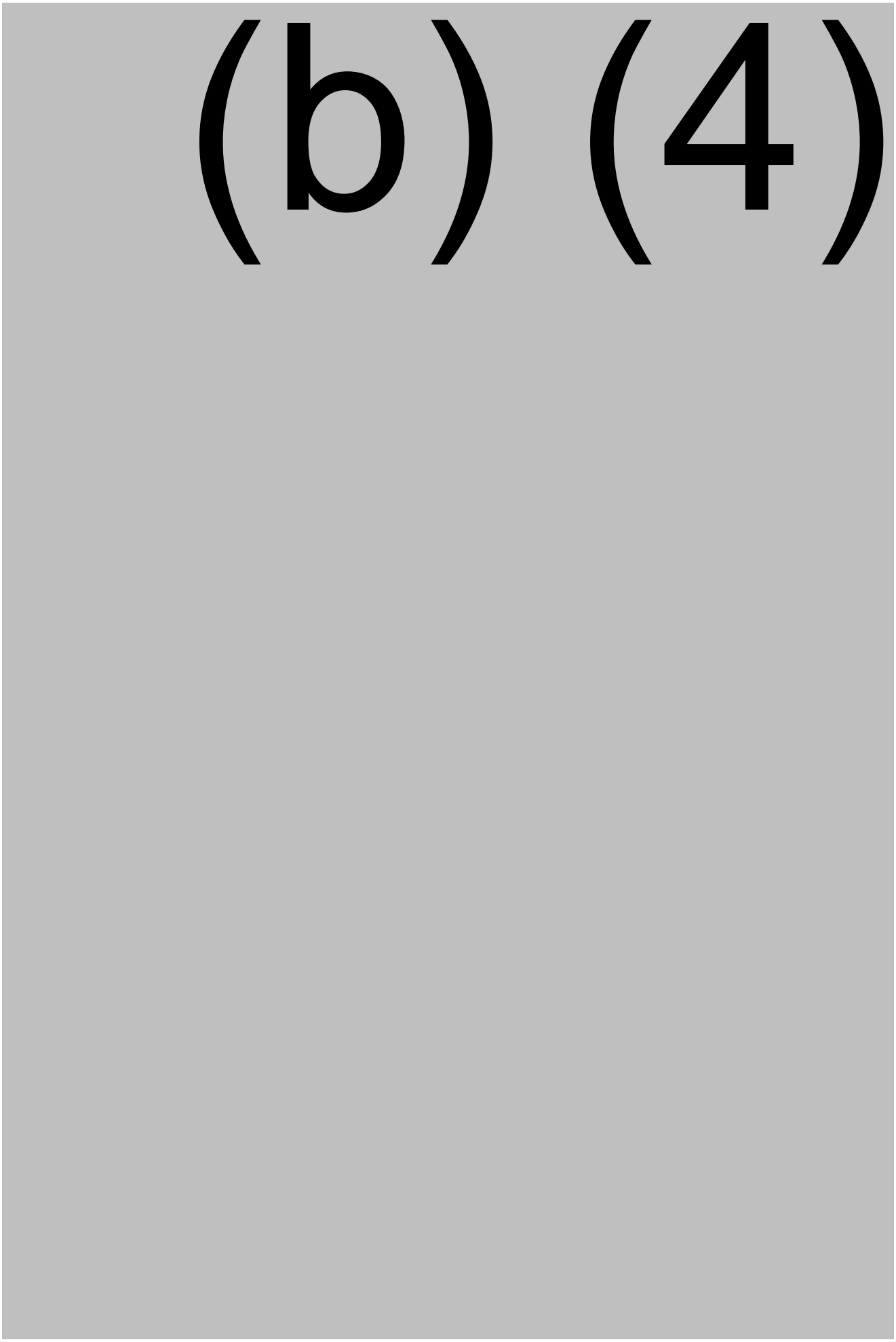

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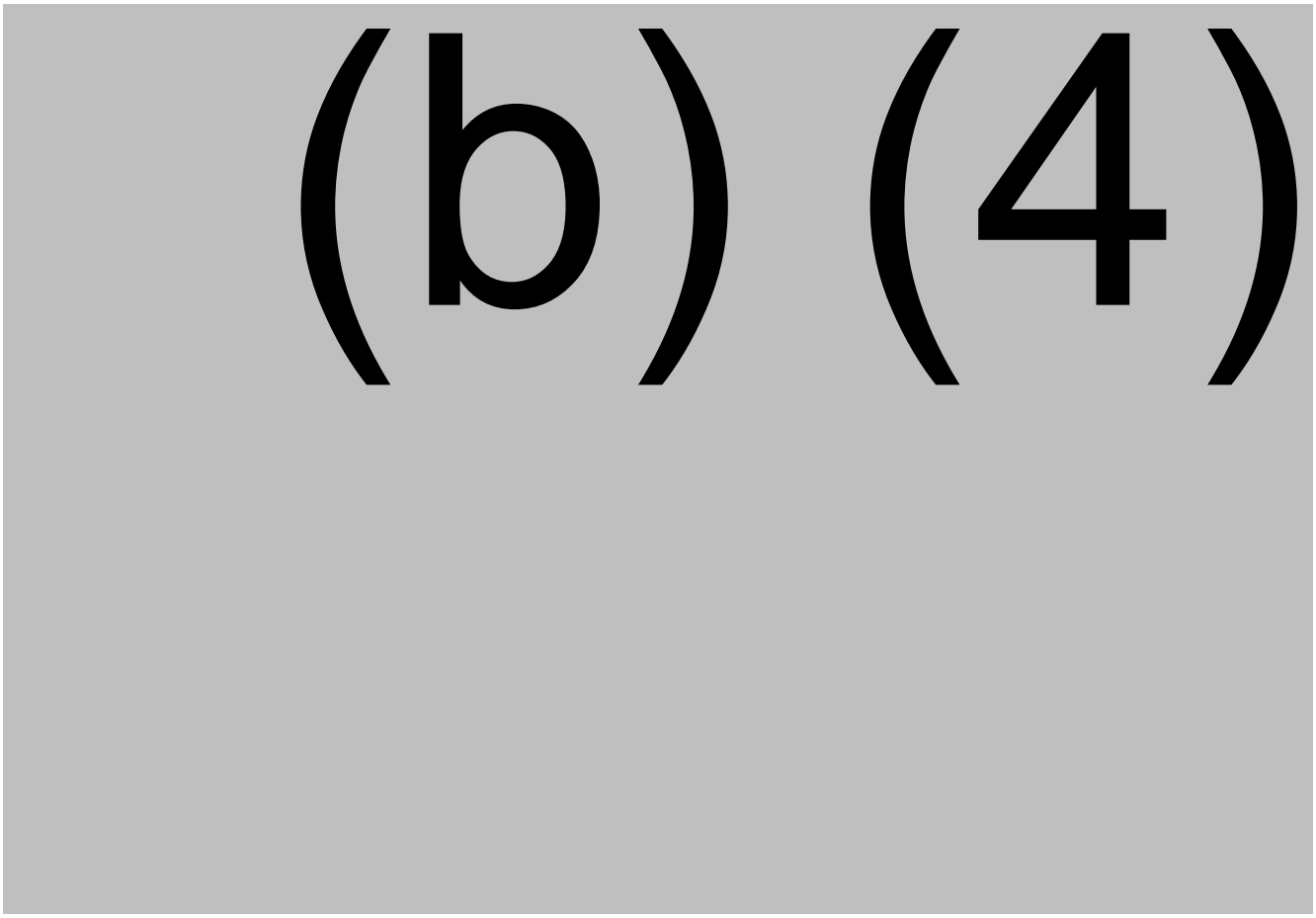

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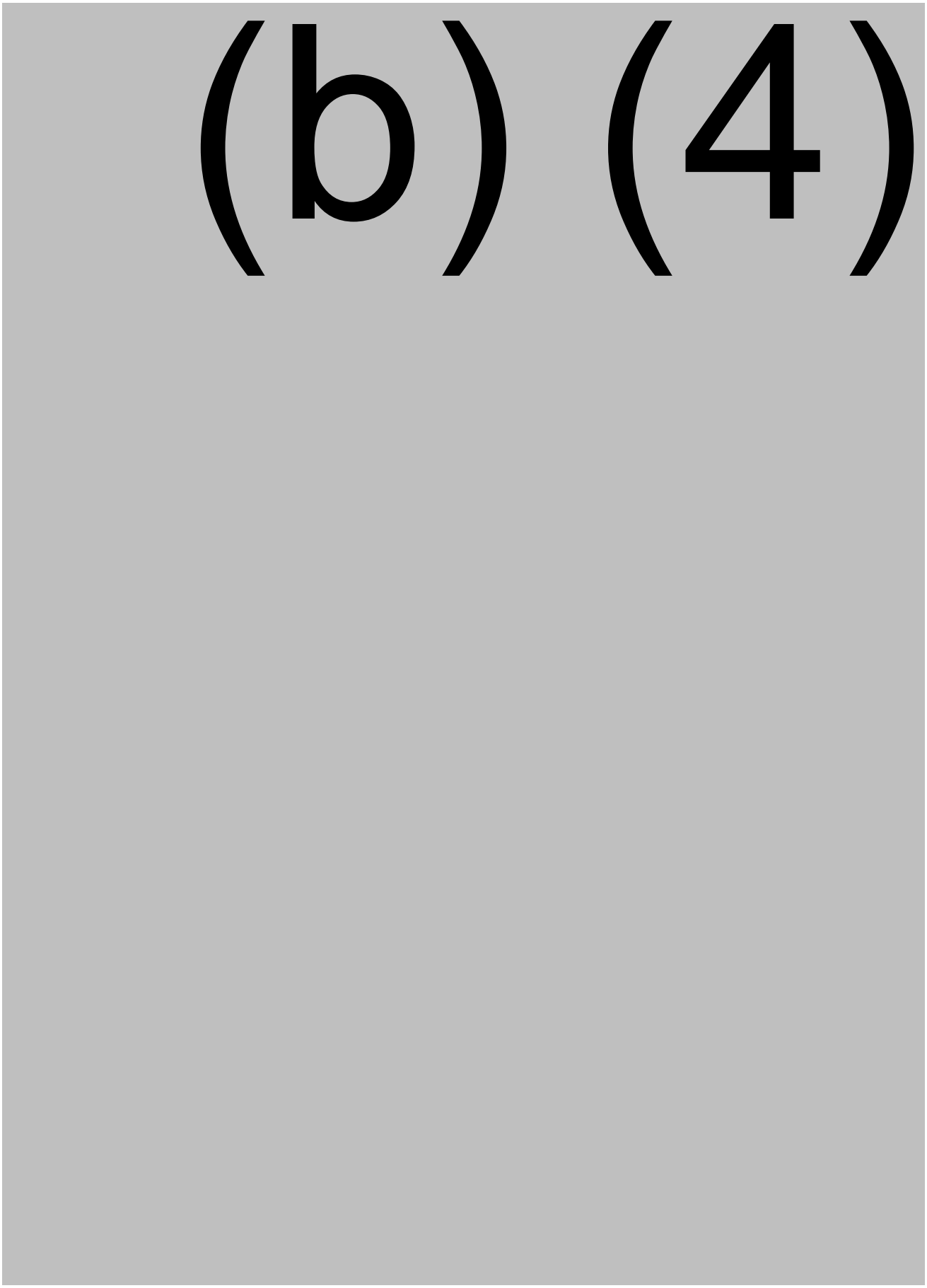

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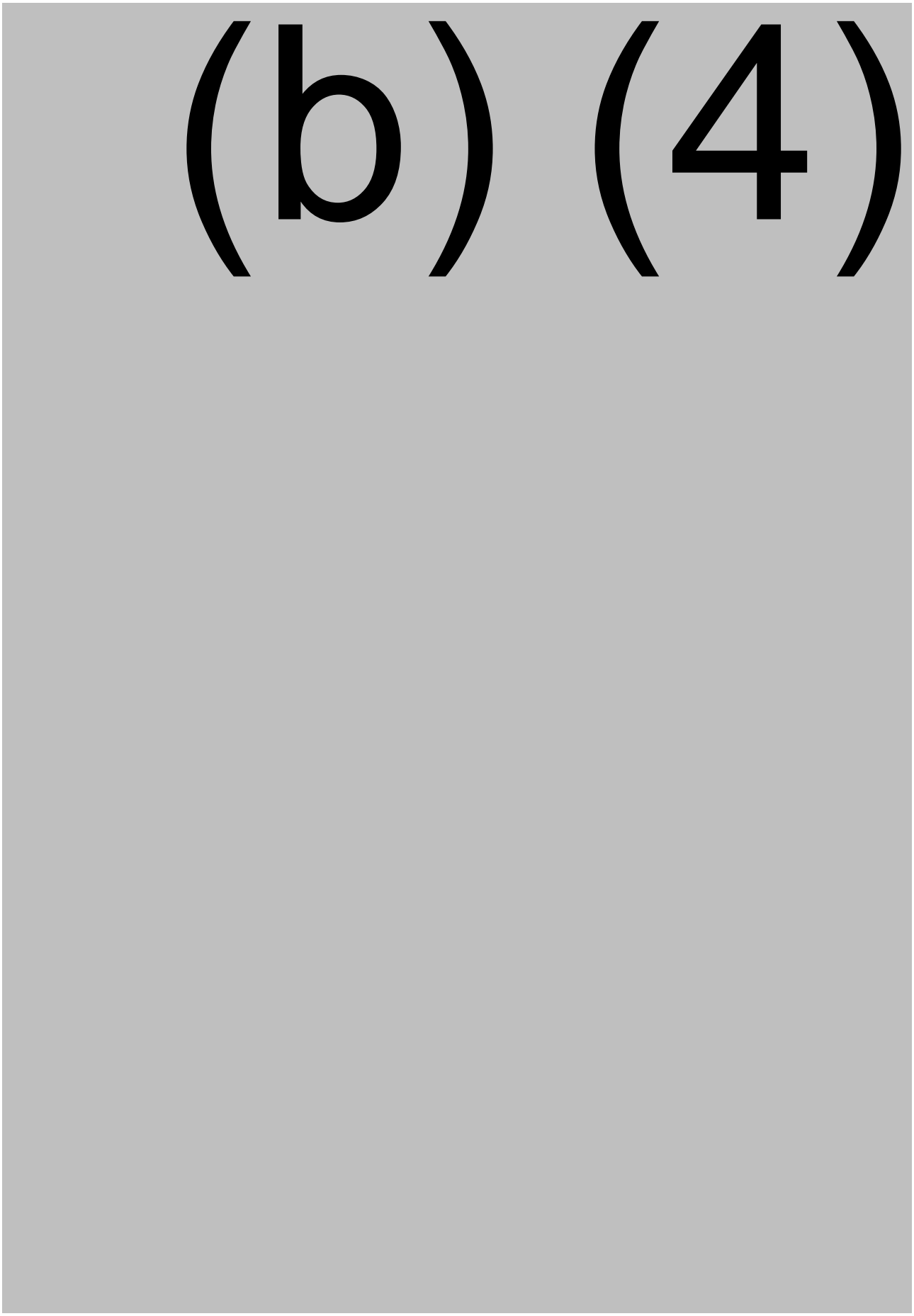


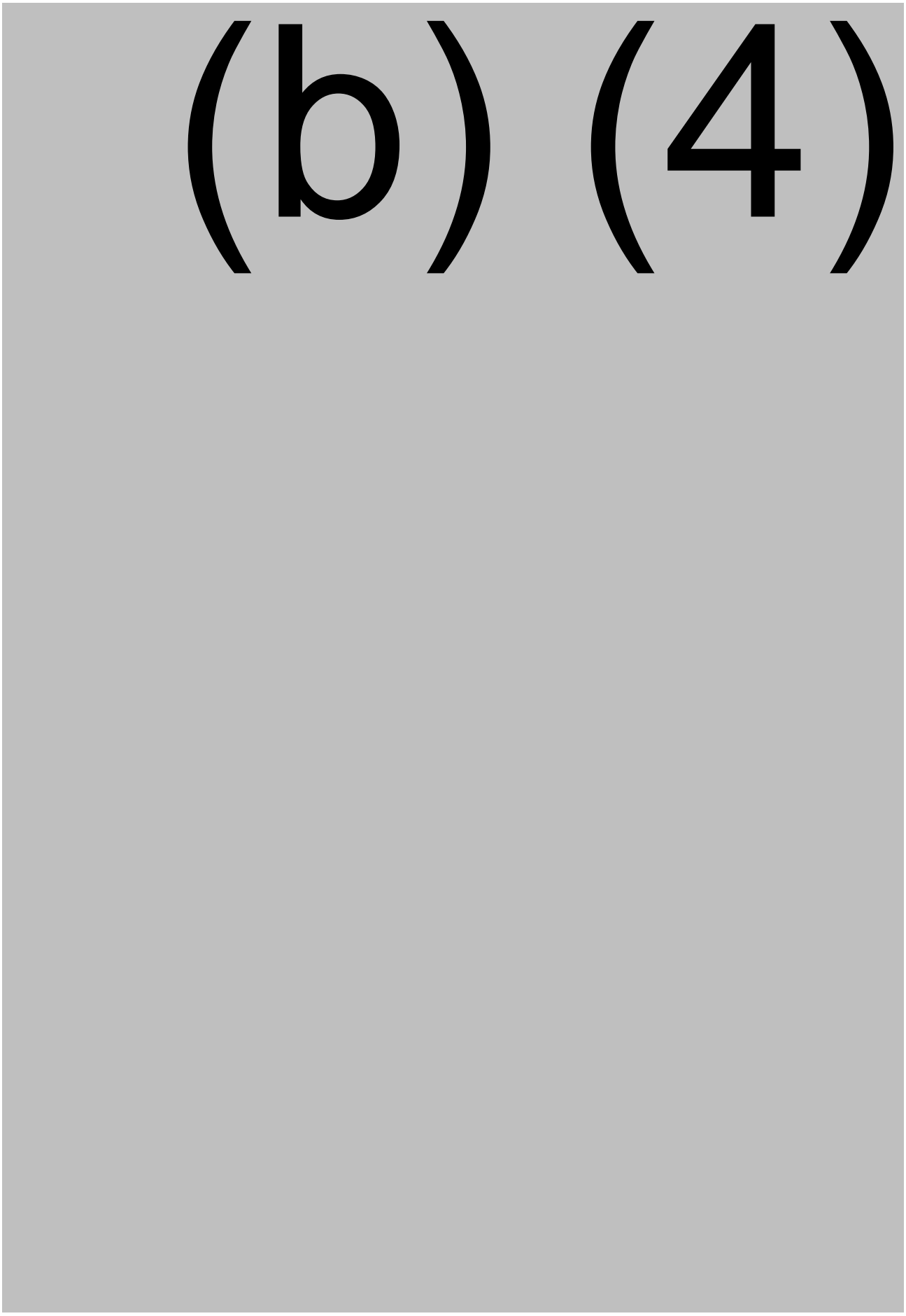
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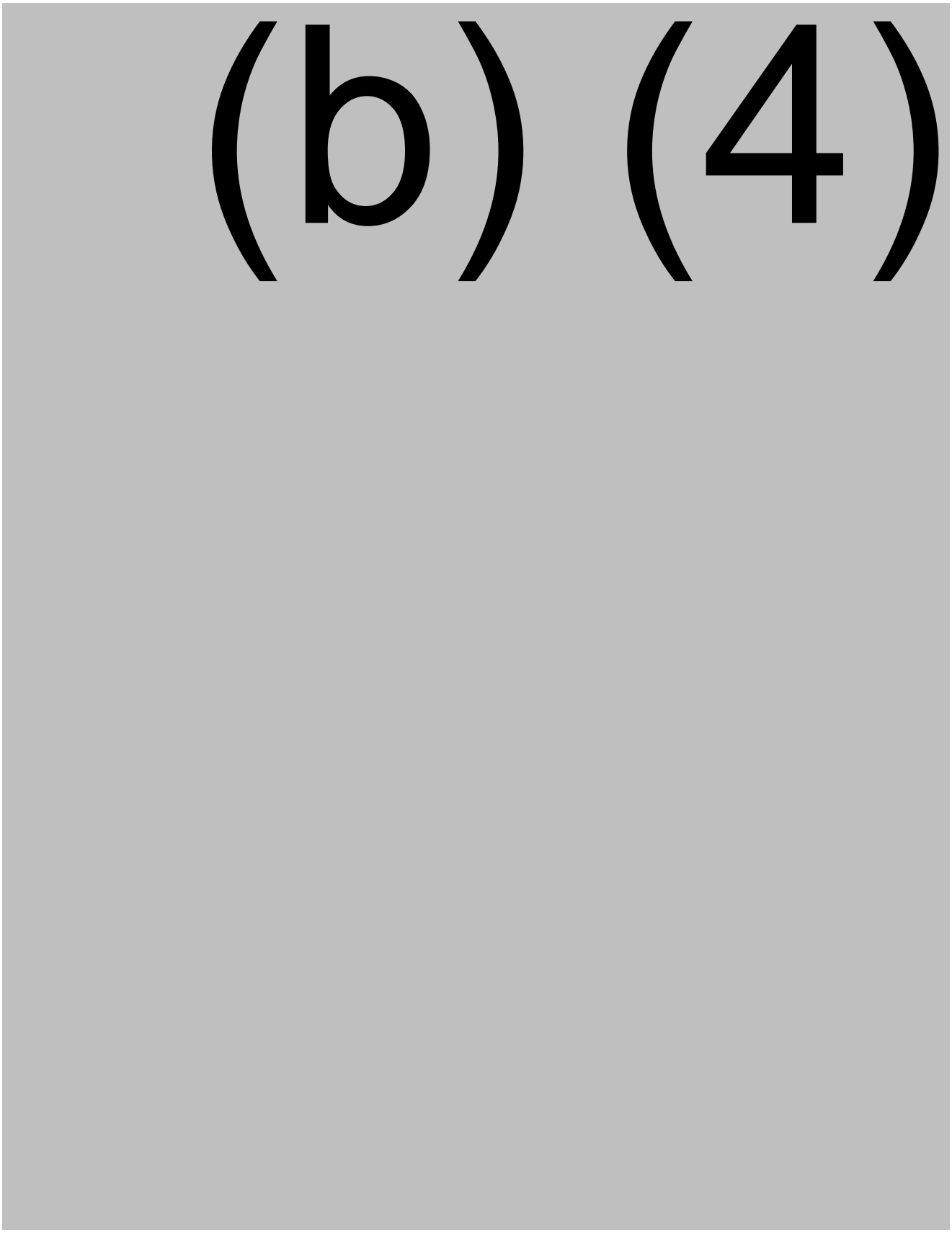
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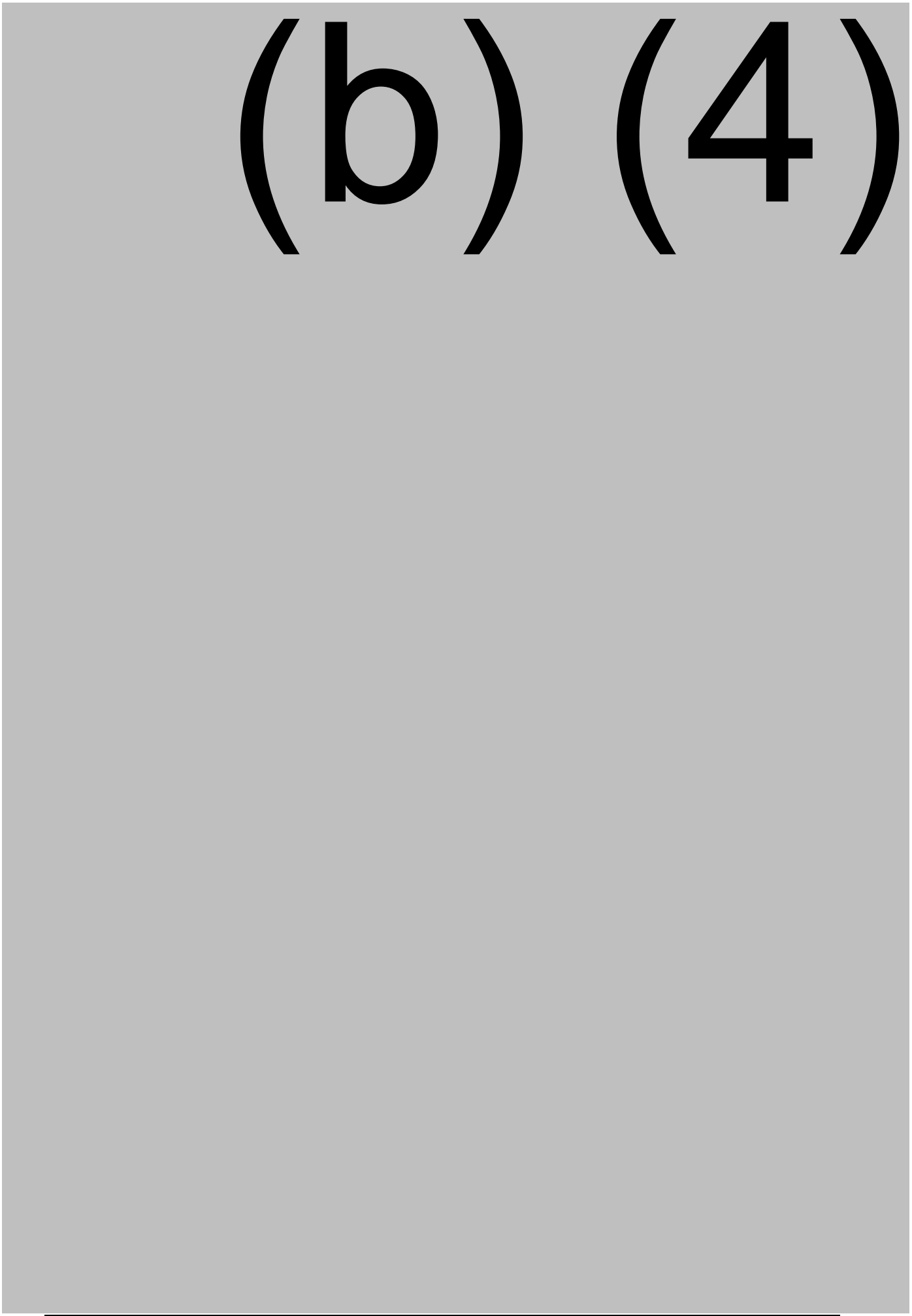

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Thompson, M., Ellison, S.L.R. and Wood, R. (2002). Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis (IUPAC Technical report). Pure Appl. Chem. 74 (5), 835-855.

Magnusson, B. and Örnemark, U. (2nd ed. 2014) Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to method Validation and Related Topics. ISBN 978-91-87461-59-0. Available from www.eurachem.org.

Signatures:

(b) (6)

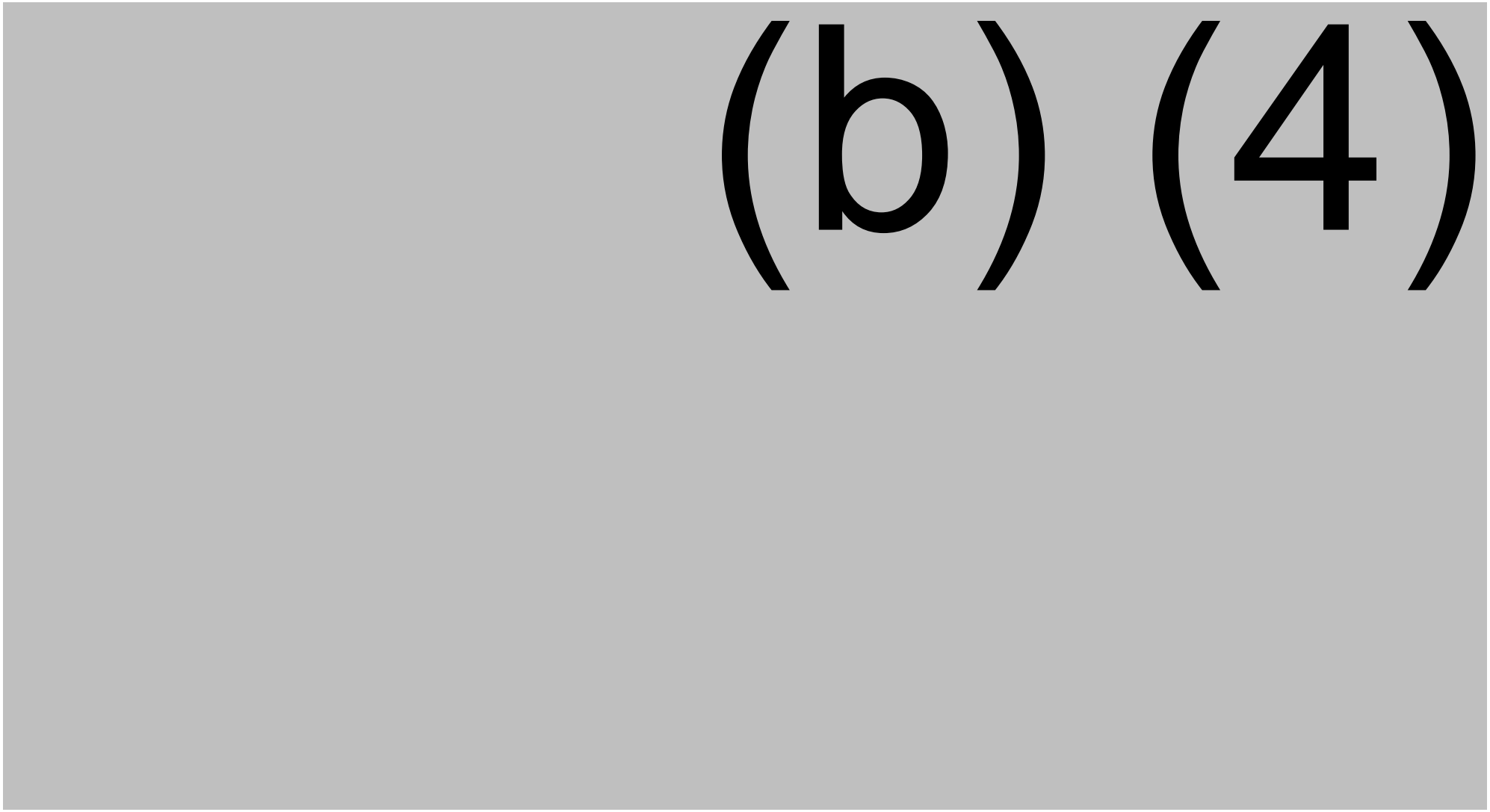
Date: 2ND MAY 2019 Date: 3 MAY 2019 Date: 3 May 2019

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ANNEXES

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ANNEX 1. VALIDATION PLAN FOR PHOTOMETRIC ASSAY OF MANNANASE ACTIVITY IN FEED ADDITIVES



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ANNEX 2. METHOD OF ANALYSIS FEN/0004/01 PROVIDED BY BASF: PHOTOMETRIC ASSAY OF MANNANASE ACTIVITY IN FEED ADDITIVES

Method of Analysis FEN/0004/01

BASF
We create chemistry

Title: **Photometric assay of mannanase activity in feed additives**

Effective date: April 20th, 2018

Prepared by: (b) (6)

Reviewed and approved: Dr. Benjamin Peikert

(b) (6)

Signature, date

1 Keywords

1.1 To be determined

Mannanase activity

1.2 Matrix

Feed additives such as

- Mannanase SD powder
- Mannanase CC
- Mannanase 8000
- Mannanase 8000 L

2 Principle

The assay procedure is specific for endo-1,4-β-D-mannanase activity. On incubation of dyed carob galactomannan with β-mannanase, the substrate is depolymerised by an endo-mechanism to produce low-molecular weight dyed fragments which remain in solution on addition of ethanol to the reaction mixture. High-molecular weight material is removed by centrifugation, and the colour of the supernatant is measured. By reference to a standard curve, endo-β-mannanase in the assay solution is determined.

3 Working range

The working range of this method is (b) (4).

4 Safety note

The method described involves the handling of hazardous substances. Attention is therefore drawn to the various provisions governing the handling of potentially dangerous material.

5 Reagents

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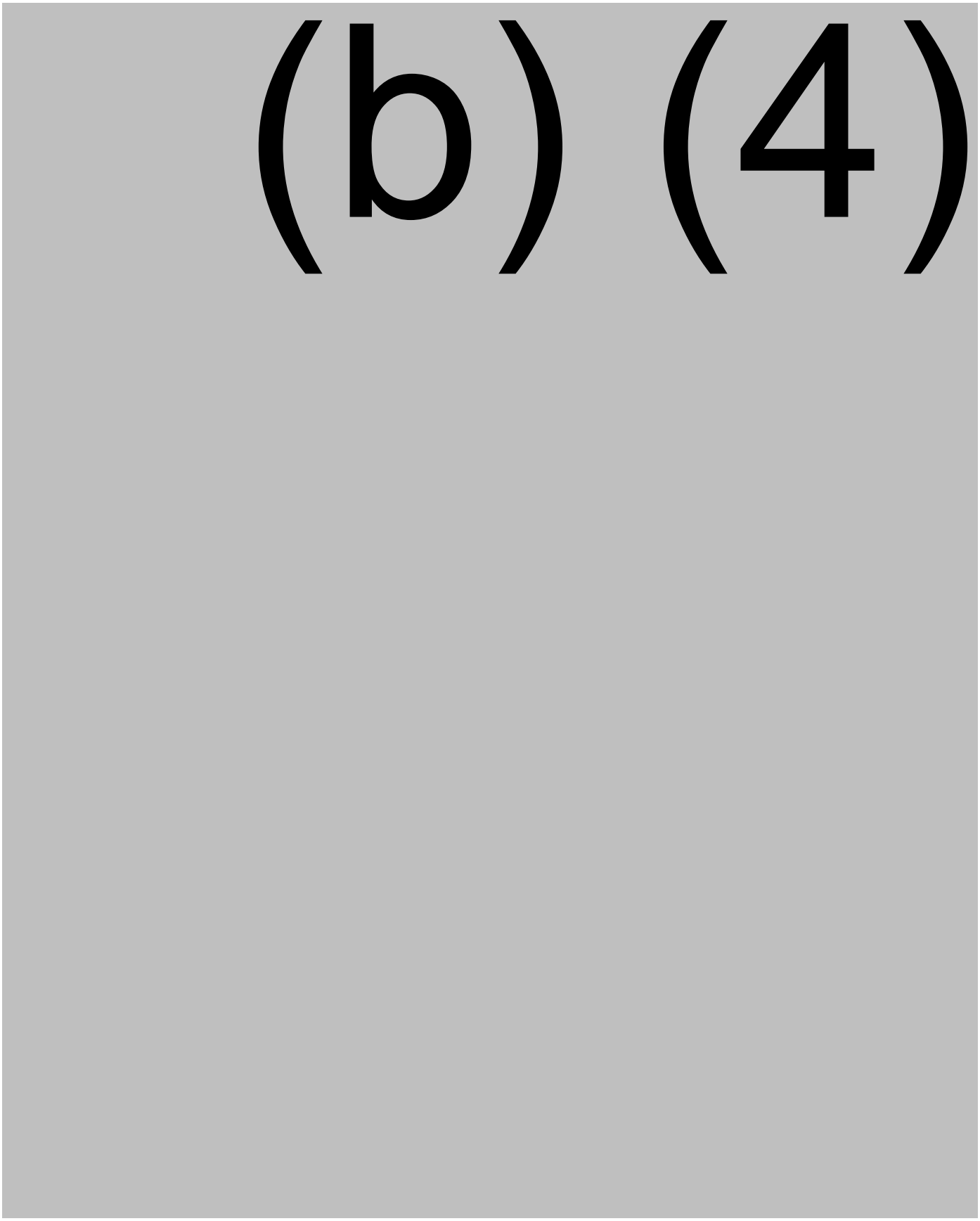
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6 Apparatus

(b) (4)

7 Preparation of standard, sample and blank solutions

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8 Procedure

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10 Measurement uncertainty

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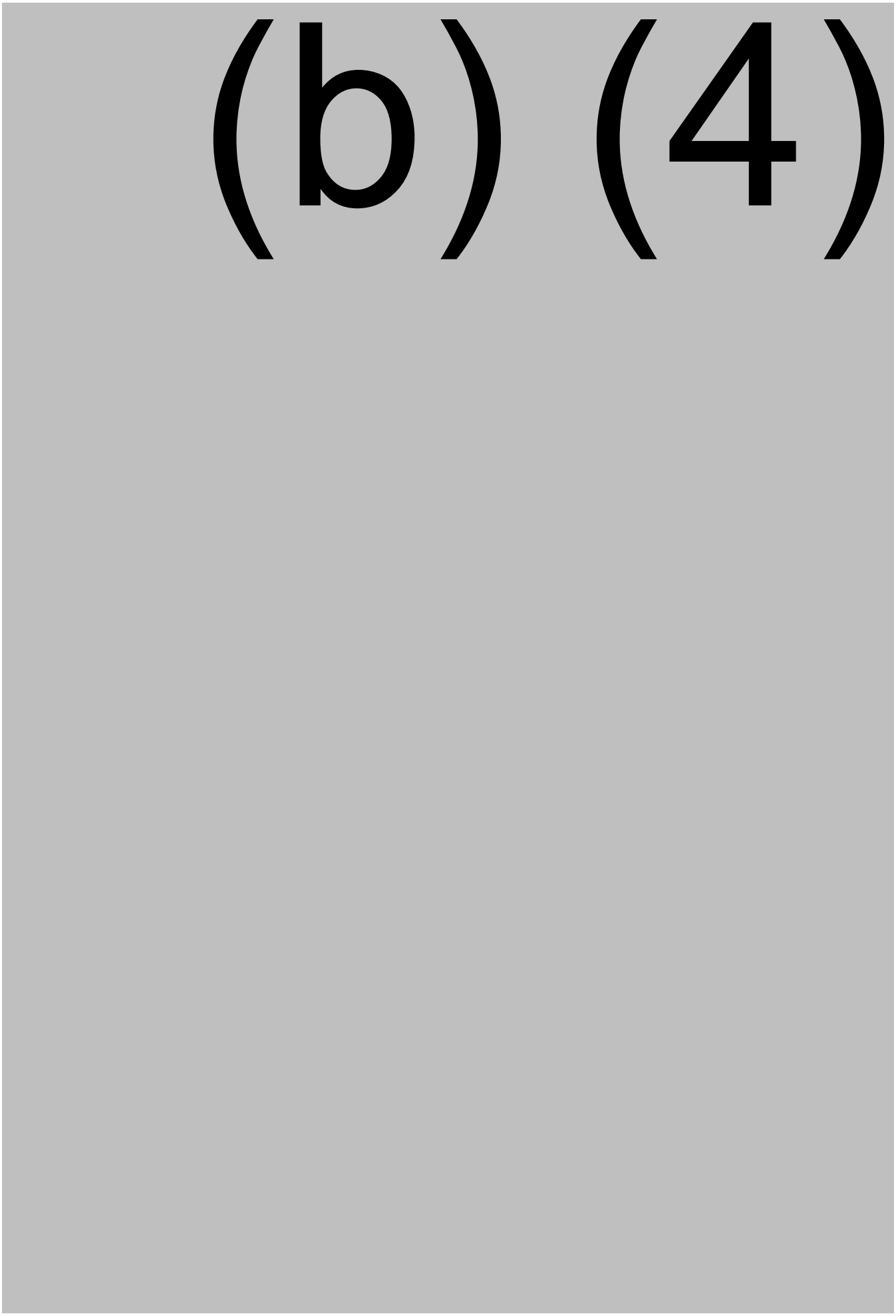
11 Comments

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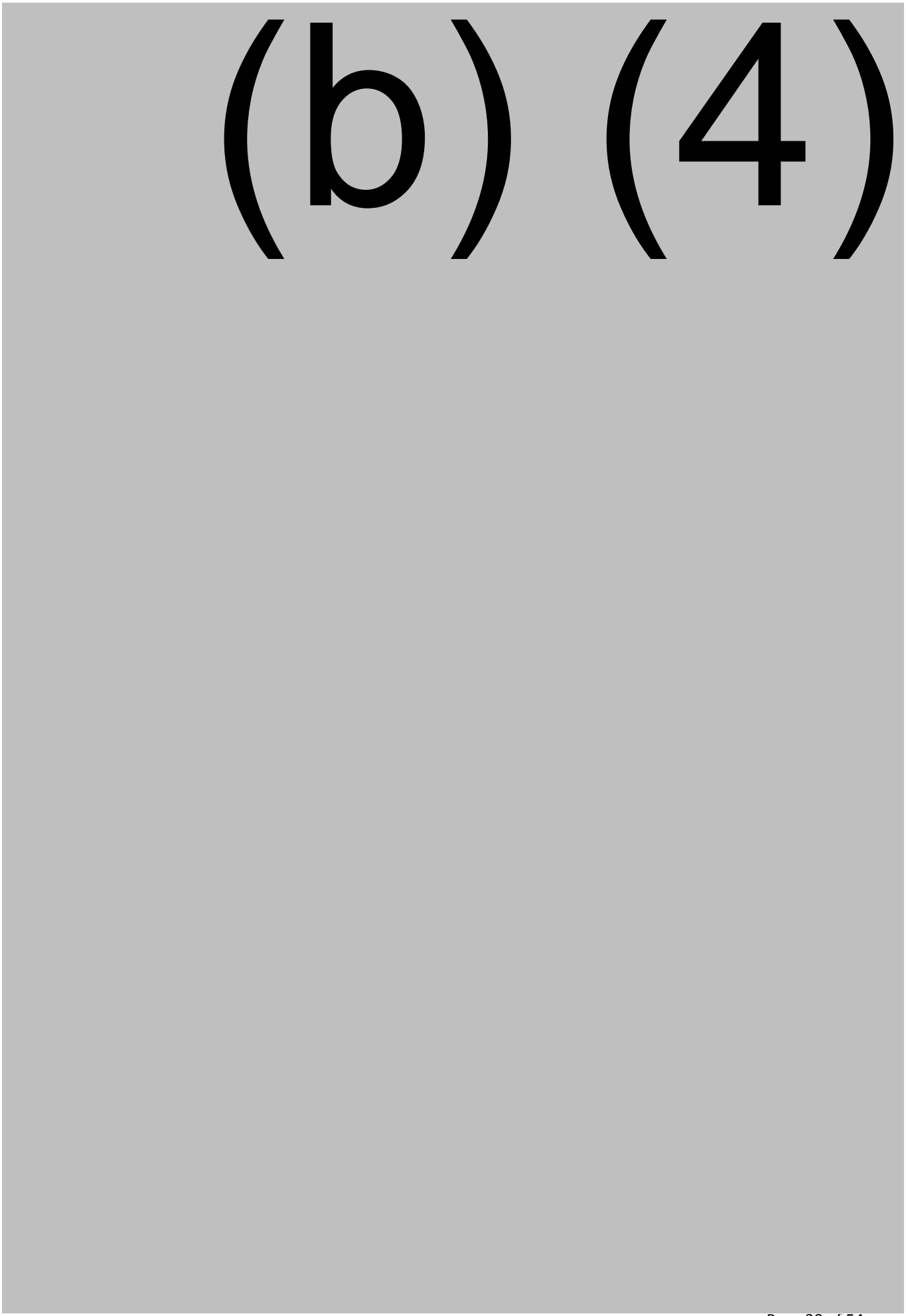
ANNEX 3: RAW DATA AND CALCULATION FOR VALIDATION OF ß-MANNANASE DETERMINATION IN FEED ADDITIVE ß-MANNANASE CC

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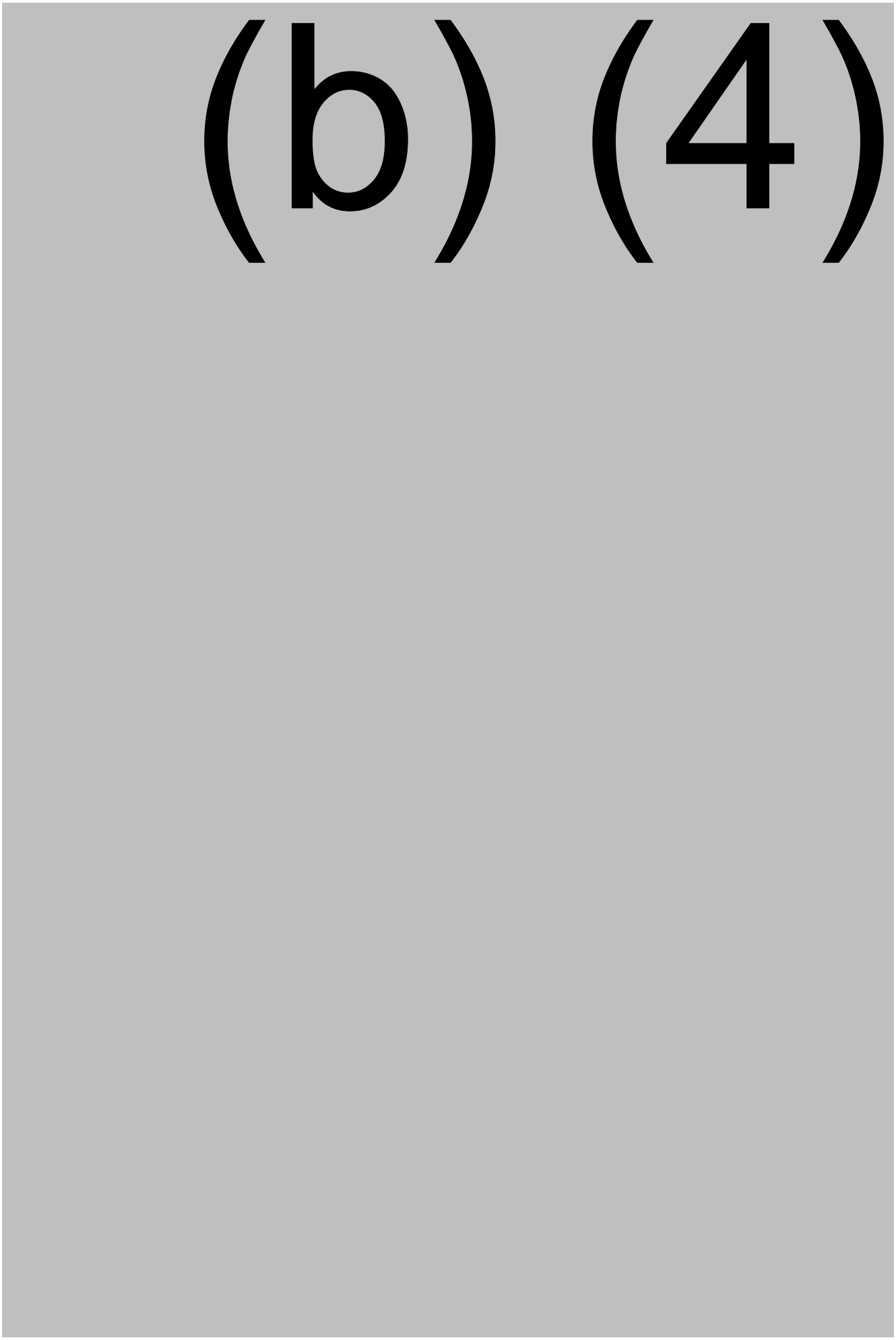


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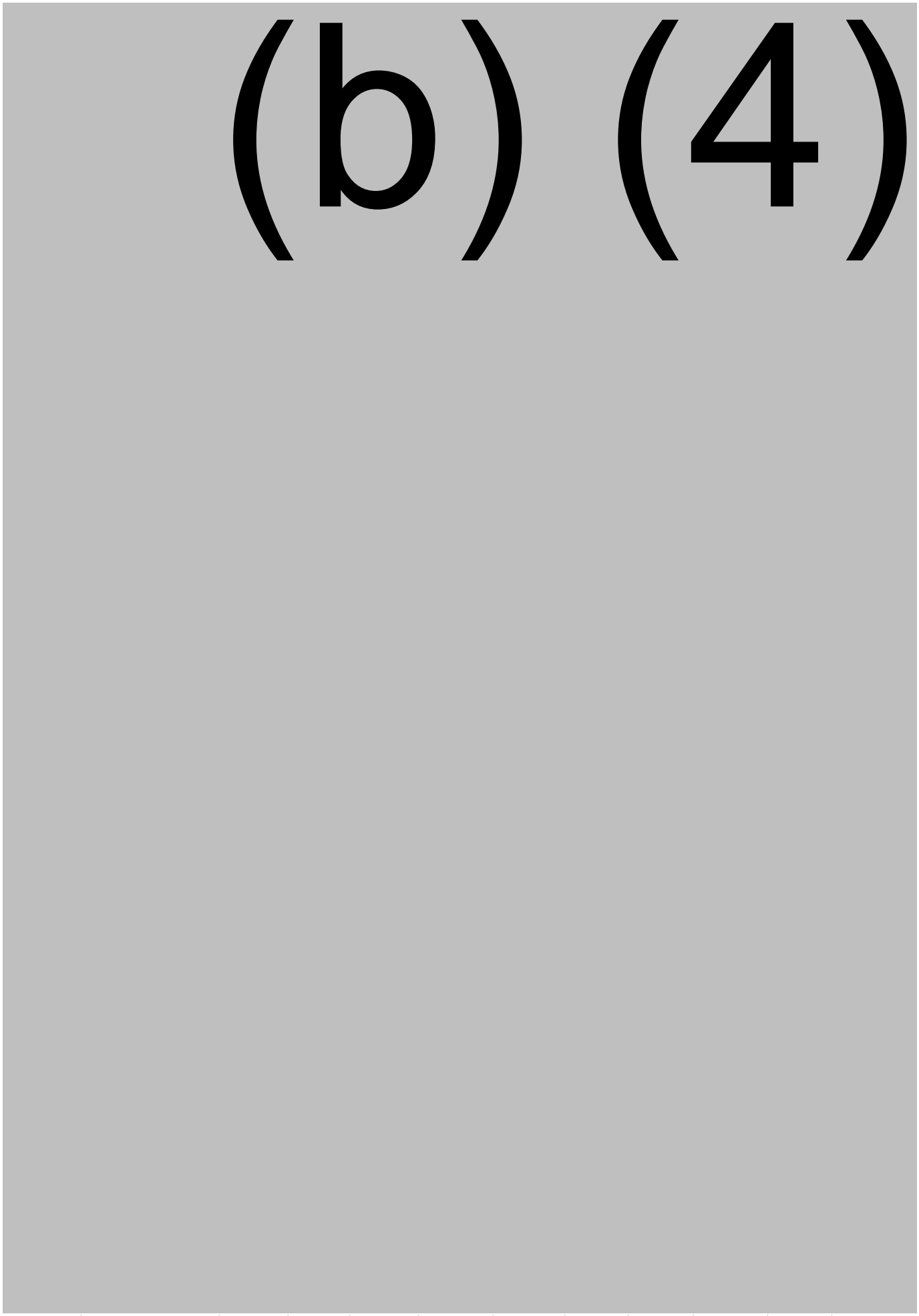


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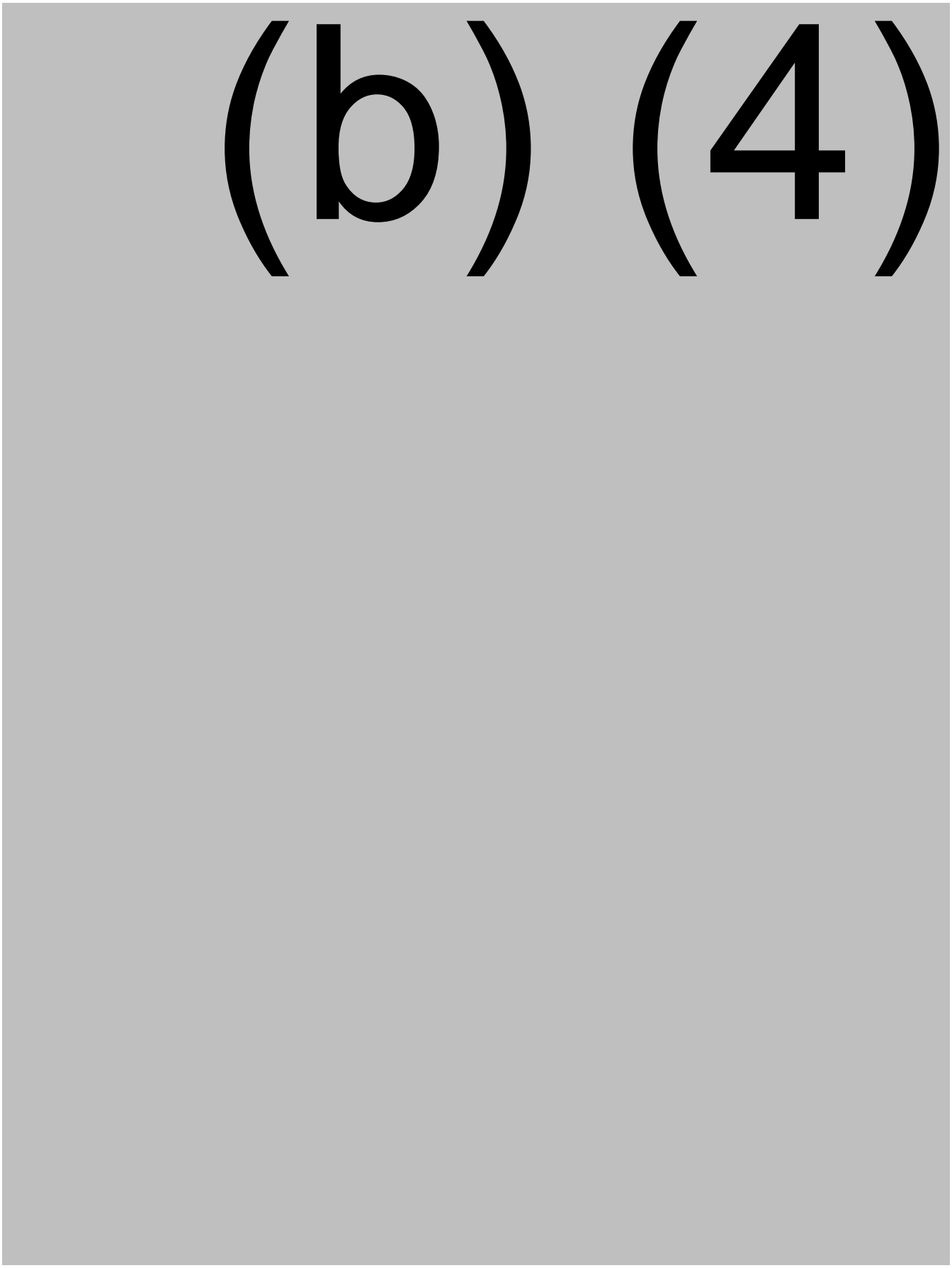
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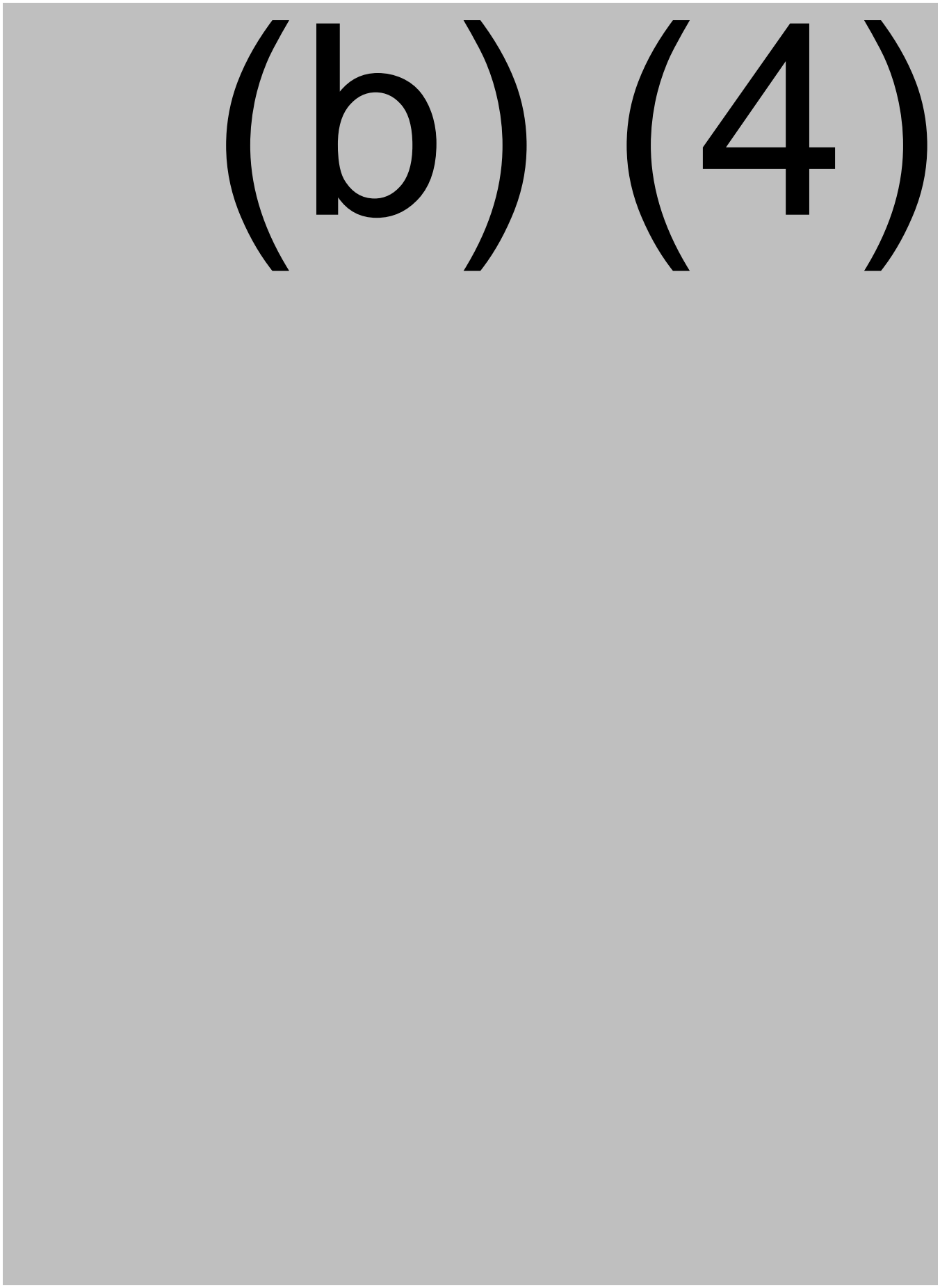
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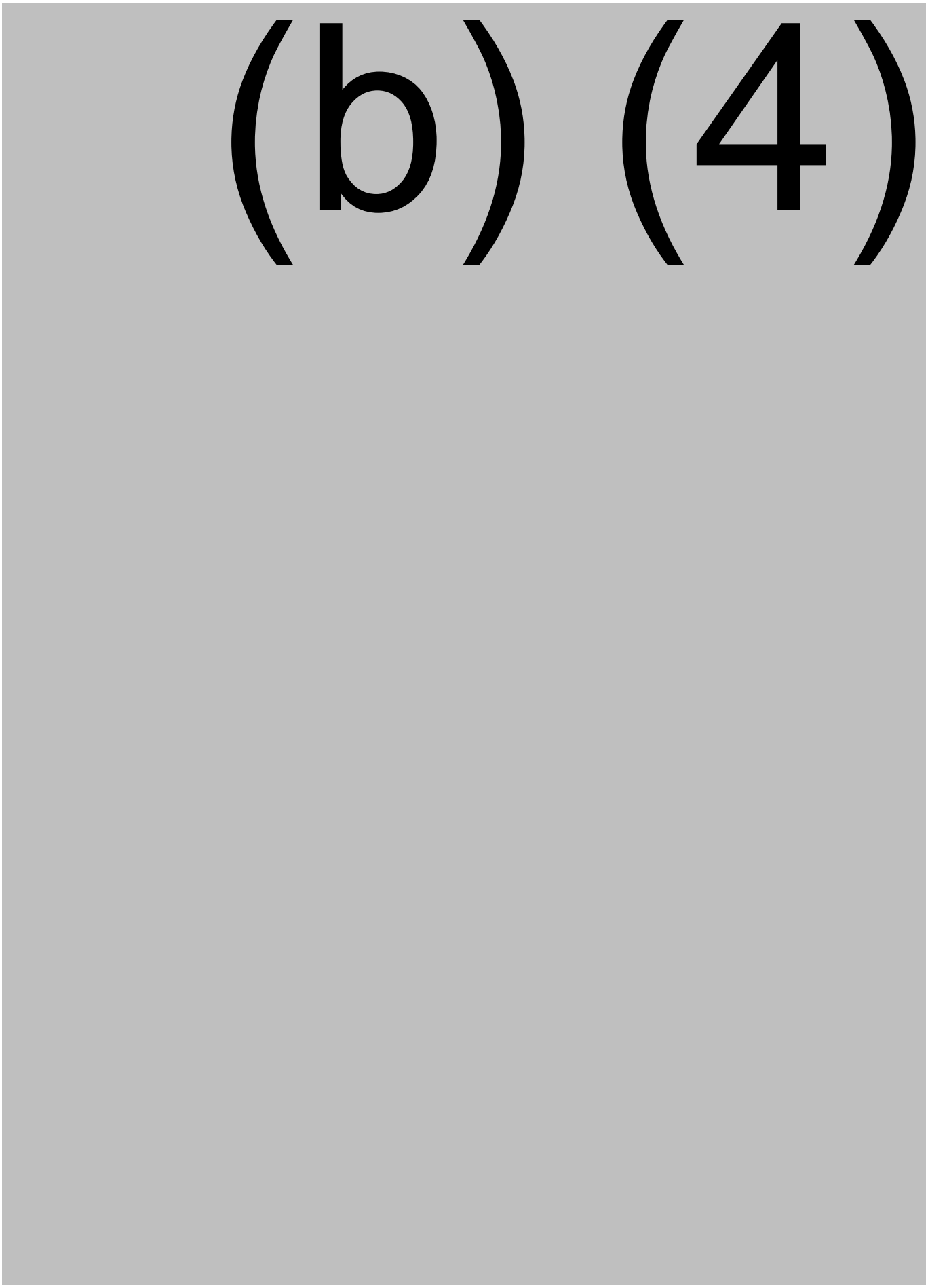
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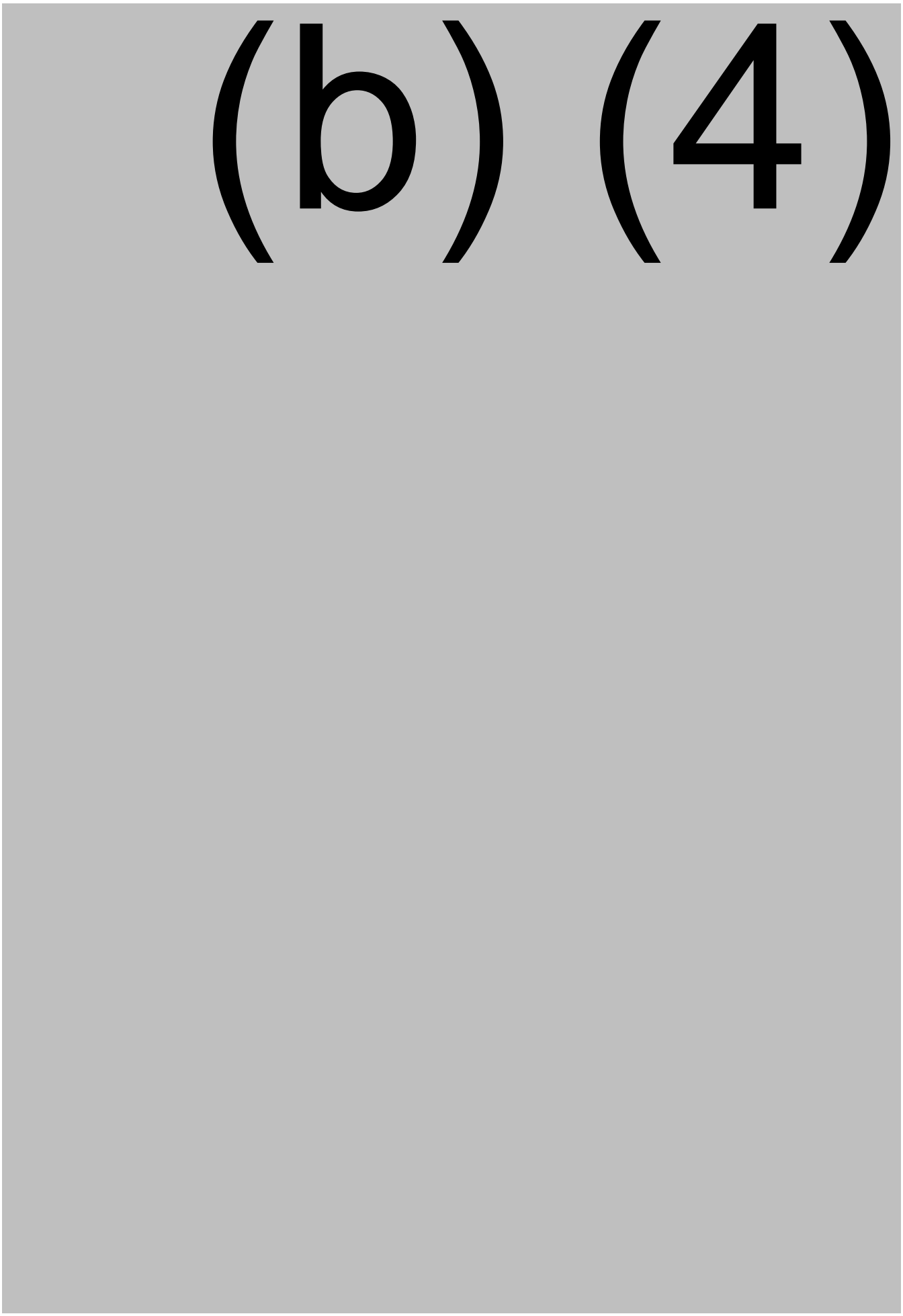
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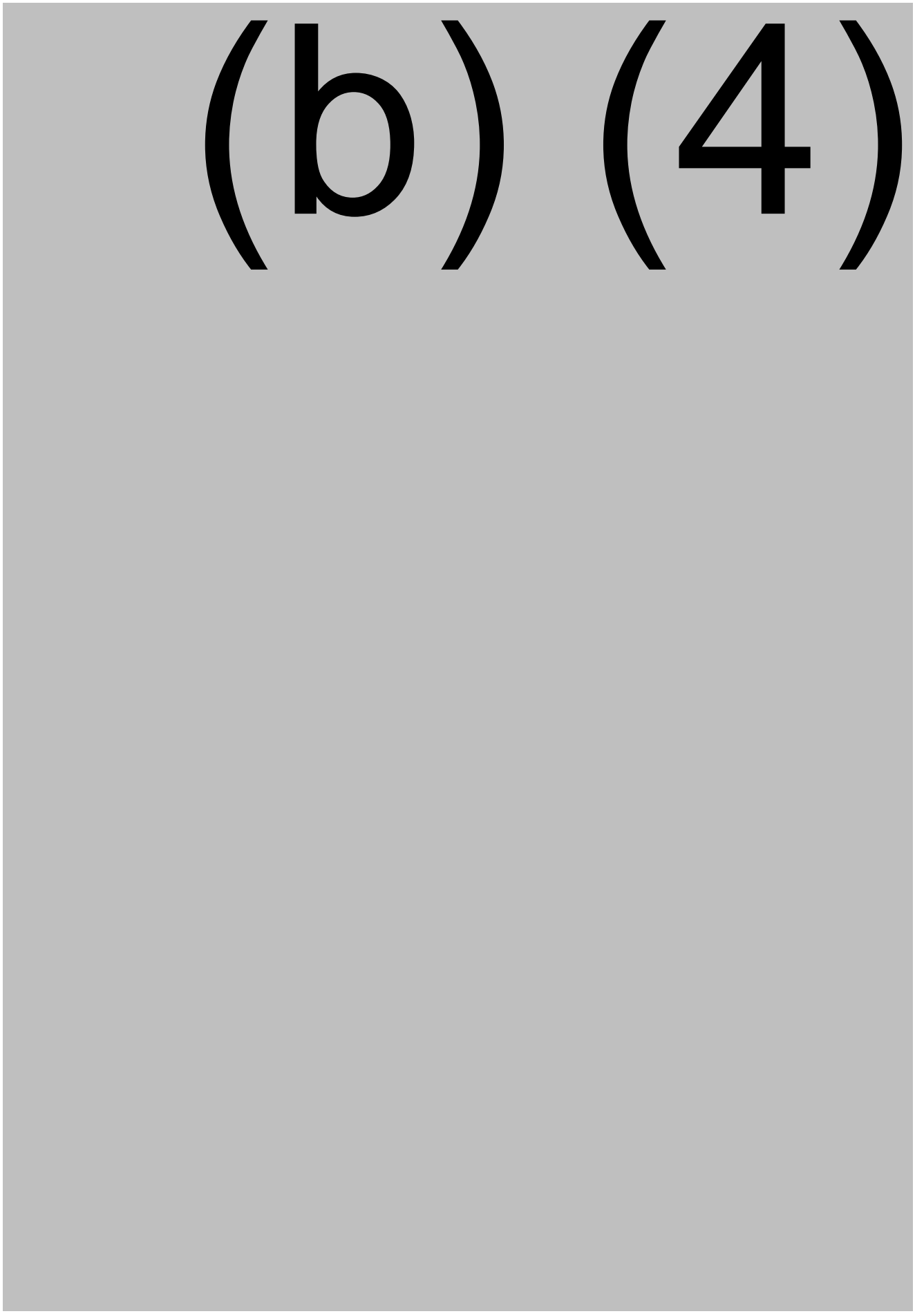
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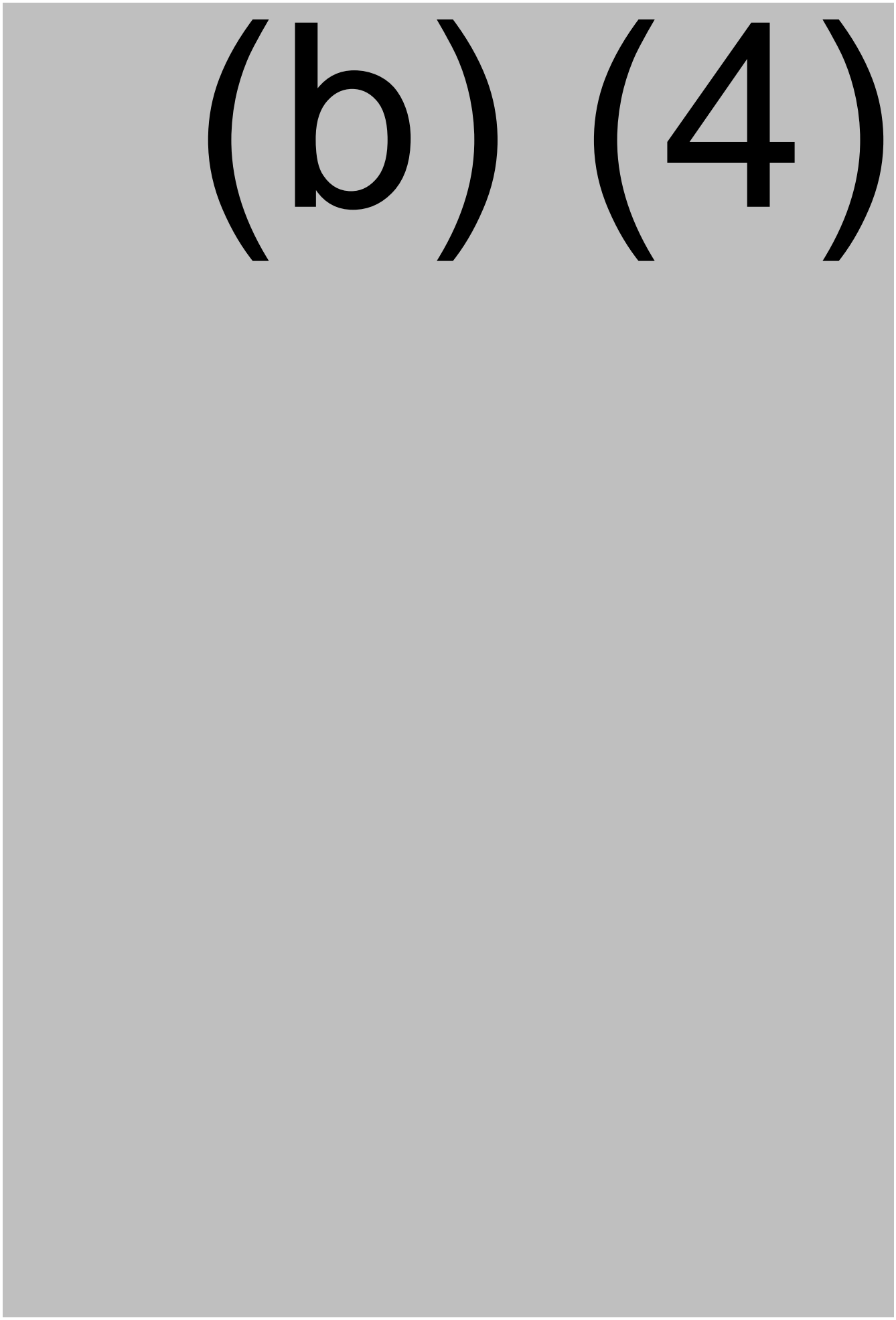
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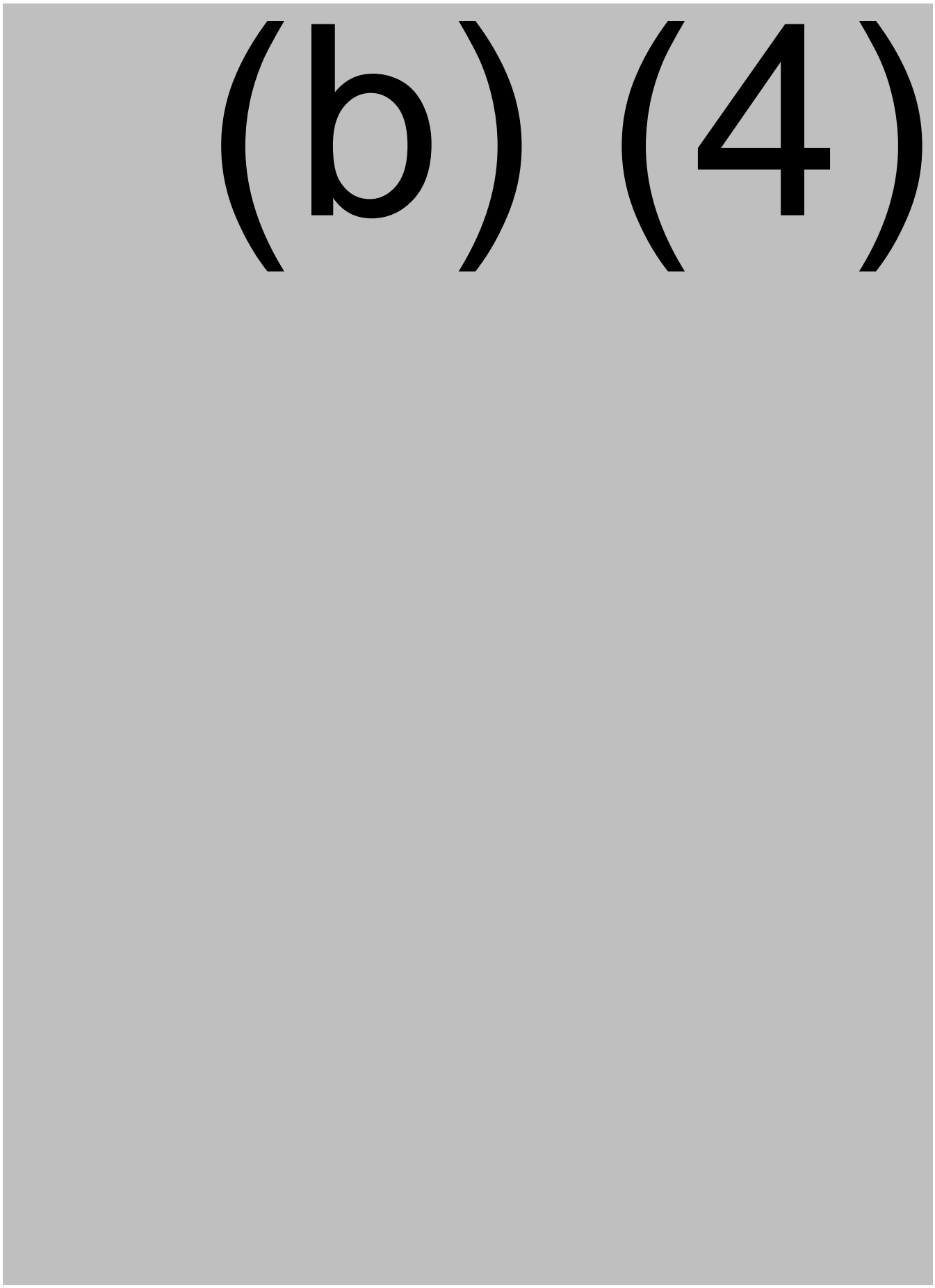
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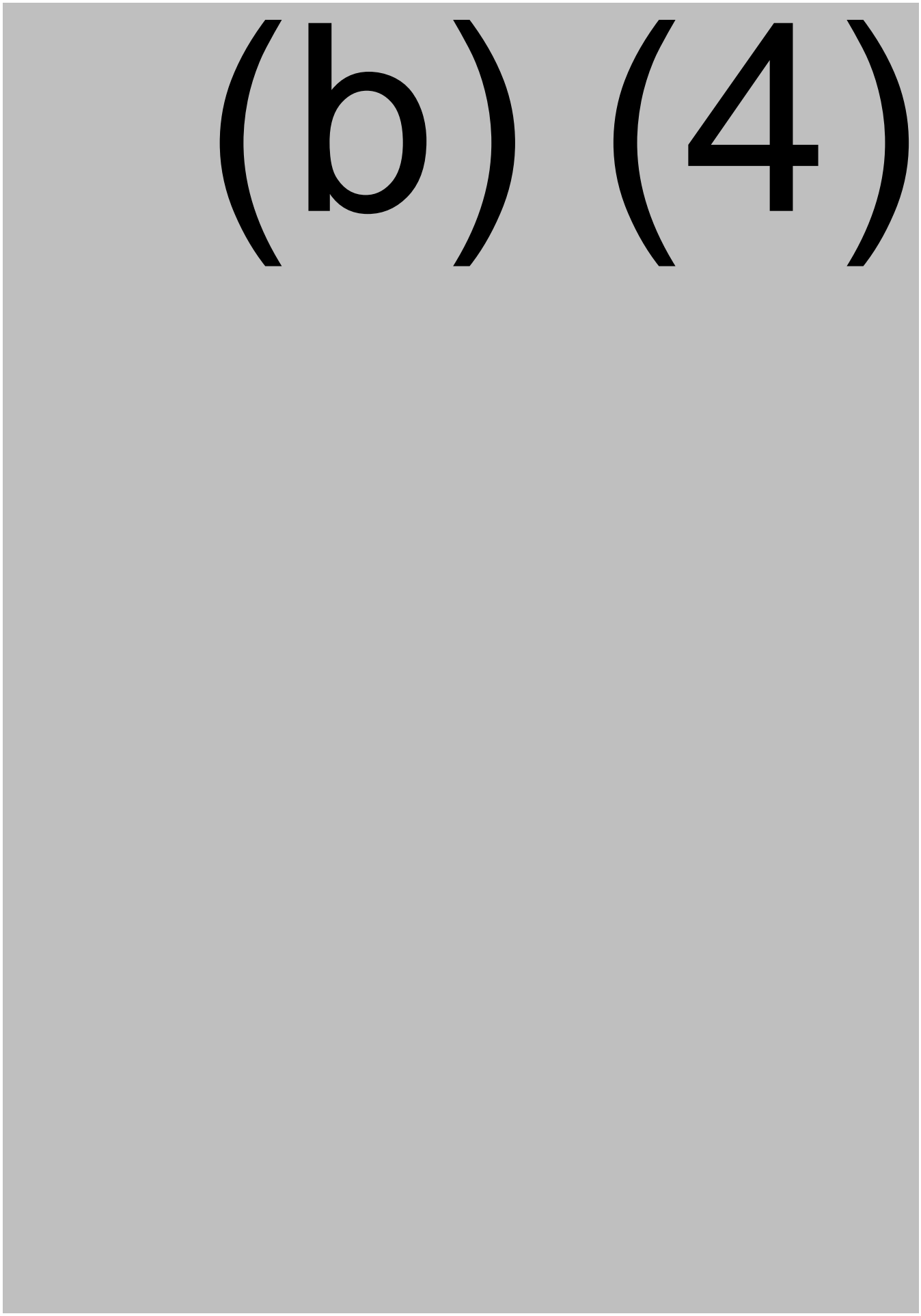
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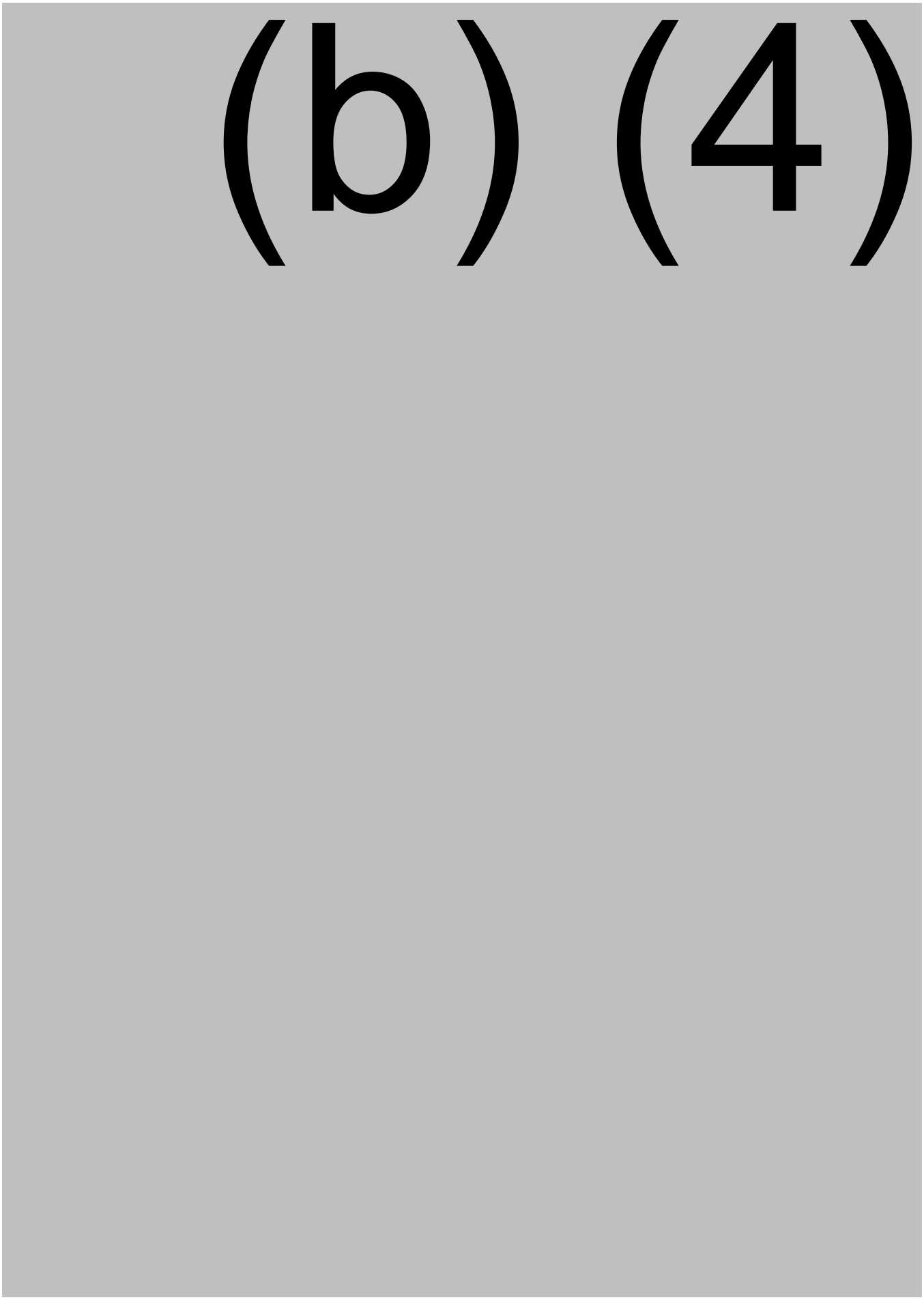
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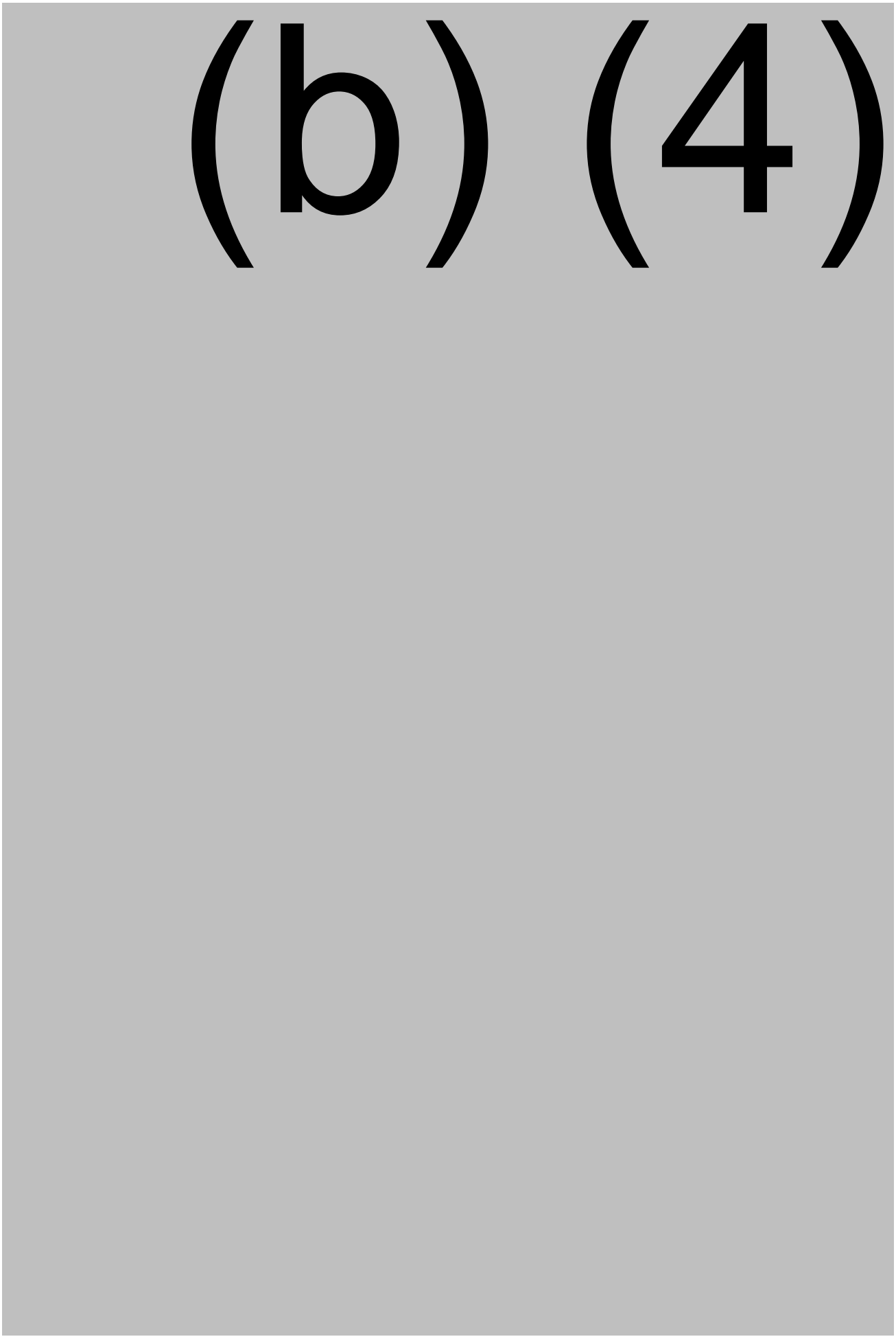
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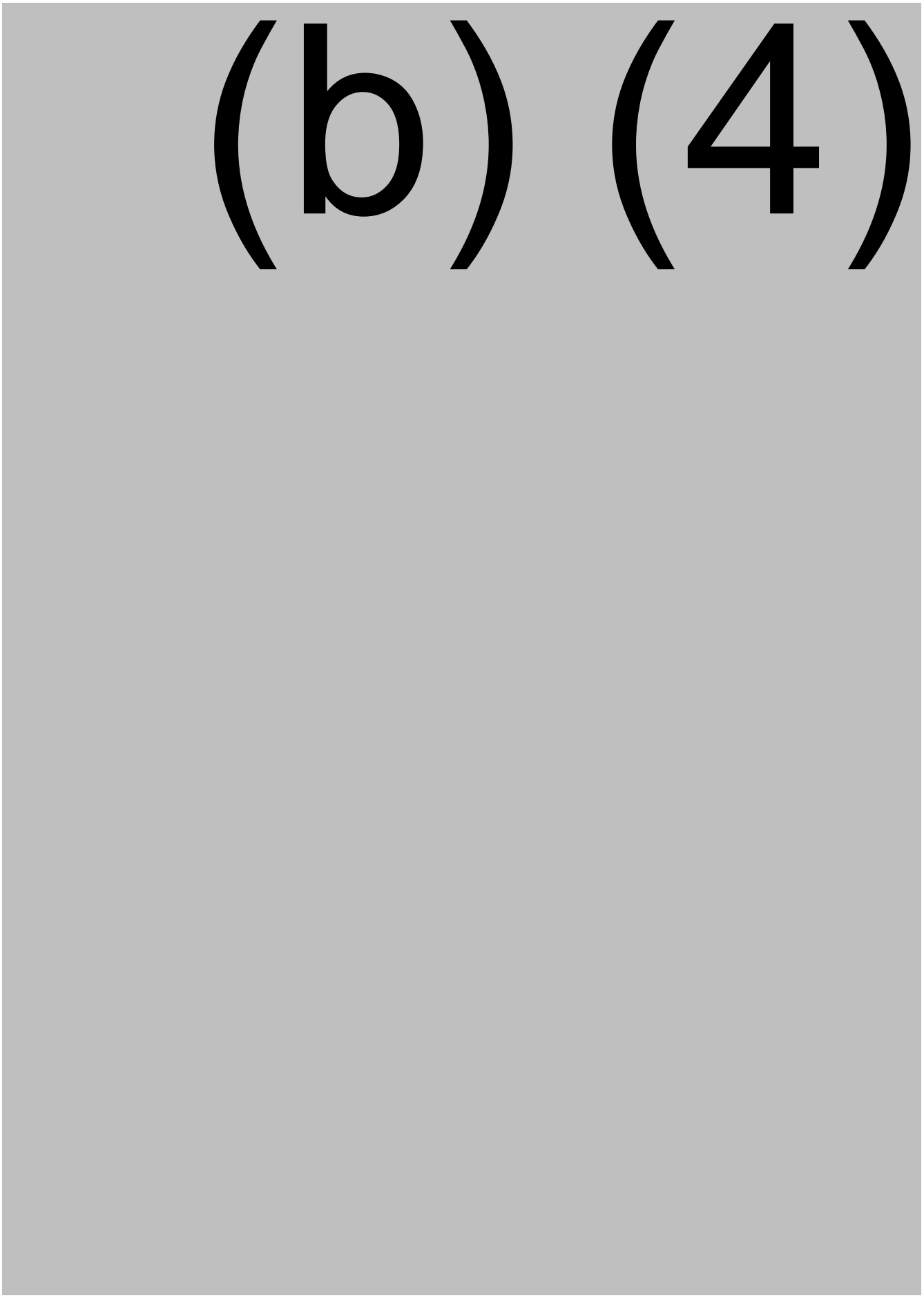
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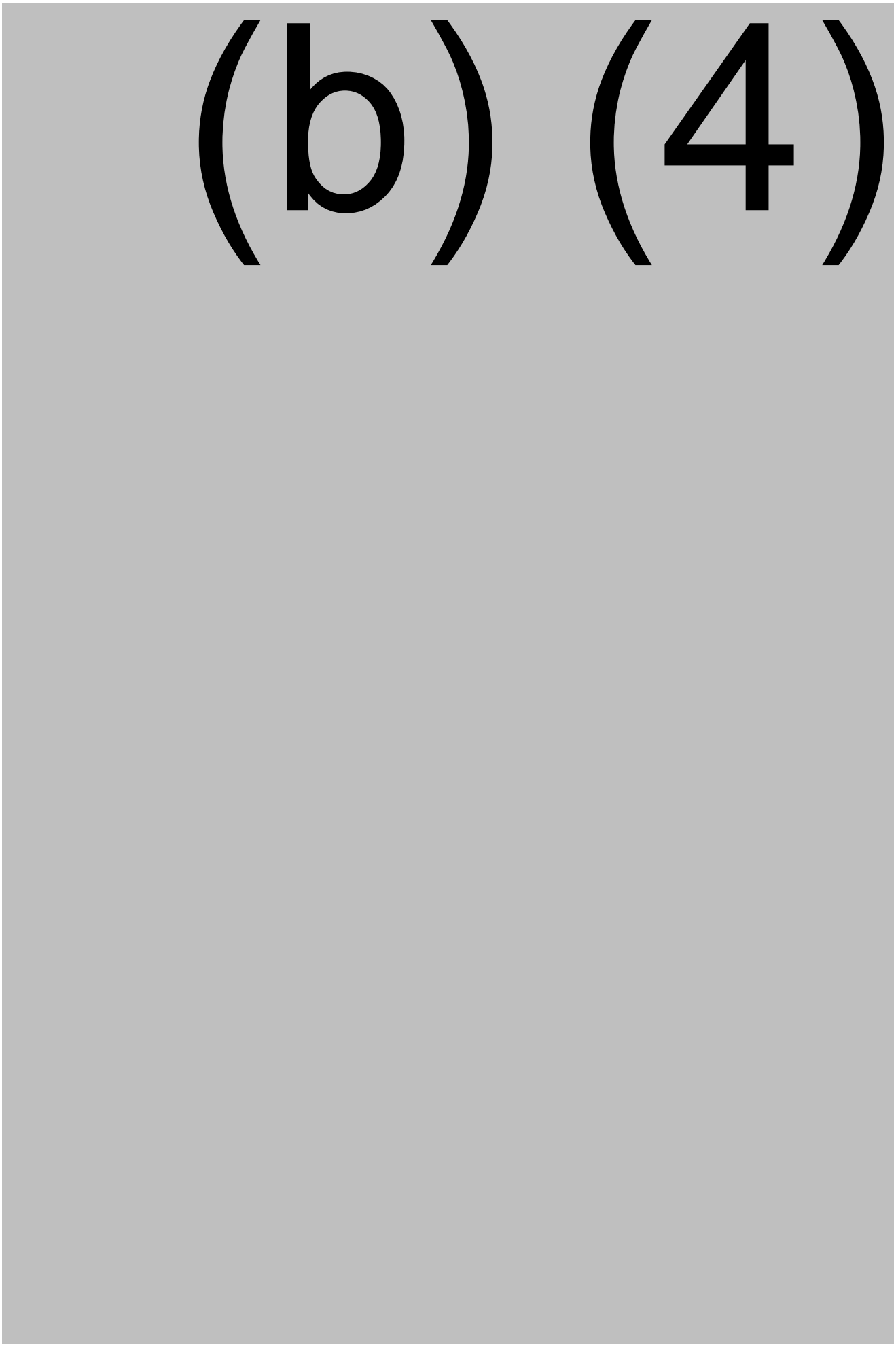
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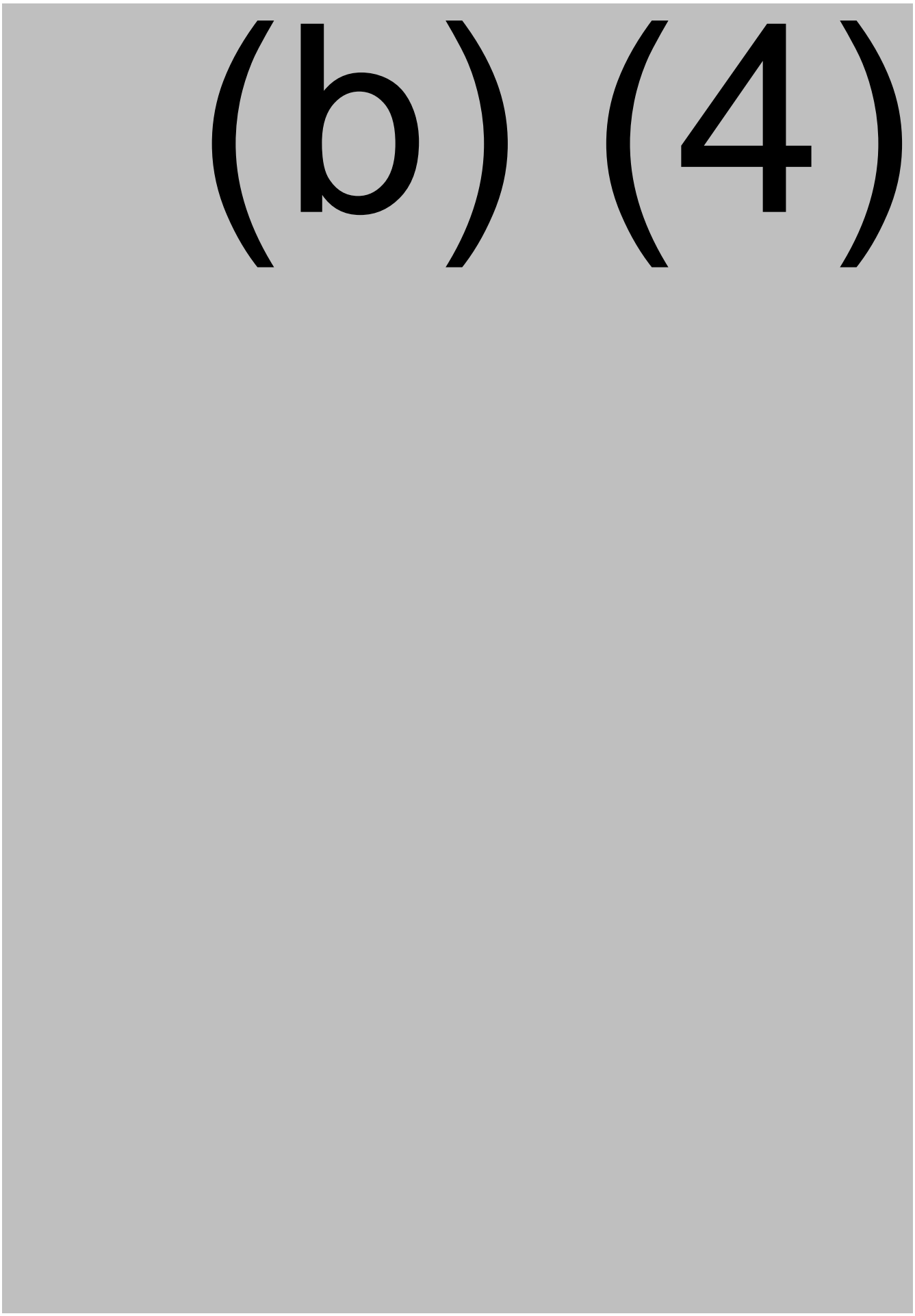
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VALIDATION OF ANALYTICAL METHODS FOR MEASURING β-MANNANASE ACTIVITY IN FEEDS

Experiment number L-251 c1

FINAL REPORT

Revision number: 0

Date: 3rd January 2019

RESEARCH ACTIVITYCONTRACTED WITH:

BASF SE
Analyt. & Appl. Technology Feed Enzymes
G-ENA/MD – A110/R0316
67056 Ludwigshafen am Rhein (Germany)

Study monitors: Benjamin Georg Peikert and (b) (6)kert

Study director: Anna Maria Pérez-Vendrell

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(b) (4)

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SUMMARY

The objective of the present study was the validation of the analytical method FEN/0005/01 for measurement of β-mannanase activity present in feeds.

The study was performed using 3 types of feeds (blank feed, feed with known β-mannanase activity, blind feed with unknown β-mannanase activity) and two thermostable endo-mannanase enzymatic products: standard A 0009/001/FEN_STD (used for preparation of calibration curves) and control 0005/001/FEN_KON.

The validation of the method for the determination of β-mannanase activity in feeds (BASF method FEN/0005/01 provided by the company) is based on the methodology using a coloured azo-substrate (azurine-crosslinked carob galactomannan). The hydrolysis of this substrate by the endo-1,4,-β-mannanase produces water soluble dyed fragments. The rate of release of these fragments is related to the enzyme activity.

The study consisted on the determination of the following quality parameters of a method of analysis: range and linearity, limits of detection (LOD) and quantification (LOQ), and accuracy and precision parameters, which included recovery, repeatability, intermediate precision and inter-laboratory reproducibility. Robustness and Uncertainty were also evaluated.

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(b) (4)

(b) (4)

RESPONSIBILITIES

Study Director

(b) (6), (b) (4)

Study monitors

(b) (6) and Benjamin Georg Peikert
BASF SE
G-ENA/MD – A110/R0316
67056 Ludwigshafen am Rhein (Germany)
Tel: + 49 621 60-52129 Fax: +49 621 60-6647447
Mobile: (b) (6)0234
E-mail: benjamin.peikert@basf.com

Analytical Laboratory

(b) (4), (b) (6)

(b) (4)

OBJECTIVE

The objective of the present study was the validation of the analytical method FEN/0005/01 for measurement of β-mannanase activity present in feeds.

QUALITY ASSURANCE

IRTA has an Integrated Quality Policy and the Institute is **ISO 9001:2015** certified for their R+D+T projects, contracts and services.

This study has been conducted in accordance with ISO 9001:2015 quality criteria, and under the global SOPs **PG-75** (Conducting Research studies).

All study staff have appropriate training and qualifications to perform their study tasks. They had been trained in Quality Systems including data recording requirements and forms for completion of the study, QC techniques, relevant SOPs and specific protocol requirements prior to study start.

SCOPE AND TEST METHOD DESCRIPTION

This procedure is for the determination of β-mannanase activity (EC 3.2.1.78) expressed as TMU/kg in compound feeds by in-house analysts.

The determination of β-mannanase activity in feeds (BASF method FEN/0005/01) is based on the methodology using a coloured azo-substrate (azurine-crosslinked carob galactomannan). The hydrolysis of this substrate by the endo-1,4,-β-mannanase produces water soluble dyed fragments. The rate of release of these fragments is related to the enzyme activity.

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DEFINITIONS

Range and Linearity

The linearity is the ability of a method (within a given range) to obtain test results that are directly proportional to the concentration of analyte in the sample (Eurachem, 1998). The range is the interval between upper and lower concentration (amounts) of analyte in the sample (including these concentrations for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity).

Limit of detection (LOD)

The limit of detection was defined as the smallest measured content from which it is possible to deduce the presence of the analyte with reasonable statistical certainty. The LOD is numerically equal to 3 times the standard deviation of the mean of blank determinations (n≥10, where n=nº of measures) performed under repeatability conditions. It can also be calculated from the slope of the calibration line as y (LOD) = a + 3 Sy/x.

Limit of Quantification (LOQ)

The limit of quantification was defined as the lowest content of analyte that can be measured with reasonable statistical certainty. The LOQ is numerically equal to 10 times the standard deviation of the mean of blank determinations (n≥10, where n=nº of measures) performed under repeatability conditions. It can also be calculated from the slope of the calibration line as y (LOQ) = a + 10 Sy/x.

Precision

The precision expresses the closeness of agreement between a series of independent measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions.

Accuracy

The accuracy expresses the closeness of agreement between the value that is accepted either as a conventional true value or an accepted reference value and the value determined by analysis.

Recovery

The Relative Percent between determined and expected values of xylanase fortified samples was calculated as an indicator of recovery.

Repeatability

The repeatability measures precision under the same operating conditions over a short interval of time. Independent test results are obtained with the same method on identical test items in the same laboratory with the same operator using the same equipment within short intervals of time. Repeatability is expressed mathematically by the coefficient of variation (relative standard deviation) of a series of different measures.

Intermediate precision

Intermediate precision expresses within-laboratory variations: e.g. different days, different analysts, different equipment, etc.

Reproducibility

The reproducibility measures precision under different operating conditions. Reproducibility relates to the agreement of test results with different operators, test apparatus, and laboratory locations. In this case it was obtained analysing samples containing unknown concentrations of the test product.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. In this case, the parameters changed have been pH, time and temperature of the enzymatic reaction.

Uncertainty

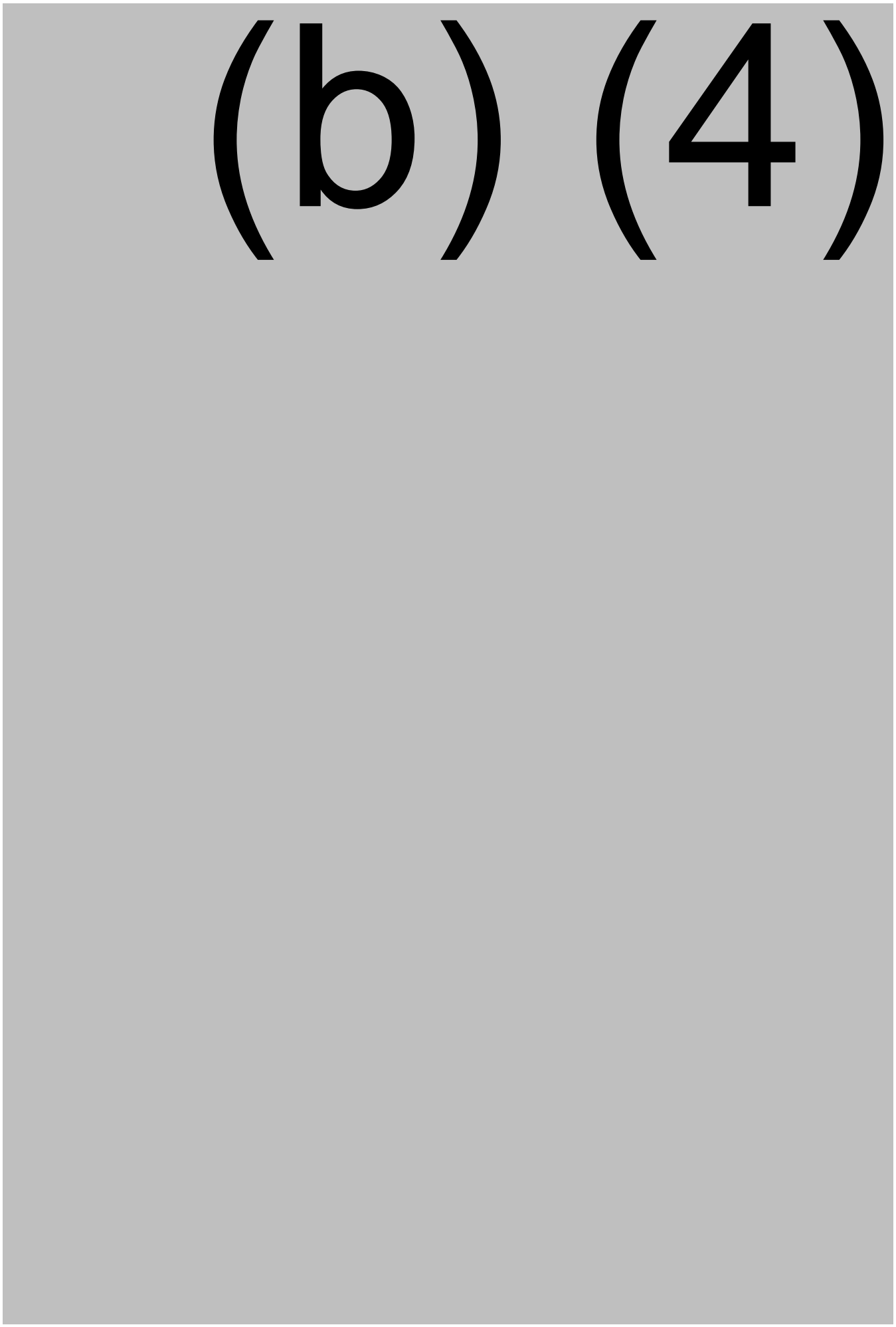
The uncertainty is the expression of the statistical dispersion of the values attributed to a measured quantity. All measurements are subject to uncertainty and a measurement result is complete only when it is accompanied by a statement of the associated uncertainty. By international agreement, this uncertainty has a probabilistic basis and reflects incomplete knowledge of the quantity value.

METHODOLOGY

Materials

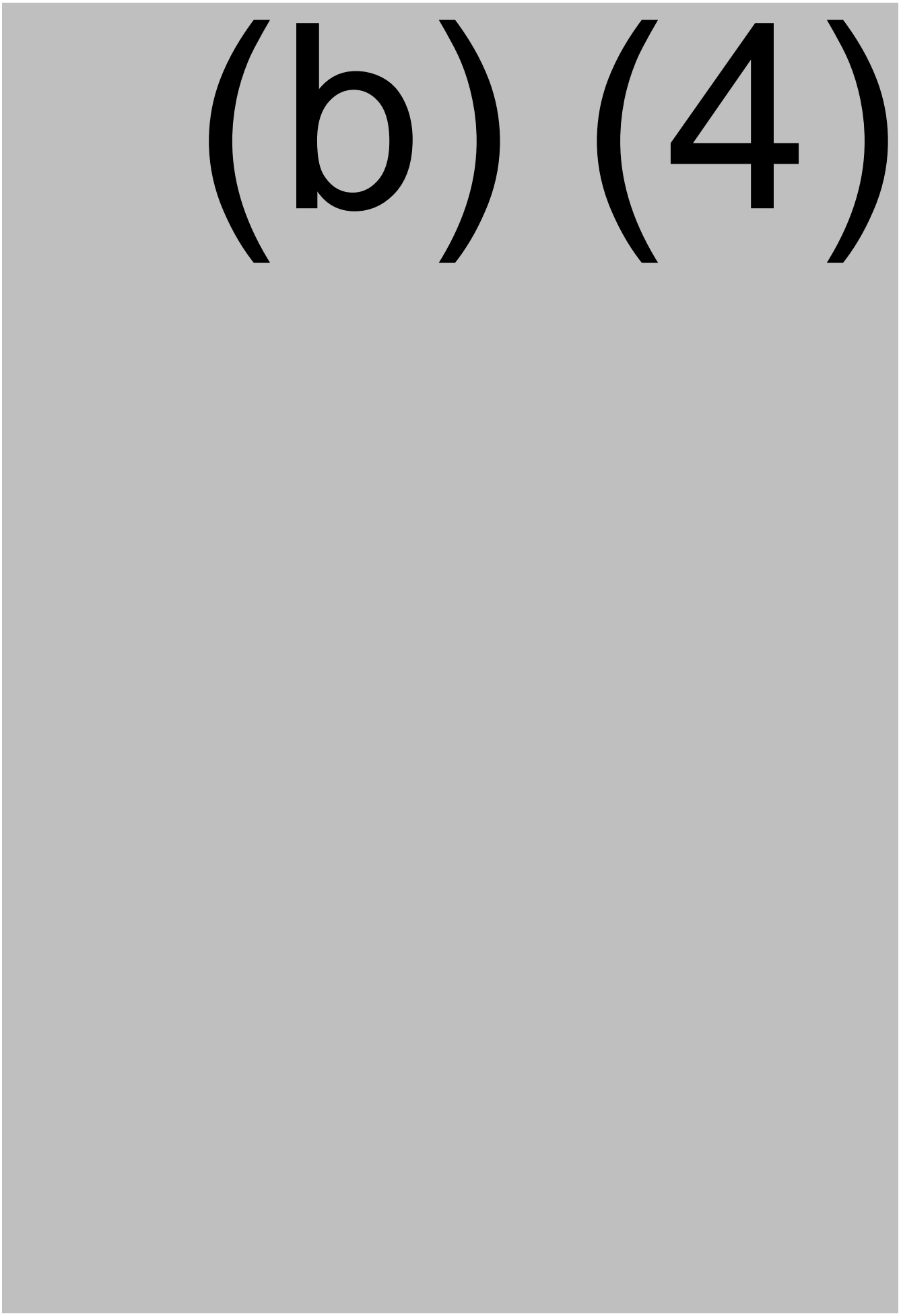
The study was performed using 3 types of feeds (blank feed, feed with known β-mannanase activity, blind feed with unknown β-mannanase activity) and two thermostable endo-mannanase enzymatic products: standard and control.

These samples are listed in Table 1 and were received on 5th November 2018.



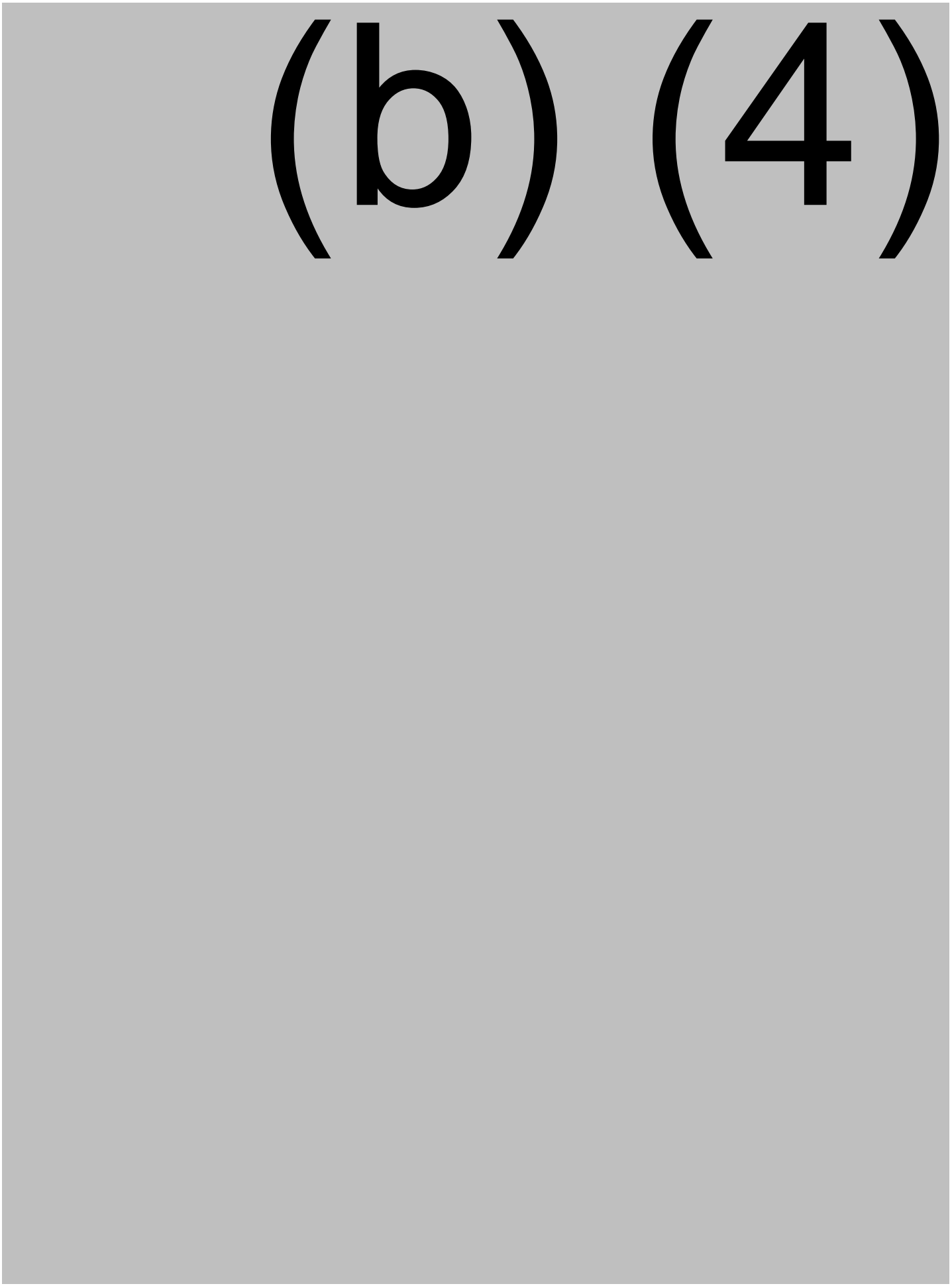
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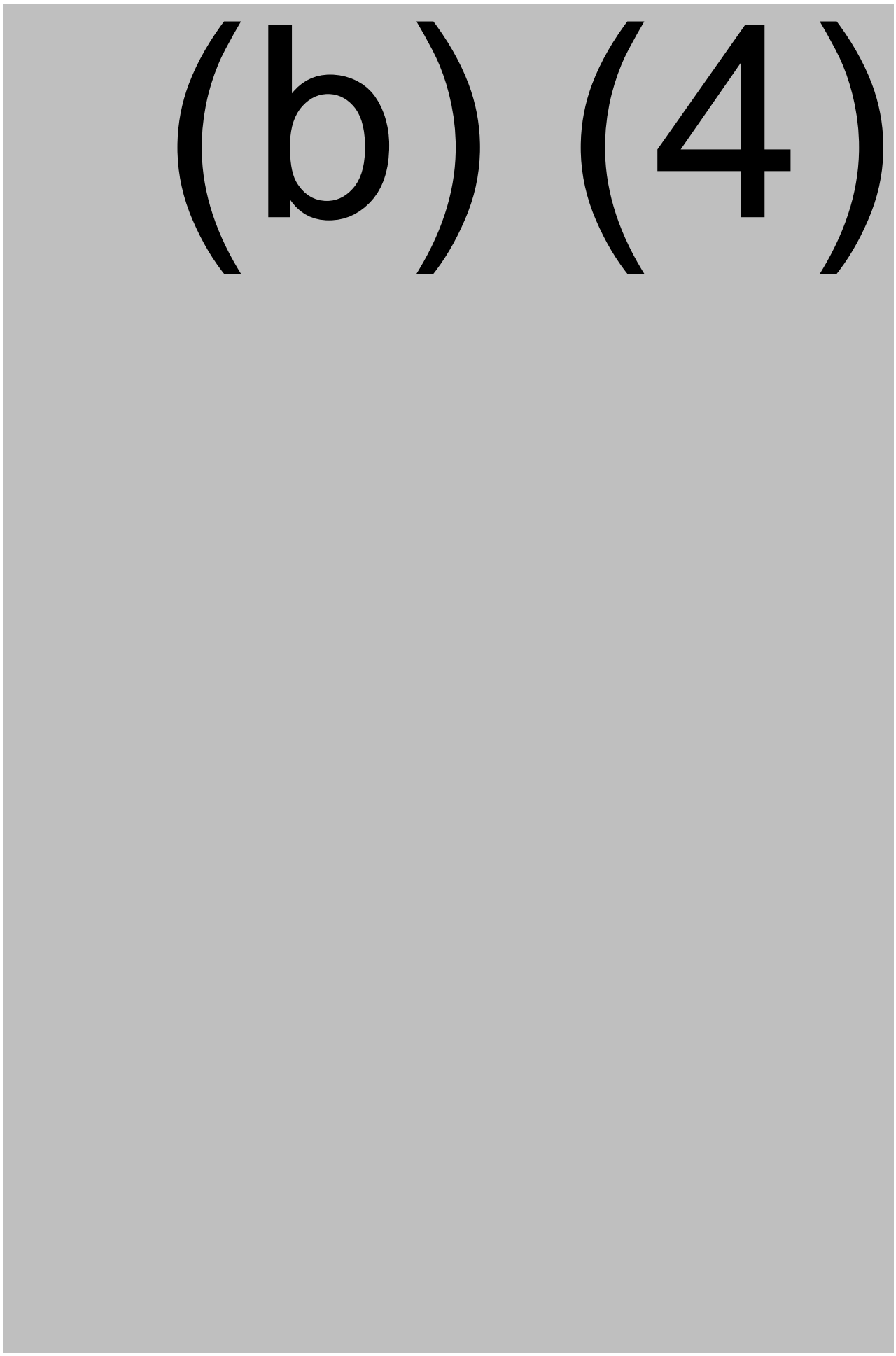
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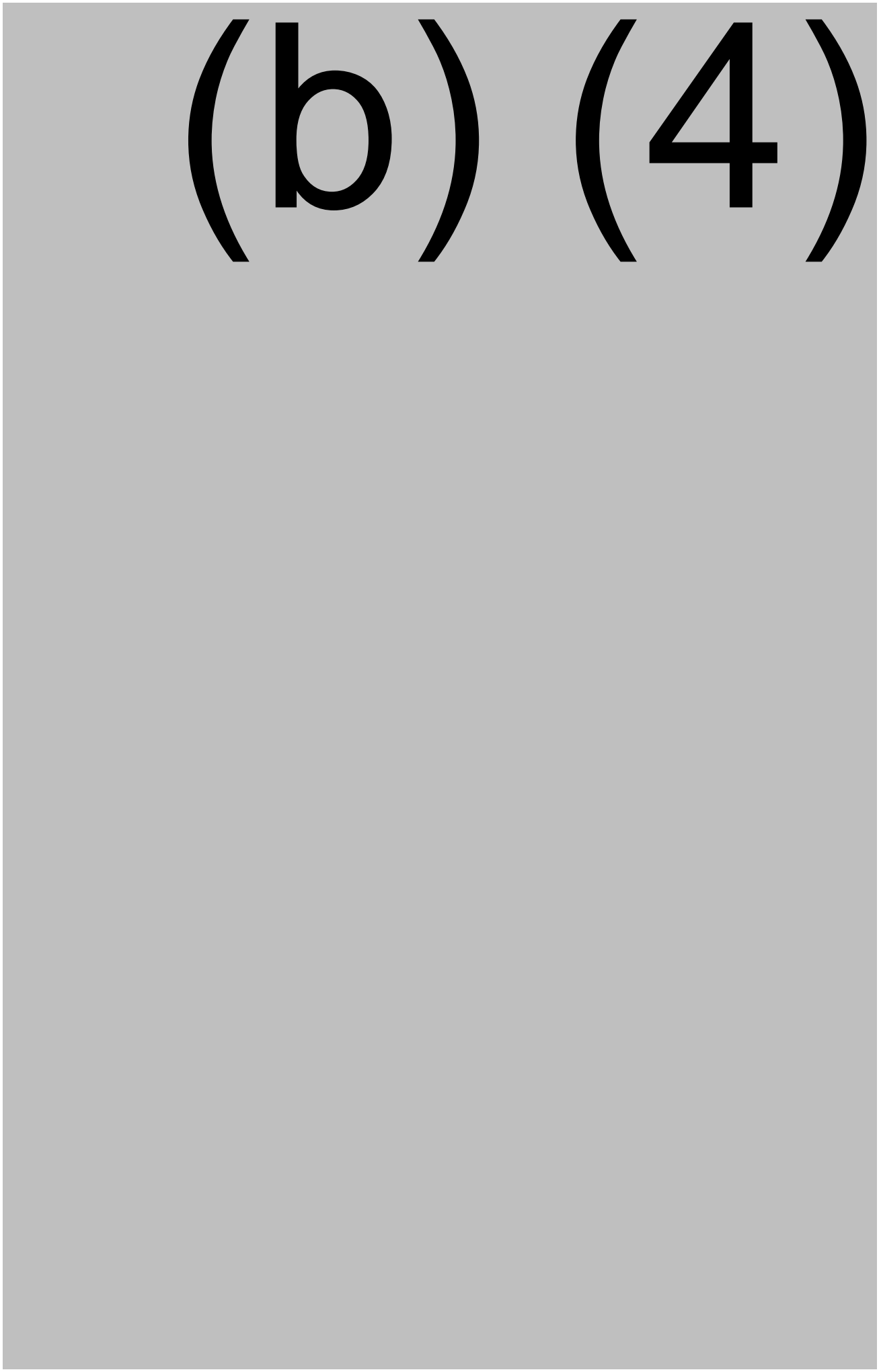
deviation (s) and relative standard deviation (%RSD_r) were calculated.



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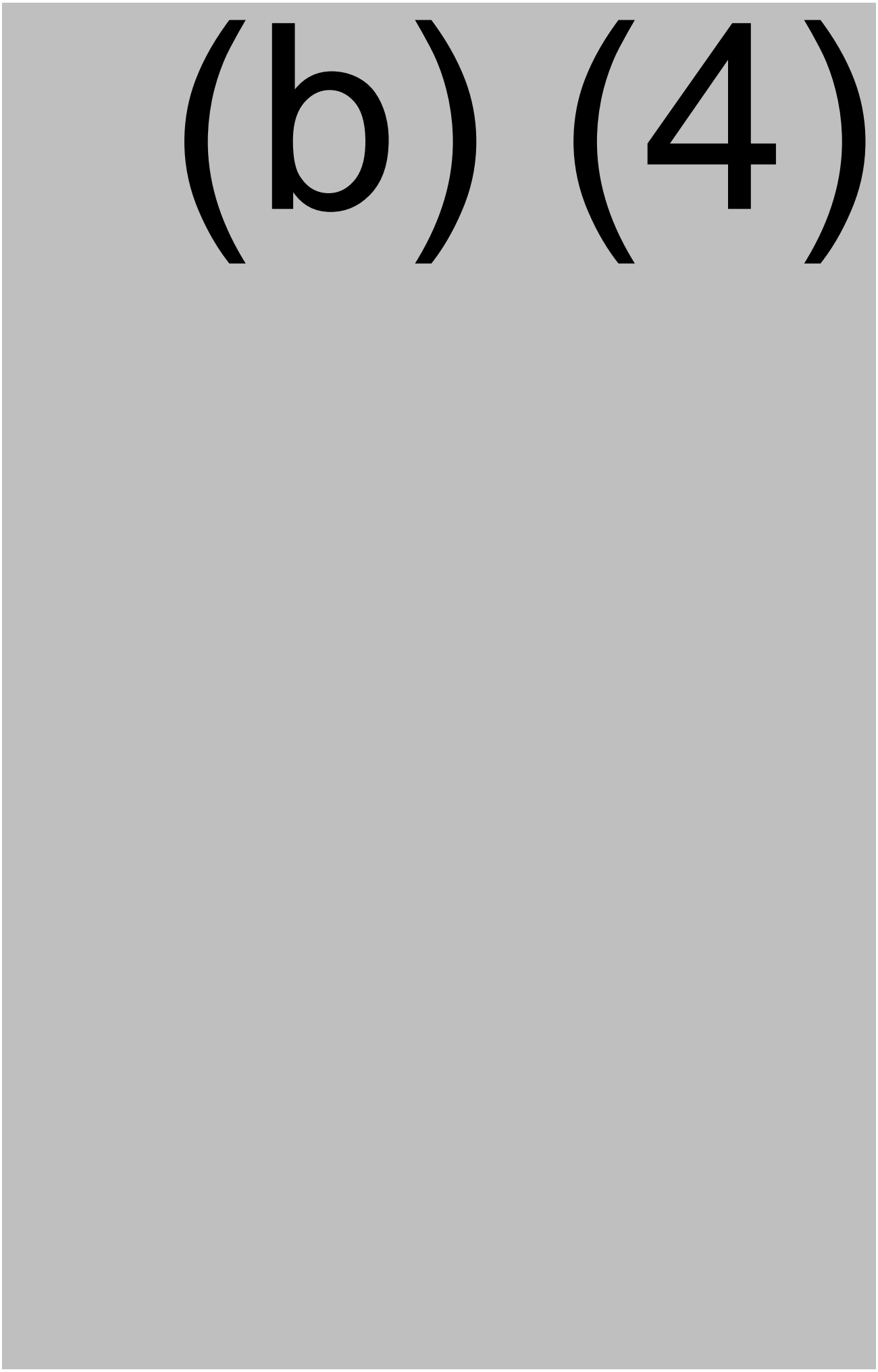
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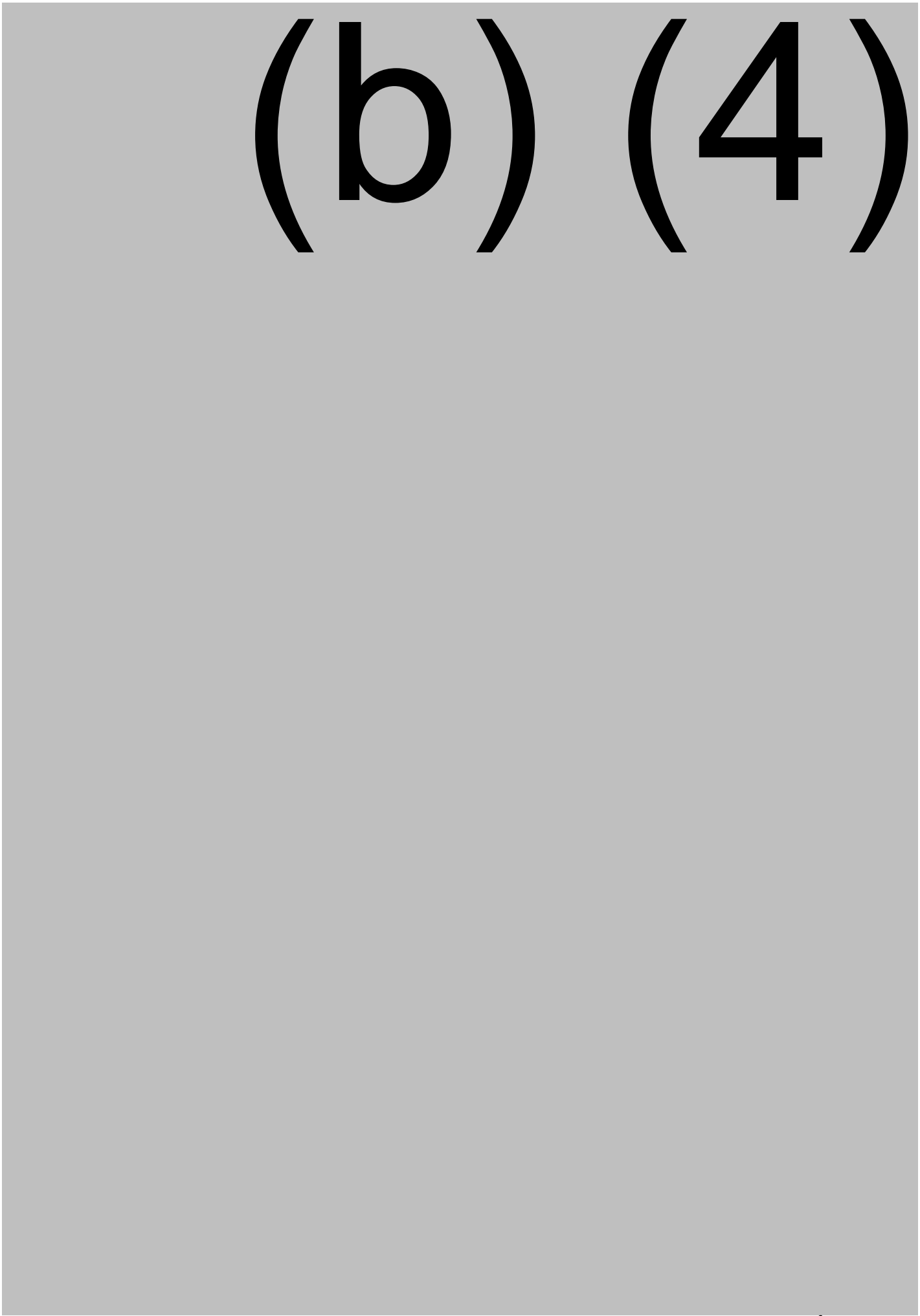

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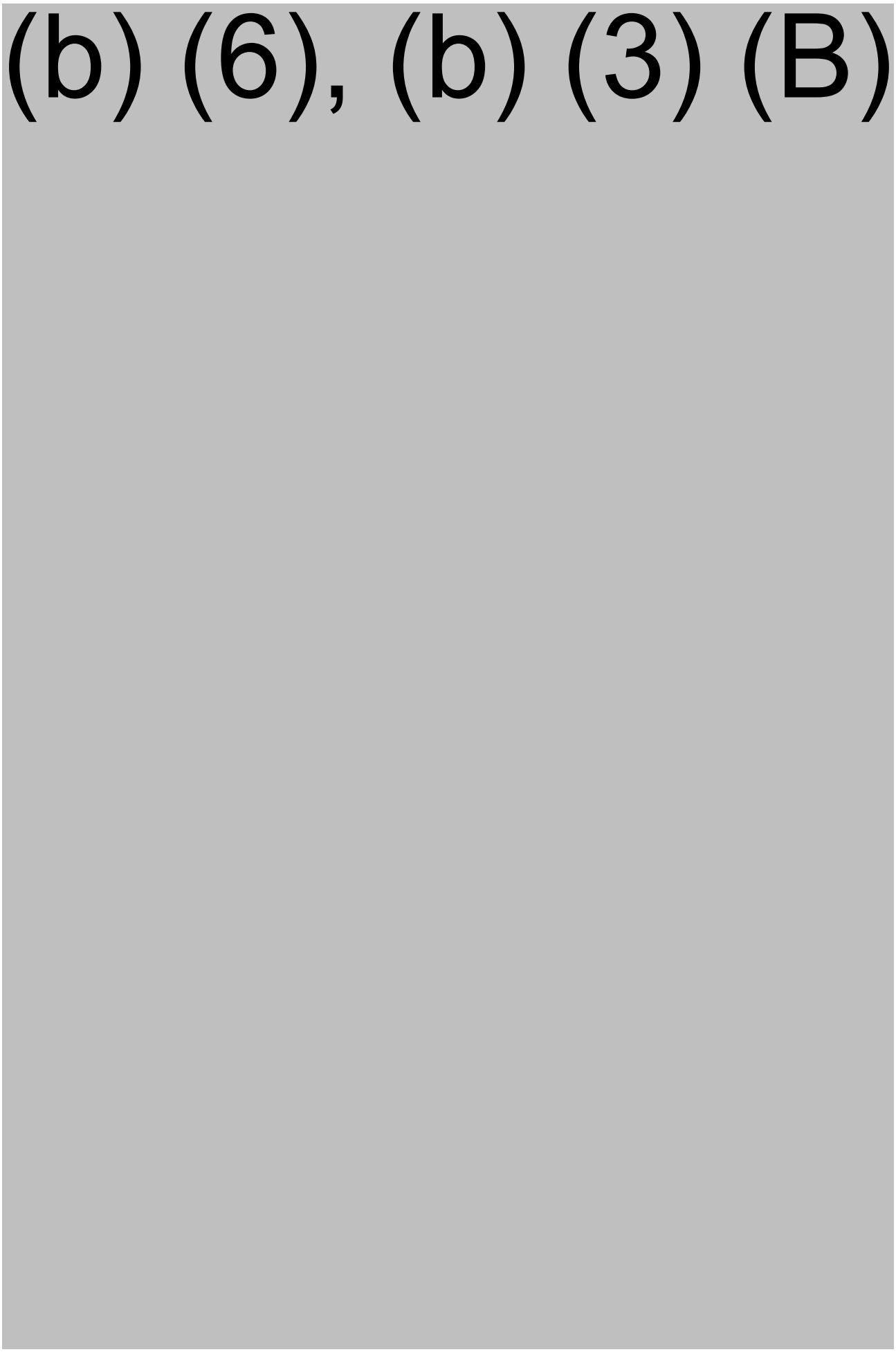


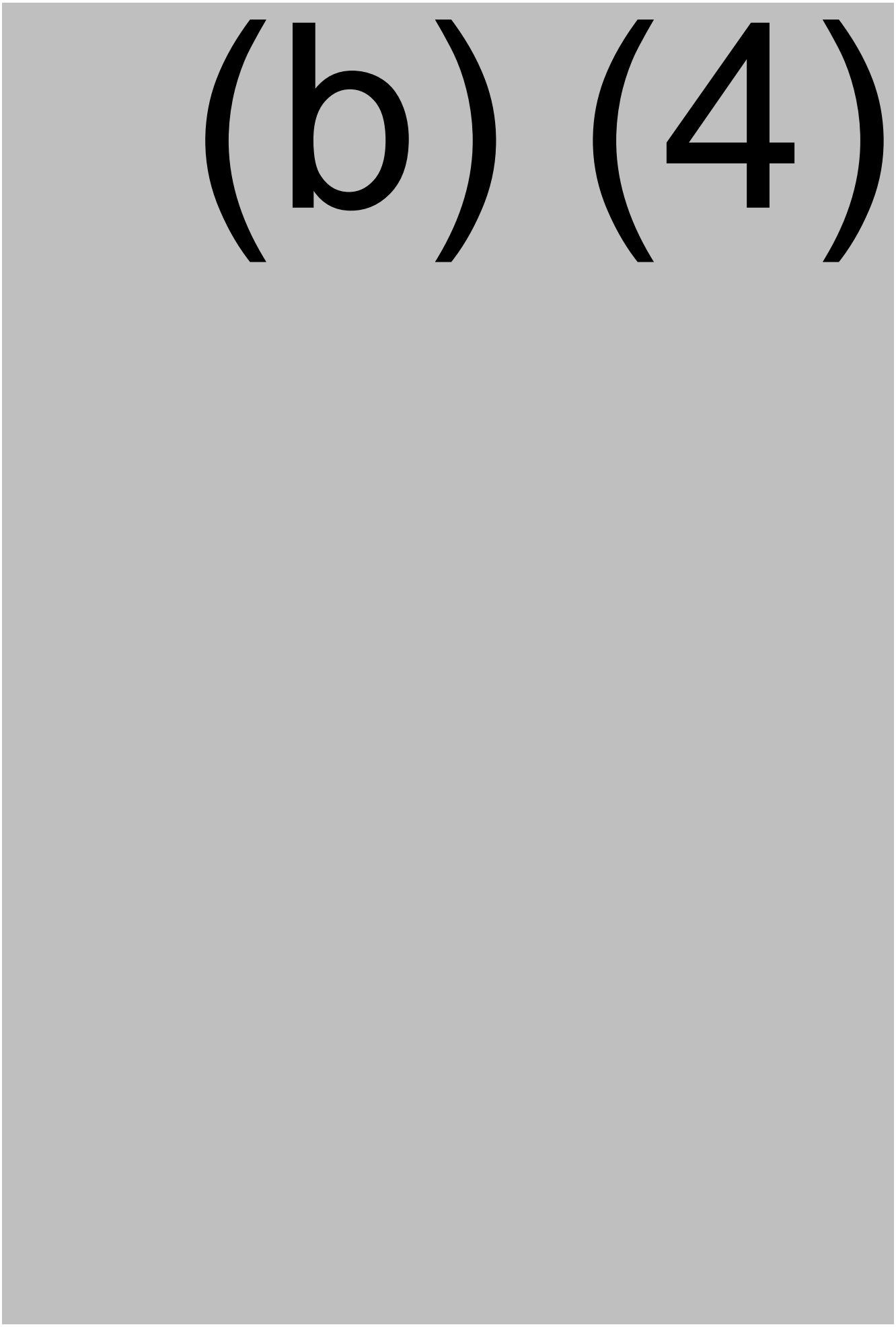
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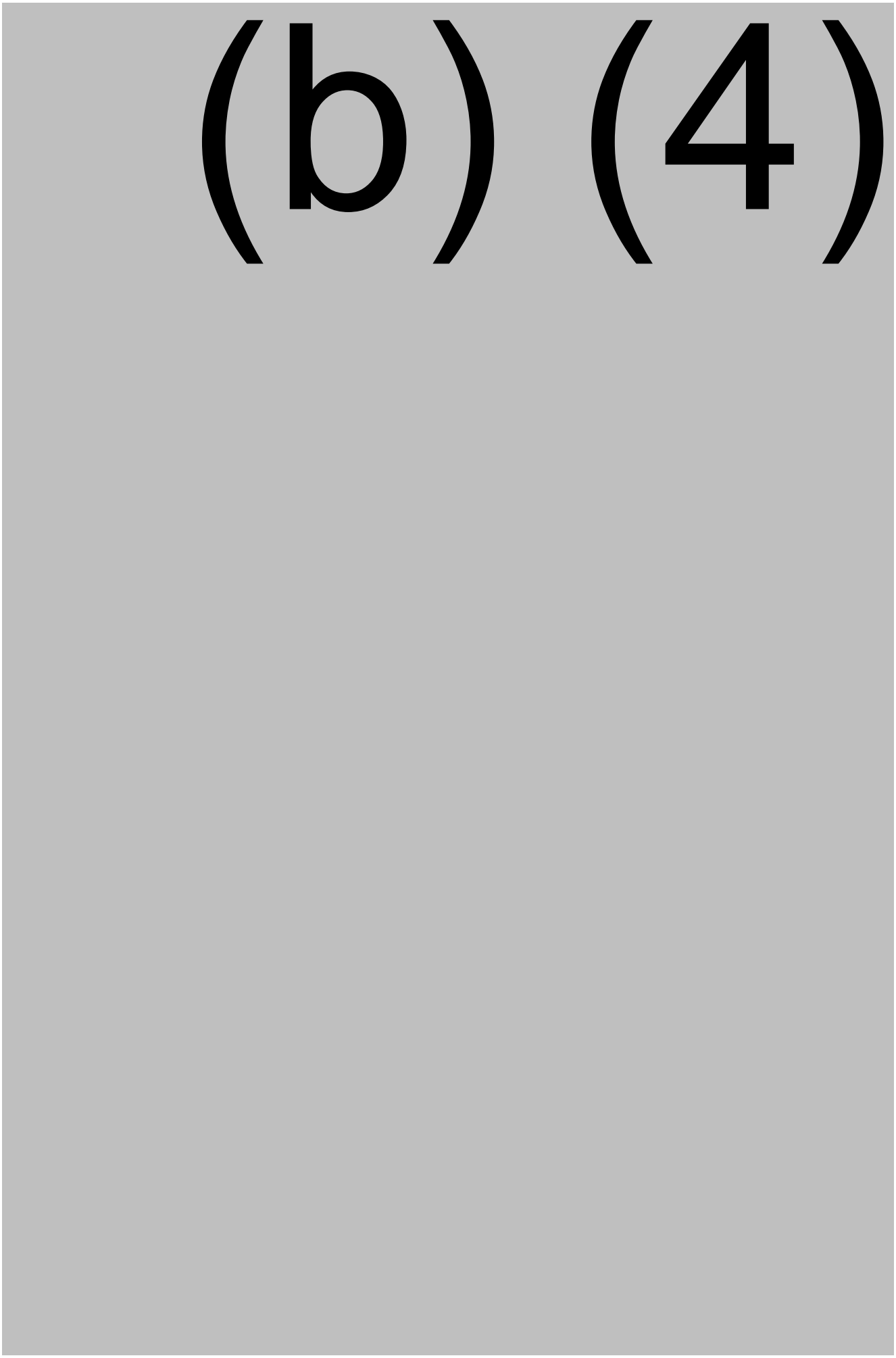
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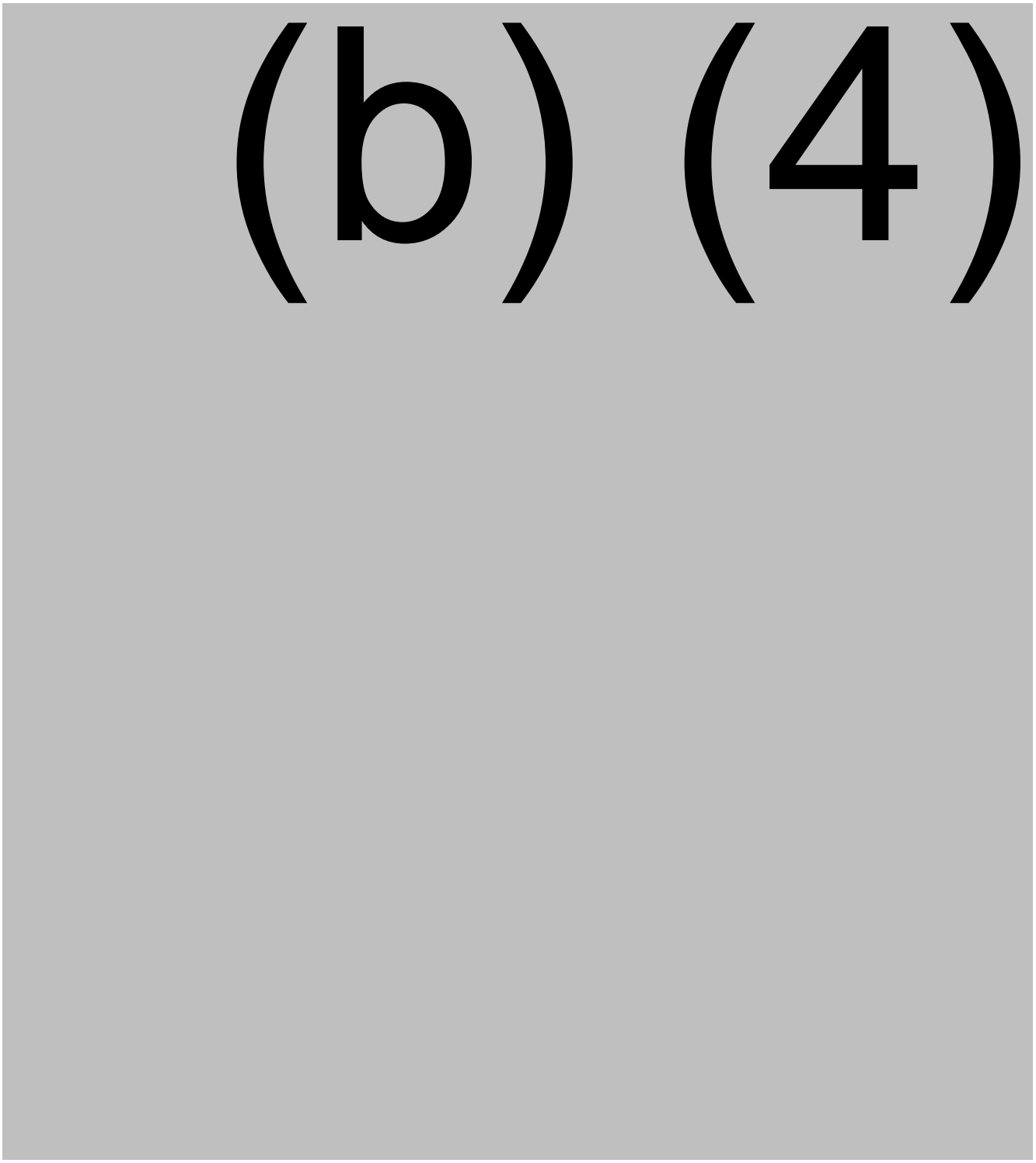

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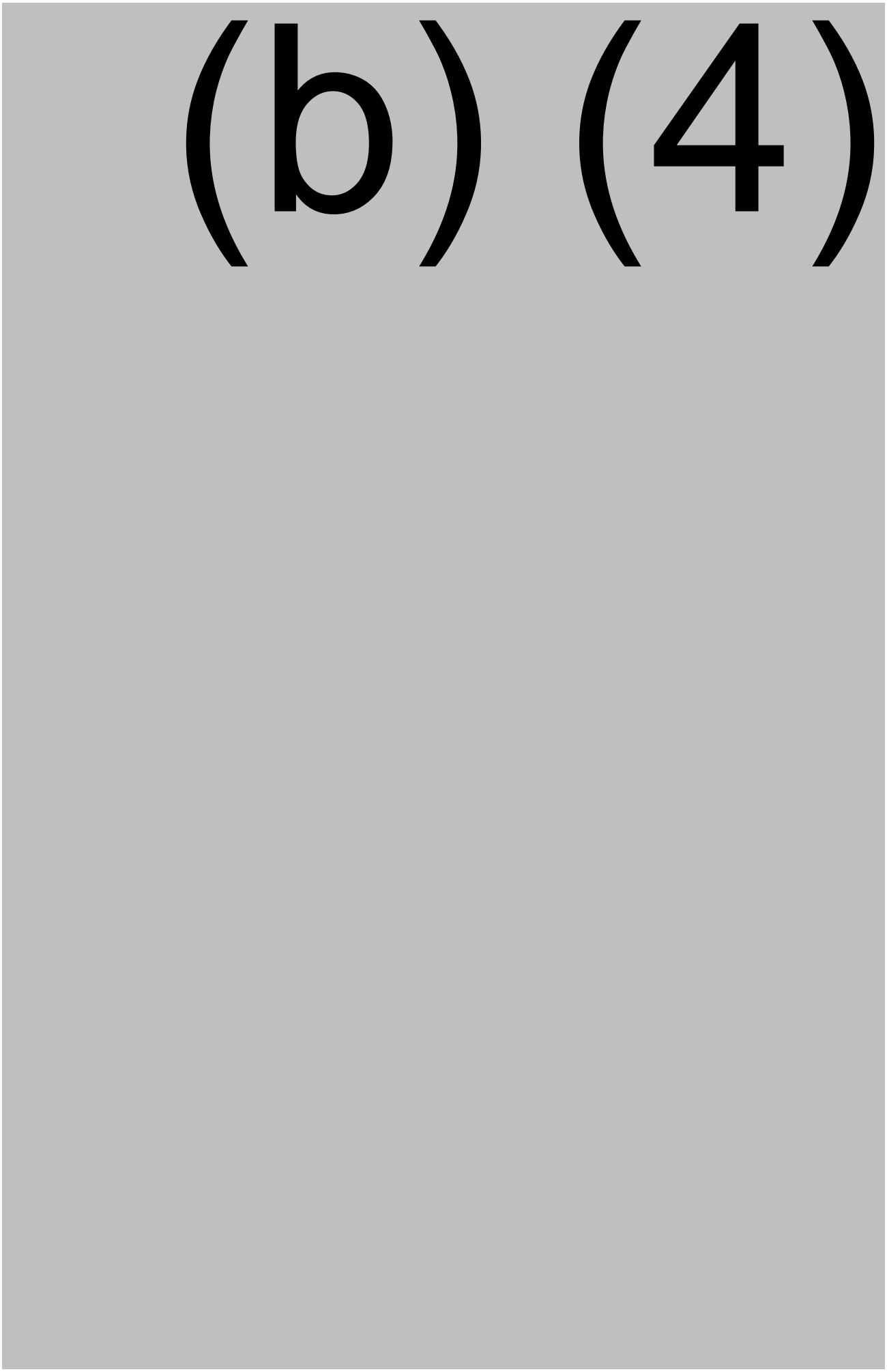
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(b) (4)

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Magnusson, B. and Örnemark, U. (2nd ed. 2014) Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to method Validation and Related Topics. ISBN 978-91-87461-59-0. Available from www.eurachem.org.

Signatures:

(b) (6)

Date: 31 - JANUARY - 2019 Date: 1 FEBRUARY 2019 Date: 4 February 2019

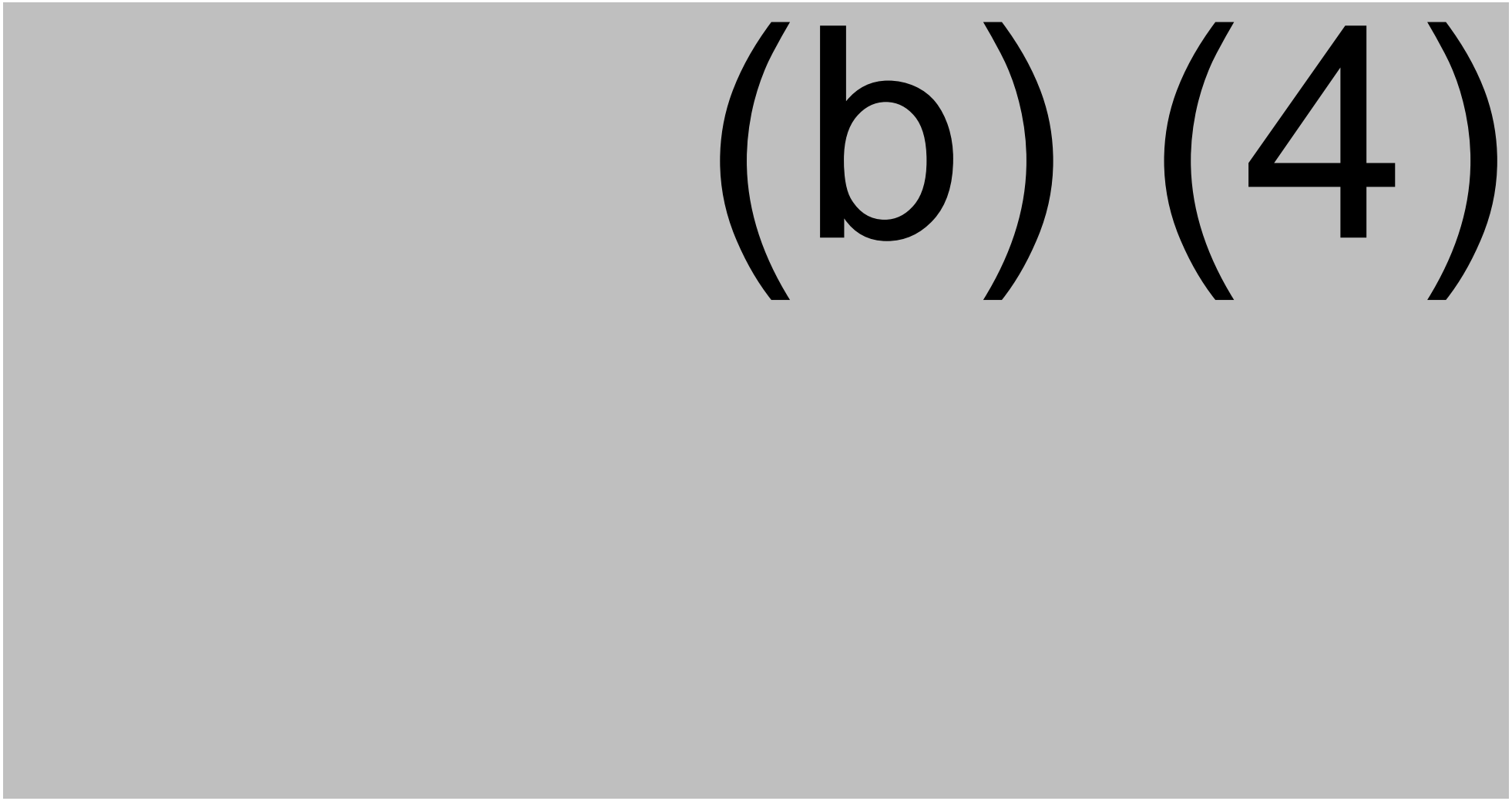
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(b) (4)

ANNEXES

(b) (4)

ANNEX 1. VALIDATION PLAN FOR MINERAL PREMIX AND FEEDS CONTAINING B-MANNANASE ACTIVITY



CONFIDENTIAL

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(b) (4)

ANNEX 2. METHOD OF ANALYSIS FEN/0005/01 PROVIDED BY BASF: PHOTOMETRIC ASSAY OF MANNANASE ACTIVITYIN FEED AND MINERAL PREMIX

Method of Analysis FEN/0005/01

BASF
We create chemistry

Title: **Photometric assay of mannanase activity in feed and mineral premix**

Effective date: May 02nd, 2018

Prepared by: (b) (6)

Reviewed and approved: Dr. Benjamin Peikert

(b) (6)

1 Keywords

1.1 To be determined

Mannanase activity

1.2 Matrix

Feed
Mineral premix

2 Principle

The method is suitable for measurement of endo-mannanase activity in feed and mineral premix. The substrate is azurine-crosslinked carob galactomannan. Hydrolysis by endo-1,4-β-mannanases produces water soluble dyed fragments. The rate of release of these (increase in absorbance at 590 nm) can be related directly to enzyme activity.

One mannanase unit (TMU) is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one µmol mannose from mannan in one minute as defined by the sugar release method.

3 Working range

The working range of this method is (b) (4).

4 Safety note

The method described involves the handling of hazardous substances. Attention is therefore drawn to the various provisions governing the handling of potentially dangerous material.

5 Reagents

(b) (4)

(b) (4)

6 Apparatus

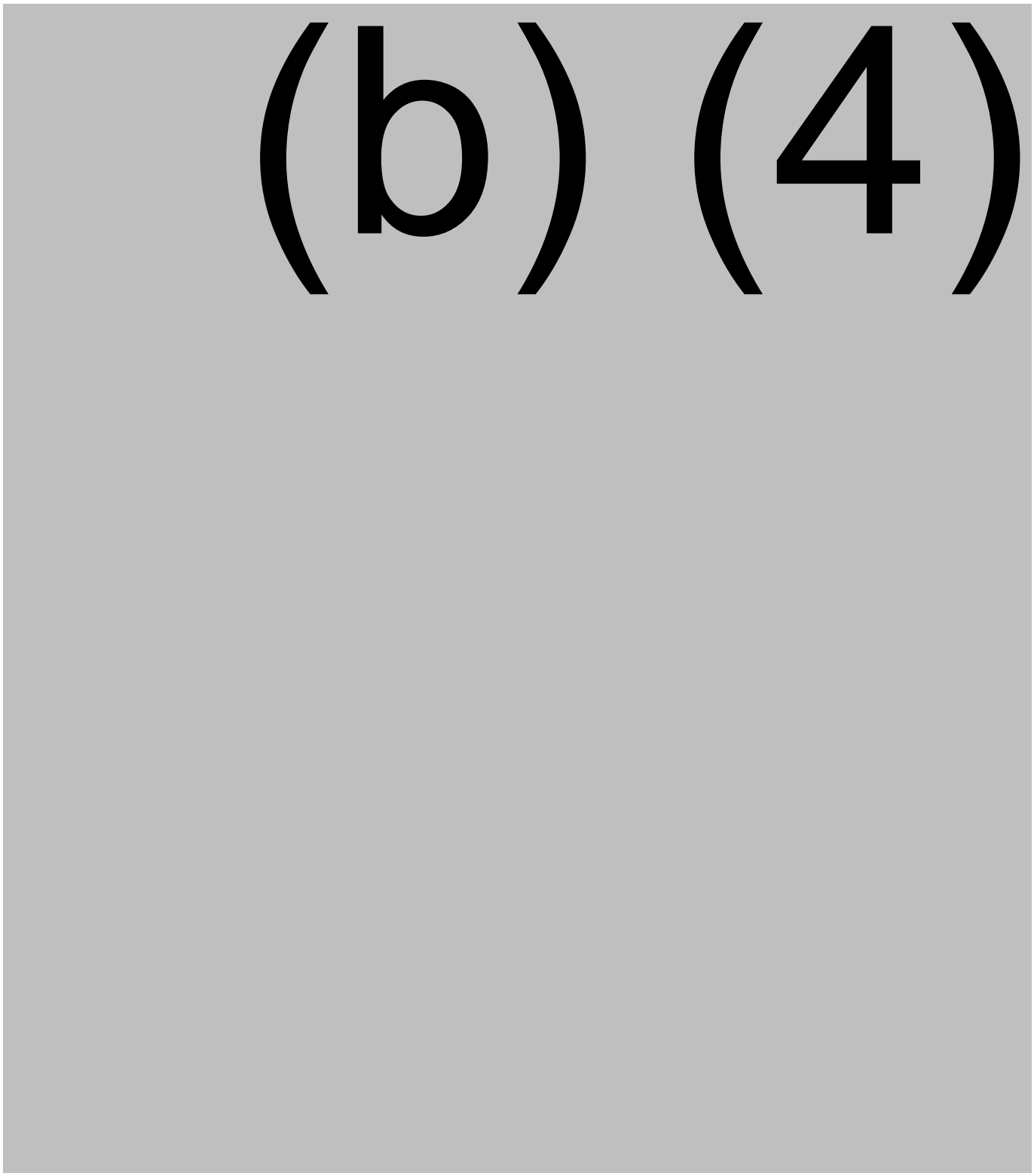
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(b) (4)

7 Preparation of standard, control and sample solutions

(b) (4)

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(b) (4)

8 Procedure

(b) (4)

9 Calculation of mannanase activity

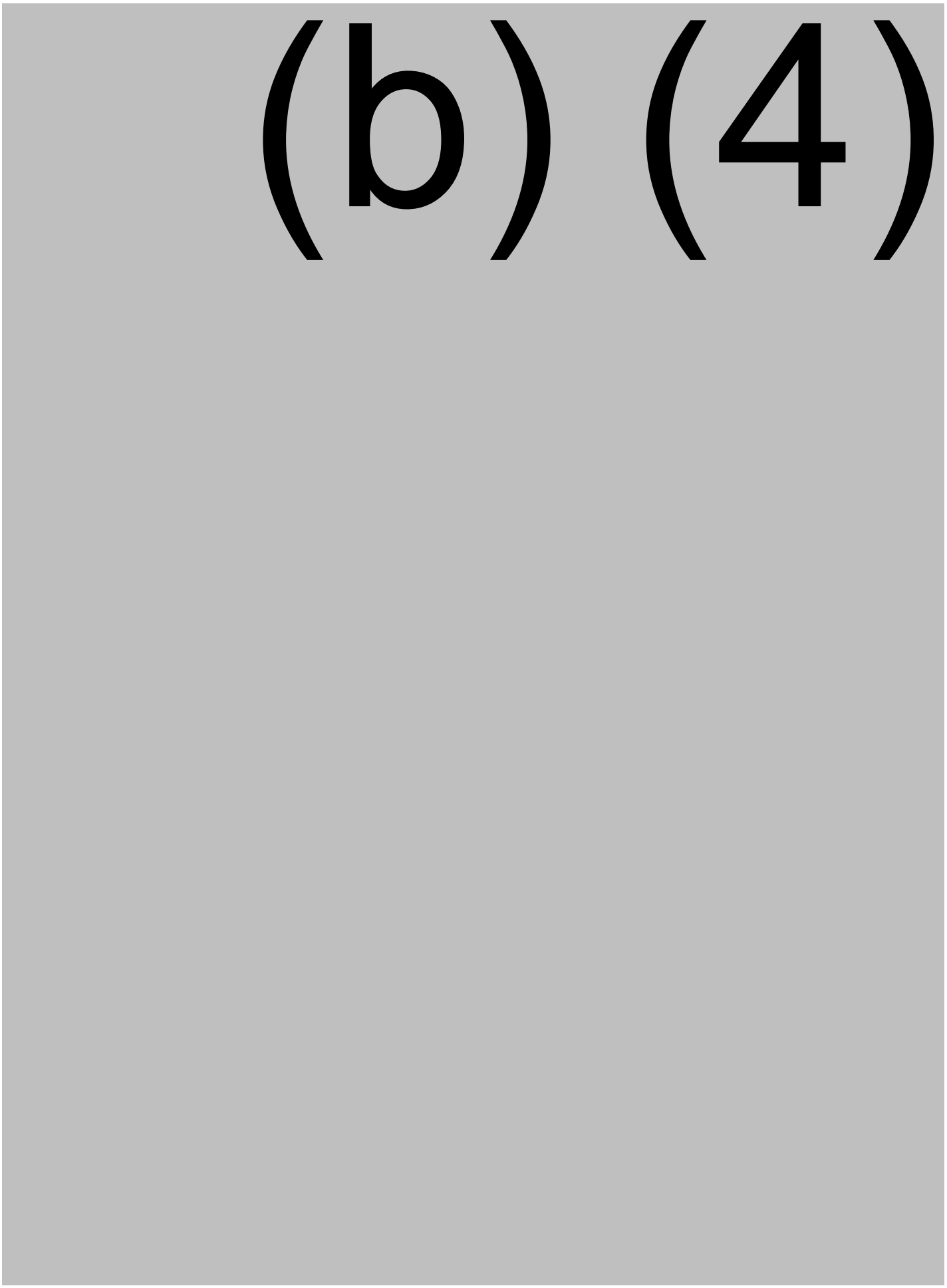
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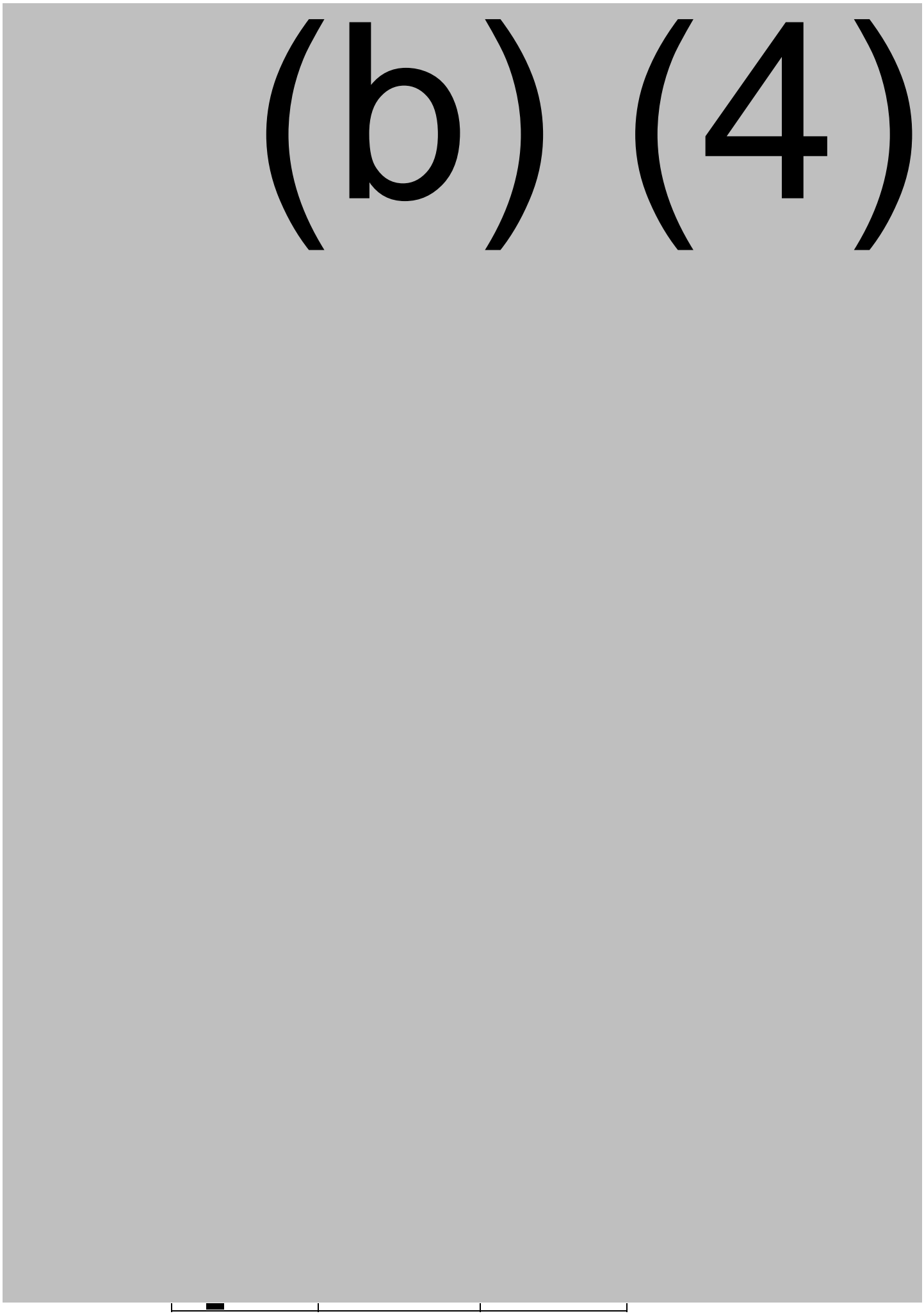
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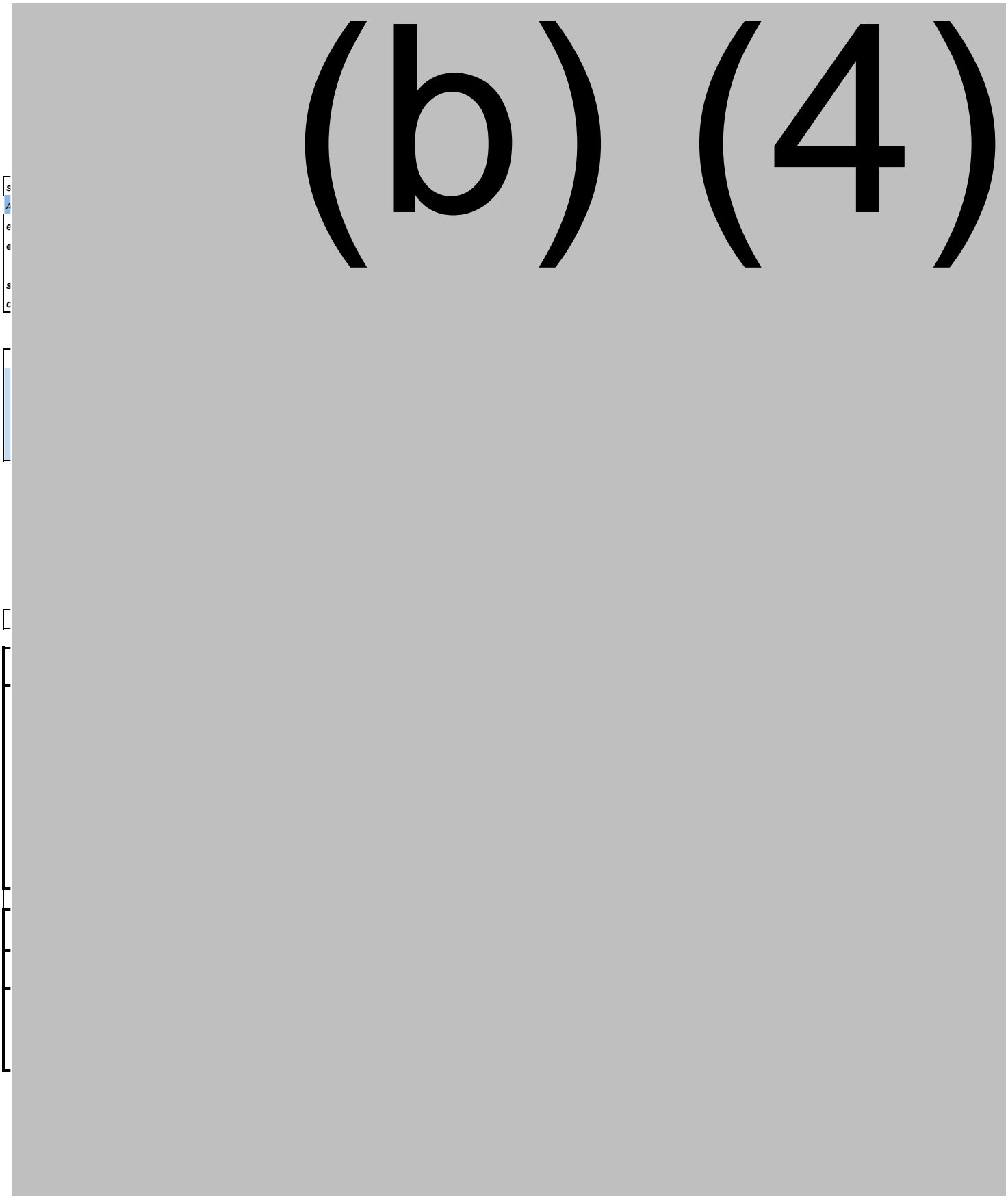
10 Comments

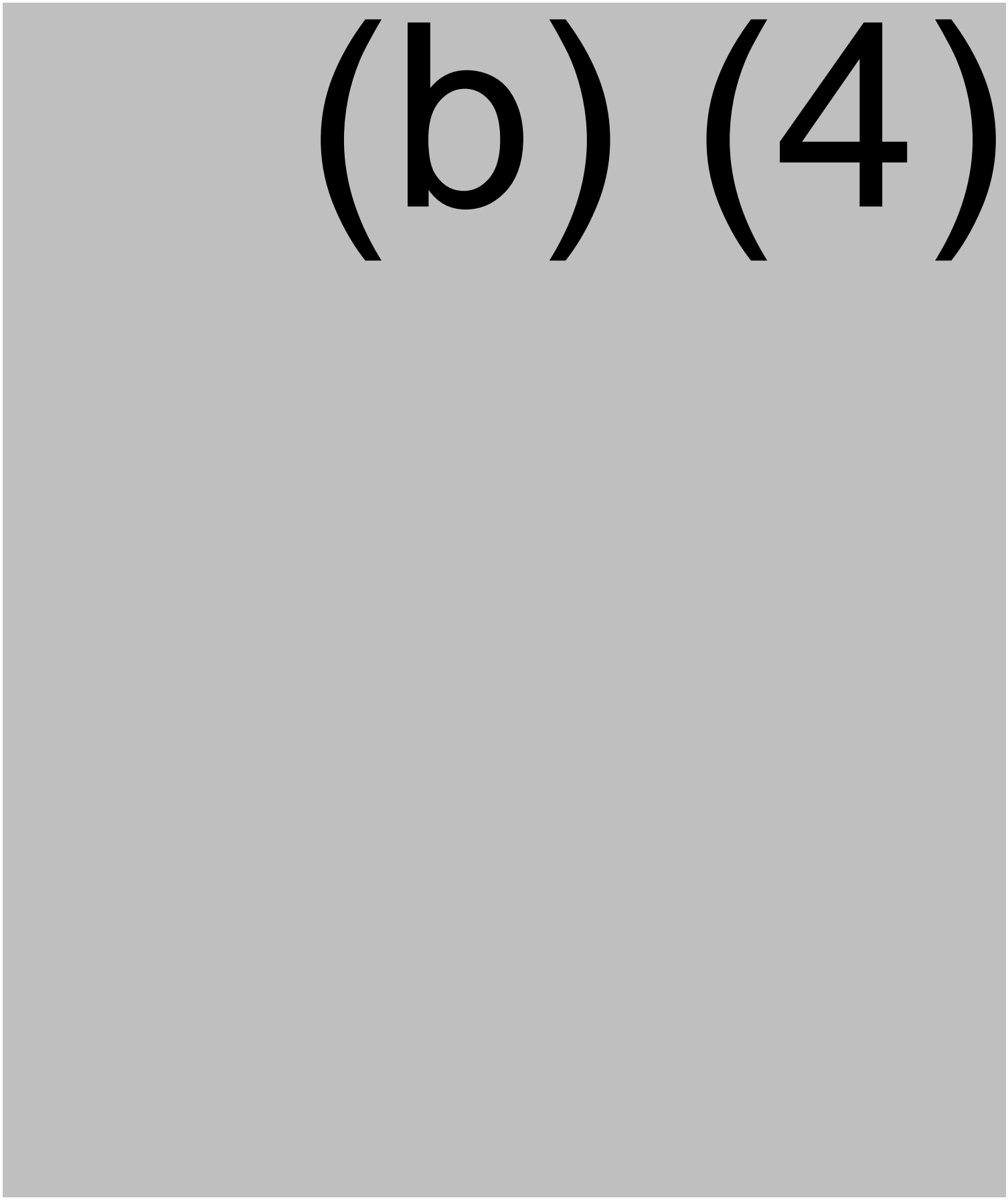
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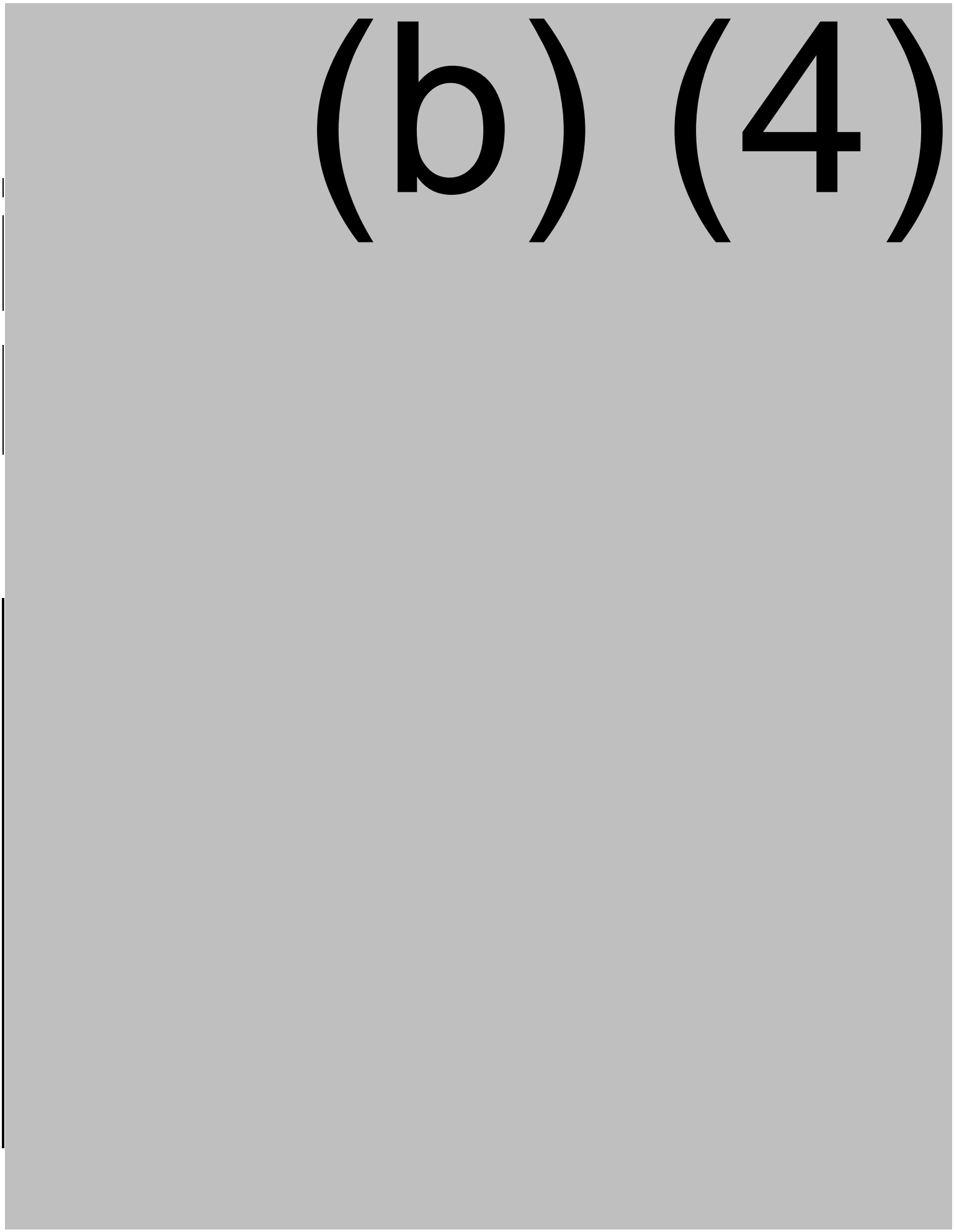
ANNEX 3. RAW DATA AND CALCULATION FOR VALIDATION OF B-MANNANASE DETERMINATION IN FEEDS

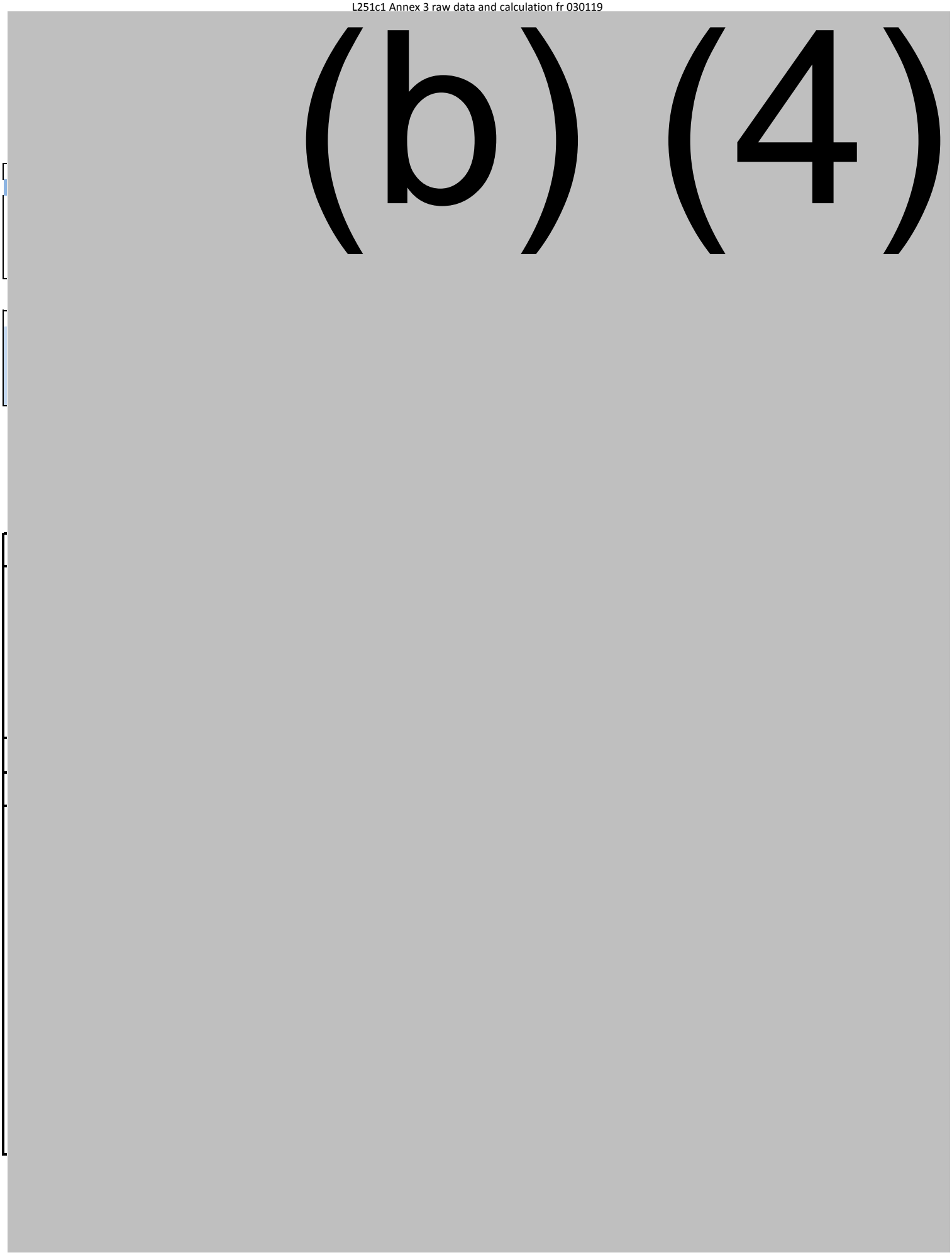

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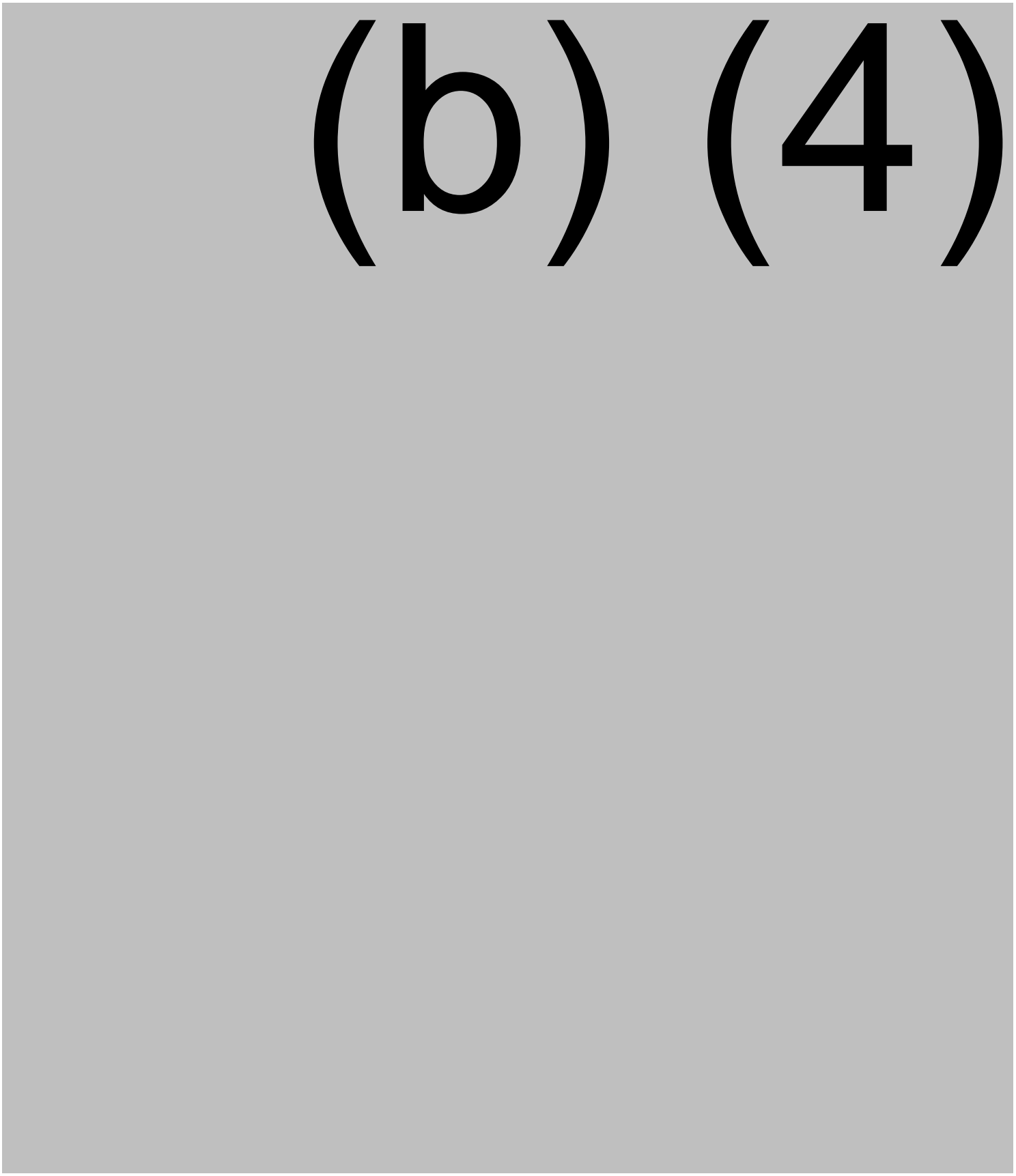

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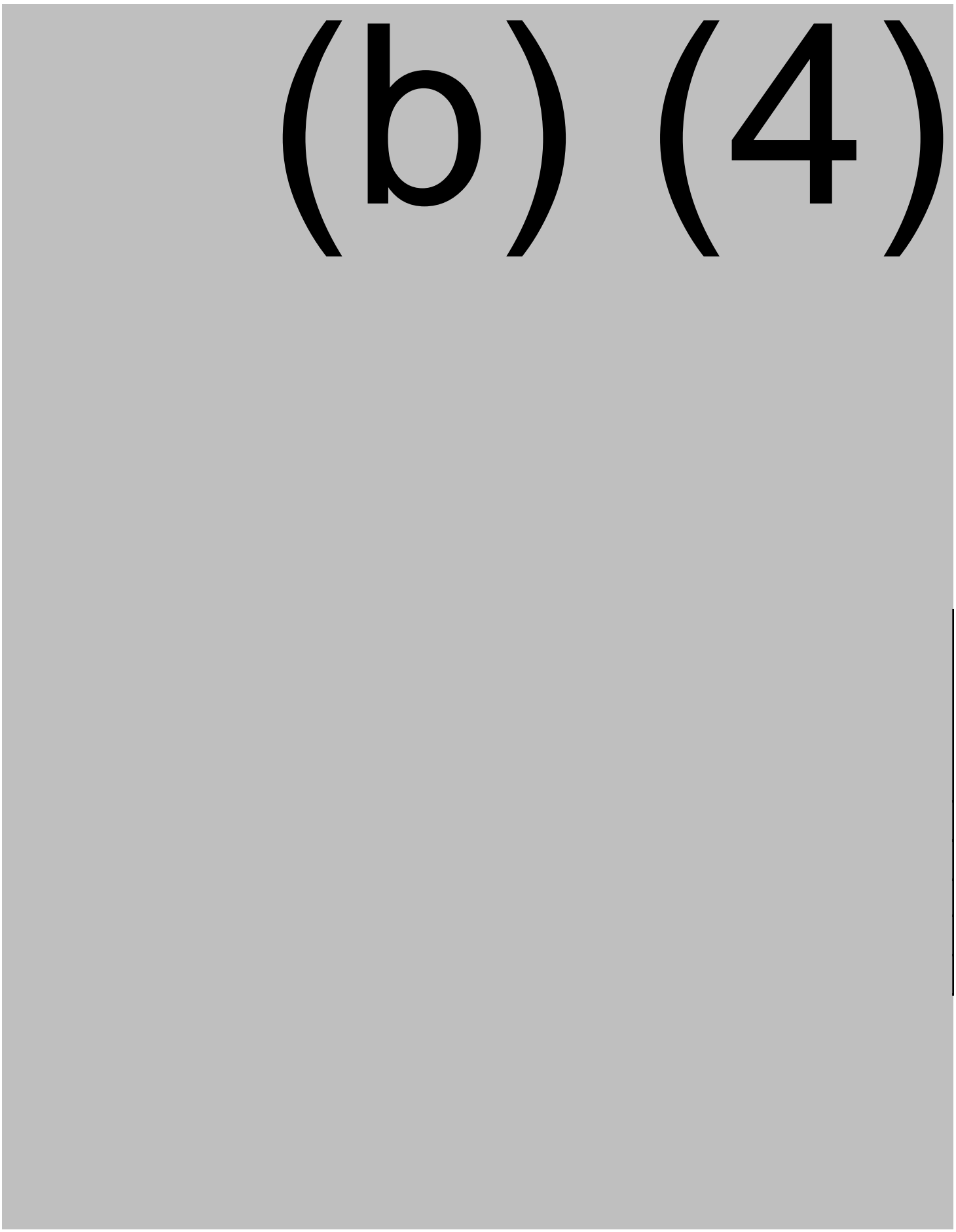

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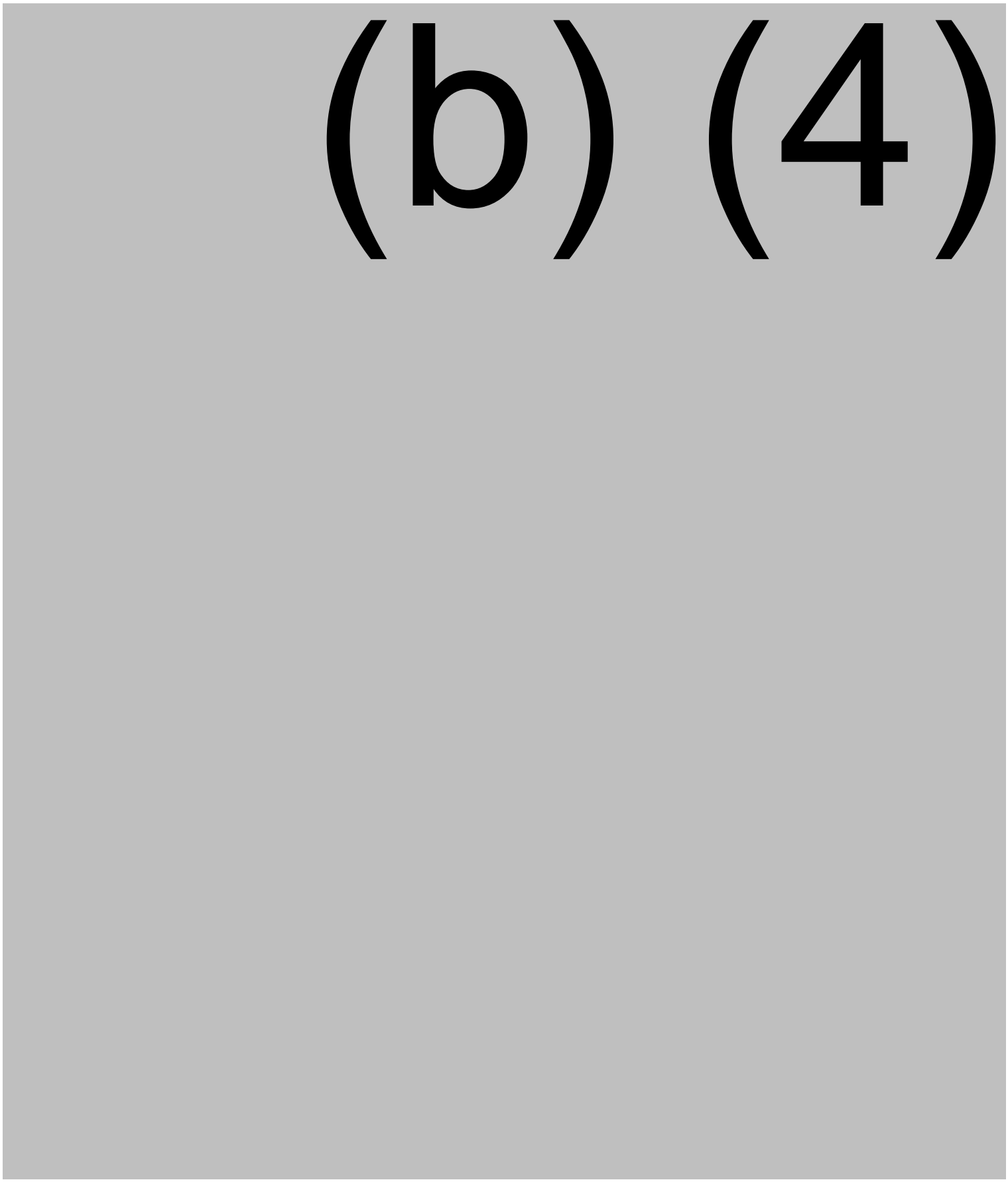

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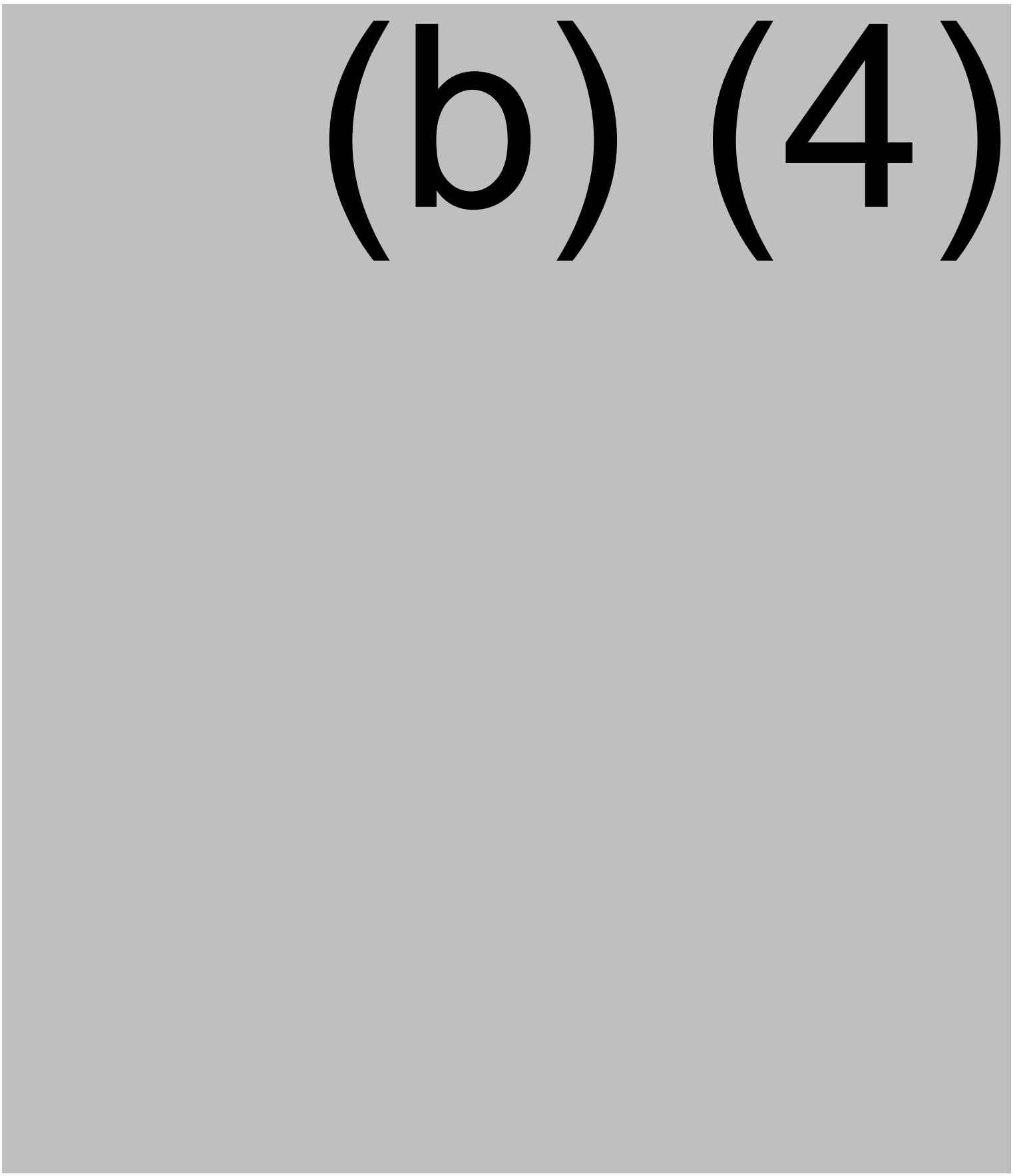

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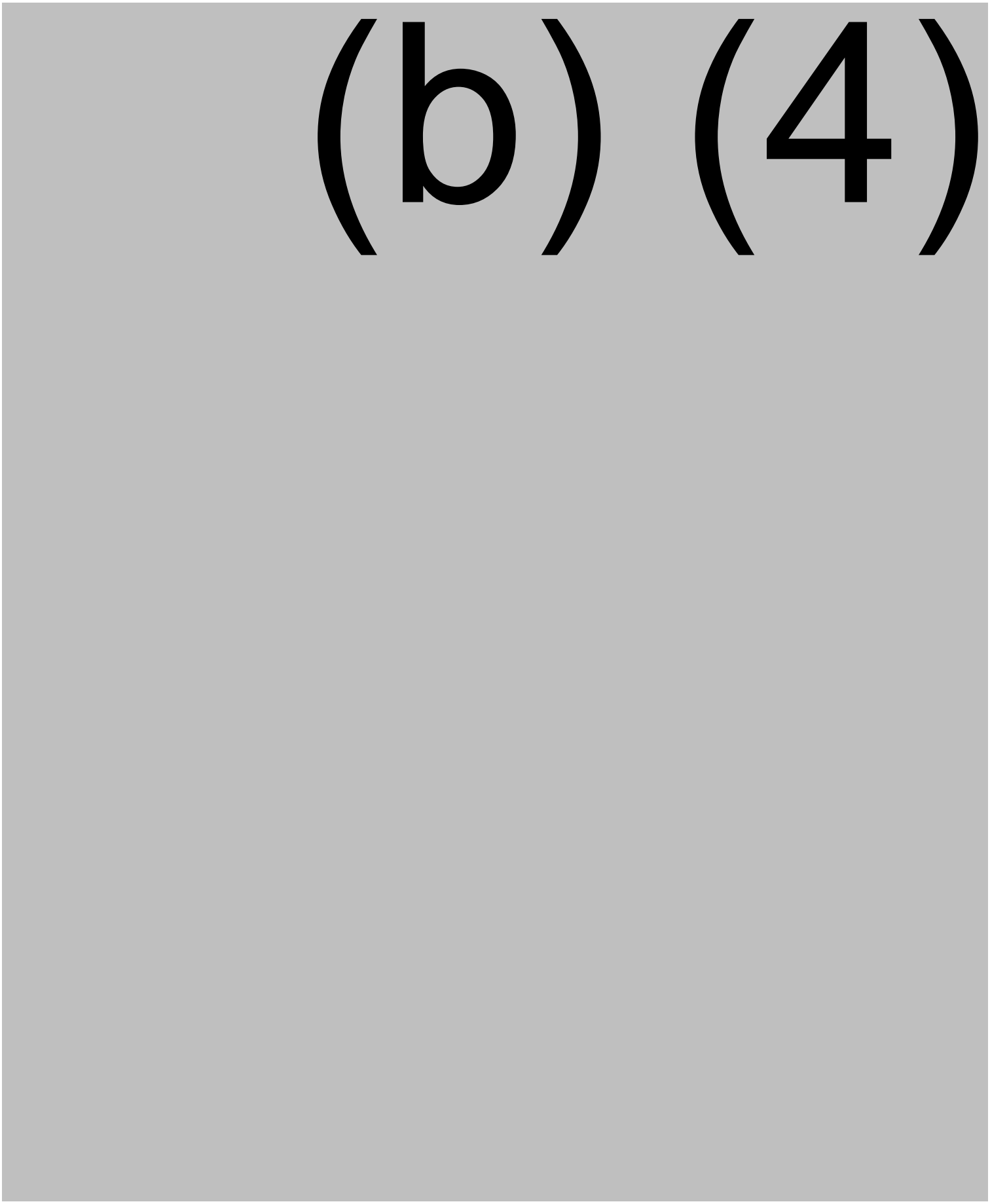

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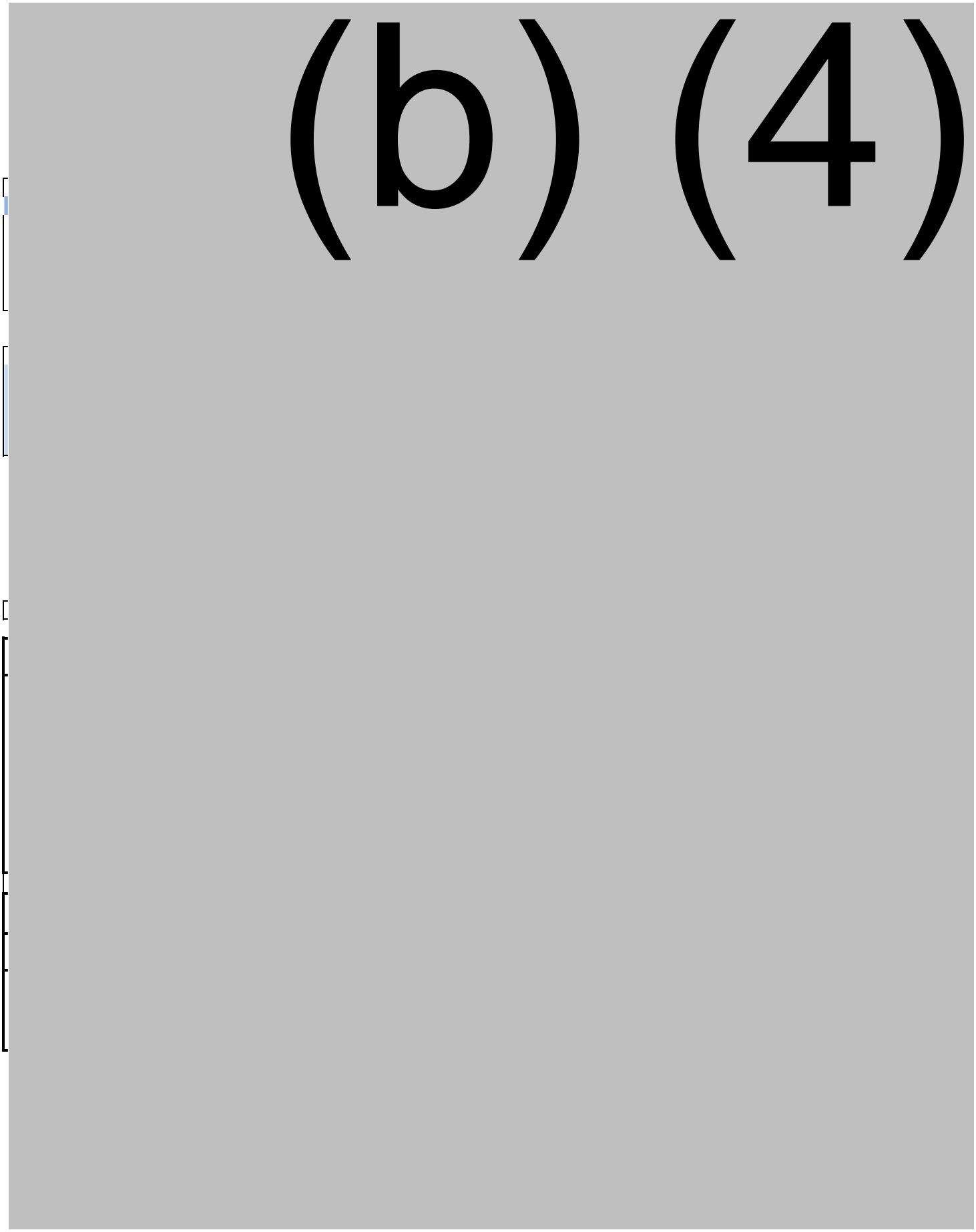

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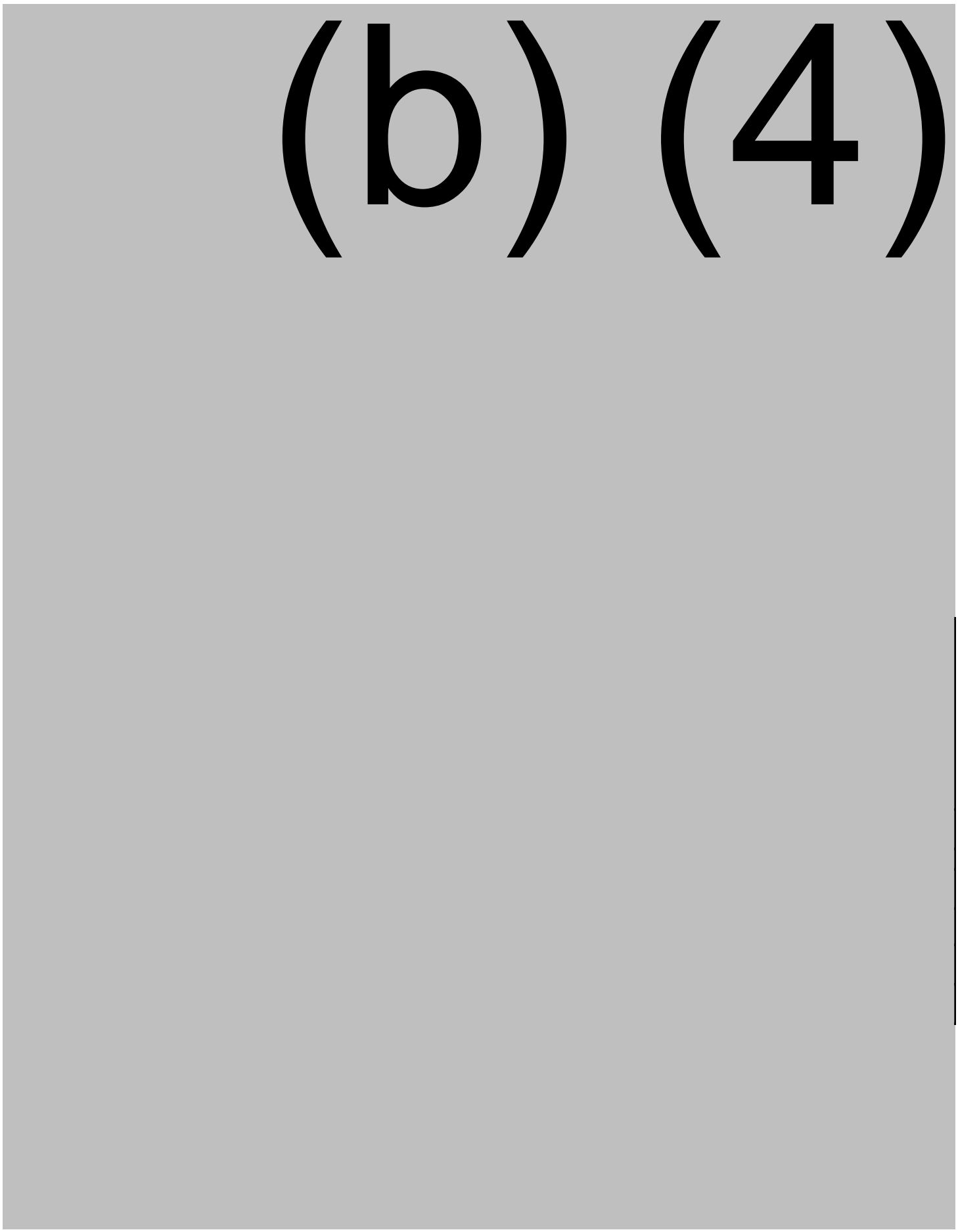

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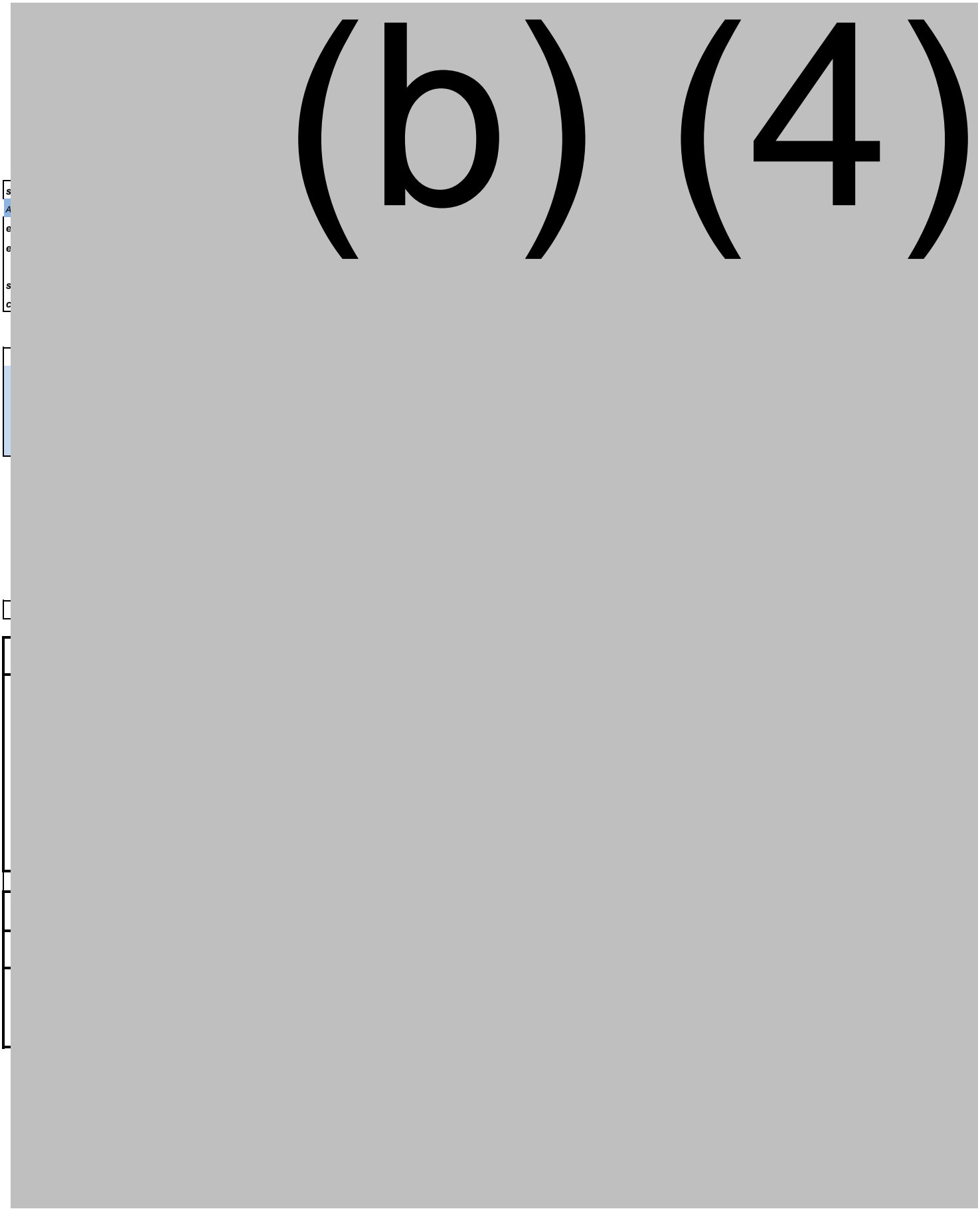

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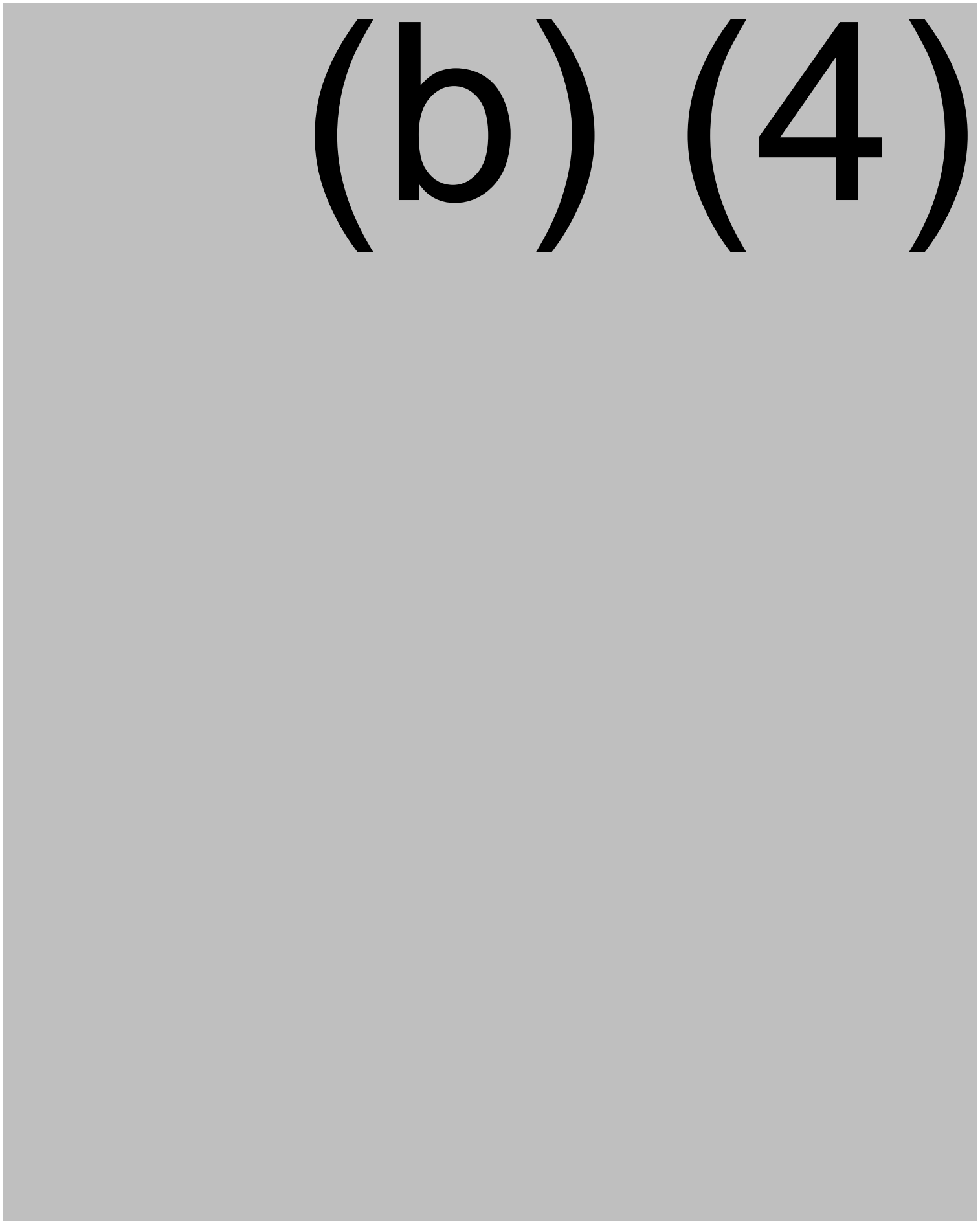
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Appendix 16: Specification and Test Results

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Introduction

Mannanase CC, the mannanase 19287 enzyme preparation concentrate, is screened for total aerobic cell counts, coliforms, antimicrobial activity, aflatoxin B1, and lead. The concentrate is the most likely source of impurities, including microbial contaminations and antimicrobial activities. Since the different final products are prepared from Mannanase CC, these results can be extrapolated to all final formulations of the mannanase 19287 enzyme preparation. Formulation ingredients for Natupulse® TS and Natupulse® TS L are food/feed grade, are GRAS for their intended purpose, or are listed in the current AAFCO OP with their common names.

In addition, twice a year the impurity information is verified via a risk-based end-product monitoring for both Natupulse® TS and Natupulse® TS L.

The following tables list the test results against the internal and final product specifications of Mannanase CC, Natupulse® TS, and Natupulse® TS L. **Table 1** reports the Mannanase CC data, **Table 2** includes the Natupulse® TS results, and Natupulse® TS L test outcomes are found in **Table 3**. Results derive from either product release documents or from Certificate of Analysis (CofAs) obtained during the end-product monitoring (EPM).

Table 1: Mannanase CC Data

Batch #		(b) (4)				
Internal ID						
File name		PP_Mannanase CC_13080536W0	PP_Mannanase CC_41646575L0	PP_Mannanase CC_12597875L0	PP_Mannanase CC_38114477L0	PP_Mannanase CC_46226977L0
TEST PARAMETER	**LIMIT**					
Enzyme activity TMU/g[1]	(b) (4)	(b) (4)				
CHEMICAL Composition						
Lead (mg/kg)[2]	≤ 5					
Dry matter [%]						
Ash [%]						
Total Organic Solids [%]	Calculate					
MICROBIAL Composition	**LIMITS**					
Total aerobic plate count (cfu/g)	≤ $5*10^4$					
Total coliform[2] (cfu/g)	≤ 30					
Salmonella (in 25g)[2]	Absent					
E. coli (in 25g)[2]	Absent					
Antimicrobial activity[2]	Absent					
Antimicrobial activity[3]	Absent					
Absence of Production Organism [CFU/g]	<1					
Mycotoxins	**LIMITS**					
Aflatoxin B1	< LoD (0.5µg/kg)					
Ochratoxin A	< LoD (5µg/kg)					
T-2 Toxin	< LoD (5µg/kg)					
Zearalenone	< LoD (1µg/kg)					
Sterigmatocystin	< LoD (10µg/kg)					
Myceliothermophins	int					
Tenuazonic acid	< LoQ (10µg/kg)					

[1] One mannanase unit (TMU) is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one µmol mannose from mannan in one minute as defined by the sugar release method (at 50ºC and pH 3.5).

[2] VDLUFA Methods Book III (2007) 28.4.1

[3] JECFA General Specifications for Enzyme Preparations (2006)

Commercial Natupulse® TS L Enzyme Product (Liquid)

At the target rate of 800 TMU/kg feed, the product is added at 100 g per metric ton feed.

Table 2: Natupulse® TS L Data

Batch #		(b) (4)							
File name		PP_NP_TS_L_34797097V0	PP_NP_TS_L_84186588Q0	PP_NP_TS_L_15025377L0	PP_NP_TS_L_46618524U0		GO-20202063	GO-20211061	GO-20234068
Release Parameter	**LIMITS**	Inspection	Inspection	Inspection	Inspection	**EPM Parameters**	EPM	EPM	EPM
Enzyme activity [TMU/g][1]	(b) (4)	(b) (4)				Aflatoxin B1 [µg/kg]	(b) (4)		
Lead (mg/kg)	≤ 5					Deoxynivalenol [µg/kg]			
pH	4.0 – 4.4					Zearalenone [µg/kg]			
Antibiotic Activity (Zone of Inhibition)	Absent					Ochratoxin A [µg/kg]			
Total aerobic plate count [CFU/g]	≤ 5*10^4					Fumonisin B1 [µg/kg]			
Total coliform [CFU/g]	≤ 30 cfu/g					Fumonisin B2 [µg/kg]			
Salmonella [in 25g]	Absent					Salmonella [in 25g]			
E. coli [in 25g]	Absent					E. coli [cfu/g]			
Tenuazonic acid	**Batch#**			(b) (4)		Lead [mg/kg]	(b) (4)		
	< LoQ (10µg/kg)			(b) (4)		Coliform bacteria [cfu/g]			

[1] One mannanase unit (TMU) is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one µmol mannose from mannan in one minute as defined by the sugar release method (at 50ºC and pH 3.5).

[2] 016445-L.5 was prepared using a lab scale process equivalent to the commercial production process; Mannanase CC production batch (b) (4) was used for formulation

[3] 016445-L.6 was prepared using a lab scale process equivalent to the commercial production process; Mannanase CC production batch (b) (4) was used for formulation

Commercial Natupulse® TS Enzyme Product (Powder)

At the target rate of 800 TMU/kg feed, the product is added at 100 g per metric ton feed.

Table 3: Natupulse® TS Data

Batch #		(b) (4)						
File name		PP_NP_TS_07589975L0	PP_NP_TS_99457956P0	PP_NP_TS_78708624U0		GO-20212060	GO-20222063	GO-20232066
Release Parameter	**LIMITS**	(b) (4)			**EPM Parameter**	(b) (4)		
Enzyme activity [TMU/g][1]	(b) (4)				Aflatoxin B1 [µg/kg]			
Loss on drying	< 10%				Deoxynivalenol [mg/kg]			
Appearance	Beige to brownish powder				Zearalenone [µg/kg]			
					Ochratoxin A [µg/kg]			
					Fumonisin B1 [µg/kg]			
					Fumonisin B2 [µg/kg]			
					Salmonella [in 25g]			
					E. coli [cfu/g]			
					Lead [mg/kg]			
Tenuazonic acid	< LoQ (10µg/kg)				Coliform bacteria [cfu/g]			

Appendix 16a: Test Article Specifications

Mannanase CC Concentrate (Test Article)

Description

Mannanase CC Concentrate is recovered from a controlled fermentation of a pure *Thermothelomyces thermophilus* DSM 33149 strain.

TEST PARAMETER	LIMIT	Methods
Enzyme activity TMU/g[1]	(b) (4)	FEN004/001
CHEMICAL Composition	**LIMITS JECFA[2]**	
Lead (mg/kg)[2]	≤ 5	VDLUFA VII 2.2.2.5
Antimicrobial activity[2]	Absent	JECFA, Volume 4 (2006)
% TOS	Run and record	JECFA, Volume 4 (2006)
Arsenic (mg/kg)	≤ 2	VDLUFA VII 2.2.2.5
Cadmium (mg/kg)	≤ 5	VDLUFA VII 2.2.2.5
Mercury (mg/kg)	≤ 0.1	VDLUFA VII 2.2.2.5
Fluoride (mg/kg)	≤ 150	BVL F 0092:2013- 04
Recombinant DNA (rDNA)	< 10 ng/mL	PCR-based method
MICROBIAL Composition		
Total aerobic plate count	≤ $5*10^4$	VDLUFA III 28.1.2, LOD <50
Total coliform[2]	≤ 30 cfu/g	DIN EN ISO 21528
Salmonella (in 25g)[2]	Absent	DIN EN ISO 6579
E. coli (in 25g)[2]	Absent	DIN EN ISO 21528
Enterobacteria	N/A	DIN EN ISO 21528
Yeast and Mold (cfu/g)	N/A	VDLUFA III 28.1.2, LOD <50
Absence of Production Organism	LoD < 5/mL*	BASF internal plate-based procedure
Mycotoxins	**LIMITS**	
Aflatoxin B1	≤ 0.01 mg/kg	VD LUFA III 16.13.1
Deoxynivalenol	≤ 5 mg/kg	VD LUFA III 16.13.1
Zearalenone	< LOD (1µg/kg)	VD LUFA III 16.13.1
HT2 toxin	< LOD (5µg/kg)	VD LUFA III 16.13.1
T2 toxin	< LOD (5µg/kg)	VD LUFA III 16.13.1
Ochratoxin A	≤ 0.1 mg/kg,	LC-MS/MS (b) (4) SP 1026
Fumonisin (FB1 + FB2)	≤ 20 mg/kg)	LC-MS/MS SP 1027
Sterigmatocystin	< LoD (10µg/kg)	(b) (4) Method CON-PV o1126, LC-MS/MS

Mannanase (b) (4) Enzyme Test Article

Description

The Mannanase (b) (4) test article is a formulated liquid mannanase 19287 enzyme preparation with a defined minimum mannanase activity manufactured from the Mannanase CC concentrate.

		Methods
TEST PARAMETER	**LIMITS**	
Enzyme activity [TMU/g][1]	(b) (4)	FEN004/001
CHEMICAL Composition	**JECFA LIMITS**[2]	
Lead (mg/kg)[2]	≤ 5	VDLUFA VII 2.2.2.5 – Test Article JECFA, Volume 4 (2006)[#]
Antibiotic Activity (Zone of Inhibition)[2]	Absent	VDLUFA III 28.4.1:2007 – Test Article JECFA, Volume 4 (2006)[#]
Recombinant DNA (rDNA)	< 10 ng/mL	PCR-based method[#]
MICROBIAL Composition		
Total aerobic plate count [CFU/ml]	≤ 5*10^4	VDLUFA III 28.1.2, LOD <50
Total coliform[2]	≤ 30 cfu/g	DIN EN ISO 21528
Salmonella [in 25g][2]	Absent	DIN EN ISO 6579
Escherichia coli [in 25g][2]	Absent	DIN EN ISO 21528
Absence of Production Organism	LoD < 5/mL*	BASF internal plate-based procedure[#]
Mycotoxins	**LIMITS**	
Aflatoxin B1	≤ 0.01 mg/kg	VD LUFA III 16.13.1
Deoxynivalenol	≤ 5 mg/kg	VD LUFA III 16.13.1
Zearalenone	< LOD (1µg/kg)	VD LUFA III 16.13.1
HT2 toxin	< LOD (5µg/kg)	VD LUFA III 16.13.1
T2 toxin	< LOD (5µg/kg)	VD LUFA III 16.13.1
Ochratoxin A	≤ 0.1 mg/kg,	LC-MS/MS (b) (4) P 1026
Fumonisin (FB1 + FB2)	≤ 20 mg/kg)	LC-MS/MS P 1027

[1] One thermostable mannanase unit (TMU) is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one µmol mannose from mannan in one minute as defined by the sugar release method (at 50ºC and pH 3.5).

[2] JECFA General Specifications for Enzyme Preparations (2006)

[#] Analysis performed on the original Mannanase CC batch

* Please note that the limit of detection (LoD) is < 3/mL in commercial manufacturing

Mannanase (b) (4) Enzyme Product (Test Article)

Description

The Mannanase (b) (4) test article is a formulated dry mannanase 19287 enzyme preparation with a defined minimum mannanase activity manufactured from the Mannanase CC concentrate.

TEST PARAMETER	LIMITS	Methods
Enzyme activity [TMU/g][1]	(b) (4)	FEN004/001
CHEMICAL Composition	**JECFA LIMITS[2]**	
Lead[2] (mg/kg)	≤ 5	VDLUFA VII 2.2.2.5 – Test Article JECFA (2006)[#]
Antimicrobial Activity[2]	Absent	VDLUFA VII 2.2.2.5 – Test Article JECFA (2006)[#]
rDNA	< 10 ng/mL	PCR-based method[#]
MICROBIAL Composition		
Total aerobic plate count [CFU/g]	< $5*10^4$	VDLUFA III 28.1.2
Total coliform[2] [CFU/g]	≤ 30	DIN EN ISO 6579
Salmonella[2] [in 25g]	Absent	DIN EN ISO 21528
Escherichia coli[2] [in 25g]	Absent	ISO 21528
Absence of Production Organism	LoD (< 5/mL)*	BASF internal plate-based procedure[#]
MYCOTOXINS	**LIMITS**	**Methods**
Aflatoxin B1	≤ 0.01 mg/kg	VD LUFA III 16.13.1
Deoxynivalenol	≤ 5 mg/kg	VD LUFA III 16.13.1
Zearalenone	< LoD (1µg/kg)	VD LUFA III 16.13.1
HT2 toxin	< LoD (5µg/kg)	VD LUFA III 16.13.1
T2 toxin	< LoD (5µg/kg)	VD LUFA III 16.13.1
Ochratoxin A	≤ 0.1 mg/kg,	LC-MS/MS (b) (4) SP 1026
Fumonisin (FB1 + FB2)	≤ 20 mg/kg)	LC-MS/MS SP 1027

[1] One thermostable mannanase unit (TMU) is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one µmol mannose from mannan in one minute as defined by the sugar release method (at 50ºC and pH 3.5).

[2] JECFA General Specifications for Enzyme Preparations (2006)

[#] Analysis performed on the original Mannanase CC batch

* Please note that the limit of detection (LoD) is < 3/mL in commercial manufacturing

Appendix 16b: Test Article Results

Mannanase CC Concentrate (Test Article)

Description

Mannanase CC Concentrate is recovered from a controlled fermentation of a pure *Thermothelomyces thermophilus* DSM 33149 strain.

		Mannanase CC	Mannanase CC	Mannanase CC
		(b) (4)		
TEST PARAMETER	**LIMIT**			
Enzyme activity TMU/g[1]	≥ (b) (4)			
CHEMICAL Composition	**LIMITS JECFA[2]**			
Lead (mg/kg)[2]	≤ 5			
Antimicrobial activity[2]	Absent			
% TOS	N/A			
Arsenic (mg/kg)	Not applicable (N/A)			
Cadmium (mg/kg)	N/A			
Mercury (mg/kg)	N/A			
Copper (mg/kg)	N/A			
Fluoride	N/A			
rDNA	< 10 ng/mL			
MICROBIAL Composition				
Total aerobic plate count	≤ $5*10^4$			
Total coliform[2]	≤ 30 cfu/g			
Salmonella (in 25g)[2]	Absent			
E. coli (in 25g)[2]	Absent			
Yeast and Mold (cfu/g)	N/A			
Absence of Production Organism	LoD < 5/mL			
Mycotoxins	**LIMITS**			
Aflatoxin B1	< LoD (0.5µg/kg)			
Fumonisin (FB1+FB2)	< LoD (20µg/kg)			
Ochratoxin A	< LoD (5µg/kg)			
Deoxynivalenol	< LoD (10µg/kg)			
T-2 Toxin	< LoD (5µg/kg)			
HT-2 Toxin	< LoD (5µg/kg)			
Zearalenone	< LoD (1µg/kg)			
Sterigmatocystin	< LoD (10µg/kg)			

(b) (4)

[1] One mannanase unit (TMU) is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one µmol mannose from mannan in one minute as defined by the sugar release method (at 50ºC and pH 3.5).

[2] JECFA General Specifications for Enzyme Preparations (2006)

* Please note that the limit of detection (LoD) is < 3/mL in commercial manufacturing

Mannanase (b) (4) Enzyme Product Test Article

Description

The Mannanase (b) (4) test article is a formulated liquid preparation with a defined minimum mannanase activity, manufactured from the Mannanase CC concentrate.

	Mannanase (b) (4)			
Test Article (produced from Mannanase CC batch)		(b) (4)		
TEST PARAMETER	**LIMITS**			
Enzyme activity [TMU/g][1]	≥ (b) (4)	(b) (4)		
CHEMICAL Composition				
Lead (mg/kg)	≤ 5			
Antibiotic Activity (Zone of Inhibition)	Absent			
rDNA	< 10 ng/mL			
MICROBIAL Composition				
Total aerobic plate count [CFU/g]	≤ 5*10^4			
Total coliform [CFU/g]	≤ 30 cfu/g			
Salmonella [in 25g]	Absent			
Escherichia coli [in 25g]	Absent			
Absence of Production Organism	LoD (< 5/mL)*			
Mycotoxins	**LIMITS**			
Aflatoxin B1	< LoD (0.5µg/kg)			
Deoxynivalenol	< LoD (10µg/kg)			
Zearalenone	< LoD (1µg/kg)			
HT2 toxin	< LoD (5µg/kg)			
T2 toxin	< LoD (5µg/kg)			
Ochratoxin A	< LoD (5µg/kg)			
Fumonisin (FB1 + FB2)	< LoD (20µg/kg)			

Mannanase (b) (4) Enzyme Test Article

Description
The Mannanase (b) (4) test article is a formulated dry mannanase 19287 enzyme preparation with a defined minimum mannanase activity, manufactured from the Mannanase CC concentrate.

	Mannanase 8000/Natupulse® TS			
Test Article (produced from Mannanase CC batch)		(b) (4)		
TEST PARAMETER	**LIMITS**			
Enzyme activity [TMU/g][1]	≥ (b) (4)	(b) (4)		
CHEMICAL Composition	**JECFA LIMITS**[2]			
Lead[2] (mg/kg)	≤ 5			
Antimicrobial Activity[2]	Absent			
rDNA (Mannanase CC)	< 10 ng/mL			
MICROBIAL Composition				
Total aerobic plate count [CFU/g]	< $5*10^4$			
Total coliform[2] [CFU/g]	≤ 30			
Salmonella[2] [in 25g]	Absent			
Escherichia coli[2] [in 25g]	Absent			
Absence of Production Organism (Mannanase CC)	LoD < 5/mL*			
MYCOTOXINS	**LIMITS**			
Aflatoxin B1 [µg/kg]	< LoD (0.5µg/kg)			
Deoxynivalenol [µg/kg] (See **note 1** below)	< LoD (10µg/kg)			
Zearalenone [µg/kg] (See **note 2** below)	< LoD (1µg/kg)			
HT2 toxin [µg/kg]	< LoD (5µg/kg)			
T2 toxin [µg/kg]	< LoD (5µg/kg)			
Ochratoxin A [µg/kg]	< LoD (5µg/kg)			
Fumonisin (FB1 + FB2) [µg/kg]	< LoD (20µg/kg)			

Note 1: The levels of DON in these test articles (0.3 ppm) were well below the 10 ppm advisory levels for the mycotoxin in feed ingredients (https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-and-fda-advisory-levels-deoxynivalenol-don-finished-wheat-products-human). At the target rate of 800 TMU/kg feed, the test article was diluted 10,000-fold in feed.

Note 2: Levels of Zearalenone in test article 013962-L.1 was 2 µg/kg or ppb. The test article was diluted 1:10,000 during feed production, resulting in a 0.2 ppt level of the mycotoxin in the final feed.

Appendix 16c: Product Specifications

Internal Product Specification *FINAL* Effective from Mar 28, 2022

Mannanase CC

PRD-No. 30686426

® = Registered trademark of BASF group ™ = Trademark of BASF group

DAWF-2022-0397 **Revision 3**

DAWF-2022-0397 / 28.03.2022 / rev3:
Löschung der Zusatzprüfung auf Hefe und Schimmelpilze

DAWF-2020-1322 / 26.11.2020 / rev2:
Mikrobiologie gekürzt, Aussehen hinzugefügt

DAWF-2019-01412 / 18.12.2019 / rev1:
shelf life von 12 auf 18 Monate

DAWF-2019-0161 / 28.03.2019 / rev0:
- Erfassung in aspect -

NOT FOR REGULATORY PURPOSES

Test parameter	Requirement	Test method
Extern relevante Prüfungen		
Enzym-Aktivität (Mannanase)	≥ 70000 TMU/g	PM02210QC
Feststoffgehalt	≥ 20.0 g/100g	PM/01110
pH-Wert (telquel)	3.5 to 4.2	Ph.Eur. 2.2.3
Mikrobiologie: Salmonellen in 25 ml	negativ	DIN EN ISO 6579 (Durchführung bei (b) (4)
Mikrobiologie: Escherichia coli in 25 ml	negativ	DIN EN ISO 21528 (Durchführung bei (b) (4)
Interne Prüfungen		
Produktionsstamm(1)	abwesend im Test	00401FF
Mikrobiologie: Gesamtkeimzahl (Aerobe Keime) in 1 ml	≤ 50000	VD LUFA 28.1.2 (Durchführung bei (b) (4)
Mikrobiologie: Coliforme in 1 ml	≤ 30	DIN EN ISO 21528 (Druchführung bei (b) (4)
Mikrobiologie: Enterobakterien und andere gramnegative in 1 ml	≤ 30	DIN EN ISO 21528 (Durchführung bei (b) (4) Speyer)
Antimikrobielle Aktivität	abwesend im Test	VD LUFA 28.4.1 (Durchführung bei (b) (4)
Aflatoxin B1	< Nachweisgrenze (0.5 µg/kg)	LC-MS/MS LUFA SP 1025 (Durchführung bei (b) (4)

Internal Product Specification *FINAL* Effective from Mar 28, 2022

Mannanase CC

PRD-No. 30686426

® = Registered trademark of BASF group ™ = Trademark of BASF group

DAWF-2022-0397 **Revision 3**

Test parameter	Requirement	Test method
Aussehen	leicht bräunliche bis dunkelbraune, leicht trübe bis klare Flüssigkeit, Partikel: besser gleich Standard	visuell / PM/01055
Cd, Cadmium	≤ 0.5 mg/kg	PM/00797
As, Arsen	≤ 2 mg/kg	PM/00797
Quecksilber	≤ 0.1 mg/kg	PM/00934
Pb, Blei	≤ 5 mg/kg	PM/00797
Cu, Kupfer	≤ 6 mg/kg	PM/00797
Zink	≤ 30 mg/kg	PM/00797
Fluor (Schöniger)	≤ 150 mg/kg	PM/01929
Zusätzliche Prüfungen		
Info für Prüfprogramm		
shelf life	18 Monate	

(1) Wert vom Betrieb übernehmen

The product meets the requirements of this specification.

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Internal Product Specification *FINAL* Effective from Nov 13, 2019

Natupulse® TS L

PRD-No. 30716461

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DAWF-2019-01205 **Revision 1**

DAWF-2019-1205 / 13.11.2019 / rev1:
shelf life von 12 auf 18 Monate

DAWF-2019-0167 / 16.05.2019 / rev0:
- Erfassung in aspect

NOT FOR REGULATORY PURPOSES

Test parameter	Requirement	Test method
Extern relevante Prüfungen		
Enzym-Aktivität (Mannanase)(1)	≥ (b) (4) TMU/g	PM02210QC
pH-Wert (telquel)(1)	4.0 to 4.4	Ph.Eur. 2.2.3
Mikrobiologie: Salmonellen in 25 ml(1)	negativ	DIN EN ISO 6579 (Durchführung bei (b) (4)
Mikrobiologie: Escherichia coli in 25 ml(1)	negativ	DIN EN ISO 21528 (Durchführung bei (b) (4)
Interne Prüfungen		
Enzym-Aktivität (Mannanase)	≥ 8800 TMU/g	PM02210QC
pH-Wert (telquel)	4.0 to 4.4	Ph. Eur. 2.2.3
Gesamt-Keimzahl (Aerobe Keime) in 1 ml	≤ 50000	VD LUFA 28.1.2 (Durchführung bei (b) (4)
Mikrobiologie: Coliforme in 1 ml	≤ 30	DIN EN ISO 21528 (Durchführung bei (b) (4)
Klarheit	klar bis leicht trüb	visuell
Fremdpartikel (visuell)	keine	PM/01055
Fluor (Schöninger)	≤ 150 mg/kg	PM/01929
Info nur für Prüfprogramm		
shelf life	18 Monate	

(1) im coa "conforms" angeben anstatt genauem Wert

The product meets the requirements of this specification.

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Internal Product Specification *FINAL* Effective from Nov 13, 2019

Natupulse® TS L

PRD-No. 30716461

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DAWF-2019-01205 **Revision 1**

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Internal Product Specification *FINAL* Effective from Nov 13, 2019

Natupulse® TS

PRD-No. 30712455

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DAWF-2019-01205 **Revision 1**

DAWF-2019-1205 / rev1 / 13.11.2019
shelf life von 12 auf 18 Monate

DAWF-2019-0165 / 30.04.2019 / rev0:
- Erfassung in aspect

NOT FOR REGULATORY PURPOSES

Test parameter	Requirement	Test method
Extern relevante Prüfungen		
Enzym Aktivität (Mannanase)(1)	≥ TMU/g	PM02210QC
Trocknungsverlust(1)	≤ 10.0 g/100g	PM/01044
Interne Prüfungen		
Enzym Aktivität (Mannanase)	≥ 9200 TMU/g	PM02210QC
Trocknungsverlust	≤ 10.0 g/100g	PM/01044
Aussehen (visuell)	beiges bis bräunliches Pulver	PM/01055
Probenahme-Protokoll von (b) (4)	muss vorliegen	
Info nur für Prüfprogramm		
shelf life	18 Monate	

(1) im coa "conforms" angeben anstatt genauem Wert

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Appendix 16d: Product Results

Quality Assurance – Release

Inspection Report

Job number (b) (4) **(confidential)**

BASF
We create chemistry

Mr. (b) (6)

G-ENB/OP (b) (4)

2019-09-13

Job coordinator
Mr. (b) (6)
(b) (6)/OQC – J550
Tel. 78204

Product short name:	**Mannanase CC**
Product name:	**Mannanase CC**
PBG no.:	**11068347**
Article Nr:	**50583313**
Batch:	
Batch indicator:	(b) (4)
Inspection lot no.:	**300020439005, 1 of 1**
Manufacturer/Supplier:	(b) (4)
Findings:	**Conforms to inspection program specification: 11068347 - 0.1 (general specification)**
Decision:	**Released, until: 2020-08-17 (released for: 12 months)**
Remark:	**Statement on 2019-09-13 16:34:37 by Mr** (b) (6)
Owner:	Dr. (b) (6), G-ENB/OP (b) (4) cost center 123670
Data:	Date of manufacture 2019-08-18; registration 2019-08-20; Disposition 2019-08-20; Findings 2019-09-03; Decision 2019-09-13; Finalization 2019-09-13
Appraisal costs:	Analysis order costs: EUR (b) (4)
Administrative:	Registered 2019-08-20 11:10 Uhr
	SAP date of expiration: 2020-08-17 SAP shelf life (in days): 365 (will be applied).

Decision issued 2019-09-13 16:34:37 by (b) (6)

Product release issued 2019-09-13 16:34:37 by (b) (6)
SAP usage decision: F

Externally Relevant Inspections:

Inspection	Method	Result	Unit	Requirement	OK
Dry Matter	PM/01110	(b) (4)		Min. 20.0	Yes

Externally Relevant Inspections:

Inspection	Method	Result	Unit	Requirement	OK
Microbiology: Salmonella	DIN EN ISO 6579 (conducted at the agricultural research and testing institute (b) (4))	(b) (4)		Negative / 25 ml	Yes
Microbiology: Escherichia coli	DIN EN ISO 21528 (conducted at (b) (4))			Negative / 25 ml	Yes
pH value (telquel)	Ph.Eur. 9th Ed., 2.2.3			Min. 3.5 Max. 4.2	Yes
Mannanase activity	PM02210QC			Min. 70,000	Yes

Internal Inspections:

Inspection	Method	Result	Unit	Specification	OK
Production strain	Apply value from plant (00401FF)	(b) (4)		Absent in test	Yes
Sterigmatocystin	JJW2Z-1 LC-MS/MS Performed at (b) (4) WEJ Contaminants			<LOD (10 µg/kg)	Yes
Antimicrobial activity	VDLUFA 28.4.1, conducted at (b) (4))			Absent in test	Yes
Microbiology: Total bacteria count (aerobic bacteria)	VDLUFA 28.1.2 (conducted at (b) (4))			Max. 5 x 10^4/ml	Yes
Microbiology: Enterobacteriaceae & other gram-negative bacteria	DIN EN ISO 21528 (conducted at (b) (4))			Max. 30	Yes
Microbiology: Coliforms	DIN EN ISO 21528 (conducted at (b) (4))			Max. 30	Yes
Ochratoxin A	LC-MS/MS (b) (4) 1026 (conducted at (b) (4))			< LOD (5 µg/kg)	Yes
T2-Toxin	VD LUFA III 16.13.1, (conducted at (b) (4))			< LOD (5 µg/kg)	Yes
Zeazeralenone	VD LUFA III 16.13.1, (conducted at (b) (4))			< LOD (1 µg/kg)	Yes
Aflatoxin B_1	Liquid chromatography-mass spectrometry (LC-MS) / mass spectrometry (MS) (b) (4) 1025 Conducted at (b) (4))			< LOD (0.5 µg/kg)	Yes
Zn, zinc	M=(PM/00797)			Max. 30	Yes
Pb, lead	M=(PM/00797)			Max. 5	Yes
As, arsenic	M=(PM/00797)			Max. 2	Yes
Cd, cadmium	M=(PM/00797)			Max. 0.5	Yes
Cu, copper	M=(PM/00797)			Max. 6	Yes

Hg, mercury	PM/00934	(b) (4)	Max. 0.1	Yes
F (Schöniger oxidation), fluorine	PM/01929		Max. 150	Yes

Additional Inspections:

Inspection	Method	Result	Unit	Reference	OK
Microbio: yeasts and moulds	VD LUFA 28.1.2, (conducted at (b) (4))	(b) (4)			
Sample draw date (allocation sample drawing, organization and release processing)	Enter under Comment: By plant/ By (b) (6) Unknown				
	Comment: By plant				

Distribution list:

(b) (6)

(b) (4), (b) (6)

Inspection Report

Job number (b) (4) **(confidential)**

BASF
We create chemistry

Mr. Dr. (b) (6)

G-ENB/OP (b) (4)

2019-09-13

Job coordinator
Mr. (b) (6)
(b) (4) – J550
Tel. 78204

Product short name:	**Mannanase CC**
Product name:	**Mannanase CC**
PBG no.:	(b) (4)
Article Nr:	
Batch:	
Batch indicator:	(b) (4)
Inspection lot no.:	(b) (4) **1 of 1**
Manufacturer/Supplier:	(b) (4)
Findings:	**Conforms to inspection program specification: 11068347 - 0.1 (general specification)**
Decision:	**Released, until: 2020-08-15 (released for: 12 months)**
Remark:	**Release on 2019-09-13 16:37:03 by Mr** (b) (6)
Owner:	Dr. (b) (6), G-ENB/OP (b) (4) cost center 123670
Data:	Date of manufacture 2019-08-16; registration 2019-08-20; Disposition 2019-08-20; Findings 2019-09-03; Decision 2019-09-13; Finalization 2019-09-13
Appraisal costs:	Analysis order costs: EUR (b) (4)
Administrative:	Registered 2019-08-20 11:11 Uhr
	SAP date of expiration: 2020-08-15 SAP shelf life (in days): 365 (will be applied).

Decision issued 2019-09-13 16:37:03 by (b) (6)

Product release issued 2019-09-13 16:37:03 by (b) (6)
SAP usage decision: F

Externally Relevant Inspections:

Inspection	Method	Result	Unit	Requirement	OK
Dry Matter	PM/01110	(b) (4)		Min. 20.0	Yes

Externally Relevant Inspections:

Inspection	Method	Result	Unit	Requirement	OK
Microbiology: Salmonella	DIN EN ISO 6579 (conducted at the agricultural research and testing institute (b) (4))	(b) (4)		Negative / 25 ml	Yes
Microbiology: Escherichia coli	DIN EN ISO 21528 (conducted at (b) (4))			Negative / 25 ml	Yes
pH value (telquel)	Ph.Eur. 9th Ed., 2.2.3			Min. 3.5 Max. 4.2	Yes
Mannanase activity	PM02210QC			Min. 70,000	Yes

Internal Inspections:

Inspection	Method	Result	Unit	Specification	OK
Production strain	Apply value from plant (00401FF)	(b) (4)		Absent in test	Yes
Sterigmatocystin	JJW2Z-1 LC-MS/MS Performed at (b) (4) WEJ Contaminants			<LOD	Yes
Antimicrobial activity	VDLUFA 28.4.1, conducted at (b) (4))			Absent in test	Yes
Microbiology: Total bacteria count (aerobic bacteria)	VDLUFA 28.1.2 (conducted at (b) (4))			Max. 5 x 10^4/ml	Yes
Microbiology: Enterobacteriaceae & other gram-negative bacteria	DIN EN ISO 21528 (conducted at (b) (4))			Max. 30	Yes
Microbiology: Coliforms	DIN EN ISO 21528 (conducted at (b) (4))			Max. 30	Yes
Ochratoxin A	LC-MS/MS (b) (4) SP 1026 (conducted at (b) (4))			< LOD (5 µg/kg)	Yes
T2-Toxin	VD LUFA III 16.13.1, (conducted at (b) (4)r)			< LOD (5 µg/kg)	Yes
Zeazeralenone	VD LUFA III 16.13.1, (conducted at (b) (4))			< LOD (1 µg/kg)	Yes
Aflatoxin B_1	Liquid chromatography-mas spectrometry (LC-MS) / mass spectrometry (MS) (b) (4) 1025 Conducted at (b) (4)			< LOD (0.5 µg/kg)	Yes
Zn, zinc	M=(PM/00797)			Max. 30	Yes
Pb, lead	M=(PM/00797)			Max. 5	Yes
As, arsenic	M=(PM/00797)			Max. 2	Yes
Cd, cadmium	M=(PM/00797)			Max. 0.5	Yes
Cu, copper	M=(PM/00797)			Max. 6	Yes

Hg, mercury	PM/00934	(b) (4)	Max. 0.1	Yes
F (Schöniger oxidation), fluorine	PM/01929		Max. 150	Yes

Additional Inspections:

Inspection	Method	Reference	OK
Microbio: yeasts and moulds	VD LUFA 28.1.2, (conducted at (b) (4)		
Sample draw date (allocation sample drawing, organization and release processing)	Enter under Comment: By plant/ By (b) (6)/ Unknown		
	Comment: By plant		

Distribution list:

(b) (4)

(b) (4), (b) (6)

Inspection Report

Job number (b) (4) **(confidential)**

BASF
We create chemistry

Mr. (b) (6)

G-ENB/OP (b) (4)

2019-09-13

Job coordinator
Mr. (b) (6)
(b) (6) – J550
Tel. 78204

Product short name:	**Mannanase CC**
Product name:	**Mannanase CC**
PBG no.:	(b) (4)
Article Nr:	
Batch:	
Batch indicator:	(b) (4)
Inspection lot no.:	(b) (4) **1 of 1**
Manufacturer/Supplier:	(b) (4)
Findings:	**Conforms to inspection program specification: 11068347 - 0.1 (general specification)**
Decision:	**Released, until: 2020-08-16 (released for: 12 months)**
Remark:	**Statement on 2019-09-13 16:40:08 by Mr** (b) (6)
Owner:	Dr. (b) (6), G-ENB/OP (b) (4) cost center 123670
Data:	Date of manufacture 2019-08-17; registration 2019-08-20; Disposition 2019-08-20; Findings 2019-09-03; Decision 2019-09-13; Finalization 2019-09-13
Appraisal costs:	Analysis order costs: EUR (b) (4)
Administrative:	Registered 2019-08-20 11:14 Uhr
	SAP date of expiration: 2020-08-15
	SAP shelf life (in days): 365 (will be applied).

Decision issued 2019-09-13 16:40:08 by (b) (6)

Product release issued 2019-09-13 16: 40:08 by (b) (6)
SAP usage decision: F

Externally Relevant Inspections:

Inspection	Method	Result	Unit	Requirement	OK
Dry Matter	PM/01110	(b) (4)		Min. 20.0	Yes

Externally Relevant Inspections:

Inspection	Method	Result	Unit	Requirement	OK
Microbiology: Salmonella	DIN EN ISO 6579 (conducted at the agricultural research and testing institute)	(b) (4)		Negative / 25 ml	Yes
Microbiology: Escherichia coli	DIN EN ISO 21528 (conducted at)			Negative / 25 ml	Yes
pH value (telquel)	Ph.Eur. 9th Ed., 2.2.3			Min. 3.5 Max. 4.2	Yes
Mannanase activity	PM02210QC			Min. 70,000	Yes

Internal Inspections:

Inspection	Method	Result	Unit	Specification	OK
Production strain	Apply value from plant (00401FF)	(b) (4)		Absent in test	Yes
Sterigmatocystin	JJW2Z-1 LC-MS/MS Performed at (b) (4) WEJ Contaminants			<LOD (10 µg/kg)	Yes
Antimicrobial activity	VDLUFA 28.4.1, conducted at (b) (4)			Absent in test	Yes
Microbiology: Total bacteria count (aerobic bacteria)	VDLUFA 28.1.2 (conducted at (b) (4))			Max. 5 x 10^4/ml	Yes
Microbiology: Enterobacteriaceae & other gram-negative bacteria	DIN EN ISO 21528 (conducted at (b) (4)			Max. 30	Yes
Microbiology: Coliforms	DIN EN ISO 21528 (conducted at (b) (4))			Max. 30	Yes
Ochratoxin A	LC-MS/MS (b) (4) 1026 (conducted at (b) (4)			< LOD (5 µg/kg)	Yes
T2-Toxin	VD LUFA III 16.13.1, (conducted at (b) (4))			< LOD (5 µg/kg)	Yes
Zeazeralenone	VD LUFA III 16.13.1, (conducted at (b) (4))			< LOD (1 µg/kg)	Yes
Aflatoxin B_1	Liquid chromatography-mass spectrometry (LC-MS) / mass spectrometry (MS) (b) (4) 1025 Conducted at (b) (4))			< LOD (0.5 µg/kg)	Yes
Zn, zinc	M=(PM/00797)			Max. 30	Yes
Pb, lead	M=(PM/00797)			Max. 5	Yes
As, arsenic	M=(PM/00797)			Max. 2	Yes
Cd, cadmium	M=(PM/00797)			Max. 0.5	Yes
Cu, copper	M=(PM/00797)			Max. 6	Yes

Hg, mercury	PM/00934		ax. 0.1	Yes
F (Schöniger oxidation), fluorine	PM/01929		ax. 150	Yes

(b) (4)

Additional Inspections:

Inspection	Method	eference	OK
Microbio: yeasts and moulds	VD LUFA 28.1.2, (conducted at (b) (4))		
Sample draw date (allocation sample drawing, organization and release processing)	Enter under Comment: By plant/ By (b) (6)/ Unknown		
	Comment: By plant		

Distribution list:

(b) (6)

(b) (4), (b) (6)

Inspection Report

Job number (b) (4) **(confidential)**

BASF
We create chemistry

To quality control J550

(b) (6) – J550

2023-07-19

Job coordinator
Mr. (b) (6)
(b) (6) – J550
Tel. 78204

Product short name:	**Mannanase CC**
Product name:	**Mannanase CC**
PBG no.:	(b) (4)
Batch:	
Batch indicator:	
Inspection lot no.:	(b) (4) **1 of 1**
Manufacturer/Supplier:	(b) (4)
Findings:	**Conforms to inspection program specification: 11068347 - 0.5 (general specification)**
Decision:	**Released, until: 2023-10-17 (released for: 18 months)**
Remark:	**None**
Owner:	Dr. (b) (6), G-ENN/EP (b) (4) cost center 123670
Data:	Date of manufacture 2022-04-20; registration 2022-04-26; materials planning 2022-04-28; findings 2022-05-18; decision 2022-05-18; fully completed 2022-05-18
Appraisal costs:	Analysis order costs: EUR (b) (4)
Administrative:	Registered 2022-04-26 at 17:32. SAP date of expiration: 2023-10-17 SAP shelf life (in days): 545 (will be applied).

Decision issued 2022-05-18, 12:07:34 by Mr. (b) (6)

Product release issued 2022-05-18, 12:07:34 by Mr. (b) (6)
SAP usage decision: F

Externally Relevant Inspections:

Inspection	Method	Result	Unit	Requirement	OK
Solids content	PM01110QC	(b) (4)		Min. 20.0	Yes

Externally Relevant Inspections:

Inspection	Method	Result	Unit	Requirement	OK
Microbiology: salmonella	DIN EN ISO 6579 (conducted at the agricultural research and testing institute)	(b) (4)		Negative / 25 ml	Yes
Microbiology: Escherichia coli	DIN EN ISO 21528 (conducted at)			Negative / 25 ml	Yes
pH value (telquel)	Ph.Eur. 10th Ed., 2.2.3			Min. 3.5 Max. 4.2	Yes
Mannanase activity	PM02210QC			Min. 70,000	Yes

Internal Inspections:

Inspection	Method	Result	Unit	Requirement	OK
Appearance	Visual, PM01055QC	(b) (4)		Slightly brownish to dark-brown, slightly cloudy to clear liquid	Yes
Appearance (particles)	Visual, PM01055QC			Better than or equal to standard	Yes
Production strain	Apply value from plant (00401FF)			Absent in test	Yes
Antimicrobial activity	VDLUFA 28.4.1, conducted at (b) (4))			Absent in test	Yes
Microbiology: Total bacteria count (aerobic bacteria)	VDLUFA 28.1.2 (conducted at (b) (4))			Max. 5 x 10^4/ml	Yes
Microbiology: Enterobacteriaceae & other gram-negative bacteria	DIN EN ISO 21528 (conducted at (b) (4))			Max. 30	Yes
Microbiology: Coliform	DIN EN ISO 21528 (conducted at (b) (4))			Max. 30	Yes
Aflatoxin B_1	Liquid chromatography-mass spectrometry (LC-MS) / mass spectrometry (MS) (b) (4) 1025 Conducted at (b) (4)			< detection limit (0.5 µg/kg)	Yes
Zn, zinc	M=(PM/00797)			Max. 30	Yes
Pb, lead	M=(PM/00797)			Max. 5	Yes
As, arsenic	M=(PM/00797)			Max. 2	Yes
Cd, cadmium	M=(PM/00797)			Max. 0.5	Yes
Cu, copper	M=(PM/00797)			Max. 6	Yes
Hg, mercury	PM/00934			Max. 0.1	Yes
F (Schöniger oxidation), fluorine	PM/01929			Max. 150	Yes
	Note: Li, B, Al, Ca and Ti can result in falsely low assay results.				

Additional Inspections:

Inspection	Method	Result	Unit	Reference	OK
Sample draw date (allocation sample drawing, organization and release processing)	Enter under Comment: By plant/ By (b) (6)/ Unknown	2022-04-20			
	Comment: By plant				

Distribution list:

(b) (6)

(b) (4)

Inspection Report

Job number (b) (4) **(confidential)**

BASF
We create chemistry

To quality control J550

(b) (6) – J550

2023-07-19

Job coordinator
Mr. (b) (6)
(b) (6) – J550
Tel. 78204

Product short name:	**Mannanase CC**
Product name:	**Mannanase CC**
PBG no.:	(b) (4)
Batch:	
Batch indicator:	(b) (4)
Inspection lot no.:	(b) (4) **1 of 1**
Manufacturer/Supplier:	(b) (4)
Findings:	**Conforms to inspection program specification: 11068347 - 0.5 (general specification)**
Decision:	**Released, until: 2023-10-22 (released for: 18 months)**
Remark:	**None**
Owner:	Dr. (b) (6), G-ENN/EP (b) (4) cost center 123670
Data:	Date of manufacture 2022-04-25; registration 2022-04-26; materials planning 2022-04-28; findings 2022-05-18; decision 2022-05-18; fully completed 2022-05-18
Appraisal costs:	Analysis order costs: EUR (b) (4)
Administrative:	Registered 2022-04-26 at 17:32. SAP date of expiration: 2023-10-22 SAP shelf life (in days): 545 (will be applied).

Decision issued 2022-05-18, 12:09:27 by Mr. (b) (6)

Product release issued 2022-05-18, 12:09:27 by Mr. (b) (6)
SAP usage decision: F

Externally Relevant Inspections:

Inspection	Method	Result	Unit	Requirement	OK
Solids content	PM01110QC	(b) (4)		Min. 20.0	Yes

Externally Relevant Inspections:

Inspection	Method	Result	Unit	Requirement	OK
Microbiology: salmonella	DIN EN ISO 6579 (conducted at the agricultura research and testing institute (b) (4))	(b) (4)		Negative / 25 ml	Yes
Microbiology: Escherichia coli	DIN EN ISO 21528 (conducted at (b) (4))			Negative / 25 ml	Yes
pH value (telquel)	Ph.Eur. 10th Ed., 2.2.3			Min. 3.5 Max. 4.2	Yes
Mannanase activity	PM02210QC			Min. 70,000	Yes

Internal Inspections:

Inspection	Method	Result	Unit	Requirement	OK
Appearance	Visual, PM01055QC	(b) (4)		Slightly brownish to dark-brown, slightly cloudy to clear liquid	Yes
Appearance (particles)	Visual, PM01055QC			Better than or equal to standard	Yes
Production strain	Apply value from plant (00401FF)			Absent in test	Yes
Antimicrobial activity	VDLUFA 28.4.1, conducted at (b) (4))			Absent in test	Yes
Microbiology: Total bacteria count (aerobic bacteria)	VDLUFA 28.1.2 (conducted at (b) (4))			Max. 5 x 10^4/ml	Yes
Microbiology: Entero-bacteriaceae & other gram-negative bacteria	DIN EN ISO 21528 (conducted at (b) (4))			Max. 30	Yes
Microbiology: Coliform	DIN EN ISO 21528 (conducted at (b) (4))			Max. 30	Yes
Aflatoxin B_1	Liquid chromatography-mass spectrometry (LC-MS) / mass spectrometry (MS) (b) (4) 1025 Conducted at (b) (4))			< detection limit (0.5 µg/kg)	Yes
Zn, zinc	M=(PM/00797)			Max. 30	Yes
Pb, lead	M=(PM/00797)			Max. 5	Yes
As, arsenic	M=(PM/00797)			Max. 2	Yes
Cd, cadmium	M=(PM/00797)			Max. 0.5	Yes
Cu, copper	M=(PM/00797)			Max. 6	Yes
Hg, mercury	PM/00934			Max. 0.1	Yes
F (Schöniger oxidation), fluorine	PM/01929			Max. 150	Yes
	Note: Li, B, Al, Ca and Ti can result in falsely low assay results.				

Additional Inspections:

Inspection	Method	Result	Unit	Reference	OK
Sample draw date (allocation sample drawing, organization and release processing)	Enter under comment: By plant/ By [redacted] / Unknown	(b) (4)			
	Comment: By plant				

Distribution list:

(b) (6)

(b) (6), (b) (4)

Inspection Report

Job number (b) (4) **(confidential)**

BASF
We create chemistry

Mr. (b) (6)

G-ENB/OP (b) (4)

2020-01-14

Job coordinator
Mr. (b) (6)
(b) (6) – J550
Tel. 78204

Product short name:	**Natupulse TS L**
Product name:	**Natupulse TS L**
PBG no.:	(b) (4)
Article Nr:	
Batch:	
Batch indicator:	(b) (4)
Inspection lot no.:	(b) (4) **1 of 1**
Manufacturer/Supplier:	(b) (4)
Findings:	**Conforms to inspection program specification: 11061154 - 0.2 (general specification)**
Decision:	**Released, until: 2021-06-03 (released for: 18 months)**
Remark:	**Statement on 2020-01-14 12:40:41 by Mr.** (b) (6) **QA Statement:** **Article Number** (b) (4) **was added later since this batch with this article number is set up in SAP.** **Batch data were inspected and are identical to the SAP requirements.** **While this batch was produced in lab scale it is still released by QA.**
Owner:	Dr. (b) (6), G-ENB/OP (b) (4) cost center 123670
Dates:	Date of manufacture 2019-12-06; registration 2019-12-10; Disposition 2019-12-11; Findings 2020-01-13; Decision 2020-01-14; Finalization 2020-01-14
Appraisal costs:	Analysis order costs: EUR (b) (4)
Administrative:	Registered 2019-12-10 15:07
	SAP date of expiration: 2021-06-03 SAP shelf life (in days): 545 (will be applied).
	Changed on 2019-12-11 14:51 by (b) (6)
	Batch # old: (b) (4)
	"
	Date of Manufacture old: "2019-11-29", new: "2019-12-06"

Changed on 2020-01-14 12:35 by (b) (6)

Artikel Nr. alt: "", neu: "50613046" Intern_Flag alt: "P", neu: "A"

Entscheid am 2020-01-14 12:40:41 von Hr. (b) (6)

Produktfreigabe am 2020-01-14 12:40:41 von (b) (6)
SAP Verwendungsentscheid: F

Externally Relevant Inspections:

Inspection	Method	Result	Unit	Requirement	OK
Mannanase activity	PM02210QC	(b) (4)		Must meet (min. 8000 TMU/g	Yes
pH value (telquel)	Ph.Eur. 9th Ed., 2.2.3			Must meet (min. 4.0 max. 4.4)	Yes
Microbiology: Salmonella	DIN EN ISO 6579 (conducted at the agricultura research and testing institute (b) (4)r)			Negative / 25 ml	Yes
Microbiology: Escherichia coli	DIN EN ISO 21528 (conducted at (b) (4)			Negative / 25 ml	Yes

Internal Inspections:

Inspection	Method	Result	Unit	Specification	OK
Clarity	Visual inspection	(b) (4)		Clear to slight turbidity	Yes
Particular matter	Visual inspection, PM/01055			None	Yes
Microbiology: Total bacteria count (aerobic bacteria)	VDLUFA 28.1.2 (conducted at (b) (4))			Max. 5 x 10^4/ml	Yes
Microbiology: Coliforms	DIN EN ISO 21528 (conducted at (b) (4))			Max. 30	Yes
pH value (telquel)	Ph.Eur. 9th Ed., 2.2.3			min. 4.0 max. 4.4	Yes
F (Schöniger), fluorine	PM/01929			Max. 150	Yes
Mannanase activity	PM02210QC			Min. 8800	Yes

Additional Inspections:

Inspection	Method	Result	Unit	Reference	OK
Sample draw date (allocation sample drawing, organization and release processing)	Enter under Comment: By plant/ By (b) (6) Unknown	(b) (4)			
	Comment: By plant				

Distribution list:

(b) (4)

(b) (4)

Inspection Report

Job number (b) (4) **(confidential)**

BASF
We create chemistry

Mr. (b) (6)

G-ENB/OP (b) (4)

2020-02-28

Job coordinator
Mr. (b) (6)
(b) (6) – J550
Tel. 78204

Product short name:	**Natupulse TS L**
Product name:	**Natupulse TS L**
PBG no.:	(b) (4)
Article Nr:	
Batch:	
Batch indicator:	**PBG Batch** (b) (4)
Inspection lot no.:	(b) (4), **1 of 1**
Manufacturer/Supplier:	(b) (4)
Findings:	**Conforms to inspection program specification: 11061154 - 0.3 (general specification)**
Decision:	**Released, until: 2021-07-25 (released for: 18 months)**
Remark:	**Statement on 2020-02-28 08:46:54 by Mr.** (b) (6)
Owner:	Dr. (b) (6), G-ENB/OP (b) (4) cost center 123670
Data:	Date of manufacture 2020-01-27; registration 2020-01-30; Disposition 2020-01-31; Findings 2020-02-27; Decision 2020-02-28; Finalization 2020-02-28
Appraisal costs:	Analysis order costs: EUR (b) (4) EUR
Administrative:	Registered 2020-01-30 16:34
	BASIS Database Date change! New :2021-07-25, on 2020-02-28 08:46:36 by (b) (6).
	Decision issued 2020-02-28 08:46:54 by (b) (6).
	Product release issued 2020-02-28 08:46:54 by (b) (6). SAP usage decision: F

Externally Relevant Inspections:

Inspection	Method	Result	Unit	Requirement	OK
Mannanase activity	PM02210QC	(b) (4)		Must meet (min. 8000 TMU/g	Yes
pH value (telquel)	Ph.Eur. 10th Ed., 2.2.3			Must meet (min. 4.0 max. 4.4)	Yes
Microbiology: Salmonella	DIN EN ISO 6579 (conducted at the agricultura research and testing institute [redacted] (b) (4))			Negative / 25 ml	Yes
Microbiology: Escherichia coli	DIN EN ISO 21528 (conducted at [redacted] (b) (4))			Negative / 25 ml	Yes

Internal Inspections:

Inspection	Method	Specification	OK
Clarity	Visual inspection	Clear to slight turbidity	Yes
Particular matter	Visual inspection, PM/01055	None	Yes
Microbiology: Total bacteria count (aerobic bacteria)	VDLUFA 28.1.2 (conducted at [redacted])	Max. 5 x 10^4/ml	Yes
Microbiology: Coliforms	DIN EN ISO 21528 (conducted at [redacted])	Max. 30	Yes
pH value (telquel)	Ph.Eur. 10th Ed., 2.2.3	min. 4.0 max. 4.4	Yes
F (Schöniger), fluorine	PM/01929	Max. 150	Yes
Mannanase activity	PM02210QC	Min. 8800	Yes

Additional Inspections:

Inspection	Method	Result	Unit	Reference	OK
Sample draw date (allocation sample drawing, organization and release processing)	Enter under Comment: By plant/ By (b) (6)/ Unknown	2020-01-30			
	Comment: By plant				

Distribution list:

(b) (6)

(b) (6), (b) (4)

Quality Assurance – Release

Inspection Report

Job number (b) (4) **(confidential)**

BASF
We create chemistry

Dr. (b) (6)

G-ENN/EP (b) (4)

2023-06-01

Job coordinator
Mr. (b) (6)
(b) (6) – J550
Tel. 78204

Product short name:	**Natupulse TS L**
Product name:	**Natupulse TS L**
PBG no.:	(b) (4)
Batch:	
Batch indicator:	(b) (4)
Inspection lot no.:	(b) (4) **1 of 1**
Manufacturer/Supplier:	(b) (4)
Findings:	**Conforms to inspection program specification: 11061154 - 0.7 (general specification)**
Decision:	**Released, until: 2025-04-17 (released for: 24 months)**
Remark:	**None**
Owner:	(b) (6), G-ENN/EP (b) (4) cost center 123670
Data:	Date of manufacture 2023-04-18; registration 2023-04-19; materials planning 2023-04-21; findings 2023-05-26; decision 2023-06-01; fully completed 2023-06-01
Appraisal costs:	Analysis order costs: EUR (b) (4)
Administrative:	Registered 2023-04-19 at 14:00. SAP date of expiration: 2025-04-17 SAP shelf life (in days): 730 (will be applied).
	Decision issued 2023-06-01, 17:32:37 by Mr. (b) (6)
	Product release issued 2023-06-01, 17:32:37 by Mr. (b) (6) SAP usage decision: F

Externally Relevant Inspections:

Inspection	Method	Result	Unit	Requirement	OK
Mannanase activity (on CoA: min. 8,000 TMU/g)	PM02210QC	(b) (4)		Min. 8,000	Yes

Externally Relevant Inspections:

Inspection	Method	Result	Unit	Requirement	OK
pH value (telquel)	Ph.Eur. 11th Ed., 2.2.3	(b) (4)		Must conform (min. 4.0; max. 4.4)	Yes
Microbiology: salmonella	Enter “conforms” (random sampling inspection)			Must conform (negative / 25 ml)	Yes
Microbiology: Escherichia coli	Enter “conforms” (random sampling inspection)			Must conform (negative / 25 ml)	Yes

Internal Inspections:

Inspection	Method	Result	Unit	Requirement	OK
Clarity	Visual, PM01055QC	(b) (4)		Clear to slightly cloudy	Yes
Foreign particles	Visual, PM01055QC			Better or equal to standard	Yes
Appearance	Visual, PM01055QC			Slight brownish to brown liquid	Yes
pH value (telquel)	Ph.Eur. 11th Ed., 2.2.3			Min. 4.0 Max. 4.4	Yes
Mannanase activity	PM02210QC			Min. 8800	Yes

Additional Inspections:

Inspection	Method	Result	Unit	Reference	OK
Sample draw date (allocation sample drawing, organization and release processing)	Enter under Comment: By plant/ By (b) (6) Unknown	(b) (4)			
	Comment: By plant				
Microbiology: salmonella	DIN EN ISO 6579 (conducted at the agricultural research and testing institute (b) (4))			Negative / 25 ml	

Additional Inspections:

Inspection	Method	Result	Unit	Reference	OK
Microbiology: Escherichia coli	DIN EN ISO 21528 (conducted at (b) (4))	(b) (4)		Negative / 25 ml	
Microbiology: Total bacteria count (aerobic bacteria)	VDLUFA 28.1.2 (conducted at (b) (4))			Max. 5 x 10^4/ml	
Microbiology: Coliform	DIN EN ISO 21528 (conducted at (b) (4))			Max. 30	

Distribution list:

(b) (6)

(b) (6), (b) (4)

Quality Assurance – Release

Inspection Report

Job number (b) (4) **(confidential)**

BASF
We create chemistry

Mr. (b) (6)

G-ENB/OP (b) (4)

2020-01-14

Job coordinator
Mr. (b) (6)
(b) (6) – J550
Tel. 78204

Product short name:	**Natupulse TS L**
Product name:	**Natupulse TS L**
PBG no.: Article Nr: Batch: Batch indicator:	(b) (4)
Inspection lot no.: Manufacturer/Supplier:	(b) (4) **1 of 1** (b) (4)
Findings:	**Conforms to inspection program specification: 11061154 - 0.3 (general specification)**
Decision:	**Released, until: 2021-07-25 (released for: 18 months)**
Remark:	**Statement on 2020-02-28 08:48:14 by Mr.** (b) (6)
Owner:	(b) (4), (b) (6), G-ENB/OP (b) (4) cost center 123670
Data:	Date of manufacture 2020-01-27; registration 2020-01-30; Disposition 2020-01-31; Findings 2020-02-27; Decision 2020-02-28; Finalization 2020-02-28
Appraisal costs:	Analysis order costs: EUR (b) (4) EUR
Administrative:	Registered 2020-01-30 16:34 SAP date of expiration: 2020-08-15 SAP shelf life (in days): 365 (will be applied).

BASIS Database Date change! New :2021-07-25, am 2020-02-28 08:47:47 by Mr. (b) (6).

Decision issued 2020-02-28 08:48:14 by Mr. (b) (6).

Product release issued 2020-02-28 08:48:14 by Mr. (b) (6).
SAP usage decision: F

Externally Relevant Inspections:

Inspection	Method	Result	Unit	Requirement	OK
Mannanase activity	PM02210QC	(b) (4)		Must meet (min. 8000 TMU/g	Yes
pH value (telquel)	Ph.Eur. 9th Ed., 2.2.3			Must meet (min. 4.0 max. 4.4)	Yes
Microbiology: Salmonella	DIN EN ISO 6579 (conducted at the agricultural research and testing institute [redacted])			Negative / 25 ml	Yes
Microbiology: Escherichia coli	DIN EN ISO 21528 (conducted at [redacted])			Negative / 25 ml	Yes

Internal Inspections:

Inspection	Method	Result	Unit	Specification	OK
Clarity	Visual inspection	(b) (4)		Clear to slight turbidity	Yes
Particular matter	Visual inspection, PM/01055			None	Yes
Microbiology: Total bacteria count (aerobic bacteria)	VDLUFA 28.1.2 (conducted at [redacted])			Max. 5 x 10^4/ml	Yes
Microbiology: Coliforms	DIN EN ISO 21528 (conducted at [redacted])			Max. 30	Yes
pH value (telquel)	Ph.Eur. 10th Ed., 2.2.3			min. 4.0 max. 4.4	Yes
F (Schöniger), fluorine	PM/01929			Max. 150	Yes
Mannanase activity	PM02210QC			Min. 8800	Yes

Additional Inspections:

Inspection	Method	Result	Unit	Reference	OK
Sample draw date (allocation sample drawing, organization and release processing)	Enter under Comment: By plant/ By (b) (6)/ Unknown	2019-08-19			
	Comment: By plant				

Distribution list:

(b) (6)

(b) (4), (b) (6)

(b) (4), (b) (6)

BASF SE
Chemiestraße 22
68623 Lampertheim

Date 15.12.2020
Customer no. 10082806

REPORT (b) (4)

The slash after the order and/or analysis number corresponds to the current version of the test report. This version replaces all previous versions of this test report. All former versions of this report should be destroyed.

Order	(b) (4)
Sample no.	
Invoice recipient	**10055545 BASF SE G-ENT/OQQ - F31**
Project	**4428 Halbjahresmonitoring**
Sample acceptance	**18.11.2020**
Date of sampling	**no information**
Customer sample description	**Natupulse TS L** **place of sampling:** (b) (6)
Packaging	**glassbottle**
Batch/Charge/Lot	(b) (4)
Item number	(b) (4)

	Unit	Result	Value in DM	result in 88% DM		Method
Nutrition values/ingredients						
Moisture/ Water content	%	(b) (4)				REG(EC) 152/2009, III, A : 2009-02
Mycotoxins						
Aflatoxine B1	µg/kg					QMP_504_KI_52_1 51 : 2020-11 (LC-MSMS)
Deoxynivalenol (DON)	µg/kg					QMP_504_KI_52_1 51 : 2020-11 (LC-MSMS)
Zearalenone (ZEA)	µg/kg					QMP_504_KI_52_1 51 : 2020-11 (LC-MSMS)
Ochratoxine A	µg/kg					QMP_504_KI_52_1 51 : 2020-11 (LC-MSMS)
Fumonisine B1	µg/kg					QMP_504_KI_52_1 38 : 2020-12 (LC-MSMS)
Fumonisine B2	µg/kg					QMP_504_KI_52_1 38 : 2020-12 (LC-MSMS)
Sum of Fumonisine B1/B2	µg/kg					calculated

x) The sum calculation is done without taking into account single values below limit of detection or limit of quantification.
pa) The detection and quantification limit had been increased because for this analysis matrix a smaller sample volume had to be used.
Explanation: The symbol "<" or n.d. in the result column means, the substance concerned is not quantifiable at the limit of quantification shown opposite.
Parameter-specific measurement uncertainties and information regarding the method of calculation will be provided upon request if the reported results are above the parameter-specific limit of quantification.

Explanation: OM = on original matter; DM = on dry matter base

es reported in this document are accredited according to DIN EN ISO/IEC 17025:2018. Only not accredited activities are identified by the symbol " *) ".

(b) (6), (b) (4)

(b) (4)

Date 15.12.2020
Customer no. 10082806

REPORT (b) (4)

Start of testing: 18.11.2020
End of testing: 15.12.2020 (extension after add. ordering and/or plausibility check)

The results are related only to the samples tested. In cases where the laboratory has not been responsible for sampling, the reported results apply to the samples as received. Duplication of this document or of parts of it requires the authorization from laboratory. In accordance our agreement in writing in the order confirmation, the results in this test report are in a simplified form in the context of DIN EN ISO/IEC 17025:2018, paragraph 7.8.1.3.

(b) (6), (b) (4)

ities reported in this document are accredited according to DIN EN ISO/IEC 17025:2018. Only not accredited activities are identified by the symbol " *) ".

BASF SE
Chemiestraße 22
68623 Lampertheim

Date 15.12.2020
Customer no. 10082806

REPORT (b) (4)

The slash after the order and/or analysis number corresponds to the current version of the test report. This version replaces all previous versions of this test report. All former versions of this report should be destroyed

Order	(b) (4)
Sample no.	
Invoice recipient	**10055545 BASF SE G-ENT/OQQ - F31**
Project	**4428 Halbjahresmonitoring**
Sample acceptance	**18.11.2020**
Date of sampling	**no information**
Customer sample description	**Natupulse TS L** **place of sampling:** (b) (6)
Packaging	**glassbottle**
Batch/Charge/Lot	(b) (4)
Item number	(b) (4)

Microbiological examinations

	Unit	Result	Value in DM	result in 88% DM			Method
Escherichia coli	cfu/g	(b) (4)					DIN ISO 16649-2 : 2009-12
Salmonella spp. in 25g							ISO 6579-1 : 2017-02

The sign "<"...."(LOD)" or n.d. in column result means, the substance concerned cannot be detected within the limit of detection. Parameter-specific measurement uncertainties and information regarding the method of calculation will be provided upon request if the reported results are above the parameter-specific limit of quantification.

Explanation: OM = on original matter; DM = on dry matter base

Remark to Escherichia coli:
A resuscitation step is done to detect stressed bacteria.
Remark to Salmonella spp.:
In case of positive Salmonella results a confirmation of Salmonella spp. was conducted by MALDI-TOF (database BDAL/7311 MSPS).

Start of testing: 18.11.2020
End of testing: 27.11.2020

The results are related only to the samples tested. In cases where the laboratory has not been responsible for sampling, the reported results apply to the samples as received. Duplication of this document or of parts of it requires the authorization from laboratory. In accordance our agreement in writing in the order confirmation, the results in this test report are in a simplified form in the context of DIN EN ISO/IEC 17025:2018, paragraph 7.8.1.3.

The activities reported in this document are accredited according to DIN EN ISO/IEC 17025:2018. Only not accredited activities are identified by the symbol " *) ".

(b) (4), (b) (6)

Date 15.12.2020
Customer no. 10082806

REPORT (b) (4)

(b) (6), (b) (4)

he activities reported in this document are accredited according to DIN EN ISO/IEC 17025:2018. Only not accredited activities are identifie

(b) (4), (b) (6)

(b) (6), (b) (4)

BASF SE
Chemiestraße 22
68623 Lampertheim

Date 15.12.2020
Customer no. 10082806

REPORT (b) (4)

The slash after the order and/or analysis number corresponds to the current version of the test report. This version replaces all previous versions of this test report. All former versions of this report should be destroyed.

Order	(b) (4)
Sample no.	
Invoice recipient	**10055545 BASF SE G-ENT/OQQ - F31**
Project	**4428 Halbjahresmonitoring**
Sample acceptance	**18.11.2020**
Date of sampling	**no information**
Customer sample description	**Natupulse TS L** **place of sampling:**
Packaging	**glassbottle**
Batch/Charge/Lot	
Item number	

	Unit	Result	Value in DM	result in 88% DM		Method
Nutrition values/ingredients						
Moisture/ Water content	%					REG(EC) 152/2009, III, A : 2009-02
Trace elements / Heavy metals / Halogenides						
Cadmium (Cd)	mg/kg					DIN EN 17053 : 2018-03
Lead (Pb)	mg/kg					DIN EN 17053 : 2018-03
Mercury (Hg)	mg/kg					DIN EN 16277 : 2012-09 (mod.)
Arsenic (As)	mg/kg					DIN EN 17053 : 2018-03

(b) (4)

Explanation: The symbol "<" or n.d. in the result column means, the substance concerned is not quantifiable at the limit of quantification shown opposite.
Parameter-specific measurement uncertainties and information regarding the method of calculation will be provided upon request if the reported results are above the parameter-specific limit of quantification.

Explanation: OM = on original matter; DM = on dry matter base

Start of testing: 18.11.2020
End of testing: 15.12.2020 (extension after add. ordering and/or plausibility check)

The results are related only to the samples tested. In cases where the laboratory has not been responsible for sampling, the reported results apply to the samples as received. Duplication of this document or of parts of it requires the authorization from laboratory. In accordance our agreement in writing in the order confirmation, the results in this test report are in a simplified form in the context of DIN EN ISO/IEC 17025:2018, paragraph 7.8.1.3.

ivities reported in this document are accredited according to DIN EN ISO/IEC 17025:2018. Only not accredited activities are identified by the symbol " *) ".

(b) (6), (b) (5)

(b) (6), (b) (4)

Date 15.12.2020
Customer no. 10082806

REPORT (b) (4)

(b) (6), (b) (4)

s reported in this document are accredited according to DIN EN ISO/IEC 17025:2018. Only not accredited activities are identifi

(b) (6), (b) (4)

(b) (6), (b) (4)

BASF SE
Chemiestraße 22
68623 Lampertheim

Date 18.06.2021
Customer no. 10082806

REPORT (b) (4)

Order	
Sample no.	
Invoice recipient	**10055545 BASF SE G-ENT/OQQ - F31**
Project	**4428 Halbjahresmonitoring**
Sample acceptance	**17.05.2021**
Date of sampling	**no information**
Customer sample description	**Natupulse TS L**
	place of sampling: (b) (6)
Packaging	**glassbottle**
Batch/Charge/Lot	(b) (4)
Item number	(b) (4)

	Unit	Result	Value in DM	result in 88% DM	Method
Nutrition values/ingredients					
Moisture/ Water content	%				REG(EC) 152/2009, III, A : 2009-02
Mycotoxins					
Aflatoxine B1	µg/kg				QMP_504_KI_52_1 51 : 2020-11 (LC-MSMS)
Deoxynivalenol (DON)	µg/kg				QMP_504_KI_52_1 51 : 2020-11 (LC-MSMS)
Zearalenone (ZEA)	µg/kg				QMP_504_KI_52_1 51 : 2020-11 (LC-MSMS)
Ochratoxine A	µg/kg				QMP_504_KI_52_1 51 : 2020-11 (LC-MSMS)
Fumonisine B1	µg/kg				QMP_504_KI_52_1 38 : 2020-12 (LC-MSMS)
Fumonisine B2	µg/kg				QMP_504_KI_52_1 38 : 2020-12 (LC-MSMS)
Sum of Fumonisine B1/B2	µg/kg				calculated

(b) (4)

x) The sum calculation is done without taking into account single values below limit of detection or limit of quantification.
pa) The detection and quantification limit had been increased because for this analysis matrix a smaller sample volume had to be used.
Explanation: The symbol "<" or n.d. in the result column means, the substance concerned is not quantifiable at the limit of quantification shown opposite.
Parameter-specific analytical measurement uncertainties and information regarding the method of calculation will be provided upon request if if the reported results are above the parameter-specific limit of quantification.

Explanation: OM = on original matter; DM = on dry matter base

ctivities reported in this document are accredited according to DIN EN ISO/IEC 17025:2018. Only not accredited activities are identified by the symbol " *) ".

(b) (6), (b) (4)

Date 18.06.2021
Customer no. 10082806

REPORT (b) (4)

Start of testing: 17.05.2021
End of testing: 18.06.2021 (extension after add. ordering and/or plausibility check)

The results are related only to the samples tested. In cases where the laboratory has not been responsible for sampling, the reported results apply to the samples as received. Duplication of this document or of parts of it requires the authorization from laboratory. In accordance our agreement in writing in the order confirmation, the results in this test report are in a simplified form in the context of DIN EN ISO/IEC 17025:2018, paragraph 7.8.1.3.

(b) (6), (b) (4)

vities reported in this document are accredited according to DIN EN ISO/IEC 17025:2018. Only not accredited activities are identified by the symbol " *) ".

BASF SE
Chemiestraße 22
68623 Lampertheim

Date 18.06.2021
Customer no. 10082806

REPORT (b) (4)

Order
Sample no.
Invoice recipient **10055545 BASF SE G-ENT/OQQ - F31**
Project **4428 Halbjahresmonitoring**
Sample acceptance **17.05.2021**
Date of sampling **no information**
Customer sample description **Natupulse TS L**
place of sampling: (b) (6)
Packaging **glassbottle**
Batch/Charge/Lot (b) (4)
Item number (b) (4)

Microbiological examinations

	Unit	Result	Value in DM	result in 88% DM			Method
Escherichia coli	cfu/g	(b) (4)					DIN ISO 16649-2 : 2009-12
Salmonella spp. in 25g							ISO 6579-1 : 2017-02

The sign "<"...."(LOD)" or n.d. in column result means, the substance concerned cannot be detected within the limit of detection. Parameter-specific analytical measurement uncertainties and information regarding the method of calculation will be provided upon request if if the reported results are above the parameter-specific limit of quantification.

Explanation: OM = on original matter; DM = on dry matter base

Remark to Escherichia coli:
A resuscitation step is done to detect stressed bacteria.

Remark to Salmonella spp.:
In case of positive Salmonella results a confirmation of Salmonella spp. was conducted by MALDI-TOF (database BDAL/7311 MSPS).

Start of testing: 17.05.2021
End of testing: 28.05.2021

The results are related only to the samples tested. In cases where the laboratory has not been responsible for sampling, the reported results apply to the samples as received. Duplication of this document or of parts of it requires the authorization from laboratory. In accordance our agreement in writing in the order confirmation, the results in this test report are in a simplified form in the context of DIN EN ISO/IEC 17025:2018, paragraph 7.8.1.3.

activities reported in this document are accredited according to DIN EN ISO/IEC 17025:2018. Only not accredited activities are identified by the symbol " *) ".

(b) (6), (b) (4)

Date 18.06.2021
Customer no. 10082806

(b) (6), (b) (4)

tivities reported in this document are accredited according to DIN EN ISO/IEC 17025:2018. Only not accredited activities are identified

(b) (6), (b) (4)

(b) (6), (b) (4)

BASF SE
Chemiestraße 22
68623 Lampertheim

The activities reported in this document are accredited according to DIN EN ISO/IEC 17025:2018. Only not accredited activities are identified by the symbol " *) ".

Date 18.06.2021
Customer no. 10082806

REPORT 2898798 - 204670

Order	**2898798 BASF GO-20211061**
Sample no.	**204670**
Invoice recipient	**10055545 BASF SE G-ENT/OQQ - F31**
Project	**4428 Halbjahresmonitoring**
Sample acceptance	**17.05.2021**
Date of sampling	**no information**
Customer sample description	**Natupulse TS L** **place of sampling:** (b) (6)
Packaging	**glassbottle**
Batch/Charge/Lot	(b) (4)
Item number	(b) (4)

	Unit		Result	Value in DM	result in 88% DM		Method
Nutrition values/ingredients							
Moisture/ Water content	%		(b) (4)				REG(EC) 152/2009, III, A : 2009-02
Trace elements / Heavy metals / Halogenides							
Lead (Pb)	mg/kg						DIN EN 17053 : 2018-03
Cadmium (Cd)	mg/kg						DIN EN 17053 : 2018-03
Mercury (Hg)	mg/kg						DIN EN 16277 : 2012-09 (mod.)
Arsenic (As)	mg/kg						DIN EN 17053 : 2018-03

Explanation: The symbol "<" or n.d. in the result column means, the substance concerned is not quantifiable at the limit of quantification shown opposite.
Parameter-specific analytical measurement uncertainties and information regarding the method of calculation will be provided upon request if if the reported results are above the parameter-specific limit of quantification.

Explanation: OM = on original matter; DM = on dry matter base

Start of testing: 17.05.2021
End of testing: 18.06.2021 (extension after add. ordering and/or plausibility check)

The results are related only to the samples tested. In cases where the laboratory has not been responsible for sampling, the reported results apply to the samples as received. Duplication of this document or of parts of it requires the authorization from laboratory. In accordance our agreement in writing in the order confirmation, the results in this test report are in a simplified form in the context of DIN EN ISO/IEC 17025:2018, paragraph 7.8.1.3.

(b) (6), (b) (4)

(b) (6), (b) (4)

Date 18.06.2021
Customer no. 10082806

REPORT 2898798 - 204670

(b) (6), (b) (4)

activities reported in this document are accredited according to DIN EN ISO/IEC 17025:2018. Only not accredited activities are identifie

(b) (6), (b) (4)

BASF SE
G-ENA/MR
Carl-Bosch-Straße 38
67056 Ludwigshafen

Date 28.12.2023
Customer no. 10082806

REPORT

Order (b) (4)
Sample no.
Invoice recipient **10085540 BASF SE G-ENA/NR, F 031**
Project **4428 Halbjahresmonitoring**
Sample acceptance **21.11.2023**
Date of sampling **no information**
Customer sample description **Natupulse TS L**
place of sampling: (b) (6)

Packaging **glassbottle**
Batch/Charge/Lot (b) (4)
Article number (b) (4)

	Unit		Result	Value in DM	result in 88% DM		Method
Nutrition values/ingredients							
Moisture/ Water content	%		(b) (4)				REG(EC) 152/2009, III, A : 2009-01
Mycotoxins							
Aflatoxine B1	µg/kg						MP-00180-DE : 2023-09 (LC-MSMS)
Deoxynivalenol (DON)	µg/kg						MP-00180-DE : 2023-09 (LC-MSMS)
Zearalenone (ZEA)	µg/kg						MP-00180-DE : 2023-09 (LC-MSMS)
Ochratoxine A	µg/kg						MP-00180-DE : 2023-09 (LC-MSMS)
Fumonisine B1	µg/kg						MP-00245-DE : 2022-03 (LC-MSMS)
Fumonisine B2	µg/kg						MP-00245-DE : 2022-03 (LC-MSMS)
Sum of Fumonisine B1/B2	µg/kg						calculated

Explanation: The symbol "<" or n.q. in the result column means, the parameter concerned is not quantifiable at the limit of quantification shown opposite.
Parameter-specific analytical measurement uncertainties and information regarding the method of calculation will be provided upon request if the reported results are above the parameter-specific limit of quantification.

Explanation: OM = on original matter; DM = on dry matter base

The activities reported in this document are accredited according to DIN EN ISO/IEC 17025:2018. Only not accredited activities are identified by the symbol " ") ".

(b) (6), (b) (4)

Date 28.12.2023
Customer no. 10082806

REPORT
Order
Sample no. (b) (4)

Start of testing: 21.11.2023
End of testing: 28.12.2023 \\(extension after add. ordering and/or plausibility check)

The results are related only to the samples tested. In cases where the laboratory has not been responsible for sampling, the reported results apply to the samples as received. The laboratory is not responsible for the information provided by the customer. The customer information, if any, presented in this test report is not subject to the accreditation of the laboratory and may affect the validity of the test results. Duplication of this document or of parts of it requires the authorization from laboratory. In accordance our agreement in writing in the order confirmation, the results in this test report are in a simplified form in the context of DIN EN ISO/IEC 17025:2018, paragraph 7.8.1.3.
In conformity assessment, the economic approach is used as the decision rule (a non-conformity exists if the measurement result is included measurement uncertainty above the specification or standard), as long as nothing else has been determined by corresponding legal or normative bases.

(b) (6), (b) (4)

tivities reported in this document are accredited according to DIN EN ISO/IEC 17025:2018. Only not accredited activities are identified by the symbol " *)."

(b) (6), (b) (4)

BASF SE
G-ENA/MR
Carl-Bosch-Straße 38
67056 Ludwigshafen

Date 28.12.2023
Customer no. 10082806

REPORT

Order (b) (4)
Sample no.
Invoice recipient **10085540 BASF SE G-ENA/NR, F 031**
Project **4428 Halbjahresmonitoring**
Sample acceptance **21.11.2023**
Date of sampling **no information**
Customer sample description **Natupulse TS L**
place of sampling: (b) (6)

Packaging **glassbottle**
Batch/Charge/Lot (b) (4)
Article number (b) (4)

Microbiological examinations

	Unit	Result	Value in DM	result in 88% DM			Method
Escherichia coli	cfu/g	(b) (4)					DIN ISO 16649-2 : 2020-12
Coliform bacteria	cfu/g						ISO 4832 : 2006-02
Salmonella spp. in 25g							ISO 6579-1 : 2017-02

The sign "<"...."(LOD)" or n.d. in column result means, the parameter concerned cannot be detected within the limit of detection. Parameter-specific analytical measurement uncertainties and information regarding the method of calculation will be provided upon request if the reported results are above the parameter-specific limit of quantification.

Explanation: OM = on original matter; DM = on dry matter base

Remark to Escherichia coli:
A resuscitation step is done to detect stressed bacteria. According to the National Footnote, these are presumptively determined ß-glucuronidase-positive Escherichia coli.
Remark to Salmonella spp.:
In case of positive Salmonella results a confirmation of Salmonella spp. was conducted by MALDI-TOF (database BDAL/7311 MSPS).

Start of testing: 21.11.2023
End of testing: 07.12.2023

The results are related only to the samples tested. In cases where the laboratory has not been responsible for sampling, the reported results apply to the samples as received. The laboratory is not responsible for the information provided by the customer. The customer information, if any, presented in this test report is not subject to the accreditation of the laboratory and may affect the validity of the test results. Duplication of this document or of parts of it requires the authorization from laboratory. In accordance our agreement in writing in the order confirmation, the results in this test report are in a simplified form in the context of DIN EN ISO/IEC 17025:2018, paragraph 7.8.1.3.
In conformity assessment, the economic approach is used as the decision rule (a non-conformity exists if the measurement result is included measurement uncertainty above the specification or standard), as long as nothing else has been determined by corresponding legal or normative bases.

The activities reported in this document are accredited according to DIN EN ISO/IEC 17025:2018. Only not accredited activities are identified by the symbol " ") ".

(b) (6), (b) (4)

Date 28.12.2023
Customer no. 10082806

REPORT
Order
Sample no. (b) (4)

(b) (6), (b) (4)

activities reported in this document are accredited according to DIN EN ISO/IEC 17025:2018. Only not accredited activities are identified by the symbol " *) ".

(b) (6), (b) (4)

BASF SE
G-ENA/MR
Carl-Bosch-Straße 38
67056 Ludwigshafen

Date 28.12.2023
Customer no. 10082806

REPORT

Order	(b) (4)
Sample no.	
Invoice recipient	**10085540 BASF SE G-ENA/NR, F 031**
Project	**4428 Halbjahresmonitoring**
Sample acceptance	**21.11.2023**
Date of sampling	**no information**
Customer sample description	**Natupulse TS L** **place of sampling:** (b) (6)
Packaging	**glassbottle**
Batch/Charge/Lot	(b) (4)
Article number	(b) (4)

	Unit	Result	Value in DM	result in 88% DM	Method
Nutrition values/ingredients					
Moisture/ Water content	%	(b) (4)			REG(EC) 152/2009, III, A : 2009-01
Trace elements / Heavy metals / Halogenides					
Lead (Pb)	mg/kg				DIN EN 17053 : 2018-03
Cadmium (Cd)	mg/kg				DIN EN 17053 : 2018-03
Mercury (Hg)	mg/kg				DIN EN 16277 : 2012-09 (mod.)
Arsenic (As)	mg/kg				DIN EN 17053 : 2018-03

he) The limit of quantification or detection could be lowered because a higher weight was used.
Explanation: The symbol "<" or n.q. in the result column means, the parameter concerned is not quantifiable at the limit of quantification shown opposite.
Parameter-specific analytical measurement uncertainties and information regarding the method of calculation will be provided upon request if the reported results are above the parameter-specific limit of quantification.

Explanation: OM = on original matter; DM = on dry matter base

Start of testing: 21.11.2023
End of testing: 11.12.2023

The results are related only to the samples tested. In cases where the laboratory has not been responsible for sampling, the reported results apply to the samples as received. The laboratory is not responsible for the information provided by the customer. The customer information, if any, presented in this test report is not subject to the accreditation of the laboratory and may affect the validity of the test results. Duplication of this document or of parts of it requires the authorization from laboratory. In accordance our agreement in writing in the order confirmation, the results in this test report are in a simplified form in the context of DIN EN ISO/IEC 17025:2018, paragraph 7.8.1.3.
In conformity assessment, the economic approach is used as the decision rule (a non-conformity exists if the measurement result is included measurement uncertainty above the specification or standard), as long as nothing else has been determined by corresponding legal or normative bases.

The activities reported in this document are accredited according to DIN EN ISO/IEC 17025:2018. Only not accredited activities are identified by the symbol " ") ".

(b) (4), (b) (6)

Date 28.12.2023
Customer no. 10082806

REPORT
Order
Sample no. (b) (4)

(b) (4), (b) (6)

activities reported in this document are accredited according to DIN EN ISO/IEC 17025:2018. Only not accredited activities are identified by the symbol " *) ".

(b) (4), (b) (6)

Quality Assurance – Release

Inspection Report

Job number (b) (4) **(confidential)**

BASF
We create chemistry

Dr. med. vet. (b) (6)

G-ENN/ED J660

2023-06-01

Job coordinator
Mr. (b) (6)
(b) (6)– J550
Tel. 78204

Product short name:	**Natupulse TS**
Product name:	**Natupulse TS**
PBG no.:	(b) (4)
Article no.:	
Batch:	
Batch indicator:	**Mixture no.:** (b) (4)
Inspection lot no.:	(b) (4) **1 of 1**
Manufacturer / Supplier:	(b) (4)
SAP supplier:	(b) (6)
Findings:	**Conforms to inspection program specification:** (b) (4) **- 0.5 (general specification)**
Decision:	**Released, until: 2025-03-16 (released for: 24 months)**
Remark:	**None**
Owner:	Dr. med. vet. (b) (6), G-ENN/ED J660, cost center 190850
Data:	Date of manufacture 2023-03-17; registration 2023-03-31; materials planning 2023-04-11; findings 2023-05-26; decision 2023-06-01; fully completed 2023-06-01
Appraisal costs:	Analysis order costs: EUR (b) (4)
Administrative:	Registered 2023-03-31 at 09:32 AM. SAP date of expiration: 2025-03-16 SAP shelf life (in days): 730 (will be applied).

Decision issued 2023-06-01 17:28:34, by Mr. (b) (6)

Product release issued 2023-06-01 17:28:34 by Mr. (b) (6)
Not for customer: 0000900864.
SAP usage decision: F

Externally Relevant Inspections:

Inspection	Method	Result	Unit	Requirement	OK
Mannanase activity (on CoA: min. 8,000 TMU/g)	PM02210QC	(b) (4)		Min. 8,000	Yes
Loss on drying	PM01044QC			Must conform (max. 10.0 g/100 g)	Yes

Internal Inspections:

Inspection	Method	Requirement	OK
Loss on drying (3 h/105°C)	PM01044QC	Max. 10.0	Yes
Sample drawing report from (b) (4)		Must be available	Yes
Appearance	Visual, PM01055QC	Beige to brownish powder	Yes
Mannanase activity	PM02210QC	Min. 10,250	Yes

Additional Inspections:

Inspection	Method	Result	Unit	Reference	OK
Sample draw date (allocation sample drawing, organization and release processing)	Enter under Comment: By plant/ By (b) (6) Unknown	2023-03-20			
	Comment: By plant				

Distribution list:

(b) (6)

(b) (6), (b) (4)

Dr. med. vet. (b) (6)

G-ENN/ED J660

2023-06-01

Job coordinator
Mr. (b) (6)
(b) (6) – J550
Tel. 78204

Page 4 of 5

Product short name:	**Natupulse TS**
Product name:	**Natupulse TS**
PBG no.:	(b) (4)
Article no.:	
Batch:	
Batch indicator:	**Mixture no.:** (b) (4)
Inspection lot no.:	(b) (4) **1 of 1**
Manufacturer / Supplier:	(b) (4)
SAP supplier:	(b) (6)
Findings:	**Conforms to inspection program specification:** (b) (4) **- 625.1** (b) (4), (b) (6)
Decision:	**Released, until: 2025-03-16 (released for: 24 months)**
Remark:	**None**
Owner:	Dr. med. vet. (b) (6), G-ENN/ED J660, cost center 190850
Data:	Date of manufacture 2023-03-17; registration 2023-03-31; materials planning 2023-04-11; findings 2023-05-26; decision 2023-06-01; fully completed 2023-06-01
Appraisal costs:	Analysis order costs: EUR (b) (4)
Administrative:	Registered 2023-03-31 at 09:32 AM.
	SAP date of expiration: 2025-03-16 SAP shelf life (in days): 730 (will be applied).
	Decision issued 2023-06-01 17:28:34 by Mr. (b) (6)
	Product release issued 2023-06-01 17:28:34 by Mr. (b) (6) Not for customer: 0000900864. SAP usage decision: F

Externally Relevant Inspections:

Inspection	Method	Result	Unit	Requirement	OK
Loss on drying (3 h/105°C)	PM01044QC	(b) (4)		Max. 10.0	Yes
Mannanase activity	PM02210QC			Min. 8,000	Yes

Additional Inspections:

Inspection	Method	Result	Unit	Reference	OK
Sample draw date (allocation sample drawing, organization and release processing)	Enter under Comment: By plant/ By (b) (6) Unknown	2023-03-20			
	Comment: By plant				

Distribution list:

(b) (6)

(b) (6), (b) (4)

Inspection Report

Job number (b) (4) **(confidential)**

BASF
We create chemistry

Dr. med. vet. (b) (6)

G-ENN/ED J660

2023-02-21

Job coordinator
Mr. (b) (6)
(b) (6) – J550
Tel. 78204

Product short name:	**Natupulse TS**
Product name:	**Natupulse TS**
PBG no.:	(b) (4)
Article no.:	
Batch:	
Batch indicator:	**Plant batch mixture no.:** (b) (4)
Inspection lot no.:	(b) (4) **1 of 1**
Manufacturer / Supplier:	(b) (4)
SAP supplier:	(b) (4)
Findings:	**Conforms to inspection program specification:** (b) (4) **- 0.5 (general specification)**
Decision:	**Released, until: 2025-02-08 (released for: 24 months)**
Remark:	**None**
Owner:	Dr. med. vet. (b) (6), G-ENN/ED J660, cost center 190850
Data:	Date of manufacture 2023-02-09; registration 2023-02-08; materials planning 2023-02-13; findings 2023-02-20; decision 2023-02-21; fully completed 2023-02-21
Appraisal costs:	Analysis order costs: EUR (b) (4)
Administrative:	Registered 2023-02-08 at 13:49. SAP date of expiration: 2025-02-08 SAP shelf life (in days): 730 (will be applied).

Decision issued 2023-02-21, 14:33:56 by Mr. (b) (6)

Product release issued 2023-02-21, 14:33:56 by Mr. (b) (6)
SAP usage decision: F

Externally Relevant Inspections:

Inspection	Method	Result	Unit	Requirement	OK
Mannanase activity (on CoA: min. 8,000 TMU/g)	PM02210QC	(b) (4)		Min. 8,000	Yes
Loss on drying	PM01044QC			Must conform (max. 10.0 g/100 g)	Yes

Internal Inspections:

Inspection	Method			Requirement	OK
Loss on drying (3 h/105°C)	PM01044QC			Max. 10.0	Yes
Sample drawing report from (b) (4)				Must be available	Yes
Appearance	Visual, PM01055QC			Beige to brownish powder	Yes
Mannanase activity	PM02210QC			Min. 10,250	Yes

Additional Inspections:

Inspection	Method	Result	Unit	Reference	OK
Sample draw date (allocation sample drawing, organization and release processing)	Enter under comment: By plant/ By (b) (6)/ Unknown	2023-02-01			
	Comment: By plant				

Distribution list:

[redacted]

[redacted]

Dr. med. vet. (b) (6)

G-ENN/ED J660

2023-02-21

Job coordinator
Mr. (b) (6)
(b) (6) – J550
Tel. 78204

Page 4 of 5

Product short name:	**Natupulse TS**
Product name:	**Natupulse TS**
PBG no.:	(b) (4)
Article no.:	
Batch:	
Batch indicator:	**Plant batch mixture no.:** (b) (4)
Inspection lot no.:	(b) (4) **1 of 1**
Manufacturer / Supplier:	(b) (4)
SAP supplier:	
Findings:	**Conforms to inspection program specification:** (b) (4) **- 625.1** (b) (4)
Decision:	**Released, until: 2025-02-08 (released for: 24 months)**
Remark:	**None**
Owner:	Dr. med. vet. (b) (6), G-ENN/ED J660, cost center 190850
Data:	Date of manufacture 2023-02-09; registration 2023-02-08; materials planning 2023-02-13; findings 2023-02-20; decision 2023-02-21; fully completed 2023-02-21
Appraisal costs:	Analysis order costs: EUR (b) (4)
Administrative:	Registered 2023-02-08 at 13:49.
	SAP date of expiration: 2025-02-08 SAP shelf life (in days): 730 (will be applied).

Decision issued 2023-02-21,14:33:56 by Mr. (b) (6)

Product release issued 2023-02-21, 14:33:56 by Mr. (b) (6)
SAP usage decision: F

Externally Relevant Inspections:

Inspection	Method	Result	Unit	Requirement	OK
Loss on drying (3 h/105°C)	PM01044QC	(b) (4)		Max. 10.0	Yes
Mannanase activity	PM02210QC			Min. 8,000	Yes

Additional Inspections:

Inspection	Method	Result	Unit	Reference	OK
Sample draw date (allocation sample drawing, organization and release processing)	Enter under Comment: By plant/ By (b) (6)/ Unknown	2023-02-01			
	Comment: By plant				

Distribution list:

(b) (6)

(b) (6), (b) (4)

Quality Assurance – Release

Inspection Report

Job number (b) (4) **(confidential)**

BASF
We create chemistry

Dr. med. vet. (b) (6)

G-ENN/ED J660

2023-06-01

Job coordinator
Mr. (b) (6)
(b) (6) – J550
Tel. 78204

Product short name:	**Natupulse TS**
Product name:	**Natupulse TS**
PBG no.:	(b) (4)
Article no.:	
Batch:	
Batch indicator:	**Mixture no.:** (b) (4)
Inspection lot no.:	(b) (4) **1 of 1**
Manufacturer / Supplier:	(b) (4)
SAP supplier:	(b) (4)
Findings:	**Conforms to inspection program specification:** (b) (4) **- 0.5 (general specification)**
Decision:	**Released, until: 2025-03-22 (released for: 24 months)**
Remark:	**None**
Owner:	Dr. med. vet. (b) (6), G-ENN/ED J660, cost center 190850
Data:	Date of manufacture 2023-03-23; registration 2023-04-06; materials planning 2023-04-26; findings 2023-05-26; decision 2023-06-01; fully completed 2023-06-01
Appraisal costs:	Analysis order costs: EUR (b) (4)
Administrative:	Registered 2023-04-06 at 08:44 AM.
	SAP date of expiration: 2025-03-22 SAP shelf life (in days): 730 (will be applied).

Decision issued 2023-06-01, 17:25:43 by Mr. (b) (6)

Product release issued 2023-06-01, 17:25:43 by Mr. (b) (6)
SAP usage decision: F

Externally Relevant Inspections:

Inspection	Method	Result	Unit	Requirement	OK
Mannanase activity (on CoA: min. 8,000 TMU/g)	PM02210QC	(b) (4)		Min. 8,000	Yes
Loss on drying	PM01044QC			Must conform (max. 10.0 g/100 g)	Yes

Internal Inspections:

Inspection	Method	Requirement	OK
Loss on drying (3 h/105°C)	PM01044QC	Max. 10.0	Yes
Sample drawing report from (b) (4)		Must be available	Yes
Appearance	Visual, PM01055QC	Beige to brownish powder	Yes
Mannanase activity	PM02210QC	Min. 10,250	Yes

Additional Inspections:

Inspection	Method	Result	Unit	Reference	OK
Sample draw date (allocation sample drawing, organization and release processing)	Enter under Comment: By plant/ By (b) (6) Unknown	2023-03-20			
	Comment: By plant				

Distribution list:

[redacted]

[redacted]

Dr. med. vet. (b) (6)

G-ENN/ED J660

2023-06-01

Job coordinator
Mr. (b) (6)
(b) (6) – J550
Tel. 78204

Page 4 of 5

Product short name:	**Natupulse TS**
Product name:	**Natupulse TS**
PBG no.:	(b) (4)
Article no.:	
Batch:	
Batch indicator:	**Mixture no.:** (b) (4)
Inspection lot no.:	(b) (4) **1 of 1**
Manufacturer / Supplier:	(b) (4)
SAP supplier:	(b) (4)
Findings:	**Conforms to inspection program specification:** (b) (4) **- 625.1** (b) (4))
Decision:	**Released, until: 2025-03-22 (released for: 24 months)**
Remark:	**None**
Owner:	Dr. med. vet. (b) (6), G-ENN/ED J660, cost center 190850
Data:	Date of manufacture 2023-03-23; registration 2023-04-06; materials planning 2023-04-26; findings 2023-05-26; decision 2023-06-01; fully completed 2023-06-01
Appraisal costs:	Analysis order costs: EUR (b) (4)
Administrative:	Registered 2023-04-06 at 08:44 AM.
	SAP date of expiration: 2025-03-22 SAP shelf life (in days): 730 (will be applied).

Decision issued 2023-06-01, 17:25:43 by Mr. (b) (6)

Product release issued 2023-06-01, 17:25:43 by Mr. (b) (6)
SAP usage decision: F

Externally Relevant Inspections:

Inspection	Method	Result	Unit	Requirement	OK
Loss on drying (3 h/105°C)	PM01044QC	(b) (4)		Max. 10.0	Yes
Mannanase activity	PM02210QC			Min. 8,000	Yes

Additional Inspections:

Inspection	Method	Result	Unit	Reference	OK
Sample draw date (allocation sample drawing, organization and release processing)	Enter under comment: By plant/ By (b) (6)/ Unknown	2023-03-20			
	Comment: By plant				

Distribution list:

(b) (6)

(b) (4), (b) (6)

BASF SE
G-ENA/MR
Chemiestraße 22
68623 Lampertheim

Date 10.12.2021
Customer no. 10082806

REPORT (b) (4)

Order
Sample no.
Project **4428 Halbjahresmonitoring**
Sample acceptance **09.11.2021**
Date of sampling **09.09.2021**
Customer sample description **Natupulse TS**
place of sampling: (b) (6)
Description of the sample **90882124U0**
Packaging **plasticcontainer**
Item number **50604289**

	Unit	Result	Value in DM	result in 88% DM		Method
Nutrition values/ingredients						
Moisture (vacuum)	%					REG(EC) 152/2009, III, A : 2009-02
Mycotoxins						
Aflatoxine B1	µg/kg					QMP_504_KI_52_1 51 : 2020-11 (LC-MSMS)
Deoxynivalenol (DON)	µg/kg					QMP_504_KI_52_1 51 : 2020-11 (LC-MSMS)
Zearalenone (ZEA)	µg/kg					QMP_504_KI_52_1 51 : 2020-11 (LC-MSMS)
Ochratoxine A	µg/kg					QMP_504_KI_52_1 51 : 2020-11 (LC-MSMS)
Fumonisine B1	µg/kg					QMP_504_KI_52_1 38 : 2020-12 (LC-MSMS)
Fumonisine B2	µg/kg					QMP_504_KI_52_1 38 : 2020-12 (LC-MSMS)
Sum of Fumonisine B1/B2	µg/kg					calculated

(b) (4)

pa) The detection and quantification limit had been increased because for this analysis matrix a smaller sample volume had to be used.
Explanation: The symbol "<" or n.d. in the result column means, the substance concerned is not quantifiable at the limit of quantification shown opposite.
Parameter-specific analytical measurement uncertainties and information regarding the method of calculation will be provided upon request if the reported results are above the parameter-specific limit of quantification.

Explanation: OM = on original matter; DM = on dry matter base

ivities reported in this document are accredited according to DIN EN ISO/IEC 17025:2018. Only not accredited activities are identified by the symbol " *) ".

(b) (6), (b) (4)

(b) (4), (b) (6)

Date 10.12.2021
Customer no. 10082806

REPORT (b) (4)

Start of testing: 09.11.2021
End of testing: 06.12.2021

The results are related only to the samples tested. In cases where the laboratory has not been responsible for sampling, the reported results apply to the samples as received. Duplication of this document or of parts of it requires the authorization from laboratory. In accordance our agreement in writing in the order confirmation, the results in this test report are in a simplified form in the context of DIN EN ISO/IEC 17025:2018, paragraph 7.8.1.3.

(b) (6), (b) (4)

he activities reported in this document are accredited according to DIN EN ISO/IEC 17025:2018. Only not accredited act

(b) (6), (b) (4)

(b) (6), (b) (4)

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G-ENA/MR
Chemiestraße 22
68623 Lampertheim

Date 10.12.2021
Customer no. 10082806

REPORT (b) (4)

Order	(b) (4)
Sample no.	
Project	**4428 Halbjahresmonitoring**
Sample acceptance	**09.11.2021**
Date of sampling	**09.09.2021**
Customer sample description	**Natupulse TS** **place of sampling:** (b) (6)
Description of the sample	**90882124U0**
Packaging	**plasticcontainer**
Item number	**50604289**

	Unit	Result	Value in DM	result in 88% DM			Method
Microbiological examinations							
Escherichia coli	cfu/g	(b) (4)					DIN ISO 16649-2 : 2020-12
Salmonella spp. in 25g		**not detected**					ISO 6579-1 : 2017-02

The sign "<"...."(LOD)" or n.d. in column result means, the substance concerned cannot be detected within the limit of detection. Parameter-specific analytical measurement uncertainties and information regarding the method of calculation will be provided upon request if the reported results are above the parameter-specific limit of quantification.

Explanation: OM = on original matter; DM = on dry matter base

Remark to Escherichia coli:
A resuscitation step is done to detect stressed bacteria. According to the National Footnote, these are presumptively determined ß-glucuronidase-positive Escherichia coli.
Remark to Salmonella spp.:
In case of positive Salmonella results a confirmation of Salmonella spp. was conducted by MALDI-TOF (database BDAL/7311 MSPS).

Start of testing: 09.11.2021
End of testing: 18.11.2021

The results are related only to the samples tested. In cases where the laboratory has not been responsible for sampling, the reported results apply to the samples as received. Duplication of this document or of parts of it requires the authorization from laboratory. In accordance our agreement in writing in the order confirmation, the results in this test report are in a simplified form in the context of DIN EN ISO/IEC 17025:2018, paragraph 7.8.1.3.

(b) (6), (b) (4)

(b) (6), (b) (4)

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(b) (6), (b) (4)

BASF SE
G-ENA/MR
Chemiestraße 22
68623 Lampertheim

Date 10.12.2021
Customer no. 10082806

REPORT (b) (4)

Order
Sample no.
Project **4428 Halbjahresmonitoring**
Sample acceptance **09.11.2021**
Date of sampling **09.09.2021**
Customer sample description **Natupulse TS**
place of sampling:
Description of the sample **90882124U0**
Packaging **plasticcontainer**
Item number **50604289**

	Unit		Result	Value in DM	result in 88% DM		Method
Nutrition values/ingredients							
Moisture (vacuum)	%		(b) (4)				REG(EC) 152/2009, III, A : 2009-02
Trace elements / Heavy metals / Halogenides							
Lead (Pb)	mg/kg						DIN EN 17053 : 2018-03
Cadmium (Cd)	mg/kg						DIN EN 17053 : 2018-03
Mercury (Hg)	mg/kg						DIN EN 16277 : 2012-09 (mod.)
Arsenic (As)	mg/kg						DIN EN 17053 : 2018-03

Explanation: The symbol "<" or n.d. in the result column means, the substance concerned is not quantifiable at the limit of quantification shown opposite.
Parameter-specific analytical measurement uncertainties and information regarding the method of calculation will be provided upon request if the reported results are above the parameter-specific limit of quantification.

Explanation: OM = on original matter; DM = on dry matter base

Start of testing: 09.11.2021
End of testing: 06.12.2021

The results are related only to the samples tested. In cases where the laboratory has not been responsible for sampling, the reported results apply to the samples as received. Duplication of this document or of parts of it requires the authorization from laboratory. In accordance our agreement in writing in the order confirmation, the results in this test report are in a simplified form in the context of DIN EN ISO/IEC 17025:2018, paragraph 7.8.1.3.

he activities reported in this document are accredited according to DIN EN ISO/IEC 17025:2018. Only not accredited activities are identified by the symbol " *) ".

(b) (6), (b) (4)

(b) (6), (b) (4)

Date 10.12.2021
Customer no. 10082806

REPORT (b) (4)

(b) (6), (b) (4)

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BASF SE
G-ENA/MR
Carl-Bosch-Straße 38
67056 Ludwigshafen

Date 20.12.2022
Customer no. 10082806

REPORT

Order (b) (4)
Sample no.
Project **4428 Halbjahresmonitoring**
Sample acceptance **04.12.2022**
Date of sampling **15.11.2022**
Customer sample description **Natupulse TS**
place of sampling: (b) (6)
Packaging **plastic container**
Batch/Charge/Lot (b) (4)
Article number (b) (4)

	Unit	Result	Value in DM	result in 88% DM	Method
Nutrition values/ingredients					
Moisture (vacuum)	%	(b) (4)			REG(EC) 152/2009, III, A : 2009-01
Mycotoxins					
Aflatoxine B1	µg/kg				MP-00180-DE : 2022-08 (LC-MSMS)
Deoxynivalenol (DON)	mg/kg				MP-00180-DE : 2022-08 (LC-MSMS)
Zearalenone (ZEA)	µg/kg				MP-00180-DE : 2022-08 (LC-MSMS)
Ochratoxine A	µg/kg				MP-00180-DE : 2022-08 (LC-MSMS)
Fumonisine B1	µg/kg				MP-00245-DE : 2022-03 (LC-MSMS)
Fumonisine B2	µg/kg				MP-00245-DE : 2022-03 (LC-MSMS)
Sum of Fumonisine B1/B2	µg/kg				calculated

pa) The detection and quantification limit had been increased because for this analysis matrix a smaller sample volume had to be used.
Explanation: The symbol "<" or n.d. in the result column means, the substance concerned is not quantifiable at the limit of quantification shown opposite.
Parameter-specific analytical measurement uncertainties and information regarding the method of calculation will be provided upon request if the reported results are above the parameter-specific limit of quantification.

Explanation: OM = on original matter; DM = on dry matter base

The activities reported in this document are accredited according to DIN EN ISO/IEC 17025:2018. Only not accredited activities are identified by the symbol " *) ".

(b) (6), (b) (4)

Date 20.12.2022
Customer no. 10082806

REPORT

Order
Sample no. (b) (4)

Start of testing: 05.12.2022
End of testing: 20.12.2022

The results are related only to the samples tested. In cases where the laboratory has not been responsible for sampling, the reported results apply to the samples as received. Duplication of this document or of parts of it requires the authorization from laboratory. In accordance our agreement in writing in the order confirmation, the results in this test report are in a simplified form in the context of DIN EN ISO/IEC 17025:2018, paragraph 7.8.1.3.

(b) (4)

e activities reported in this document are accredited according to DIN EN ISO/IEC 17025:2018. Only not accredited activities are identified by the sy

(b) (6), (b) (4)

(b) (6), (b) (4)

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G-ENA/MR
Carl-Bosch-Straße 38
67056 Ludwigshafen

Date 20.12.2022
Customer no. 10082806

REPORT

Order (b) (4)
Sample no.
Project **4428 Halbjahresmonitoring**
Sample acceptance **04.12.2022**
Date of sampling **15.11.2022**
Customer sample description **Natupulse TS**
place of sampling: (b) (6)
Packaging **plastic container**
Batch/Charge/Lot (b) (4)
Article number (b) (4)

	Unit	Result	Value in DM	result in 88% DM		Method
Nutrition values/ingredients						
Moisture (vacuum)	%	(b) (4)				REG(EC) 152/2009, III, A : 2009-01
Trace elements / Heavy metals / Halogenides						
Lead (Pb)	mg/kg					DIN EN 17053 : 2018-03
Cadmium (Cd)	mg/kg					DIN EN 17053 : 2018-03
Mercury (Hg)	mg/kg					DIN EN 16277 : 2012-09 (mod.)
Arsenic (As)	mg/kg					DIN EN 17053 : 2018-03

Explanation: The symbol "<" or n.d. in the result column means, the substance concerned is not quantifiable at the limit of quantification shown opposite.
Parameter-specific analytical measurement uncertainties and information regarding the method of calculation will be provided upon request if the reported results are above the parameter-specific limit of quantification.

Explanation: OM = on original matter; DM = on dry matter base

Start of testing: 05.12.2022
End of testing: 20.12.2022

The results are related only to the samples tested. In cases where the laboratory has not been responsible for sampling, the reported results apply to the samples as received. Duplication of this document or of parts of it requires the authorization from laboratory. In accordance our agreement in writing in the order confirmation, the results in this test report are in a simplified form in the context of DIN EN ISO/IEC 17025:2018, paragraph 7.8.1.3.

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DOC-12-17676736-EN-P1

(b) (4), (b) (6)

Date 20.12.2022
Customer no. 10082806

REPORT
Order
Sample no. (b) (4)

(b) (6), (b) (4)

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BASF SE
G-ENA/MR
Carl-Bosch-Straße 38
67056 Ludwigshafen

Date 20.12.2022
Customer no. 10082806

REPORT

Order (b) (4)
Sample no.
Project **4428 Halbjahresmonitoring**
Sample acceptance **04.12.2022**
Date of sampling **15.11.2022**
Customer sample description **Natupulse TS**
place of sampling: (b) (6)
Packaging **plastic container**
Batch/Charge/Lot (b) (4)
Article number (b) (4)

Microbiological examinations

	Unit		Result	Value in DM	result in 88% DM			Method
Escherichia coli	cfu/g		(b) (4)					DIN ISO 16649-2 : 2020-12
Coliform bacteria	cfu/g							ISO 4832 : 2006-02
Salmonella spp. in 25g								ISO 6579-1 : 2017-02

The sign "<"...."(LOD)" or n.d. in column result means, the substance concerned cannot be detected within the limit of detection. Parameter-specific analytical measurement uncertainties and information regarding the method of calculation will be provided upon request if the reported results are above the parameter-specific limit of quantification.

Explanation: OM = on original matter; DM = on dry matter base

Remark to Escherichia coli:
A resuscitation step is done to detect stressed bacteria. According to the National Footnote, these are presumptively determined ß-glucuronidase-positive Escherichia coli.
Remark to Salmonella spp.:
In case of positive Salmonella results a confirmation of Salmonella spp. was conducted by MALDI-TOF (database BDAL/7311 MSPS).

Start of testing: 05.12.2022
End of testing: 11.12.2022

The results are related only to the samples tested. In cases where the laboratory has not been responsible for sampling, the reported results apply to the samples as received. Duplication of this document or of parts of it requires the authorization from laboratory. In accordance our agreement in writing in the order confirmation, the results in this test report are in a simplified form in the context of DIN EN ISO/IEC 17025:2018, paragraph 7.8.1.3.

ties reported in this document are accredited according to DIN EN ISO/IEC 17025:2018. Only not accredited activities are identified by the symbol " *) ".

Date 20.12.2022
Customer no. 10082806

REPORT
Order
Sample no. (b) (4)

(b) (6), (b) (4)

he activities reported in this document are accredited according to DIN EN ISO/IEC 17025:2018. Only not accredited activities are identified by the symbol " *) ".

BASF SE
G-ENA/MR
Carl-Bosch-Straße 38
67056 Ludwigshafen

Date 14.06.2023
Customer no. 10082806

REPORT

Order (b) (4)
Sample no.
Invoice recipient **10085540 BASF SE G-ENA/SE, F 31**
Project **4428 Halbjahresmonitoring**
Sample acceptance **19.05.2023**
Date of sampling **no information**
Customer sample description **Natupulse TS**
place of sampling: (b) (6)

Packaging **plastic container**
Batch/Charge/Lot (b) (4)
Article number (b) (4)

	Unit	Result	Value in DM	result in 88% DM	Method
Nutrition values/ingredients					
Moisture (vacuum)	%	(b) (4)			REG(EC) 152/2009, III, A : 2009-01
Mycotoxins					
Aflatoxine B1	µg/kg				MP-00180-DE : 2022-08 (LC-MSMS)
Deoxynivalenol (DON)	mg/kg				MP-00180-DE : 2022-08 (LC-MSMS)
Zearalenone (ZEA)	µg/kg				MP-00180-DE : 2022-08 (LC-MSMS)
Ochratoxine A	µg/kg				MP-00180-DE : 2022-08 (LC-MSMS)
Fumonisine B1	µg/kg				MP-00245-DE : 2022-03 (LC-MSMS)
Fumonisine B2	µg/kg				MP-00245-DE : 2022-03 (LC-MSMS)
Sum of Fumonisine B1/B2	µg/kg				calculated

pa) The detection and quantification limit had been increased because for this analysis matrix a smaller sample volume had to be used.
Explanation: The symbol "<" or n.q. in the result column means, the substance concerned is not quantifiable at the limit of quantification shown opposite.
Parameter-specific analytical measurement uncertainties and information regarding the method of calculation will be provided upon request if the reported results are above the parameter-specific limit of quantification.

Explanation: OM = on original matter; DM = on dry matter base

The activities reported in this document are accredited according to DIN EN ISO/IEC 17025:2018. Only not accredited activities are identified by the symbol " ") ".

(b) (6), (b) (4)

Date 14.06.2023
Customer no. 10082806

REPORT
Order (b) (4)
Sample no.

Start of testing: 23.05.2023
End of testing: 12.06.2023

The results are related only to the samples tested. In cases where the laboratory has not been responsible for sampling, the reported results apply to the samples as received. Duplication of this document or of parts of it requires the authorization from laboratory. In accordance our agreement in writing in the order confirmation, the results in this test report are in a simplified form in the context of DIN EN ISO/IEC 17025:2018, paragraph 7.8.1.3.
In conformity assessment, the economic approach is used as the decision rule (a non-conformity exists if the measurement result is included measurement uncertainty above the specification or standard), as long as nothing else has been determined by corresponding legal or normative bases.

(b) (6), (b) (4)

The activities reported in this document are accredited according to DIN EN ISO/IEC 17025:2018. Only not accredited activities are iden

(b) (6)

BASF SE
G-ENA/MR
Carl-Bosch-Straße 38
67056 Ludwigshafen

Date 14.06.2023
Customer no. 10082806

REPORT

Order (b) (4)
Sample no.
Invoice recipient **10085540 BASF SE G-ENA/SE, F 31**
Project **4428 Halbjahresmonitoring**
Sample acceptance **19.05.2023**
Date of sampling **no information**
Customer sample description **Natupulse TS**
place of sampling: (b) (6)
Packaging **plastic container**
Batch/Charge/Lot (b) (4)
Article number

Microbiological examinations

	Unit		Result	Value in DM	result in 88% DM			Method
Escherichia coli	cfu/g		(b) (4)					DIN ISO 16649-2 : 2020-12
Salmonella spp. in 25g								ISO 6579-1 : 2017-02

The sign "<"...."(LOD)" or n.d. in column result means, the substance concerned cannot be detected within the limit of detection. Parameter-specific analytical measurement uncertainties and information regarding the method of calculation will be provided upon request if the reported results are above the parameter-specific limit of quantification.

Explanation: OM = on original matter; DM = on dry matter base

Remark to Escherichia coli:
A resuscitation step is done to detect stressed bacteria. According to the National Footnote, these are presumptively determined ß-glucuronidase-positive Escherichia coli.
Remark to Salmonella spp.:
In case of positive Salmonella results a confirmation of Salmonella spp. was conducted by MALDI-TOF (database BDAL/7311 MSPS).

Start of testing: 23.05.2023
End of testing: 03.06.2023

The results are related only to the samples tested. In cases where the laboratory has not been responsible for sampling, the reported results apply to the samples as received. Duplication of this document or of parts of it requires the authorization from laboratory. In accordance our agreement in writing in the order confirmation, the results in this test report are in a simplified form in the context of DIN EN ISO/IEC 17025:2018, paragraph 7.8.1.3.
In conformity assessment, the economic approach is used as the decision rule (a non-conformity exists if the measurement result is included measurement uncertainty above the specification or standard), as long as nothing else has been determined by corresponding legal or normative bases.

The activities reported in this document are accredited according to DIN EN ISO/IEC 17025:2018. Only not accredited activities are identified by the symbol " *) ".

Date 14.06.2023
Customer no. 10082806

REPORT
Order
Sample no. (b) (4)

(b) (6), (b) (4)

he activities reported in this document are accredited according to DIN EN ISO/IEC 17025:2018. Only not accredited activities are identified by the symbol " *) ".

BASF SE
G-ENA/MR
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67056 Ludwigshafen

Date 14.06.2023
Customer no. 10082806

REPORT

Order (b) (4)
Sample no.
Invoice recipient **10085540 BASF SE G-ENA/SE, F 31**
Project **4428 Halbjahresmonitoring**
Sample acceptance **19.05.2023**
Date of sampling **no information**
Customer sample description **Natupulse TS**
place of sampling:
Packaging **plastic container**
Batch/Charge/Lot (b) (4)
Article number

	Unit	Result	Value in DM	result in 88% DM	Method
Nutrition values/ingredients					
Moisture (vacuum)	%				REG(EC) 152/2009, III, A : 2009-01
Trace elements / Heavy metals / Halogenides					
Lead (Pb)	mg/kg				DIN EN 17053 : 2018-03
Cadmium (Cd)	mg/kg				DIN EN 17053 : 2018-03
Mercury (Hg)	mg/kg				DIN EN 16277 : 2012-09 (mod.)
Arsenic (As)	mg/kg				DIN EN 17053 : 2018-03

(b) (4)

Explanation: The symbol "<" or n.q. in the result column means, the substance concerned is not quantifiable at the limit of quantification shown opposite.
Parameter-specific analytical measurement uncertainties and information regarding the method of calculation will be provided upon request if the reported results are above the parameter-specific limit of quantification.

Explanation: OM = on original matter; DM = on dry matter base

Start of testing: 23.05.2023
End of testing: 08.06.2023

The results are related only to the samples tested. In cases where the laboratory has not been responsible for sampling, the reported results apply to the samples as received. Duplication of this document or of parts of it requires the authorization from laboratory. In accordance our agreement in writing in the order confirmation, the results in this test report are in a simplified form in the context of DIN EN ISO/IEC 17025:2018, paragraph 7.8.1.3.
In conformity assessment, the economic approach is used as the decision rule (a non-conformity exists if the measurement result is included measurement uncertainty above the specification or standard), as long as nothing else has been determined by corresponding legal or normative bases.

he activities reported in this document are accredited according to DIN EN ISO/IEC 17025:2018. Only not accredited activities are identified by the symbol " *) ".

(b) (6), (b) (4)

Date 14.06.2023
Customer no. 10082806

REPORT
Order (b) (4)
Sample no.

(b) (4), (b) (6)

The activities reported in this document are accredited according to DIN EN ISO/IEC 17025:2018. Only not accredited activities are identified by the symbol " *) ".

Appendix 16e: (b) (4) **Test Reports**

(b) (6), (b) (4)

(b) (6), (b) (4)

BASF SE
Carl-Bosch-Straße 38

67056 Ludwigshafen am Rhein
Deutschland

Date: 07.08.2023

Analysis Report No.: **234368**

Product:	Mannanase CC
Date of reception:	01.08.2023 parcel service ((b) (4)
Order of:	26.07.2023 written by (b) (6)
Assignment:	Tenuazonic acid
Labelling:	Mannanase CC
	Art.: (b) (4)
	Batch: (b) (4)
Quantity:	0,5 kg
Packaging:	PE-HD-bottle
Sampling date:	
Temperature at reception / storage:	2,3°C / <= -18°C
Start / End of analysis:	03.08.2023 / 07.08.2023

(b) (6), (b) (4)

Results of Analysis:

Tenuazonic acid

Parameter	Result	Unit
Tenuazonic acid	(b) (4)	

Methods applied:
Determination of tenuazonic acid in feedstuff with LC-MS/MS (PAW 029, 2022-11). Limit of quantification: 10 µg/kg. #

(b) (6)

The results refer solely to the samples analysed.
An uncomplete copy of this report requires in advance our written approval.
The methods labelled with # are non-accredited test methods.

(b) (6), (b) (4)

(b) (6), (b) (4)

BASF SE
Carl-Bosch-Straße 38

67056 Ludwigshafen am Rhein
Deutschland

Date: 07.08.2023

Analysis Report No.: **234369**

Product:	Mannanase CC
Date of reception:	01.08.2023 parcel service (
Order of:	26.07.2023 written by (b) (6)
Assignment:	Tenuazonic acid
Labelling:	Mannanase CC Art.: 83012427 Batch:
Quantity:	0,5 kg
Packaging:	PE-HD-bottle
Sampling date:	
Temperature at reception / storage:	2,3°C / <= -18°C
Start / End of analysis:	03.08.2023 / 07.08.2023

(b) (6), (b) (4)

Results of Analysis:

Tenuazonic acid

Parameter	Result	Unit
Tenuazonic acid	(b) (4)	

Methods applied:
Determination of tenuazonic acid in feedstuff with LC-MS/MS (PAW 029, 2022-11). Limit of quantification: 10 µg/kg. #

(b) (6)

The results refer solely to the samples analysed.
An uncomplete copy of this report requires in advance our written approval.
The methods labelled with # are non-accredited test methods.

(b) (6), (b) (4)

(b) (6), (b) (4)

BASF SE
Carl-Bosch-Straße 38

67056 Ludwigshafen am Rhein
Deutschland

Analysis Report No.: **233890-A**
This Analysis Report replaces Analysis Report No. 233890; Change: Report language

Product:	Natupulse TS (Feed additive)
Date of reception:	04.07.2023 parcel service ((b) (4)
Order of:	30.06.2023 written by (b) (6)
Assignment:	Tenuazonic acid
Labelling:	Natupulse TS
	Lot: (b) (4)
Quantity:	0,5 kg
Packaging:	PE-HD-bottle
Sampling date:	Sampling was carried out by customer
Temperature at reception / storage:	Room temperature / Room temperature
Start / End of analysis:	05.07.2023 / 17.07.2023

(b) (6), (b) (4)

Results of Analysis:

Tenuazonic acid

Parameter	Result	Unit
Tenuazonic acid	(b) (4)	

Methods applied:
Determination of tenuazonic acid in feedstuff with LC-MS/MS (PAW 029, 2022-11). Limit of quantification: 10 µg/kg.#

(b) (6)

(b) (6)

The results refer solely to the samples analysed.
An uncomplete copy of this report requires in advance our written approval.
The methods labelled with # are non-accredited test methods.

(b) (6), (b) (4)
BASF SE
Carl-Bosch-Straße 38

67056 Ludwigshafen am Rhein
Deutschland

Analysis Report No.: **233891-A**
This Analysis Report replaces Analysis Report No. 233891; Change: Report language

Product:	Natupulse TS (Feed additive)
Date of reception:	04.07.2023 parcel service (
Order of:	30.06.2023 written by
Assignment:	Tenuazonic acid
Labelling:	Natupulse TS
	Lot: (b) (4)
Quantity:	0,5 kg
Packaging:	PE-HD-bottle
Sampling date:	Sampling was carried out by customer
Temperature at reception / storage:	Room temperature / Room temperature
Start / End of analysis:	05.07.2023 / 17.07.2023

Results of Analysis:

Tenuazonic acid

Parameter	Result	Unit
Tenuazonic acid	(b) (4)	

Methods applied:
Determination of tenuazonic acid in feedstuff with LC-MS/MS (PAW 029, 2022-11). Limit of quantification: 10 µg/kg.#

(b) (6)

The results refer solely to the samples analysed.
An uncomplete copy of this report requires in advance our written approval.
The methods labelled with # are non-accredited test methods.

(b) (6)

(b) (6), (b) (4)

BASF SE
Carl-Bosch-Straße 38

67056 Ludwigshafen am Rhein
Deutschland

Analysis Report No.: **233892-A1**

This Analysis Report replaces Analysis Report No. 233892-A; Change of customer address

Product:	Natupulse TS (Feed additive)
Date of reception:	04.07.2023 parcel service ((b) (4)
Order of:	30.06.2023 written by (b) (6)
Assignment:	Tenuazonic acid
Labelling:	Natupulse TS
	Lot: (b) (4)
Quantity:	0,5 kg
Packaging:	PE-HD-bottle
Sampling date:	Sampling was carried out by customer
Temperature at reception / storage:	Room temperature / Room temperature
Start / End of analysis:	05.07.2023 / 17.07.2023

(b) (6), (b) (4)

Results of Analysis:

Tenuazonic acid

Parameter	Result	Unit
Tenuazonic acid	(b) (4)	

Methods applied:
Determination of tenuazonic acid in feedstuff with LC-MS/MS (PAW 029, 2022-11). Limit of quantification: 10 µg/kg.#

(b) (6)

The results refer solely to the samples analysed.
An uncomplete copy of this report requires in advance our written approval.
The methods labelled with # are non-accredited test methods.

(b) (6), (b) (4)

(b) (6), (b) (4)

BASF SE
Carl-Bosch-Straße 38

67056 Ludwigshafen am Rhein
Deutschland

Analysis Report No.: **233893-A**

This Analysis Report replaces Analysis Report No. 233893; Change: Report language

Product:	Natupulse TS L (Feed additive)
Date of reception:	04.07.2023 parcel service (
Order of:	30.06.2023 written by
Assignment:	Tenuazonic acid
Labelling:	Natupulse TS L
	Lot:
Quantity:	0,5 kg
Packaging:	PE-HD-bottle
Sampling date:	Sampling was carried out by customer
Temperature at reception / storage:	Room temperature / Room temperature
Start / End of analysis:	05.07.2023 / 17.07.2023

(b) (6), (b) (4)

Results of Analysis:

Tenuazonic acid

Parameter	Result	Unit
Tenuazonic acid	(b) (4)	

Methods applied:
Determination of tenuazonic acid in feedstuff with LC-MS/MS (PAW 029, 2022-11). Limit of quantification: 10 µg/kg.#

(b) (6)

The results refer solely to the samples analysed.
An uncomplete copy of this report requires in advance our written approval.
The methods labelled with # are non-accredited test methods.

(b) (4), (b) (6)

(b) (4), (b) (6)

BASF SE
Carl-Bosch-Straße 38

67056 Ludwigshafen am Rhein
Deutschland

Analysis Report No.: **233894-A**

This Analysis Report replaces Analysis Report No. 233894: Change: Report language

Product:	Natupulse TS L (Feed additive)
Date of reception:	04.07.2023 parcel service (
Order of:	30.06.2023 written by (b) (6)
Assignment:	Tenuazonic acid
Labelling:	Natupulse TS L
	Lot: (b) (4)
Quantity:	0,5 kg
Packaging:	PE-HD-bottle
Sampling date:	Sampling was carried out by customer
Temperature at reception / storage:	Room temperature / Room temperature
Start / End of analysis:	05.07.2023 / 17.07.2023

(b) (4)

Results of Analysis:

Tenuazonic acid

Parameter	Result	Unit
Tenuazonic acid	(b) (4)	

Methods applied:
Determination of tenuazonic acid in feedstuff with LC-MS/MS (PAW 029, 2022-11). Limit of quantification: 10 µg/kg.#

(b) (6)

The results refer solely to the samples analysed.
An uncomplete copy of this report requires in advance our written approval.
The methods labelled with # are non-accredited test methods.

(b) (6), (b) (4)

(b) (6), (b) (4)

BASF SE
Carl-Bosch-Straße 38

67056 Ludwigshafen am Rhein
Deutschland

Analysis Report No.: **233895-A**

This Analysis Report replaces Analysis Report No. 233895: Change: Report language

Product:	Natupulse TS L (Feed additive)
Date of reception:	04.07.2023 parcel service (
Order of:	30.06.2023 written by
Assignment:	Tenuazonic acid
Labelling:	Natupulse TS L
	Lot: (b) (4)
Quantity:	0,5 kg
Packaging:	PE-HD-bottle
Sampling date:	Sampling was carried out by customer
Temperature at reception / storage:	Room temperature / Room temperature
Start / End of analysis:	05.07.2023 / 17.07.2023

(b) (6), (b) (4)

Results of Analysis:

Tenuazonic acid

Parameter	Result	Unit
Tenuazonic acid	(b) (4)	

Methods applied:
Determination of tenuazonic acid in feedstuff with LC-MS/MS (PAW 029, 2022-11). Limit of quantification: 10 µg/kg.#

(b) (6)

The results refer solely to the samples analysed.
An uncomplete copy of this report requires in advance our written approval.
The methods labelled with # are non-accredited test methods.

Appendix 18: Product Stability in Feed or Premix

Table of Contents

Appendix 18a: Natupulse® TS in Mash and Pellet 2
Appendix 18b: Natupulse® TS L in Feed after PPLA 12
Appendix 18c: (b) (4) in Mineral Premix 20

Appendix 18a: Natupulse® TS in Mash and Pellet

Stability in feed mash and pellets

Stability of Natupulse® TS in feed mash and pellets after storage at (b) (4) .h.
(TID-51C-19)

BASF
The Chemical Company

September 1, 2020
Dr. Benjamin Peikert
G-ENA/MD - A 110
Tel.: 0621/60 52129

1. Introduction

The stability of Natupulse® TS containing the feed additive Mannanase produced by Thermothelomyces thermophila strain LU19287 (DSM 33149), was established in feed mash and pellets. In order to examine the stability of the feed additive after the feed manufacturing three different lots were mixed in a typical poultry feed and stored under different conditions.

2. Material and methods

2.1. Climate chambers

(b) (4)

2.2. Feed additive

(b) (4)

(b) (4)

2.3. Diets

(b) (4)

2.4. Sampling

(b) (4)

2.5. Chemical analysis

(b) (4)

2.6. Calculation

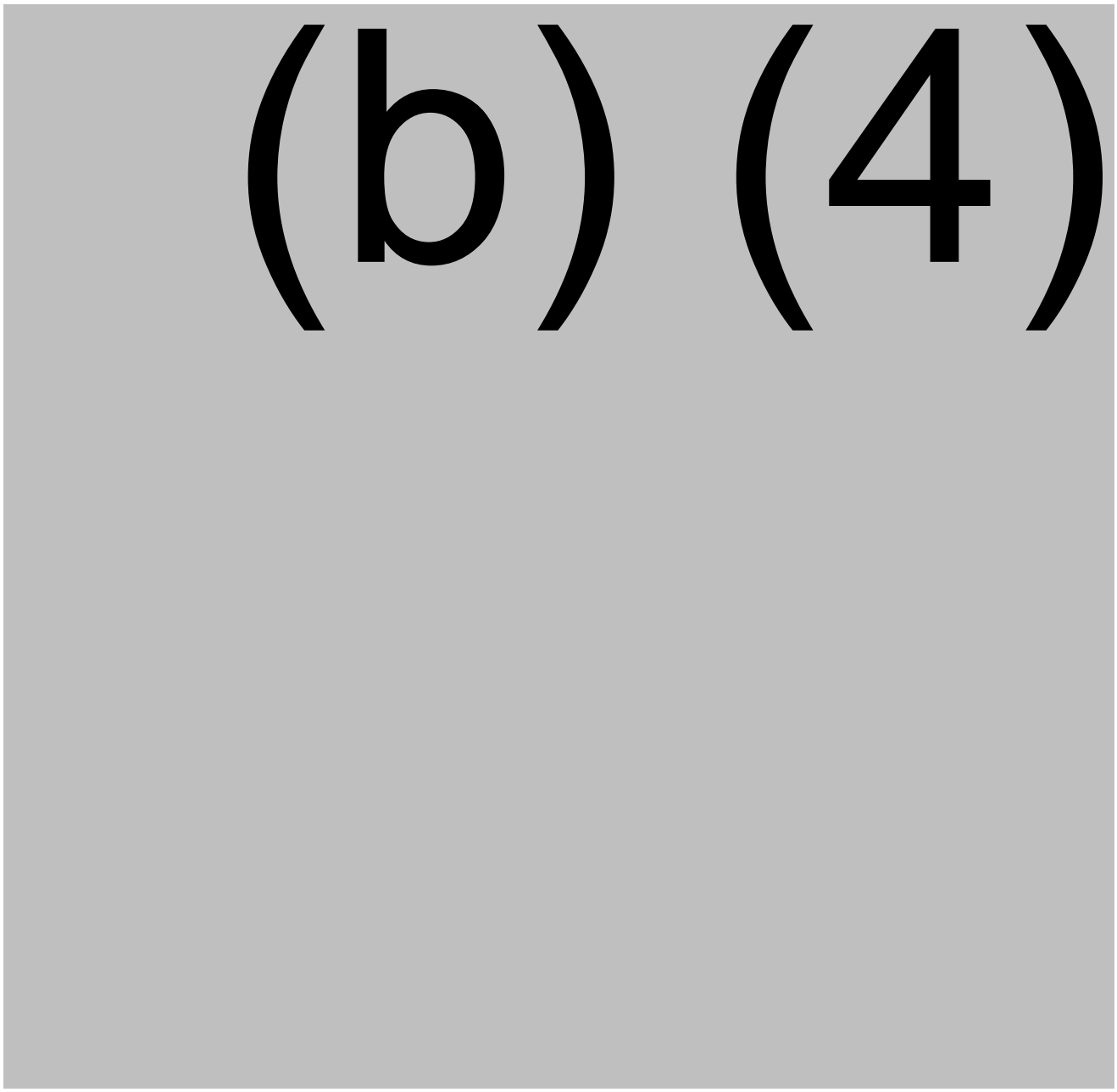
The retention was calculated according to the following formula

$$\text{Retention (\%)} = \frac{\text{analyzed activity of feed sample after x months (TMU/kg feed)}}{\text{analyzed activity of feed sample after 0 months (TMU/kg feed)}} \times 100$$

2.7. Mash feed

(b) (4)

2.8. Feed pellets



3. Conclusions

Retention of Mannanase activity after mixing of Natupulse TS in a typical poultry mash feed ranges from (b) (4) after storage for 3 months at (b) (4).
After the same storage period, however, but at (b) (4) r.h., the retention of mannanase activity ranged from (b) (4)

Retention of Mannanase activity after pelleting a typical poultry feed and storage for 3 months at 20°C ranges from (b) (4)
After the same storage period, however, but at 30 °C/75 % r.h., the retention of mannanase activity ranged from (b) (4)%.

In summary, it can be concluded that Natupulse TS shows good stability in feed.

Date of issue: September 1, 2020

BASF SE
G-ENA/MD
Analytics & Application Technology Feed Enzymes

(b) (4)
Dr. Benjamin Peikert

4. Annexes

Annex 1 Composition and nutrient contents of the feed

(b) (4)

Annex 2 Summary of the methods used

FEN/0004 Photometric assay of mannanase activity in feed additives

FEN/0005 Photometric assay of mannanase activity in feed and mineral premix

Annex 3 Analyzed activity of mannanase in mash feed (TMU/kg)

Batch	Product	Storage conditions	Storage duration (months)	Result 1	Result 2	Average	STDEV%	Unit
1	mash	0	AW	(b) (4)				
2	mash	0	AW					
3	mash	0	AW					
1	mash	(b) (4)	1					
1	mash		2					
	mash		3					
2	mash		1					
2	mash		2					
	mash		3					
3	mash		1					
3	mash		2					
	mash		3					
1	mash		1					
1	mash		2					
	mash		3					
2	mash		1					
2	mash		2					
	mash		3					
3	mash		1					
3	mash		2					
3	mash		3					

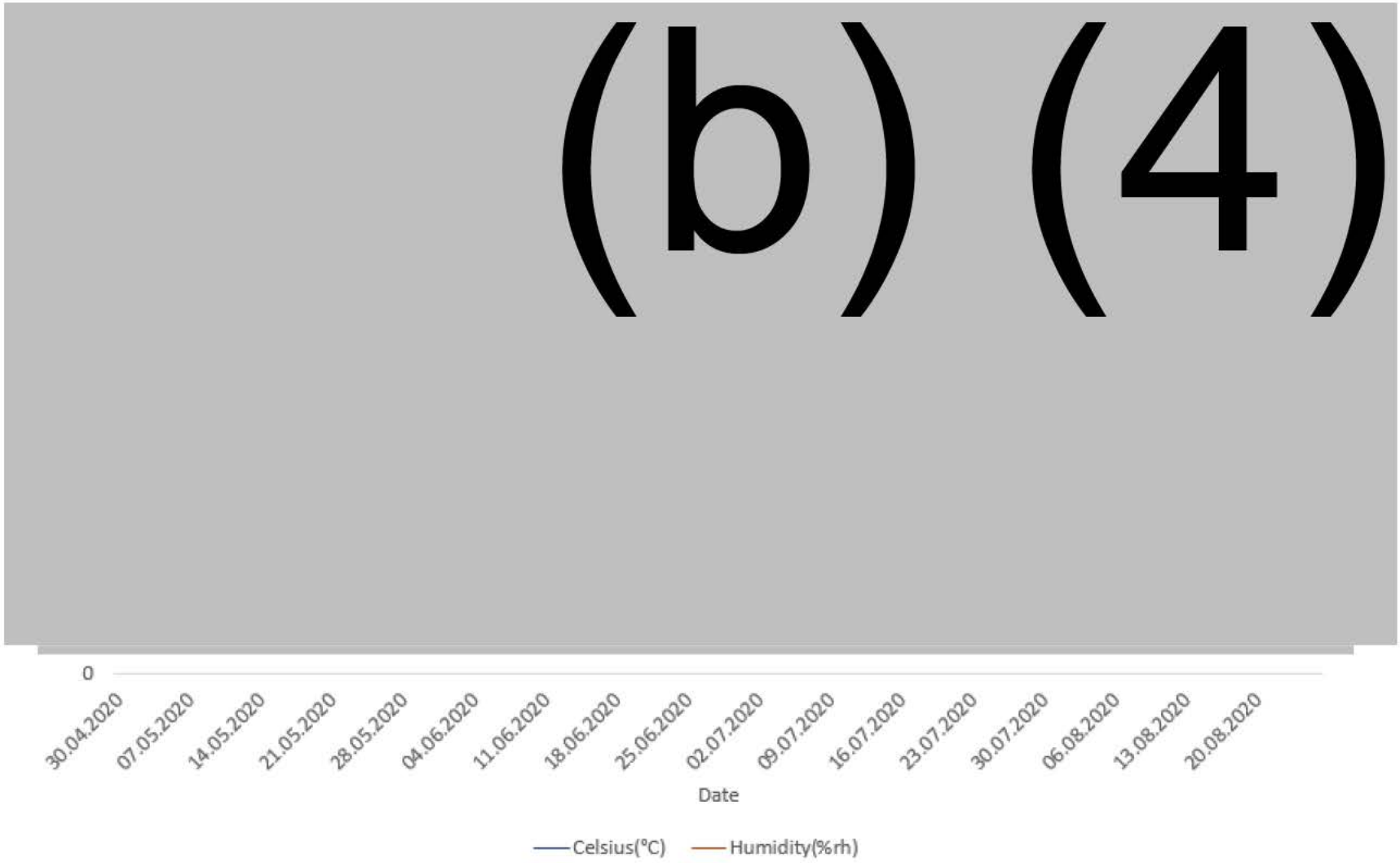
LIMS Nr (b) (4)

Annex 4 Analyzed activity of mannanase in feed pellets (TMU/kg)

Batch	Product	Storage conditions	Storage duration (months)	Result 1	Result 2	Average	STDEV%	Unit
1	pellets	0	AW	(b) (4)				
2	pellets	0	AW					
3	pellets	0	AW					
1	pellets	(b) (4)	1					
1	pellets		2					
	pellets		3					
2	pellets		1					
2	pellets		2					
	pellets		3					
3	pellets		1					
3	pellets		2					
	pellets		3					
1	pellets		1					
1	pellets		2					
	pellets		3					
2	pellets		1					
2	pellets		2					
	pellets		3					
3	pellets		1					
3	pellets		2					
3	pellets		3					

LIMS Nr 20F00145 / 20F00206 / 20F00209 / 20F00212

Annex 8: Humidity and temperature in climate chamber 2 set at 20°C without humidity regulation (30th April – 28th August 2020)



Appendix 18b: Natupulse® TS L in Feed after PPLA

Stability in feed after PPLA

Stability of Natupulse® TS L after post pellet liquid application after storage at (b) (4) r.h.
(TID-51C-19)

BASF
The Chemical Company

September 17, 2020
Dr. Benjamin Peikert
G-ENA/MD - A 110
Tel.: 0621/60 52129

1. Introduction

The stability of a Natupulse® TS L containing the feed additive Mannanase produced by Thermothelomyces thermophila strain LU19287 (DSM 33149), was established after spraying on a pelletized feeding stuff via a Post Pelleting Liquid Application (PPLA) System. In order to examine the stability of the feed additive after the PPLA process three different lots were sprayed on a typical poultry feed and stored under different conditions.

2. Material and methods

2.1. Climate chambers

(b) (4)

Table 1 Information about Natupulse® TS L batches used

Sample identification	Formulation batch	Production date	Activity [TMU/g]	Date of analysis	LIMS Nr
Natupulse® TS L	(b) (4)	27.Feb.2018	9010	Febr 27th, 2020	(b) (4)
Natupulse® TS L		04.Jun.2019	8940	Febr 27th, 2020	
Natupulse® TS L		04.Jun.2019	8610	Febr 27th, 2020	

2.3. Diets

2.3.1. Pelleting process

(b) (4)

2.3.2. Post Pellet Liquid Application

(b) (4)

2.4. Sampling

(b) (4)

(b) (4)

2.5. Chemical analysis

(b) (4)

2.6. Calculation

The retention was calculated according to the following formula

$$\text{Retention (\%)} = \frac{\text{analyzed activity of feed sample after x months (TMU/kg feed)}}{\text{analyzed activity of feed sample after 0 months (TMU/kg feed)}} \times 100$$

3. Results and discussion

(b) (4)

Table 4 Retention (%) of mannanase activity in feed after storage at (b) (4) r.h.

(b) (4)

4. Conclusions

Activity of Natupulse® TS L, sprayed on pelletized poultry feed via a Post Pellet Liquid Application, decreased by (b) (4) storage at (b) (4) for 3 months.
After the same storage period, however, but at (b) (4) r.h., the retention of mannanase activity decreased by 1(b) (4)%.

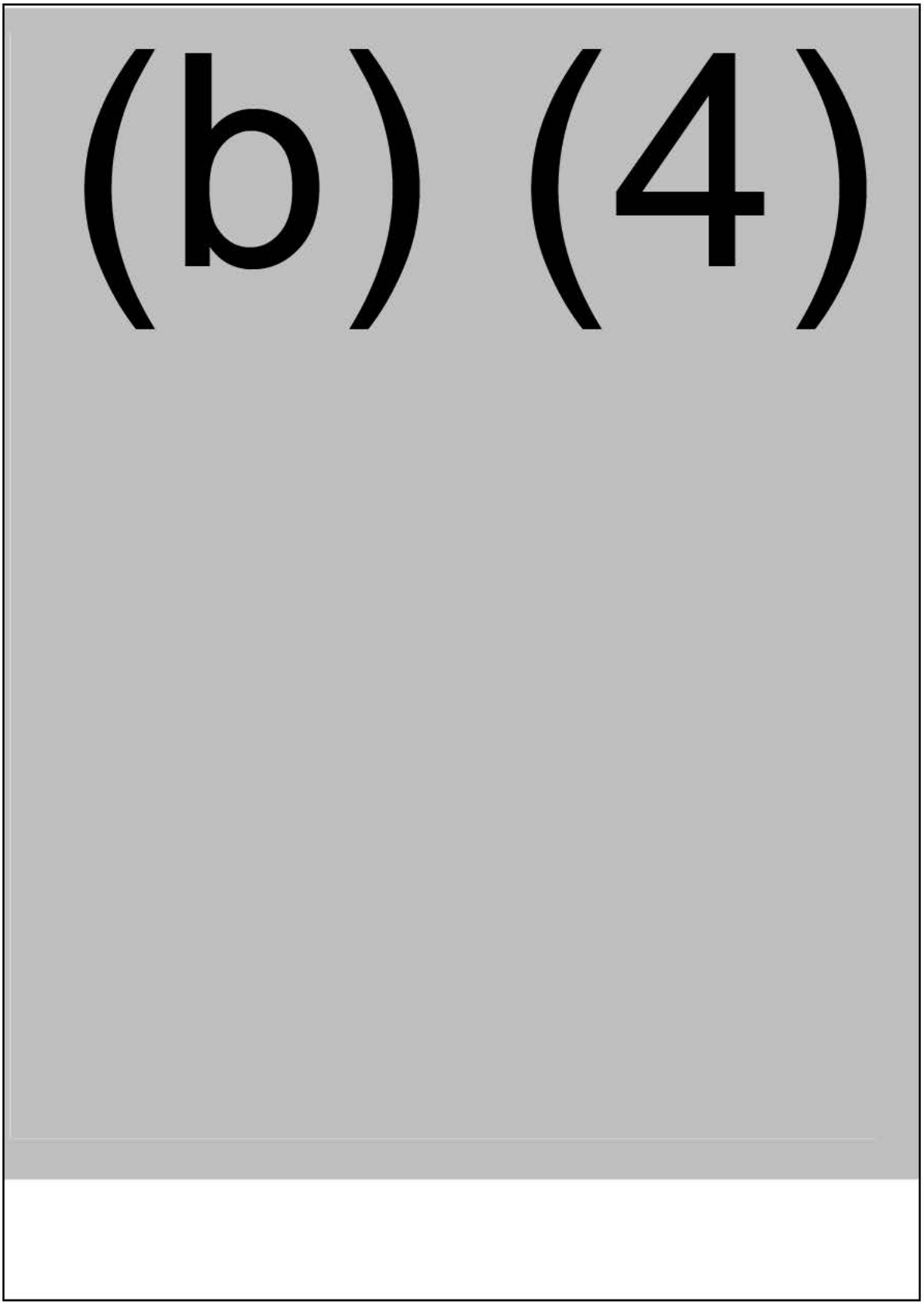
Date of issue: September 17, 2020

BASF SE
G-ENA/MD
Analytics & Application Technology Feed Enzymes

(b) (6)
Dr. Benjamin Peikert

5. Annexes

Annex 1 Composition and nutrient contents of the feed



Annex 2 Summary of the methods used

FEN/0004 Photometric assay of mannanase activity in feed additives

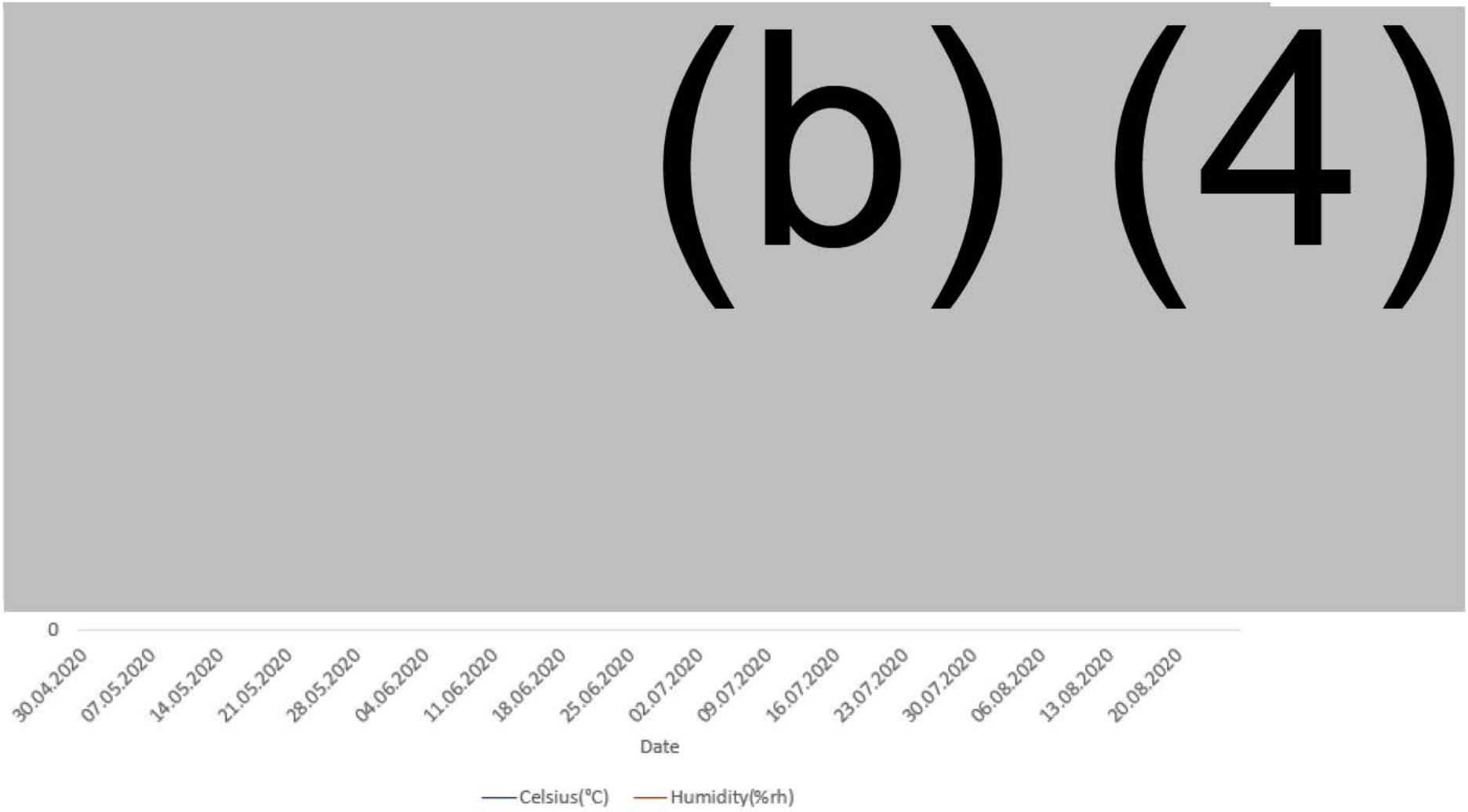
FEN/0005 Photometric assay of mannanase activity in feed and mineral premix

Annex 3 Analyzed activity of mannanase in feed pellets after PPLA

Batch	Product	Storage conditions	Storage duration (months)	Result 1	Result 2	Average	STDEV%	Unit
1	PPLA	0	AW	(b) (4)				
2	PPLA	0	AW					
3	PPLA	0	AW					
1	PPLA	(b) (4)	1					
1	PPLA		2					
	PPLA		3					
2	PPLA		1					
2	PPLA		2					
	PPLA		3					
3	PPLA		1					
3	PPLA		2					
	PPLA		3					
1	PPLA		1					
1	PPLA		2					
	PPLA		3					
2	PPLA		1					
2	PPLA		2					
	PPLA		3					
3	PPLA		1					
3	PPLA		2					
3	PPLA		3					

LIMS Nr 20F00146 / 20F00207 /20F00210 /20F00213 /

Annex 8: Humidity and temperature in climate chamber 2 set at 20°C without humidity regulation (30th April – 28th August 2020)



Appendix 18c: (b) (4) Powder in Mineral Premix

Stability in Mineral Premix

Stability of Natupulse® Powder Formulations in Mineral Premix (TID-104-18)

BASF
The Chemical Company

16.06.2020

Dr. Benjamin Peikert
G-ENA/MD - A 110
Tel.: 0621/60 52129

Introduction

The stability of Natupulse® Powder Formulations containing the feed-additive Mannanase produced by Thermothelomyces thermophila strain LU19287 (DSM 33149), was established in a mineral premix. In order to examine the stability of the feed additive after the premixing process three different lots were mixed in a typical broiler premix and stored under different conditions.

Material and methods

(b) (4)

Diets

(b) (4)

Calculation of stability

The retention of enzyme activity (remaining activity) characterizing the effect of storage of the enzymes was calculated for each storage condition conducted. The retention was calculated according to the following formula:

$$\text{retention (\%)} = \frac{\text{mean of analyzed activity of sample after x months (TMU/kg)}}{\text{mean of analyzed activity of sample after 0 months (TMU/kg)}} \times 100$$

The retention of Mannanase (b) (4) in mineral premix before and after storage is shown in tables and figures 1 to 3, respectively.

Table 1: Retention (%) of Mannanase (b) (4) in mineral premix at (b) (4)

	retention [%]
(b) (4)	

Figure 1: Retention (%) of Mannanase (b) (4) in mineral premix at (b) °C

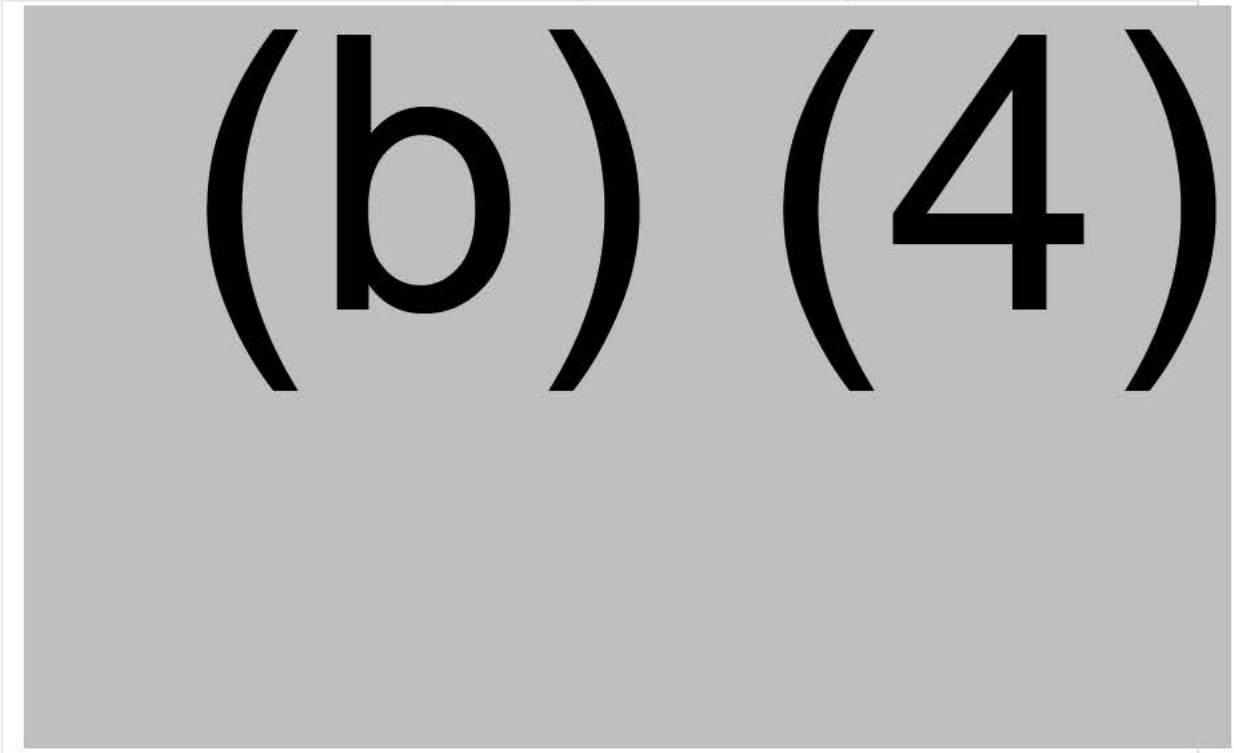


Figure 2: Retention (%) of (b) (4) powder in mineral premix at (b)(4) °C

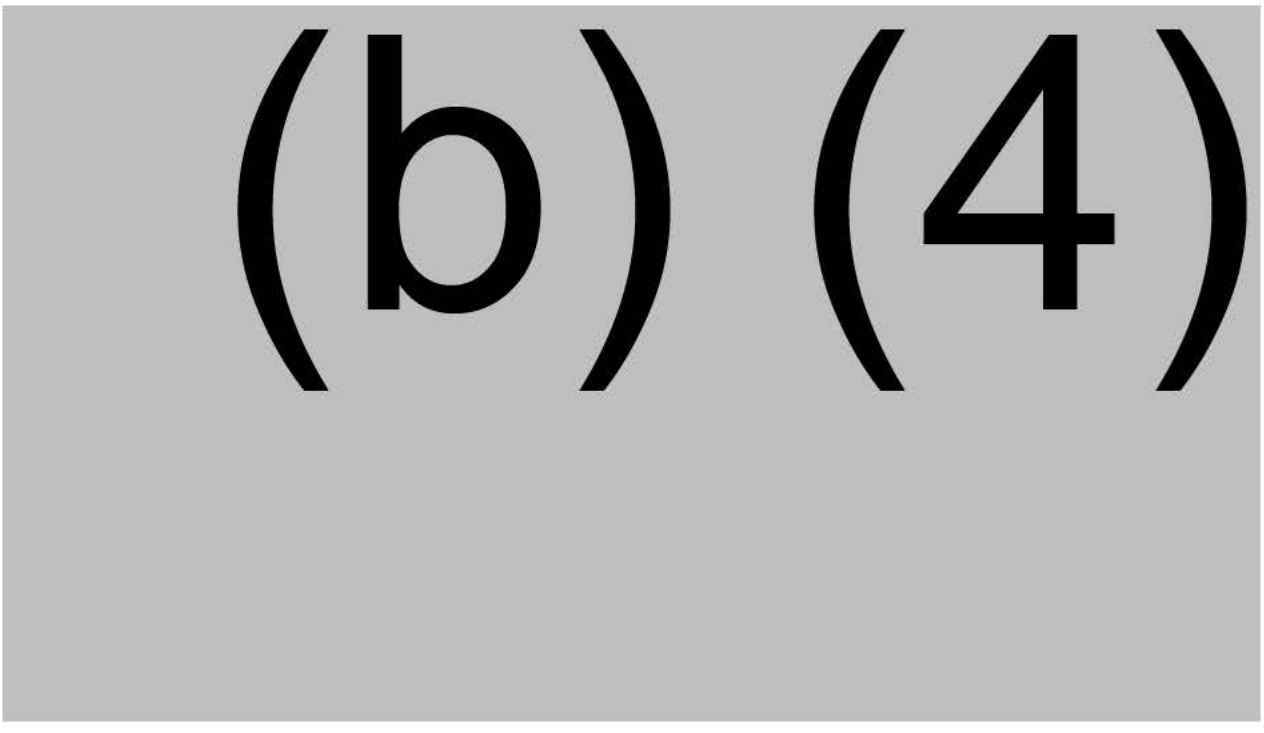
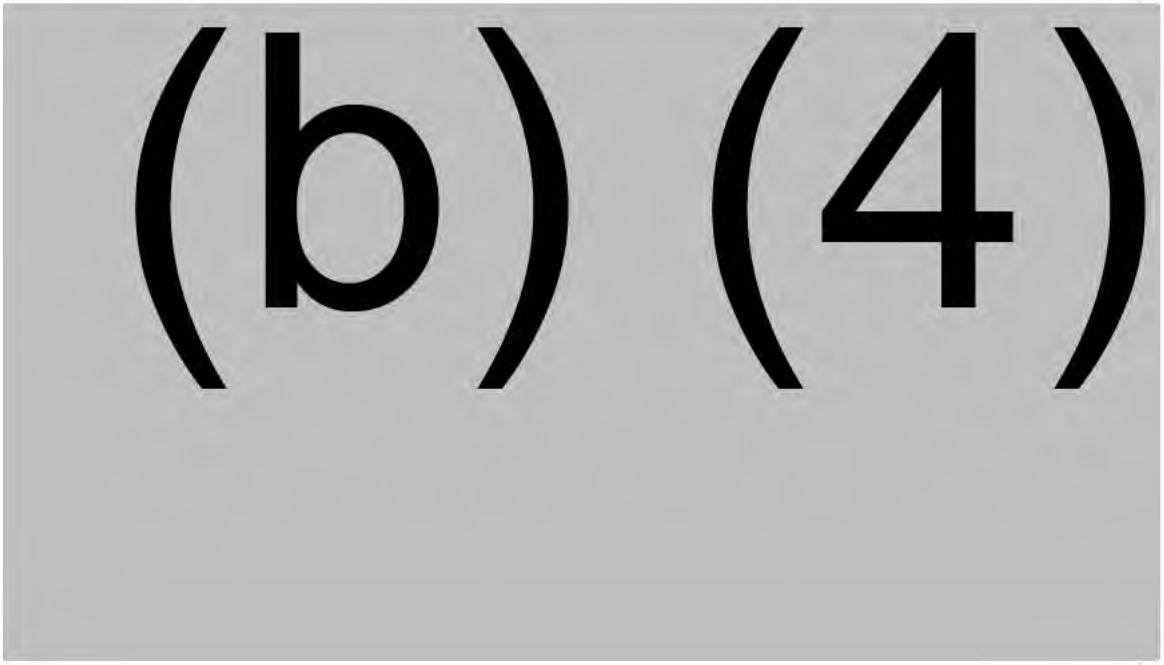


Figure 3: Retention (%) of (b) (6), (b) (4), (b) (5) powder in mineral premix at (b) (3) (B)



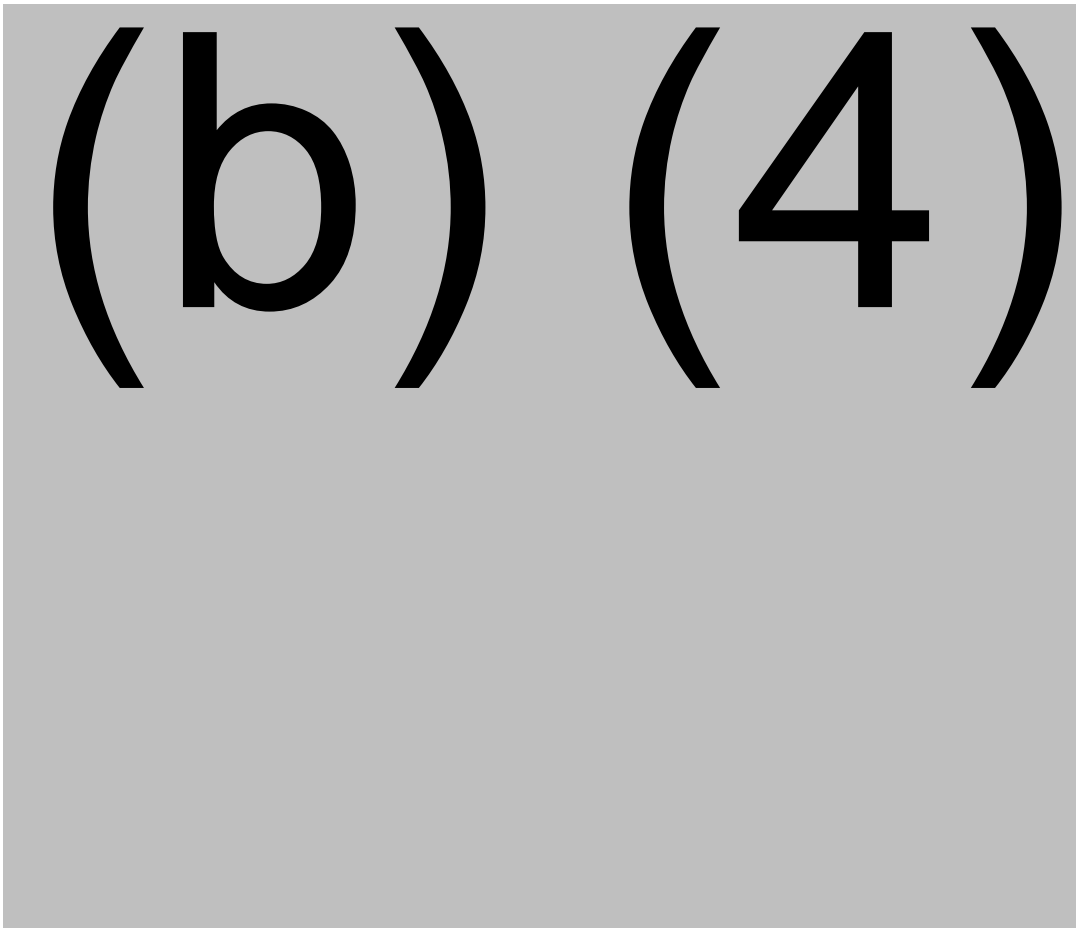
The retention of Mannanase activity after 6 months of storage in a typical mineral broiler premix stored at (b) (4) °C was (b) (4) From four to six months of storage the stability for mineral premix batch 2 seems to increase. This observation can be explained by the challenging matrix resulting in a high coefficient of variation. For comparison, the intermediate precision (one analyst, two days) is 10 % as presented by the validation report for mineral premix.
During storage in the refrigerator (6°C) activity losses were higher (b) (4) in comparison with storage losses at (b) (4) Most probably an additional water update due to condensation effects reduced the stability in the refrigerator.
Finally, after storage at (b) (4) %r.h. the retention after 6 months of storage was still (b) (4) %.

Date of issue: 2020-06-16

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(b) (4)
Dr. Benjamin Peikert

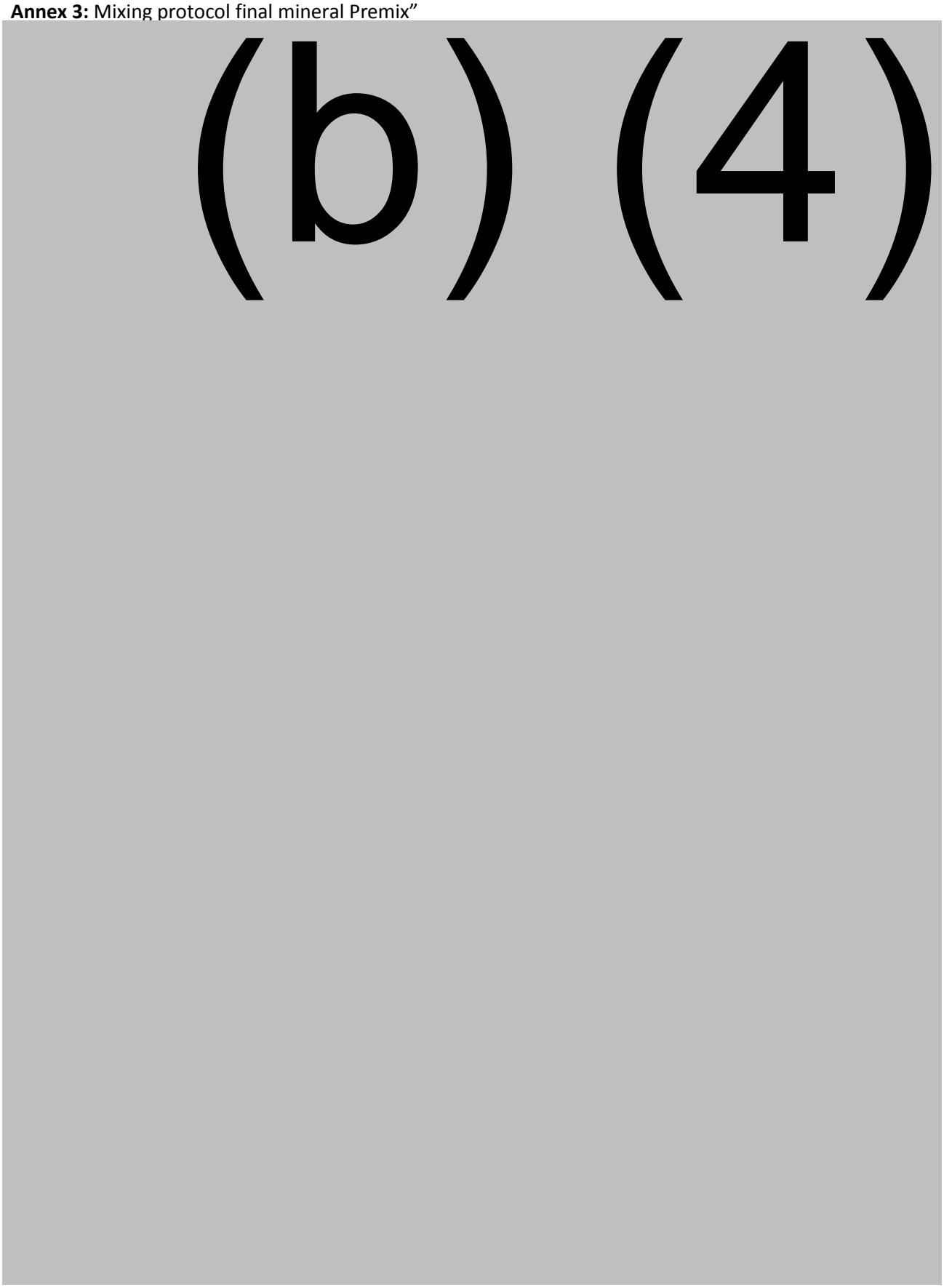
Annex 1: Composition and nutrient contents of “Base-Mix”



Annex 2: Mixing protocol "Base Mix"

(b) (4)

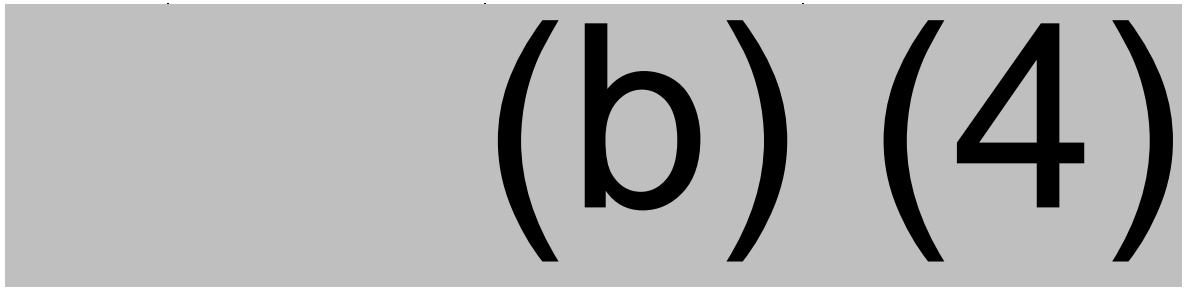
Annex 3: Mixing protocol final mineral Premix”



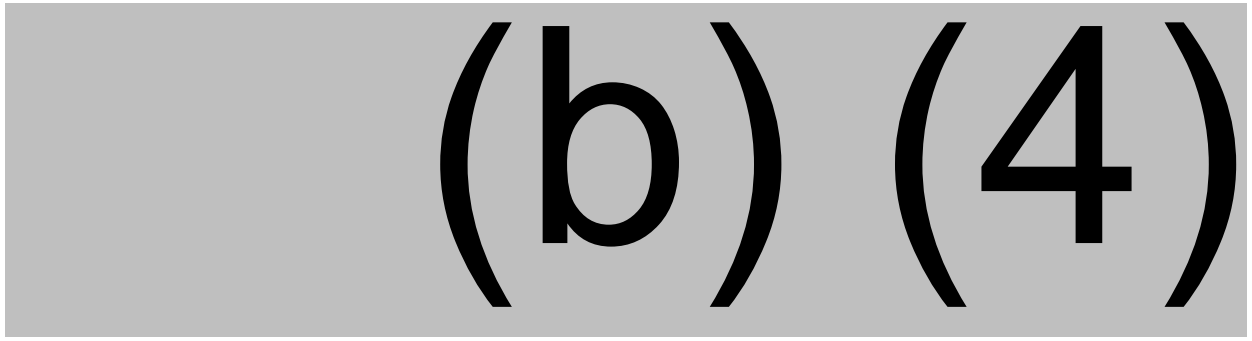
Annex 4: Summary of the methods used

FEN/0004	Photometric assay of mannanase activity in feed additives
FEN/0005	Photometric assay of mannanase activity in feed and mineral premix

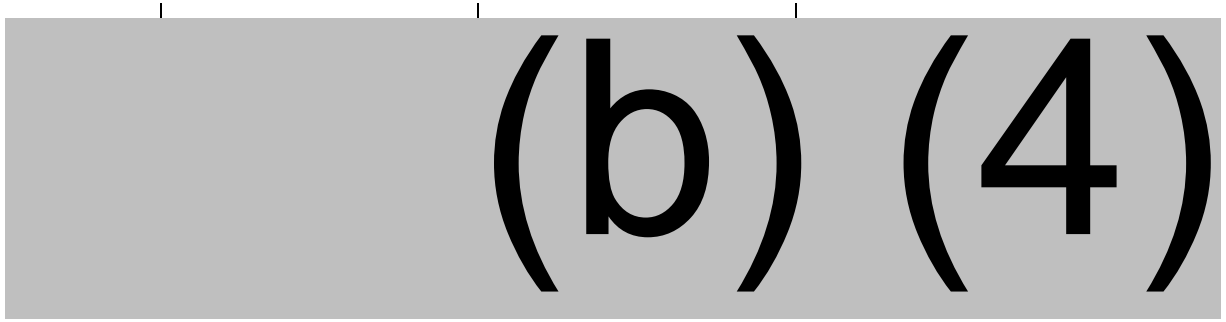
Annex 5: Analyzed activity of mineral premix batch 1 produced with Mannanase (b) (4) (Order Nr. 20F00026)



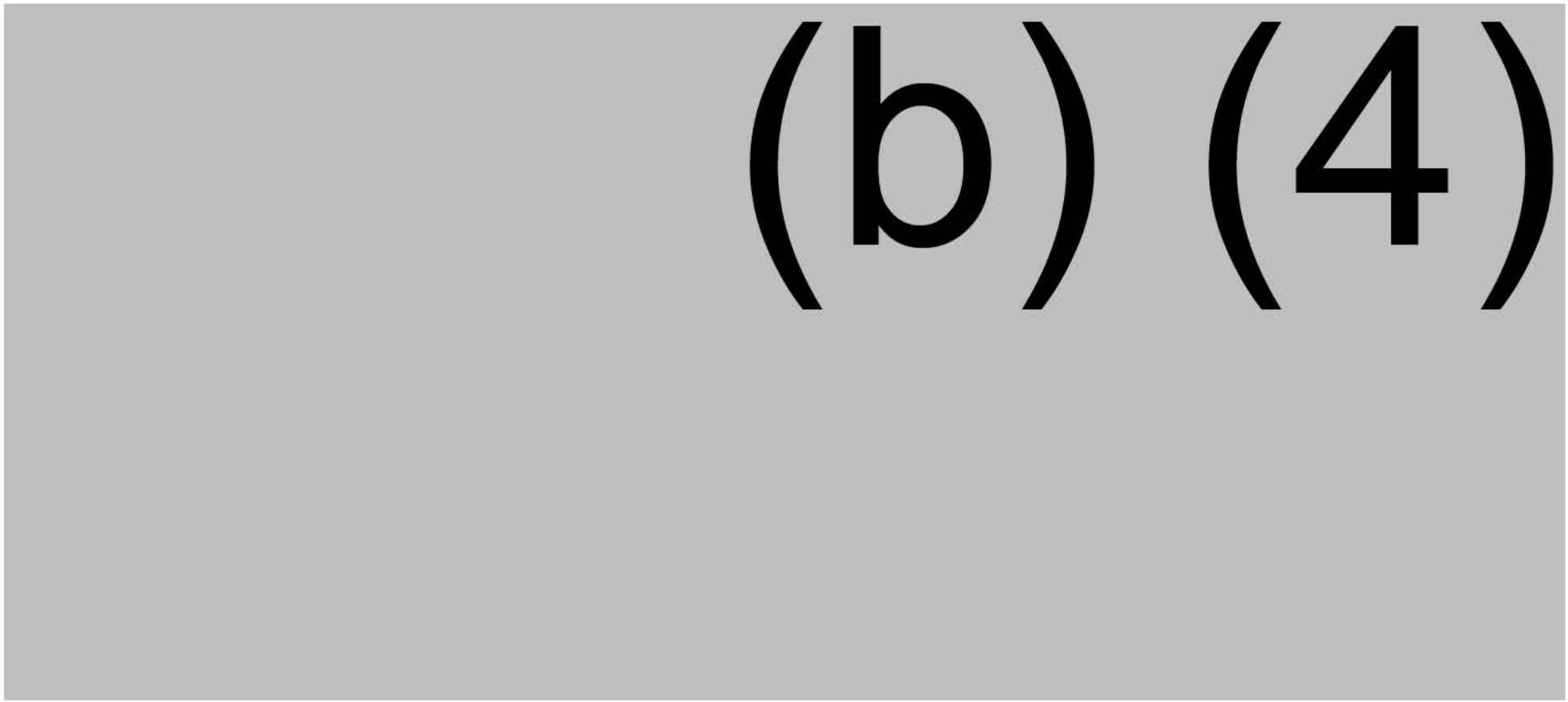
Annex 6: Analyzed activity of mineral premix batch 2 produced with Mannanase (b) (4) (Order Nr. 20F00027)



Annex 7: Analyzed activity of mineral premix batch 3 produced with Mannanase (b) (6) (Order Nr. 20F00028)



Annex 8: Humidity and temperature in climate chamber 2 set at (b) (4) without humidity regulation



Appendix 19: Pelleting Thermostability in Feed

Pelleting Stability of Natupulse® TS

Pelleting stability of Natupulse® TS in feed
(TID-51A-19)

BASF
The Chemical Company

September 9, 2020
Dr. Benjamin Peikert
G-ENA/MD - A 110
Tel.: 0621/60 52129

1. Introduction

The stability during pelleting process of Natupulse® TS, a powder formulation containing the feed additive Mannanase produced by Thermothelomyces thermophila strain LU19287 (DSM 33149) has been established. Three different lots of a typical poultry feed were prepared and pelleted.

2. Material and methods

(b) (4)

2.1. Diets

(b) (4)

2.2. Sampling and pelleting temperature

(b) (4)

2.3. Chemical analysis

(b) (4)

2.4. Calculation

Characterizing the mixing process and the losses after pelleting of a feed additive in feed, the retention (pelleting yield) was calculated according to the following formula:

$$\text{retention (\%)} = \frac{\text{analyzed activity of pelleted feed (TMU/kg)}}{\text{analyzed activity of mash feed (TMU/kg)}} \times 100$$

Results and discussion

During mixing process no disturbances occurred. The intended pelleting temperatures were reached showing a low variation (see Table 3)

Table 3 Measured pelleting temperatures

(b) (4)

A summary of the analyzed activities is shown in Table 4 to Table 6 (detailed data presented in Annex **3**). The three poultry mash feed batches have an activity of (b) (4) kg, respectively. This results in a dosing accuracy of 100-108 %. The coefficient of variation of all analyzed treatments lies in an acceptable range of 0.8-10.8 %.
While the retention of mannanase activity ranges between (b) (4) % for feed samples pelleted at 80 and 85 °C, only (b) (4) % mannanase activity is retained at 90°C. Although enzyme activity decreases with pelleting temperature, Natupulse TS shows good pelleting stability.

Table 4 Activity, coefficient of variance (CV) and retention of Mannanase in feed

(b) (4)

Table 5 Activity, coefficient of variance (CV) and retention of Mannanase in feed

(b) (4)

Table 6 Activity, coefficient of variance (CV) and retention of Mannanase in feed

(b) (4)

3. Conclusions

It was demonstrated that Natupulse TS shows good pelleting stability under pelleting conditions commonly practiced in the feed industry.
While the retention of mannanase activity ranges between (b) (4) for feed samples pelleted at 80 and 85 °C, only (b) (4) mannanase activity is retained at 90°C.

Date of issue: September 9, 2020

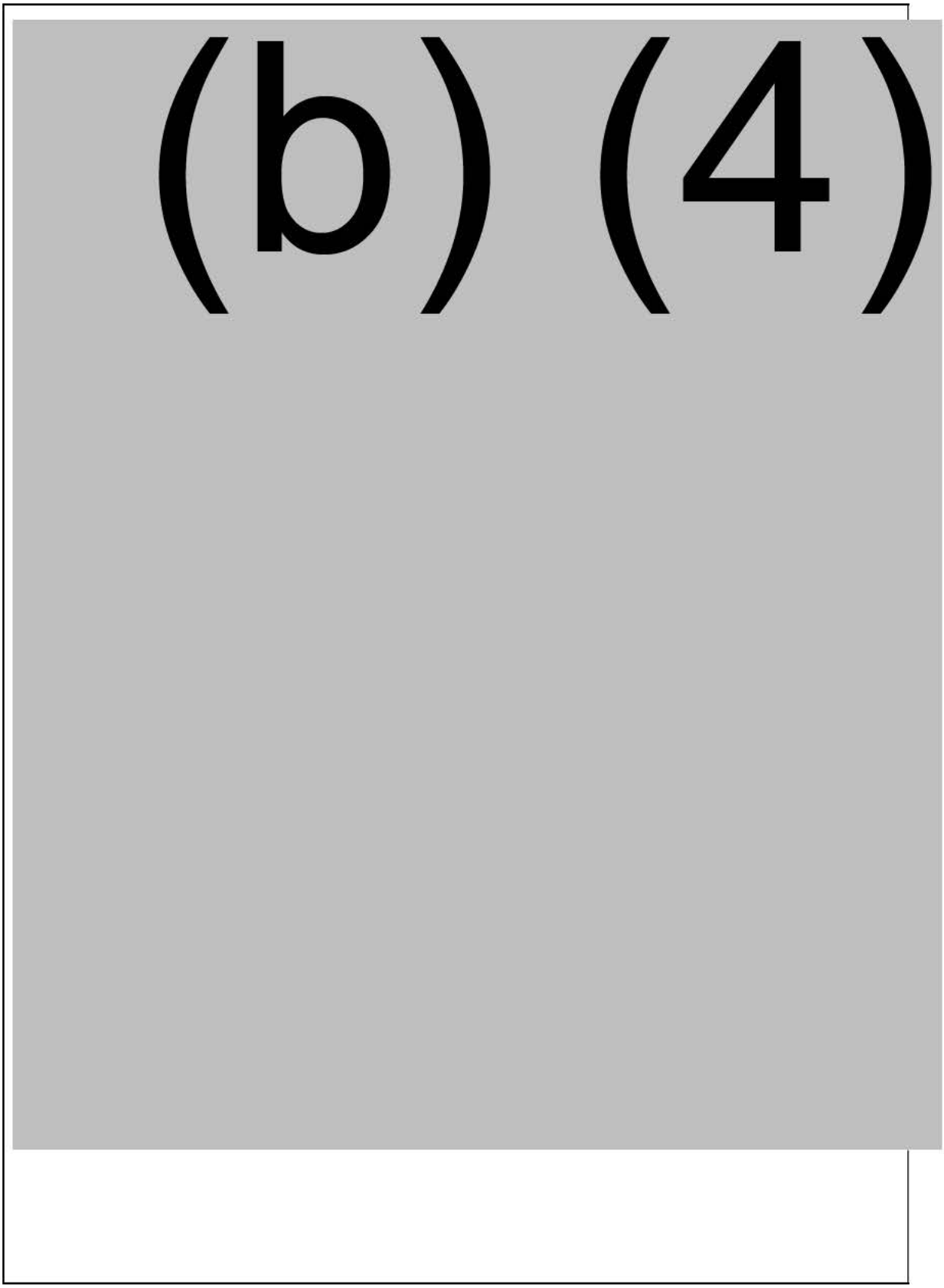
BASF SE
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Analytics & Application Technology Feed Enzymes

(b) (6)

Dr. Benjamin Peikert

4. Annexes

Annex 1 Composition and nutrient contents of the feed



Annex 2 Summary of the methods used

FEN/0004 Photometric assay of mannanase activity in feed additives

FEN/0005 Photometric assay of mannanase activity in feed and mineral premix

Annex 3 Analyzed activity in feed pellets (TMU/kg)

	sample A	sample B	sample C	Average	CV	Retention
	(b) (4)					

Appendix 20: Homogeneity

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Appendix 20a: Natupulse® Homogeneity in Feed or Premix

Appendix 20a- 1: Natupulse® TS in Mash and Pellet

Homogeneity in feed

Homogeneity of Natupulse® TS in feed
(TID-51D-19)

BASF
The Chemical Company

August 18, 2020
Dr. Benjamin Peikert
G-ENA/MD - A 110
Tel.: 0621/60 52129

1. Introduction

The homogeneous distribution of Natupulse® TS, a powder formulation containing the feed additive Mannanase produced by Thermothelomyces thermophila strain LU19287 (DSM 33149), was established in feed. In order to examine the homogeneity during the production process, three different lots were mixed in a typical poultry feed and pelleted.

2. Material and methods

(b) (4)

2.1. Diets

(b) (4)

(b) (4)

2.2. Sampling

(b) (4)

2.3. Chemical analysis

(b) (4)

2.4. Calculation

The following parameters characterizing the mixing process and the homogeneous distribution of a feed additive in feed were calculated.

- Relative standard deviation (coefficient of variation) [%]
- Recovery (mixing yield) [%]

Recovery was calculated according to the following formula based on 12 feed samples:

$$\text{recovery (\%)} = \frac{\text{analyzed activity of feed sample (TMU/kg feed)}}{\text{analyzed activity of product (TMU/g) x dosage of product (g/kg feed)}} \text{ x } 100$$

3. Results and discussion

3.1. Mash feed

(b) (4)

Table 3 Activity, coefficient of variance (CV) and recovery of Mannanase in poultry mash feed

(b) (4)

3.2. Feed pellets

(b) (4)

4. Conclusions

It was demonstrated that a homogeneous distribution of Natupulse TS in a feed can be obtained by using standard mixing procedures. Coefficient of variation and recovery have been acceptable for Natupulse TS in mash feed and pelleted feed.

Date of issue: August 18, 2020

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(b) (6)
Dr. Benjamin Peikert

5. Annexes

Annex 1 Composition and nutrient contents of the feed

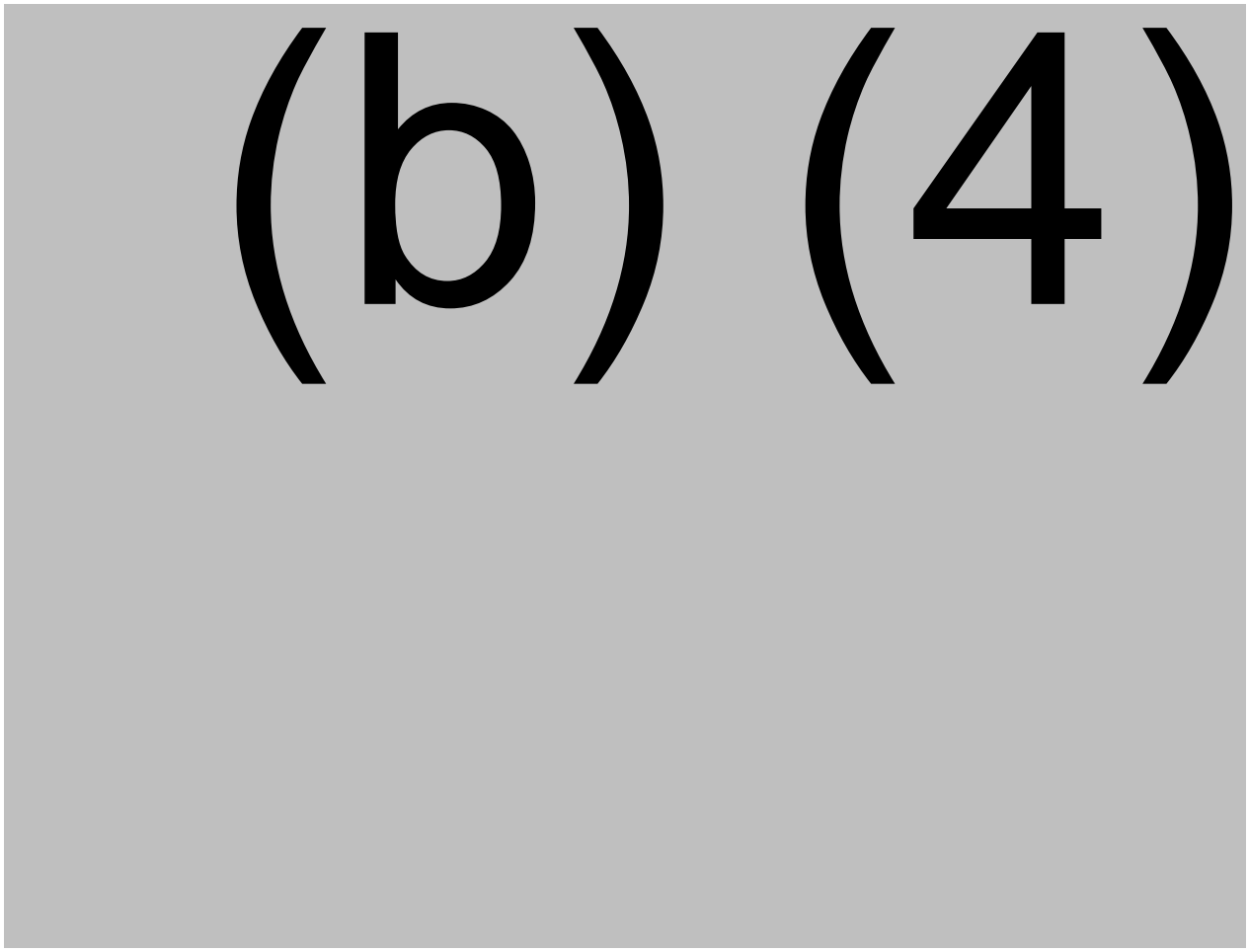
(b) (4)

Annex 2 Summary of the methods used

FEN/0004 Photometric assay of mannanase activity in feed additives

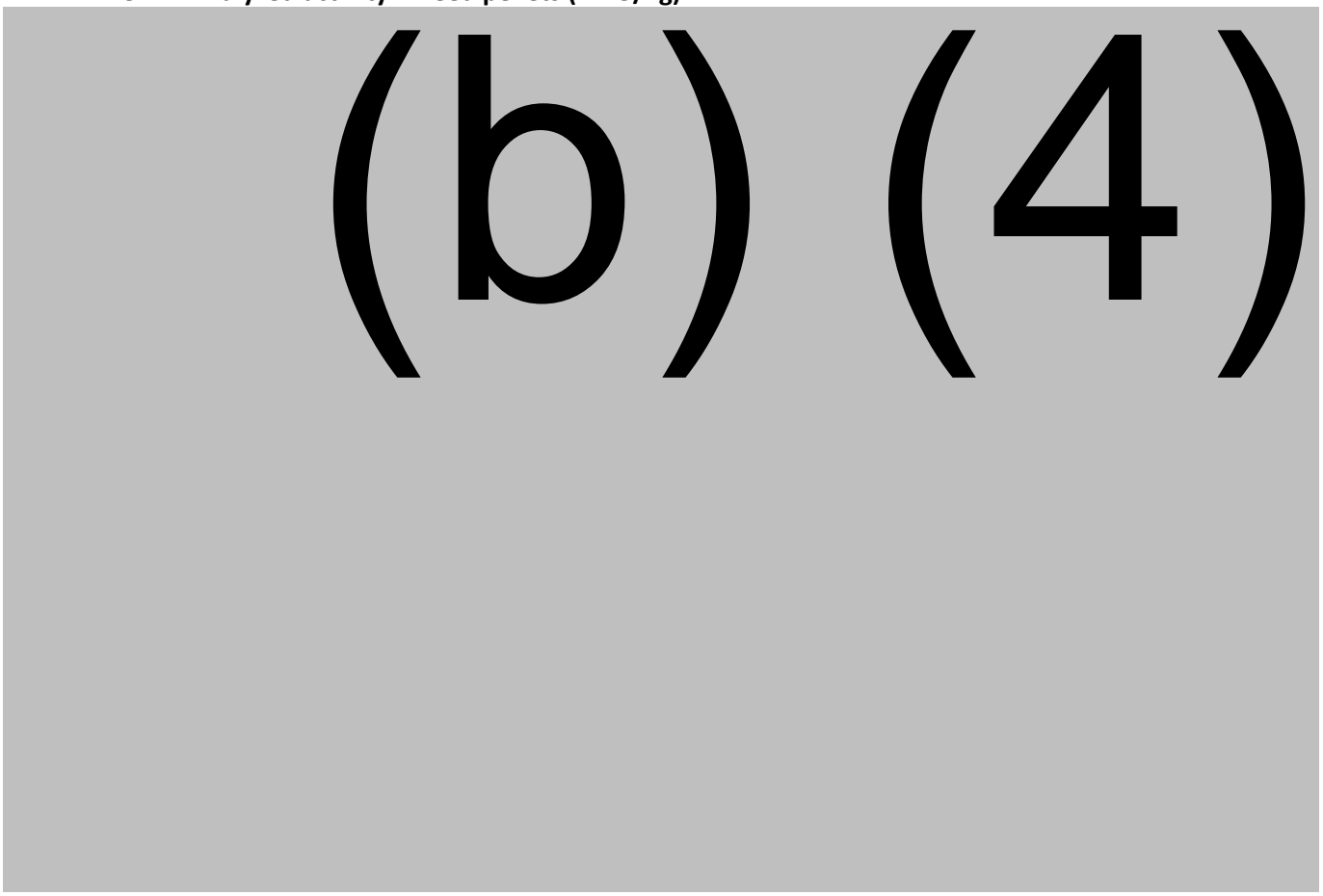
FEN/0005 Photometric assay of mannanase activity in feed and mineral premix

Annex 3 Analyzed activity in mash feed (TMU/kg)



[1] Outlier according to Grubbs's test for outliers

Annex 4 Analyzed activity in feed pellets (TMU/kg)



Appendix 20a- 2: Natupulse® TS L in Feed after PLLA

Homogeneity in feed after PPLA

Homogeneity of Natupulse® TS L after post pellet liquid application (TID-51D-19)

BASF
The Chemical Company

July 24, 2020
Dr. Benjamin Peikert
G-ENA/MD - A 110
Tel.: 0621/60 52129

1. Introduction

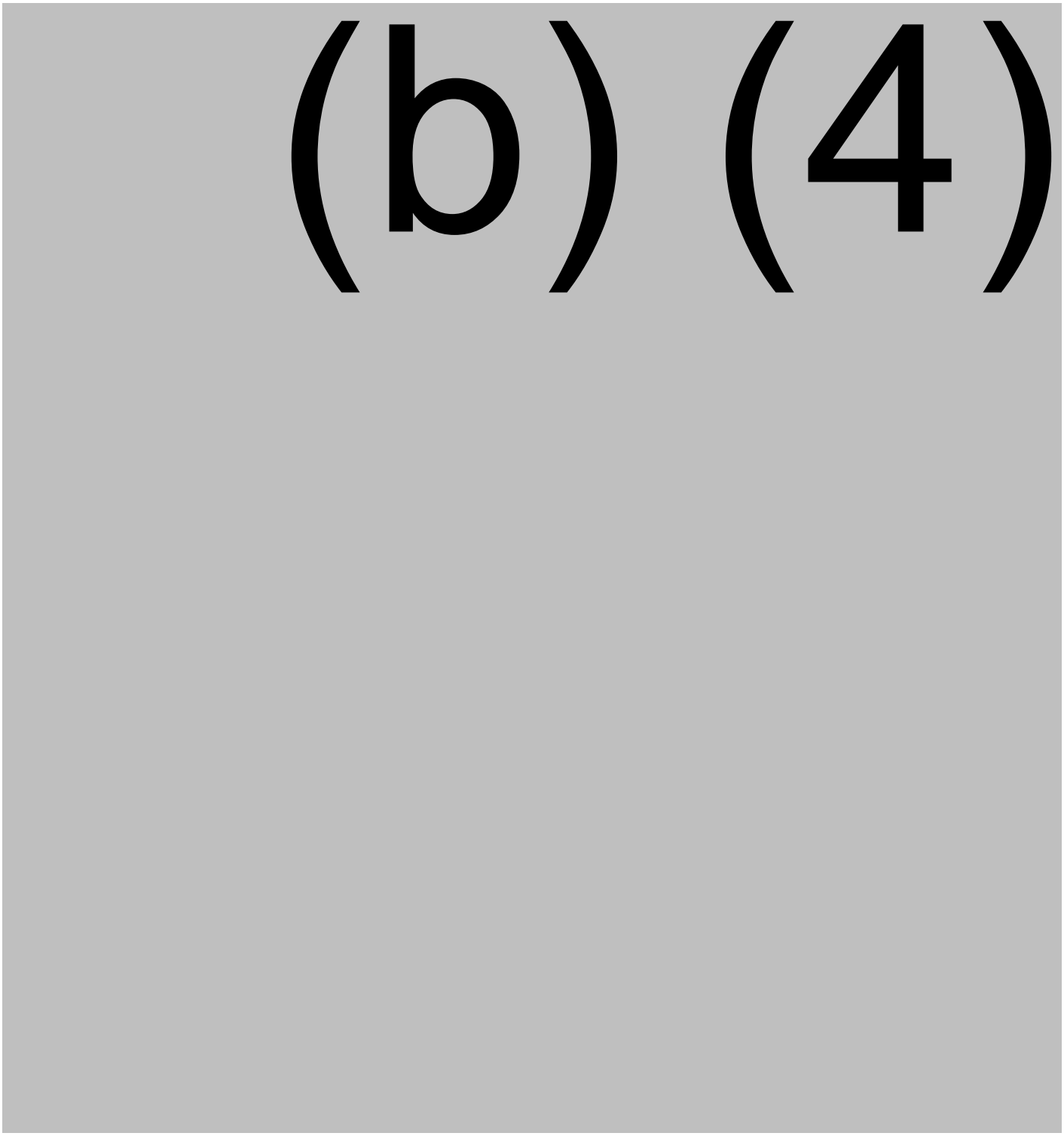
The homogeneous distribution of a Natupulse® TS L, a liquid formulation containing the feed additive Mannanase produced by Thermothelomyces thermophila strain LU19287 (DSM 33149), was established after spraying on a pelletized feeding stuff via a Post Pelleting Liquid Application System (PPLA). In order to examine the homogeneity during the production process, three different lots were of a typical poultry feed were prepared.

2. Material and methods

(b) (4)

2.1. Diets

2.1.1. Pelleting process



2.4. Calculation

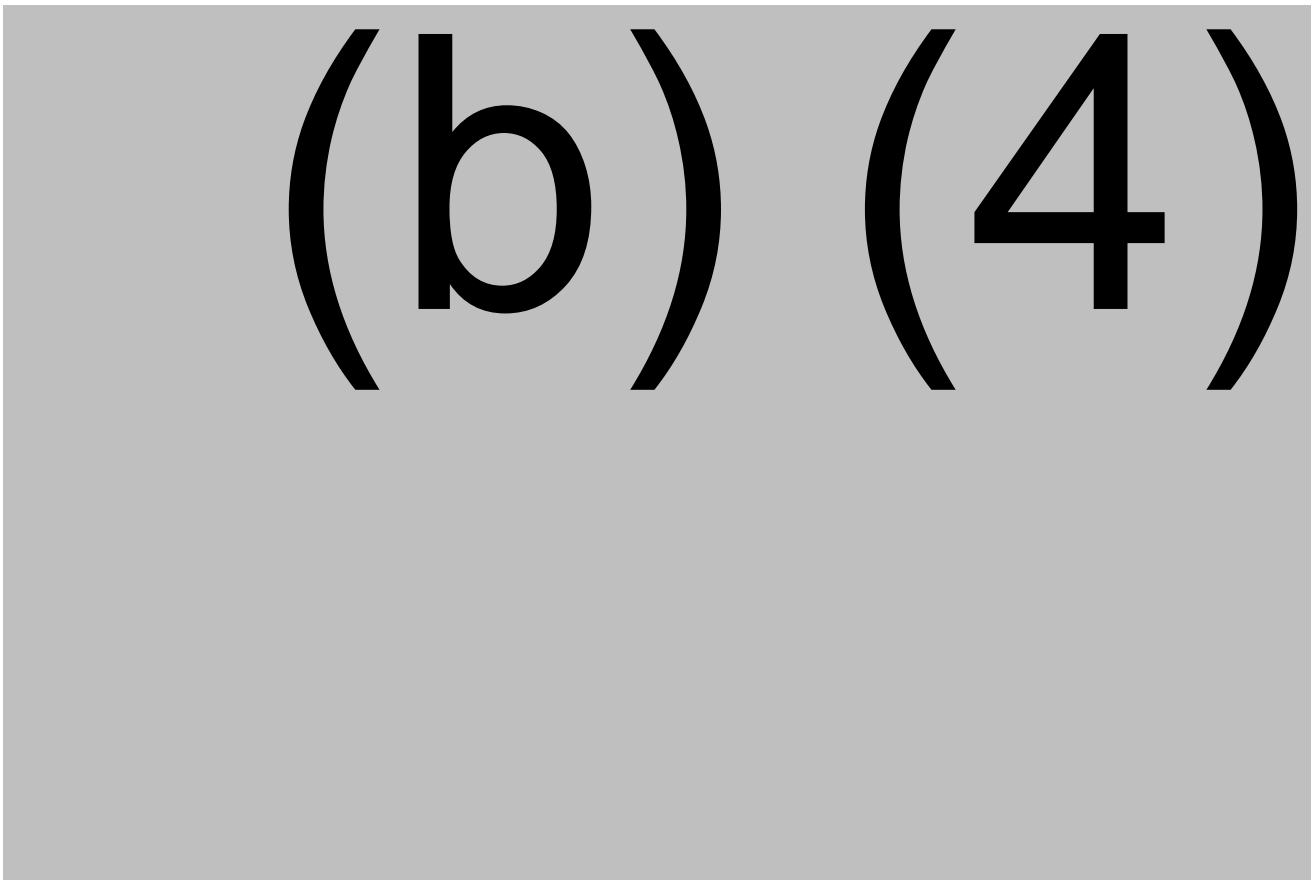
The following parameters characterizing the mixing process and the homogeneous distribution of a feed additive in feed were calculated.

- Relative standard deviation (coefficient of variation) [%]
- Recovery (mixing yield) [%]

Recovery was calculated according to the following formula based on 12 feed samples:

$$\text{recovery (\%)} = \frac{\text{analyzed activity of feed sample (TMU/kg feed)}}{\text{analyzed activity of product (TMU/g) x dosage of product (g/kg feed)}} \text{ x } 100$$

3. Results and discussion



4. Conclusions

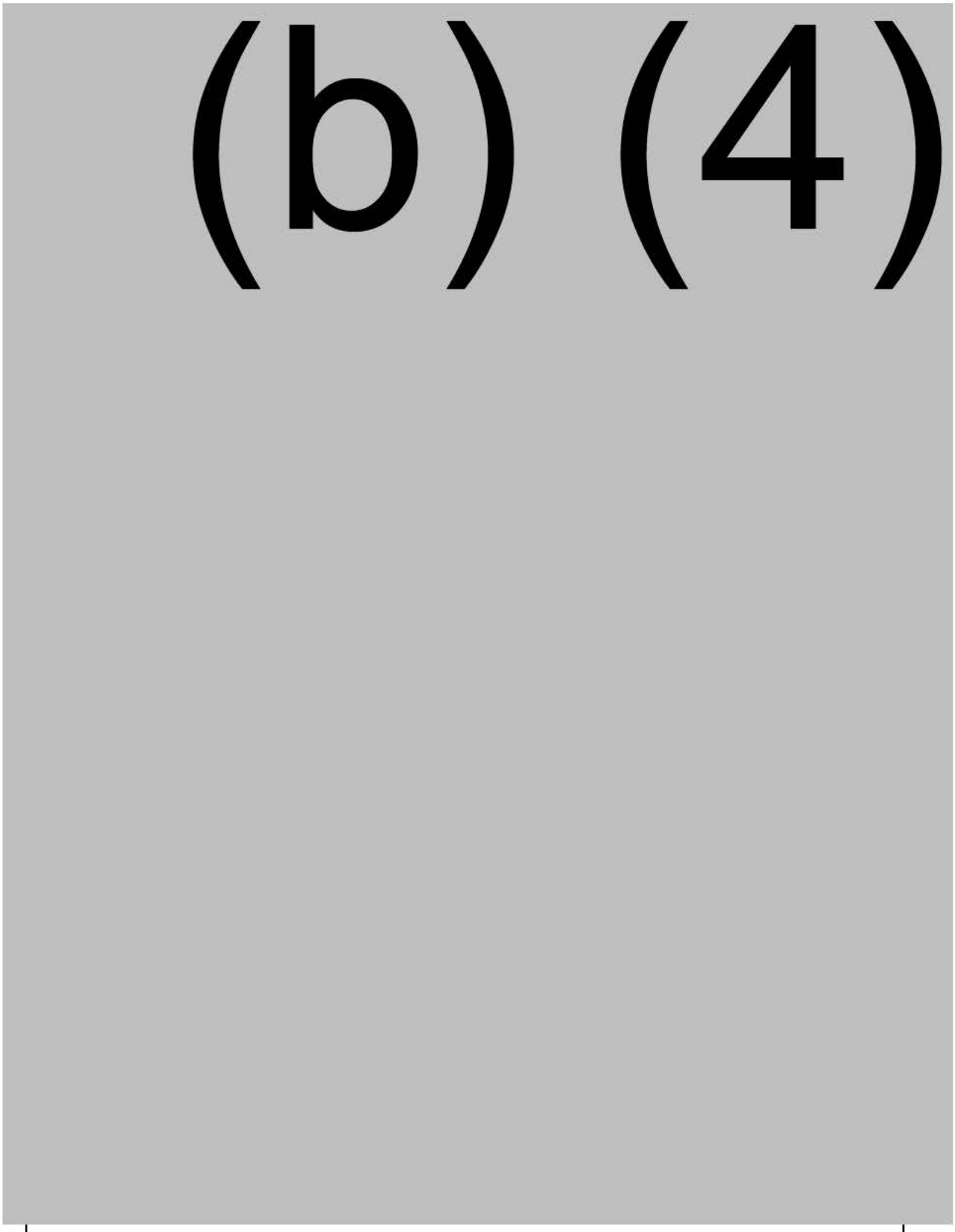
It was demonstrated Natupulse TS L can be homogeneously distributed on feed pellets using a Post Pellet Liquid Application system.

Date of issue: July 24, 2020

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Analytics & Application Technology Feed Enzymes
(b) (6)

5. Annexes

Annex 1 Composition and nutrient contents of the feed

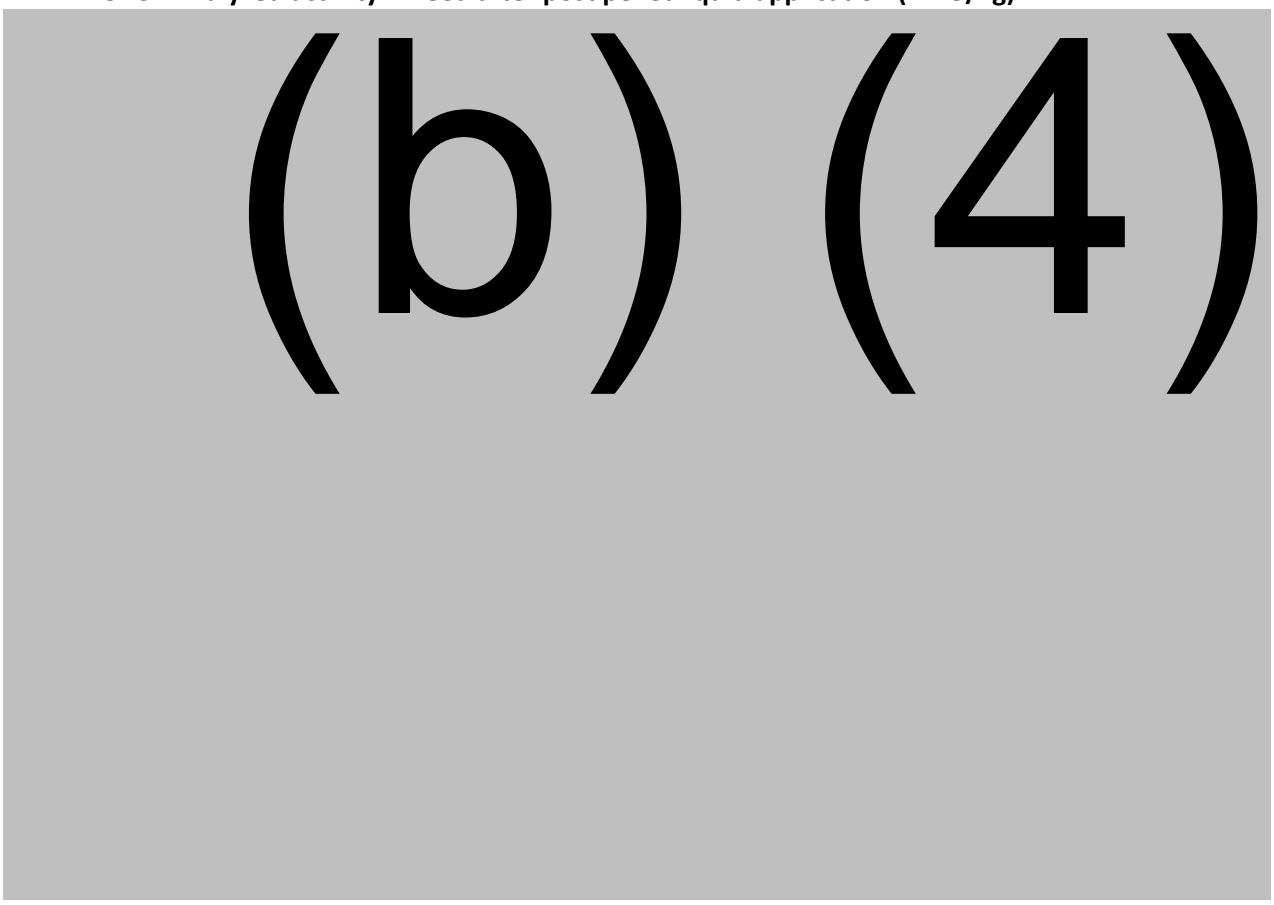


Annex 2 Summary of the methods used

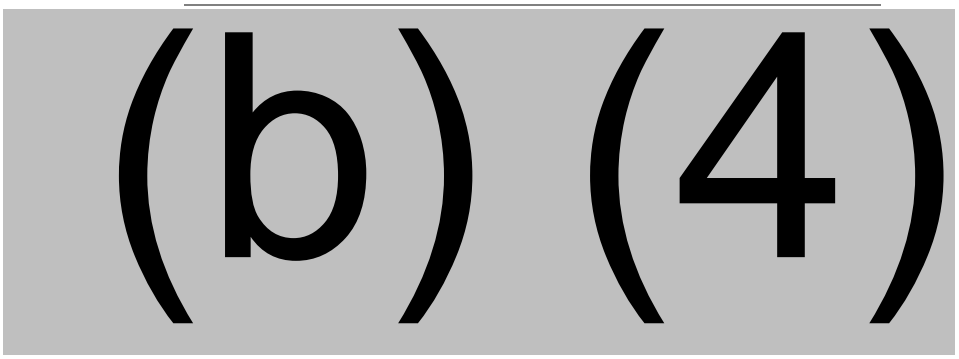
FEN/0004 Photometric assay of mannanase activity in feed additives

FEN/0005 Photometric assay of mannanase activity in feed and mineral premix

Annex 3 Analyzed activity in feed after post pellet liquid application (TMU/kg)



Annex 4 Activity and recovery in feed after post pellet liquid application (TMU/kg)



Appendix 20a- 3: (b) (4) in Premix

Homogeneity in Vitamin and Mineral Premix

Homogeneity of Natupulse® TS in
Vitamin and Mineral Premix (TID-104-18)

BASF
The Chemical Company

September 1, 2020
Dr. Benjamin Peikert
G-ENA/MD - A 110
Tel.: 0621/60 52129

Introduction

The homogeneous distribution of Natupulse® TS containing the feed additive Mannanase produced by Thermothelomyces thermophila strain LU19287 (DSM 33149), was established in a vitamin and mineral premix. In order to examine the homogeneity of the feed additive during production process three different lots were mixed in a typical broiler premix.

Material and methods

(b) (4)

Diets

(b) (4)

Sampling

(b) (4)

Chemical analysis

(b) (4)

Calculation of parameters

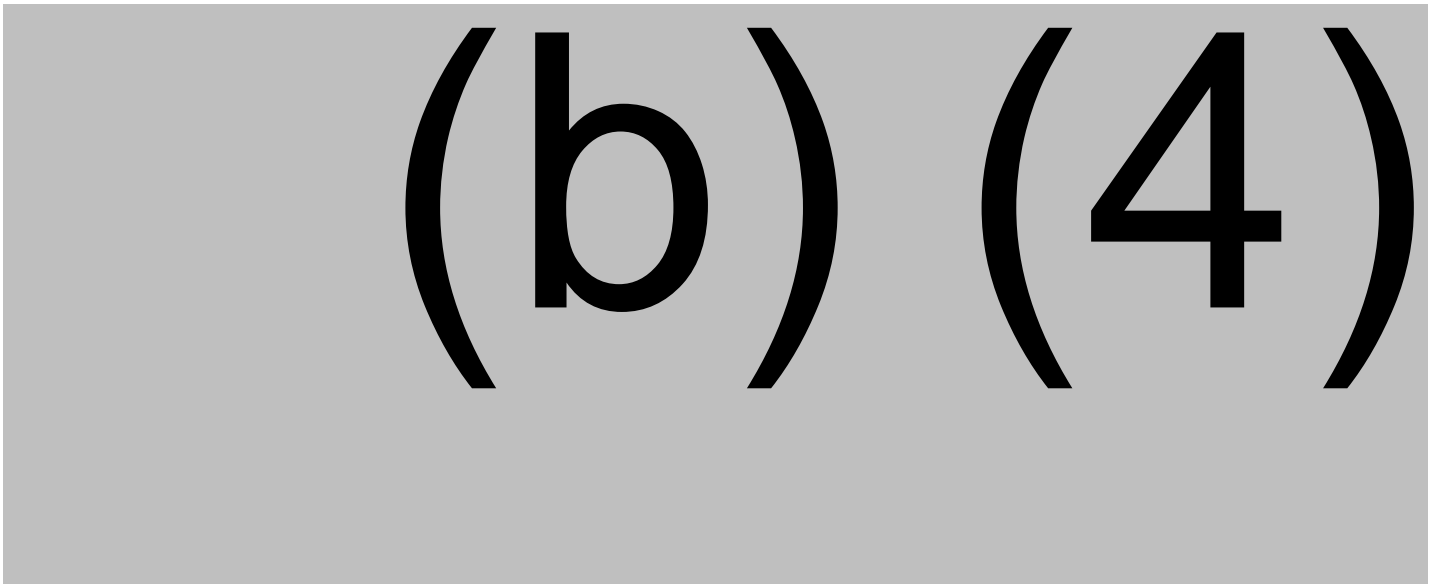
The following parameters characterizing the mixing process and the homogeneous distribution of a feed additive in a mineral premix were calculated.

- Relative standard deviation (coefficient of variation) [%]
- Recovery (mixing yield) [%]

Recovery was calculated according to the following formula based on 12 premix samples:

$$\text{recovery (\%)} = \frac{\text{analyzed activity of premix sample (TMU/kg premix)}}{\text{analyzed activity of product (TMU/g) x dosage of product (g/kg premix)}} \text{ x } 100$$

Results and discussion



Conclusions

It was demonstrated that a homogeneous distribution of Natupulse TS in a vitamin and mineral premix can be obtained by using standard mixing procedures. The coefficient of variation of mineral premixes containing thermostable mannanase from Natupulse was in the range of (b) (4) %. Additionally, the mixing yield (recovery) was in an acceptable range (b) (4).

Date of issue: September 1, 2020

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Analytics & Application Technology Feed Enzymes

(b) (6)

Annex 1: Composition and nutrient contents of "Base-Mix"

Ingredients	Common name	Content	Unit
Calcium	Calcium carbonate	832.7	g/kg
Feed additives			
Vitamin A 1000	Vitamin A acetate	2.0	g/kg
Vitamin D3 500	Cholecalciferol	1.0	g/kg
Vitamin E (50%)	Tocopherol	20.0	g/kg
Vitamin K (44%)	Menadione	0.68	g/kg
Vitamin B1 (100%)	Thiamine hydrochloride	0.4	g/kg
Vitamin B2 (80%)	Riboflavin	1.88	g/kg
Vitamin B12 (1%)	Vitamin B12 supplement	0.4	g/kg
Nicotinic Acid (Niacin) (100%)	Niacin	7.0	g/kg
Pantothenic Acid (98%)	Calcium-pantothenate	2.4	g/kg
Vitamin B6 (Pyridoxine) (100%)	Pyridoxine-hydrochloride	0.7	g/kg
Folic Acid (90%)	Folic acid	0.2	g/kg
Biotin (2%)	Biotin	2.0	g/kg
KI (68% I)	Potassium iodide	0.3	g/kg
Na_2SeO_3 premix (4.5% Se)	Sodium selenite	1.1	g/kg
$FeSO_4$ x 7 H_2O (20% Fe)	Ferrous sulfate	50.0	g/kg
$CuSO_4$ x 5 H_2O (25% Cu)	Copper sulfate	16.0	g/kg
MnO (60% Mn)	Manganese oxide	33.4	g/kg
Zn-oxide (72% Zn)	Zinc oxide	27.8	g/kg

Annex 2: Mixing protocol "Base Mix"

With:

Zuiver mg/kg = targeted amount of feed additive per kg mineral premix

Prep % = amount of active ingredient in feed additive (e.g. B2 = 80% active ingredient)

Omger mg/kg = sample weight of feed additive added to the mineral premix

Kg/g = amount necessary for total mineral premix weight "Hoeveelheid" (e.g. 15kg)

MENGRECEPTFORMULIER

Formulier : RDS/ALG/002 F02
Revisie : 2
Datum : 14/11/2003

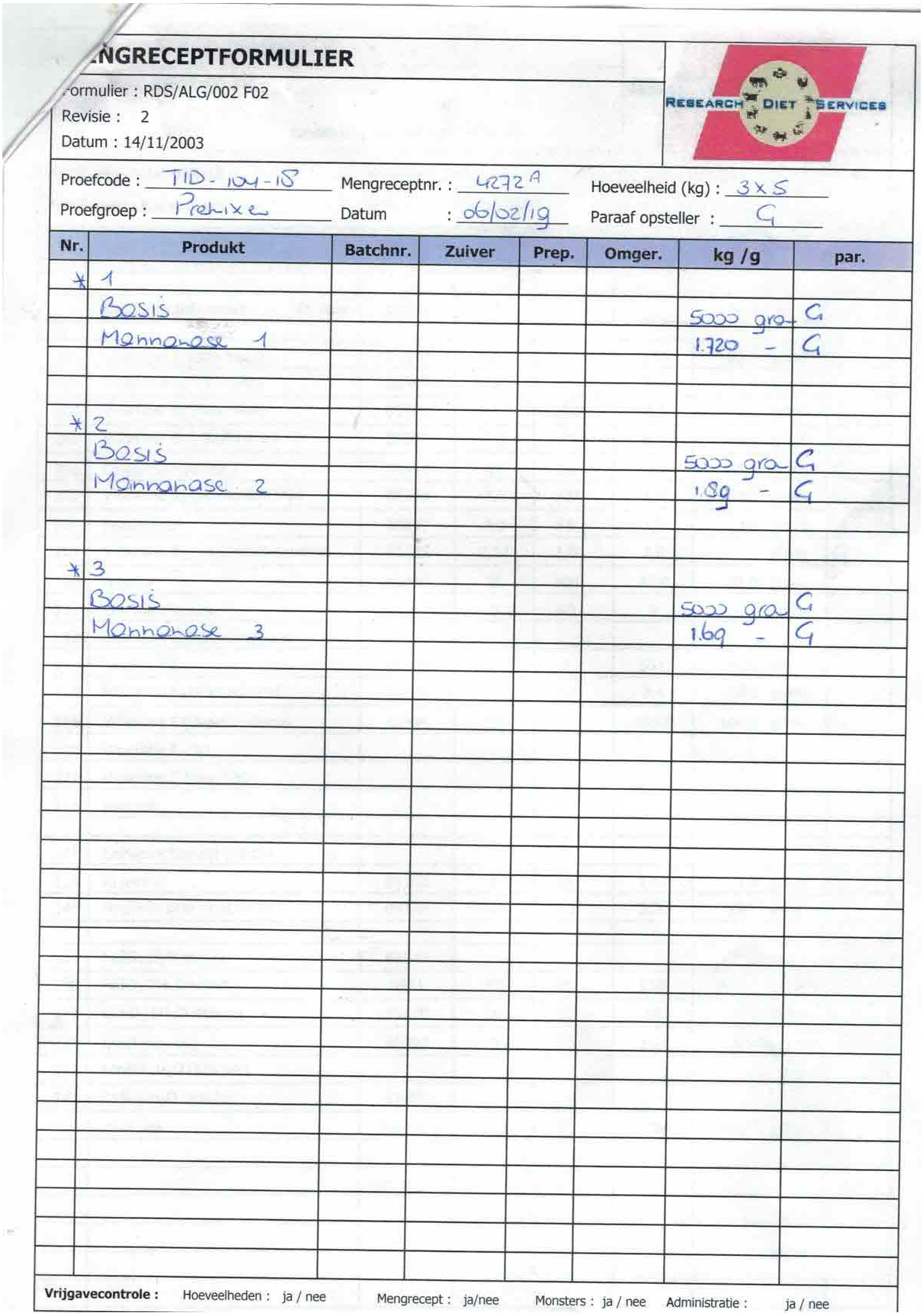
Dosering eindvoer : 0,5 %

RESEARCH DIET SERVICES

Proefcode : TID-104-18 Mengreceptnr. : 4272a Hoeveelheid (kg) : 15 -> 3000 kg
Proefgroep : Basispremix Datum : 06/02/2019 Paraaf opsteller :

Nr.	Produktnaam	Batchnr.	Zuiver mg/kg	Prep. %	Omger. mg/kg	kg /g	par.
	Calciumcarbonaat Drager	**B3427**				12,49 kg	
201	Vitamine A 1000 IU/mg	B3384	10		10,0	30,0 gram	
202	Vitamine D_3 500 IU/mg	B3367	5		5,0	15,0 gram	
204	Vitamine B_1 (thiamine)	B3320	2	100	2,0	6,0 gram	
205	Vitamine B_2 (riboflavine)	B3357	7,5	80	9,4	28,1 gram	
206	Ca-pantotheenzuur	B3419	12	98	12,2	36,7 gram	
207	Vitamine B_6 (pyridoxine HCl)	B3073	3,5	100	3,5	10,5 gram	
208	Foliumzuur	B3277	0,9	90	1,0	3,0 gram	
209	Vitamine B_{12} (cyanocobalamine)	B3282	0,02	1,0	2,0	6,0 gram	
210	Niacine	B3405	35	100	35,0	105,0 gram	
211	Choline chloride		0	50	0	- gram	
212	Vitamine C (ascorbinezuur)						
215	Biotine 2%	B3366	0,2	2	10,0	30,0 gram	
218	Vitamine K_3 (menadione)	B3198	1,5	44	3,4	10,2 gram	
219	Vitamine E (dl-a-tocoferol)	B3396	50	50	100,0	300,0 gram	
220	Vitamine E - SD						
221	Vitamine C Stay-C 35						
216	Inositol						
147	Cobaltcarbonaat (5% Co)						
116	KJ (68% J)	B3163	1	68	1,47	4,4 gram	
146	Na_2SeO_3 premix (4,5% Se)	B3179	0,25	4,5	5,56	16,7 gram	
137	$FeSO_4.H_2O$ (30% Fe)	B3345					
138	$FeSO_4.7H_2O$ (20% Fe)	B931	50	20	250	750,0 gram	
121	$CuSO_4.5H_2O$ (25% Cu)	B3370	20	25	80	240 gram	
125	MnO (60% Mn)	B3322	100	60	167	500 gram	
126	$MnSO_4.H_2O$ (32% Mn)						
141	$ZnSO_4.H_2O$ (36% Zn)	B3257					
	Zn-oxide	B3139	100	72	139	416,7 gram	

Annex 3: Mixing protocol final mineral Premix"



.NGRECEPTFORMULIER

-ormulier : RDS/ALG/002 F02
Revisie : 2
Datum : 14/11/2003

RESEARCH DIET SERVICES

Proefcode : TID - 104 - 18 Mengreceptnr. : 4272 A Hoeveelheid (kg) : 3 x 5
Proefgroep : Premixen Datum : 06/02/19 Paraaf opsteller : G

Nr.	Produkt	Batchnr.	Zuiver	Prep.	Omger.	kg /g	par.
*	1						
	Basis					5000 gra	G
	Mannanase 1					1.720 -	G
*	2						
	Basis					5000 gra	G
	Mannanase 2					1.89 -	G
*	3						
	Basis					5000 gra	G
	Mannanase 3					1.69 -	G

Vrijgavecontrole : Hoeveelheden : ja / nee Mengrecept : ja/nee Monsters : ja / nee Administratie : ja / nee

Annex 4: Summary of the methods used

FEN/0004 Photometric assay of mannanase activity in feed additives

FEN/0005 Photometric assay of mannanase activity in feed and mineral premix

Annex 5: Analyzed activity of mineral premix (TMU/kg)

Product	Mannanase Premix Batch 1	Mannanase Premix Batch 2	Mannanase Premix Batch 3
Lot Nr	012695-L.A	012704-L.A	012706-L.A
Dosage	1.72 g / 5 kg	1.89 g / 5 kg	1.69 g / 5 kg
Activity Product [TMU/g]	236270	221120	244150
Activity Premix 1 [TMU/kg]	82356	80900	92882
Activity Premix 2 [TMU/kg]	69766	76220	98556
Activity Premix 3 [TMU/kg]	91079	72022	81689
Activity Premix 4 [TMU/kg]	77904	84283	86042
Activity Premix 5 [TMU/kg]	75926	64828	69185
Activity Premix 6 [TMU/kg]	79638	84175	67716
Activity Premix 7 [TMU/kg]	94474	78621	95653
Activity Premix 8 [TMU/kg]	86192	84885	62675
Activity Premix 9 [TMU/kg]	79689	89265	86306
Activity Premix 10 [TMU/kg]	94528	86393	80408
Activity Premix 11 [TMU/kg]	88914	77026	88351
Activity Premix 12 [TMU/kg]	93401	62018	88928
Average Premix [TMU/kg]	84489	78386	82132
Stabw. [TMU/kg]	9221	10233	14160
CV [%]	10.9	13.1	17.2
Range [%]	42.7	44.8	67.8
Recovery [%]	104%	112%	100%

LIMS Nr: 20F00026 / 20F00027 / 20F00028

Appendix 20b: Homogeneity of Broiler Study

2020-10-01

BASF SE
G-ENA/MD – A 110
Dr. Benjamin Peikert
Carl-Bosch-Strasse 38
67056 Ludwigshafen
Germany

COLORADO QUALITY RESEARCH, INC.
400 East County Road 72
Wellington, Colorado 80549
USA

Date of order: 2020-08-05
2020-08-10
2020-08-13
2020-08-21

Order number: 20F00248
20F00249
20F00250
20F00251
20F00252

TID-50-20 (BF-20-1) **Efficacy of β-Mannanase on Intestinal Digesta Viscosity in Broiler When Fed Diets Containing Guar Meal**

Diet code	Phase	Feed Type	Diet Description	Expected value [TMU/kg]*	Analytical result [TMU/kg]
T-1	Starter	Crumbles	PC	-	<100
T-2	Starter	Crumbles	NC	-	<100
T-3	Starter	Crumbles	NC + Mannanase (400 TMU/kg)	400	440
T-4	Starter	Crumbles	NC + Mannanase (800 TMU/kg)	800	890
T-5	Starter	Crumbles	NC + Mannanase (1200 TMU/kg)	1200	1500
T-1	Grower	Pellets	PC	-	<100
T-2	Grower	Pellets	NC	-	<100
T-3	Grower	Pellets	NC + Mannanase (400 TMU/kg)	400	440
T-4	Grower	Pellets	NC + Mannanase (800 TMU/kg)	800	1020
T-5	Grower	Pellets	NC + Mannanase (1200 TMU/kg)	1200	1470
T-1	Finisher	Pellets	PC	-	<100
T-2	Finisher	Pellets	NC	-	<100
T-3	Finisher	Pellets	NC + Mannanase (400 TMU/kg)	400	470
T-4	Finisher	Pellets	NC + Mannanase (800 TMU/kg)	800	1060
T-5	Finisher	Pellets	NC + Mannanase (1200 TMU/kg)	1200	1430

The aforementioned data shall constitute the agreed contractual quality of the product at the time of passing of risk. The data are controlled at regular intervals as part of our assurance program. Neither these data nor the properties of product specimens shall imply any legally binding guarantee of certain properties or of fitness for a specific purpose. No liability of ours can be derived therefrom.

Analytical results for Starter, Grower and Finisher are based on duplicates. Mannanase activity was determined according to analytical method FEN/0005.

Additionally, homogeneity was analyzed for Starter T-4 and Grower T-4.

Diet code	Phase	Feed Type	Diet Description	Expected value [TMU/kg]*	Analytical result [TMU/kg]
T-4	Starter	Crumbles	NC + Mannanase (800 TMU/kg)	**800**	1020
T-4	Starter	Crumbles	NC + Mannanase (800 TMU/kg)	**800**	900
T-4	Starter	Crumbles	NC + Mannanase (800 TMU/kg)	**800**	820
T-4	Starter	Crumbles	NC + Mannanase (800 TMU/kg)	**800**	900
T-4	Starter	Crumbles	NC + Mannanase (800 TMU/kg)	**800**	980
T-4	Starter	Crumbles	NC + Mannanase (800 TMU/kg)	**800**	1010
T-4	Starter	Crumbles	NC + Mannanase (800 TMU/kg)	**800**	880
T-4	Starter	Crumbles	NC + Mannanase (800 TMU/kg)	**800**	1020
T-4	Starter	Crumbles	NC + Mannanase (800 TMU/kg)	**800**	940
T-4	Starter	Crumbles	NC + Mannanase (800 TMU/kg)	**800**	980
T-4	Starter	Crumbles	NC + Mannanase (800 TMU/kg)	**800**	940
T-4	Starter	Crumbles	NC + Mannanase (800 TMU/kg)	**800**	950

Average	950
coefficient of variance	6.5 %
Recovery rel. to activity of dosed feed additive	102 %

Diet code	Phase	Feed Type	Diet Description	Expected value [TMU/kg]*	Analytical result [TMU/kg]
T-4	Grower	Crumbles	NC + Mannanase (800 TMU/kg)	**800**	960
T-4	Grower	Crumbles	NC + Mannanase (800 TMU/kg)	**800**	1050
T-4	Grower	Crumbles	NC + Mannanase (800 TMU/kg)	**800**	870
T-4	Grower	Crumbles	NC + Mannanase (800 TMU/kg)	**800**	960
T-4	Grower	Crumbles	NC + Mannanase (800 TMU/kg)	**800**	~~1196~~[1]
T-4	Grower	Crumbles	NC + Mannanase (800 TMU/kg)	**800**	930
T-4	Grower	Crumbles	NC + Mannanase (800 TMU/kg)	**800**	910
T-4	Grower	Crumbles	NC + Mannanase (800 TMU/kg)	**800**	1020
T-4	Grower	Crumbles	NC + Mannanase (800 TMU/kg)	**800**	970
T-4	Grower	Crumbles	NC + Mannanase (800 TMU/kg)	**800**	820
T-4	Grower	Crumbles	NC + Mannanase (800 TMU/kg)	**800**	850
T-4	Grower	Crumbles	NC + Mannanase (800 TMU/kg)	**800**	900

Average	930
coefficient of variance	7.5 %
Recovery rel. to activity of dosed feed additive	100

[1] Outlier according to Grubb's outlier test

The aforementioned data shall constitute the agreed contractual quality of the product at the time of passing of risk. The data are controlled at regular intervals as part of our assurance program. Neither these data nor the properties of product specimens shall imply any legally binding guarantee of certain properties or of fitness for a specific purpose. No liability of ours can be derived therefrom.

* One Thermostable Mannanase Unit (TMU) is defined as the amount of enzyme that liberates 1 µmol reducing sugars determined as Mannose per minute under the conditions of the test method (pH 3.5 and 50°C).

For questions regarding the analytical data please contact Mr. Dirk Ruby (Analytics & Application Technology Feed Enzymes Tel +49 621 60 55778; Mail: ddirk.ruby@basf.com;)

BASF SE
G-ENA/MD
Analytics & Application Technology Feed Enzymes



Dr. Benjamin Peikert

The aforementioned data shall constitute the agreed contractual quality of the product at the time of passing of risk. The data are controlled at regular intervals as part of our assurance program. Neither these data nor the properties of product specimens shall imply any legally binding guarantee of certain properties or of fitness for a specific purpose. No liability of ours can be derived therefrom.

Appendix 21: Feed Ingredients

Ingredients	Common name	AAFCO ID 21 CFR Citation
Maize	Ground corn	48.4
Soybean meal (44% CP)	Soybean Meal	84.7
Extruded full fat soybean meal	Ground Extruded Whole Soybeans	84.15
Soybean oil	Vegetable Oil	33.2
Animal fat (mainly lard)	Animal Fat	33.1
L-lysine-HCl	L-Lysine	6.4
L-threonine	L-Threonine	6.5
DL-methionine	DL-Methionine	6.2
Salt	Salt	57.31
Calcium carbonate	Calcium carbonate	57.1
Dicalcium phosphate	Dicalcium phosphate	57.71
Choline chloride	Choline Chloride	90.25
Vitamin-mineral premix[1]	(see below)	N/A
Noxyfeed®,[2]		
Maxiban® G160[3]		
Titanium dioxide	Titanium dioxide	87.146
Wheat middlings[4]	Wheat Bran	93.1
[1]Vitamin-mineral premix		
vitamin A (3a672)	Vitamin A acetate	90.25
vitamin D3 (E 671)	Vitamin D3	21 CFR 582.5953
vitamin E (alfa-tocopherol, 3a700)	Tocopherol	90.25
vitamin B1 (thiamine mononitrate 3a821)	Thiamine mononitrate	90.25
vitamin B2	Riboflavin	90.25
vitamin B6 (3a831)	Pyridoxine hydrochloride	90.26
vitamin B12	Vitamin B12 supplement	90.11
vitamin K3 (menadione sodium bisulphate, 3a710)	Menadione sodium bisulfite complex	90.25
calcium pantothenate (3a841)	Calcium pantothenate	90.25
nicotinic acid (3a314)	Nicotinic acid	90.25
folic acid (3a316)	Folic acid	90.25
biotin (3a880)	Biotin	90.25
Fe (E-1) (from FeSO4·H2O)	ferrous sulfate monohydrate	57.83
I (3b202) (from Ca(IO3)2)	calcium iodate	57.53
Cu (E-4) (from CuSO4·5H2O)	copper sulfate pentahydrate	57.69
Mn (E-5) (from MnO)	manganese oxide	57.97
Zn (3b603) (from ZnO)	zinc oxide	57.111
Se (E-8) (from Na2SeO3)	sodium selenite	57.119

2 Containing butylated hydroxytoluene, propyl gallate(56%) and citric acid (14%).

3 Providing 50 g narasin + 50 g nicarbazin/metric ton of feed

4 Wheat middlings were replaced by Mannanase 8000 premix at 0.005% (T-2), 0.010% (T-3), 0.015% (T-4) and 1.25% (T-5).

Table 1. Ingredient and nutrient composition of basal feeds

Ingredients (%)	Starter 0-14 d	Grower 14-28 d	Finisher 28-35 d
Maize	49.87	51.30	54.84
Soybean meal (44% CP)	35.15	32.33	26.33
Extruded full-fat soybean meal	6.69	7.63	8.90
Soybean oil	3.00	4.00	2.00
Animal fat (mainly lard)	-	-	3.00
L-lysine-HCl	0.194	0.098	0.102
L-threonine	0.096	0.038	0.030
DL-methionine	0.315	0.256	0.235
Common salt	0.383	0.356	0.353
Calcium carbonate	0.667	0.604	0.549
Dicalcium phosphate	1.962	1.727	1.555
Choline chloride	0.046	0.034	0.039
Vitamin-mineral premix[1]	0.300	0.300	0.300
Noxyfeed[2]	0.020	0.020	0.020
Maxiban G160[3]	0.063	0.063	-
Titanium dioxide	-	-	0.500
Wheat middling[4]	1.250	1.250	1.250
Calculated nutrient levels (g/kg)			
ME (kcal/kg)	2940	3038	3136
Crude protein	225	215	195
Ash	61.4	57.2	57.4
Crude fibre	35.2	34.3	31.9
Crude fat	60.3	72.0	84.4
Digestible lysine	12.54	11.27	10.09
Digestible threonine	8.43	7.55	6.76
Digestible methionine	6.17	5.51	5.05
Digestible Met+Cys	9.31	8.53	7.84
Total calcium	9.60	8.70	7.90
Total phosphorus	7.39	6.89	6.38
Non-phytate phosphorus	4.80	4.35	3.95

[1]The premix provides per kg feed: vitamin A (E 672) 10000 UI; vitamin D_3 (E 671) 4800 UI; vitamin E (alfa tocopherol) 45mg; vitamin B_1 3 mg; vitamin B_2 9mg; vitamin B_6 4.5mg; vitamin B_{12} 40µg; vitamin K_3 3mg; calcium panthotenate 16.5 mg; nicotinic acid 51mg; folic acid 1.8mg; biotin 0.15mg; Fe(E1) (from $FeSO_4 \cdot H_2O$) 54 mg; I(E2) (from $Ca(IO_3)2$) 1.2mg; Cu(E4) (from $CuSO_4 \cdot 5H_2O$)12mg; Mn(E5) (from MnO) 90 mg; Zn(E6) (from ZnO) 66mg; Se(E8) (from Na_2SeO_3) 0.18mg

[2]Contains BHT+ propyl gallate (56%) and citric acid (14%)

[3]Providing 50g narasin + 50g nicarbazin/ton of feed

[4]Wheat middling was replaced by Natupulse® TS or Mannanase product at 0.005% (T-2), 0.010% (T-3), 0.015% (T-4) and 1.25% (T-5)

Appendix 22: Broiler Tolerance Study

(b) (6), (b) (4)

Dose response and tolerance study with a new Mannanase in broiler chickens

(b) (4)

ADAPTED FINAL REPORT

Revision number: Rev. 0

Date: 16th June 2023

RESEARCH ACTIVITY CONTRACTED WITH:

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Study monitor: (b) (4)

(b) (6), (b) (4)

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1. SUMMARY

A trial was conducted to evaluate the dose related effects of Mannanase (b) (4) enzyme product supplementation on performance and apparent ileal digestibility (AID) of dry matter, organic matter, crude protein and ether extract, and on AIDE (apparent ileal digestible energy) in broiler chickens fed a maize-soybean meal based diet. In addition, the tolerance of broiler chickens to this Mannanase product, when administered at more than (b) (4) the maximum recommended dose was evaluated during 35 days. A total of 1845 day-of-hatch male broiler chickens of the Ross 308 strain were allocated to 5 dietary treatments, replicated 9 times each with 41 chickens per replicate, in a randomized complete block design. The treatments consisted of a negative control diet supplemented with Mannanase (b) (4)/kg feed. Feeds were supplied as crumbles from 1-14 d and in pellets of 3 mm afterwards. Starter and grower diets contained narasin and nicarbazin as coccidiostats. Chickens and feed were weighed at (b) (4)d of age, and performance was calculated for each period and for the overall experiment. Apparent ileal digestibility (AID) of dry matter, organic matter, crude protein, ether extract and energy (AIDE) were measured at 36 d of age. Health status of the chickens and mortality were monitored throughout the study. The study was a completely randomized block design of 5 treatments with 9 replications of 41 birds. Data were subjected to ANOVA, and the Tukey adjustment was used to perform multiple comparisons. In addition, polynomial contrasts with the appropriate coefficients were used to determine linear and quadratic responses to increasing doses of mannanase (up to the maximum recommended dose of 1200 TMU/kg). (b) (4)

(b) (4)

2. OBJECTIVES

The objectives of this study were:

- To evaluate the dose related effects of dietary administration of Mannanase (b) (4) enzyme product on performance and apparent ileal digestibility (AID) of dry matter, organic matter, crude protein and ether extract, and on apparent ileal digestible energy (AIDE), in broiler chickens fed a maize-soybean meal based diet.
- To evaluate the tolerance of broiler chickens to Mannanase 8000 enzyme product, when administered at more than (b) (4) the maximum recommended dose during 35 days.

3. MATERIALS AND METHODS

3.1. Animal welfare and quality assurance

(b) (4)

3.2. Animals, housing and husbandry

3.2.1. Animals

Animals: 1845 fattening chickens	Breed/Strain: Ross 308 broiler chickens
(b) (4)	

3.2.2. Husbandry conditions

(b) (4)

(b) (4)

3.2.3. Diets

Feeding program

(b) (4)

Feeds preparation and test products addition

(b) (4)

(b) (4)

(b) (4)

Feeds sampling and analysis

(b) (4)

(b) (4)

(b) (4)

3.3. Study design

3.3.1. Study location

(b) (6), (b) (4)

3.3.2. Dates

The study started at farm on the 12th April 2018 and it finished on the 18th May 2018.

3.3.3. Key study personnel

(b) (4), (b) (6)

3.3.4. Study duration

The study lasted 36 days

(b) (4)

3.3.5. Design of the study

(b) (4)

3.3.6. Experimental groups

(b) (4)

3.3.7. Experimental unit

(b) (4)it.

3.3.8. Basis for the different measurements (e.g. individual animal, pen)

(b) (4)

3.3.9. Randomisation and blinding

Allocation of animals to treatment groups

(b) (4)

(b) (4)

Allocation of treatments groups to experimental units

(b) (4)

Blinding

(b) (4)

3.3.10. Test item

(b) (4)

3.4. Experimental procedures

3.4.1. Zootechnical performance parameters

(b) (4)

(b) (4)

(b) (4)

3.4.2. Digestibility measurement

(b) (4)

(b) (4)

(b) (4)

3.4.3. Health of the animals, morbidity and mortality

(b) (4)

3.5. Statistical methods

3.5.1. Power analysis

(b) (4)

3.5.2. Statistical analysis

(b) (4)

(b) (4)

(b) (4)

4. RESULTS AND DISCUSSION

4.1. Feed analysis and concentration of the active substance in feeds

(b) (4)

(b) (4)

4.2. Performance

(b) (4)

4.3. Apparent ileal digestibility of nutrients

(b) (4)

4.4. Health status, mortality and culling

(b) (4)

According to the inspection performed by the veterinarian practitioner, the probable causes of death were:

Date	Age (d)	Pen	TRT	Body weight (g)	Omphalitis	Chronic respiratory disease	Ascites syndrome	Sudden death syndrome	Decom-posed	Other
16/4/2018	(b) (4)									
16/4/2018										
16/4/2018										
16/4/2018										
16/4/2018										
16/4/2018										
19/4/2018										
21/4/2018										
23/4/2018										
30/4/2018										
30/4/2018										
30/4/2018										
30/4/2018										
30/4/2018										
2/5/2018										
2/5/2018										
4/5/2018										
5/5/2018										
7/5/2018										
11/5/2018										
11/5/2018										
11/5/2018										
14/5/2018										
14/5/2018										

[1]Elargement bursa of Fabricius. [2]Kidney inflammation. [3]Unkow

A total of 16 birds were culled during the trial. The inventory of culled birds by treatment and the reason for culling were:

Date	Age (d)	Pen	TRT	Body weight (g)	Reason
23/4/2018	(b) (4)				
2/5/2018					
4/5/2018					
14/5/2018					
24/4/2018					
17/4/2018					
4/5/2018					
8/5/2018					
16/4/2018					
30/4/2018					
2/5/2018					
16/5/2018					
30/4/2018					
30/4/2018					
2/5/2018					
8/5/2018					

(b) (4)

(b) (4)

(b) (4)

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B.O.E. number 34, Real Decreto 53/2013, de 1 febrero, por el que se establecen las normas básicas aplicables para la protección de los animales utilizados para experimentación y otros fines científicos, incluyendo la docencia.

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Signatures:

(b) (6)

(b) (6)

APPENDIX 1. RESULTS

(b) (4)

Table 1A. Composition and calculated nutrient composition of basal diets

Ingredients (%)	Starter 1-14 d	Grower 15-28 d	Finisher 29-35 d
Maize	49.87	51.30	54.84
Soybean meal (44% CP)	35.15	32.33	26.33
Extruded full fat soybean meal	6.69	7.63	8.90
Soybean oil	3.00	4.00	2.00
Animal fat (mainly lard)	-	-	3.00
L-lysine-HCl	0.194	0.098	0.102
L-threonine	0.096	0.038	0.030
DL-methionine	0.315	0.256	0.235
Salt	0.383	0.356	0.353
Calcium carbonate	0.667	0.604	0.549
Dicalcium phosphate	1.962	1.727	1.555
Choline chloride	0.046	0.034	0.039
Vitamin-mineral premix[1]	0.300	0.300	0.300
Noxyfeed[2]	0.020	0.020	0.020
Maxiban-G160[3]	0.063	0.063	-
Titanium dioxide	-	-	0.500
Wheat middlings[4]	1.250	1.250	1.250
Calculated nutrient levels (g/kg)			
Metabolisable energy (kcal/kg)	2940	3038	3136
			(b) (4)
Crude protein	225	215	195
Ash	61.4	57.2	57.4
Crude fibre	35.2	34.3	31.9
Crude fat	60.3	72.0	84.4
Digestible lysine	12.54	11.27	10.09
Digestible threonine	8.43	7.55	6.76
Digestible methionine	6.17	5.51	5.05
Digestible Met+Cys	9.31	8.53	7.84
Total calcium	9.60	8.70	7.90
Total phosphorus	7.39	6.89	6.38
Non-phytate phosphorus	4.80	4.35	3.95

[1]The vitamin and mineral provided per kg feed: vitamin A (3a672a) 10000 UI; vitamin D3 (3a671) 4800 UI; vitamin E (3a700) 45 mg; vitamin B_1 (3a821) 3 mg; vitamin B_2 9 mg; vitamin B_6 (3a831) 4.5 mg; vitamin B_{12} 40 µg; vitamin K_3 (3a710) 3 mg; calcium panthotenate (3a841) 16.5 mg; nicotinamide (3a315) 51 mg; folic acid (3a316) 1.8 mg; biotin (3a880) 0.15 mg; Fe (3b103) (from FeSO4•H2O) 54 mg; I (3b202) (from $Ca(IO_3)_2$) 1.2 mg; Cu (E4) (from CuSO4•5H2O) 12 mg; Mn (3b502) (from MnO) 90 mg; Zn (3b603) (from ZnO) 66 mg; Se (E 8) (from Na2SeO3) 0.18 mg.

[2] Contains BHT+ propyl galate (56%) and citric acid (14%).

[3] Providing 50 g narasin + 50 g nicarbazin/tonne of feed.

[4] Wheat middlings were replaced by Mannanase 8000 premix at 0.005% (T-2), 0.010% (T-3), 0.015% (T-4) and 1.25% (T-5).

(b) (4)

Table 1B. Composition of the vitamin and mineral premix

	Kg of premix
Vitamins	(b) (4)
Vitamin A (retinyl acetate 3a672a)	
Vitamin D3 (cholecalciferol 3a671)	
Vitamin E (all-rac-alfa-tocopheryl acetate 3a700)	
Vitamin B1 (thiamine mononitrate 3a821)	
Vitamin B2	
Vitamin B6 (pyridoxine chlorhydrate 3a831)	
Vitamin B12	
Vitamin K3 (menadione sodium bisulphate 3a710)	
Calcium D-pantothenate (3a841)	
Nicotinamide (3a315)	
Folic acid (3a316)	
Biotin (3a880)	
Trace elements	
Fe (3b103) (from FeSO4·H2O)	
I (3b202) (from Ca(IO3)2)	
Cu (E4) (from CuSO4·5H2O)	
Mn (3b502) (from MnO)	
Zn (3b603) (from ZnO)	
Se (E-8) (from Na2SeO3)	
Antioxidant	
BHT(E321)	
Anti-caking agents	
Colloidal silica (E551b)	
Sepiolite (E562)	
Preservatives	
Fumaric acid (1a297)	
D,L-malic acid (E296)	
Carrier	
Calcium carbonate	

(b) (4)

Table 2. Feed analysis (as fed)

Treatments	Dry matter (g/kg)	Crude protein (g/kg)	Ether extract (g/kg)	Ash (g/kg)	Gross energy (kcal/kg)
Starter					
T-1					
T-2					
T-3					
T-4					
T-5					
Grower					
T-1					
T-2					
T-3					
T-4					
T-5					
Finisher					
T-1					
T-2					
T-3					
T-4					
T-5					

(b) (4)

Table 3. In-feed mannanase recoveries

	Starter		Grower		Finisher	
	Expected TMU*/kg	Measured TMU/kg	Recovery %	Measured TMU/kg	Recovery %	Measured TMU/kg	Recovery %
T-1							
T-2							
T-3							
T-4							
T-5							

(b) (4)

*One mannanase unit (TMU) is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one µmol mannose from locust bean gum (0.3 g/100 ml buffer solution) in one minute under the assay conditions of 50.0 +/-0.1 °C and pH 3.5.

(b) (4)

Table 4. Effect of dietary treatment on performance of broiler chickens from 1-14 d of age

	Average body weight 1 d (g)	Average body weight 14 d (g)	Average daily gain (g)	Average daily feed intake (g)	Feed to gain ratio (g feed/ g gain)
Negative control (NC)	(b) (4)				
NC + 400 TMU/kg feed					
NC + 800 TMU/kg feed					
NC + 1200 TMU/kg feed					
NC + 100000 TMU/kg feed					
Standard error					
P-values					
Treatment effect					
NC vs. mannanase					
Mannanase effect[1]					
Linear					
Quadratic					

Values are least-square means of 9 replicates of 41 chickens per treatment at the beginning of the study.
[1]Using 0, 400, 800 and 1200 TMU/kg.

Table 5. Effect of dietary treatment on performance of broiler chickens from 15-28 d of age

	Average body weight 14 d (g)	Average body weight 28 d (g)	Average daily gain (g)	Average daily feed intake (g)	Feed to gain ratio (g feed/ g gain)
Negative control (NC)	(b) (4)				
NC + 400 TMU/kg feed					
NC + 800 TMU/kg feed					
NC + 1200 TMU/kg feed					
NC + 100000 TMU/kg feed					
Standard error					
P-values					
Treatment effect					
NC vs. mannanase					
Mannanase effect[1]					
Linear					
Quadratic					

Values are least-square means of 9 replicates of 41 chickens per treatment at the beginning of the study.

(b) (4)

(b) (4)

Table 6. Effect of dietary treatment on performance of broiler chickens from 29-35 d of age

	Average body weight 28 d (g)	Average body weight 35 d (g)	Average daily gain (g)	Average daily feed intake (g)	Feed to gain ratio (g feed/ g gain)
Negative control (NC)	(b) (4)				
NC + 400 TMU/kg feed					
NC + 800 TMU/kg feed					
NC + 1200 TMU/kg feed					
NC + 100000 TMU/kg feed					
Standard error					
P-values					
Treatment effect					
NC vs. mannanase					
Mannanase effect[1]					
Linear					
Quadratic					

(b) (4)

Table 7. Effect of dietary treatment on performance of broiler chickens from 1-35 d of age

	Average body weight 1 d (g)	Average body weight 35 d (g)	Average daily gain (g)	Average daily feed intake (g)	Feed to gain ratio (g feed/ g gain)	EPEF[2]
Negative control (NC)	(b) (4)					
NC + 400 TMU/kg feed						
NC + 800 TMU/kg feed						
NC + 1200 TMU/kg feed						
NC + 100000 TMU/kg feed						
Standard error						
P-values						
Treatment effect						
NC vs. mannanase						
Mannanase effect[1]						
Linear						
Quadratic						

Values are least-square means of 9 replicates of 41 chickens per treatment at the beginning of the study.
[1]Using 0, 400, 800 and 1200 TMU/kg.

(b) (4)

Table 8. Effect of dietary treatment on apparent ileal digestibility of nutrients at 36 d of age

	Apparent ileal digestibility (%)					AIDE[2] (kcal/kg)
	Dry matter	Organic matter	Crude protein	Ether extract	Gross energy	
Negative control (NC)	(b) (4)					
NC + 400 TMU/kg feed						
NC + 800 TMU/kg feed						
NC + 1200 TMU/kg feed						
NC + 100000 TMU/kg feed						
Standard error						
P-values						
Treatment effect						
NC vs. mannanase						
Mannanase effect[1]						
Linear						
Quadratic						

Values are least-square means of 9 replicates (pool from 4 chickens per replicate) per treatment.
[1]Using 0, 400, 800 and 1200 TMU/kg.
[2]AIDE=apparent ileal digestible energy.

Table 9. Effect of dietary treatment on percentage of dead and culled birds trough the study

	% Dead birds				% Culled birds			
	1-14 d	15-28 d	29-35 d	1-35 d	1-14 d	15-28 d	29-35 d	1-35 d
Negative control (NC)	(b) (4)							
NC + 400 TMU/kg feed								
NC + 800 TMU/kg feed								
NC + 1200 TMU/kg feed								
NC + 100000 TMU/kg feed								
Standard error								
P-values								
Treatment effect								
NC vs. mannanase								
Mannanase effect[1]								
Linear								
Quadratic								

Values are least-square means of 9 replicates of 41 chickens per treatment at the beginning of the study.
[1]Using 0, 400, 800 and 1200 TMU/kg.

APPENDIX II. CERTIFICATE OF ANALYSIS

(b) (4)

BASF
We create chemistry

Analytical Report

2019-08-12

BASF SE
G-ENA/MD – A 110
Dr. Benjamin Peikert
Carl-Bosch-Strasse 38
67056 Ludwigshafen
Germany

(b) (6), (b) (4)

Date of order: 2018-05-02

Order number: 19F00260

(b) (6), (b) (4) **Dose response and tolerance study with a new Mannanase in broiler chickens**

Sample identification	Mannanase CC batch	Formulation batch	Parameter	Analytical Result	Unit
Mannanase	(b) (4)		Mannanase activity	(b) (4)	TMU/g*
Mannanase			Mannanase activity		TMU/g*
Mannanase			Mannanase activity		TMU/g*
Mannanase			Mannanase activity		TMU/g*

* One Thermostable Mannanase Unit (TMU) is defined as the amount of enzyme that liberates 1 µmol reducing sugars determined as Mannose per minute under the conditions of the test method.

Analysis was performed according to FEN/0004. Analytical results were presented as mean values based on duplicates.

For questions regarding the analytical data please contact (b) (6)
(b) (6)

BASF SE
G-ENA/MD
Analytics & Application Technology Feed Enzymes
(b) (6)
Dr. Benjamin Peikert

The aforementioned data shall constitute the agreed contractual quality of the product at the time of passing of risk. The data are controlled at regular intervals as part of our assurance program. Neither these data nor the properties of product specimens shall imply any legally binding guarantee of certain properties or of fitness for a specific purpose. No liability of ours can be derived therefrom.

(b) (6)

BASF
We create chemistry

Analytical Report

2019-08-12

BASF SE
G-ENA/MD – A 110
Dr. Benjamin Peikert
Carl-Bosch-Strasse 38
67056 Ludwigshafen
Germany

(b) (6), (b) (4)

Date of order: 2018-05-07
2018-05-08
2018-05-30
2018-07-10

Order number: 19F00261
19N01036
19F00263

(b) (4) **Dose response and tolerance study with a new Mannanase in broiler chickens**

Diet code	Phase	Diet Description	Expected [TMU/kg]*	Average [TMU/kg]*
T-1	Starter	(b) (4)		
T-2	Starter			
T-3	Starter			
T-4	Starter			
T-5	Starter			
T-1	Grower			
T-2	Grower			
T-3	Grower			
T-4	Grower			
T-5	Grower			
T-1	Finisher			
T-2	Finisher			
T-3	Finisher			
T-4	Finisher			
T-5	Finisher			

Analytical results are based on duplicates. For T3 (Grower) the analytical result is based on six values. Mannanase activity was determined according to analytical method FEN/0005.

* One Thermostable Mannanase Unit (TMU) is defined as the amount of enzyme that liberates 1 µmol reducing sugars determined as Mannose per minute under the conditions of the test method (pH 3.5 and 50°C).

The aforementioned data shall constitute the agreed contractual quality of the product at the time of passing of risk. The data are controlled at regular intervals as part of our assurance program. Neither these data nor the properties of product specimens shall imply any legally binding guarantee of certain properties or of fitness for a specific purpose. No liability of ours can be derived therefrom.

APPENDIX III. RAW DATA

(b) (4)

Table 10. Performance from 1-14 d of age raw data

PEN	TRT	BLOCK	Body weight D1 (g)	Body weight D14 (g)	Average daily gain (g/d)	Average daily feed intake (g/d)	FCR (g feed/g gain)	Dead birds (%)	Culled birds (%)
(b) (4)									

(b) (4)

Table 11. Performance from 15-28 d of age raw data

PEN	TRT	BLOCK	Body weight D14 (g)	Body weight D28 (g)	Average daily gain (g/d)	Average daily feed intake (g/d)	FCR (g feed/g gain)	Dead birds (%)	Culled birds (%)
(b) (4)									

(b) (4)

Table 12. Performance from 29-35 d of age raw data

PEN	TRT	BLOCK	Body weight D28 (g)	Body weight D35 (g)	Average daily gain (g/d)	Average daily feed intake (g/d)	FCR (g feed/g gain)	Dead birds (%)	Culled birds (%)
(b) (4)									

(b) (4)

Table 13. Performance from 1-35 d of age raw data

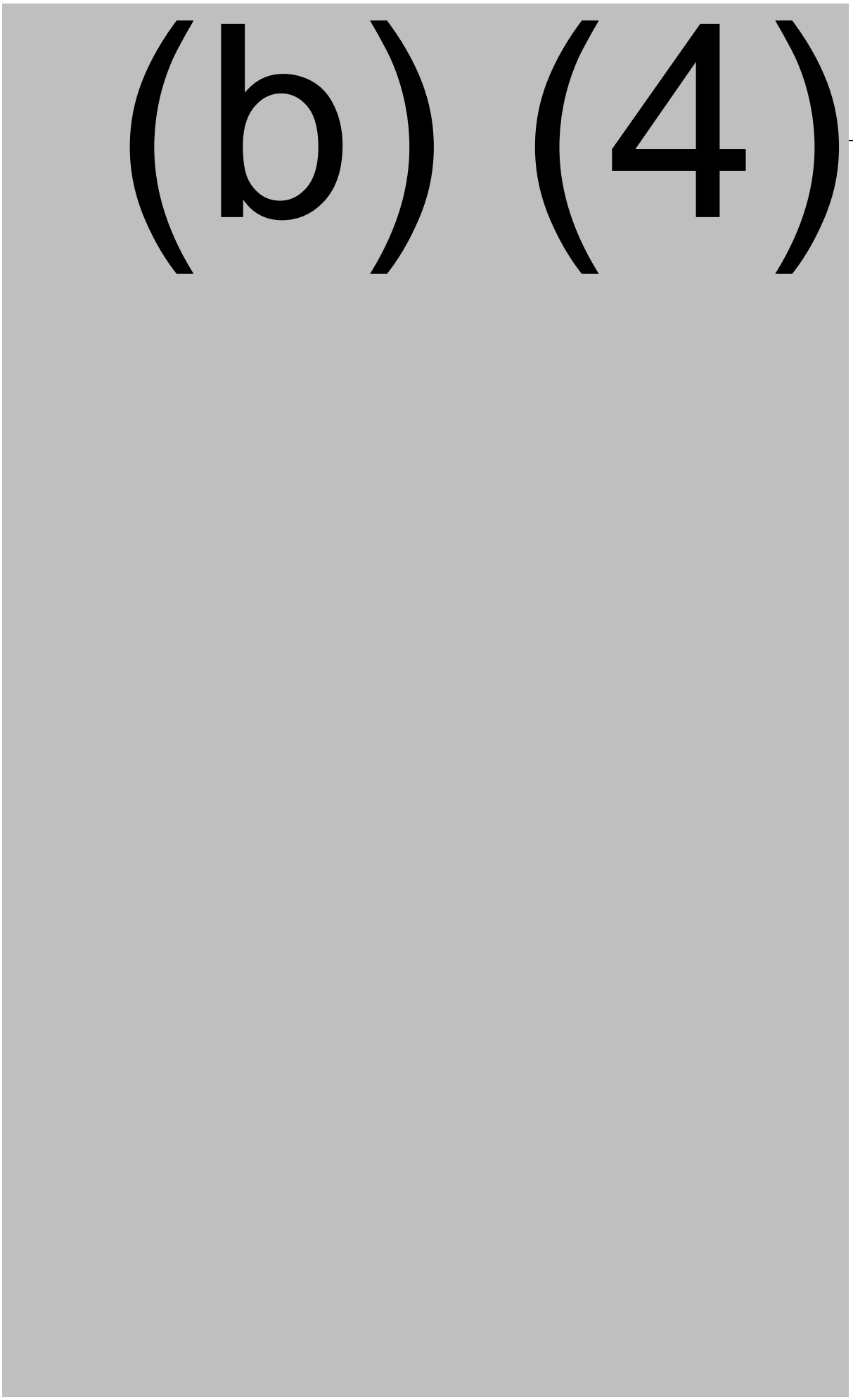
PEN	TRT	BLOCK	Body weight D1 (g)	Body weight D35 (g)	Average daily gain (g/d)	Average daily feed intake (g/d)	FCR (g feed/g gain)	European production efficiency factor	Dead birds (%)	Culled birds (%)
(b) (4)										

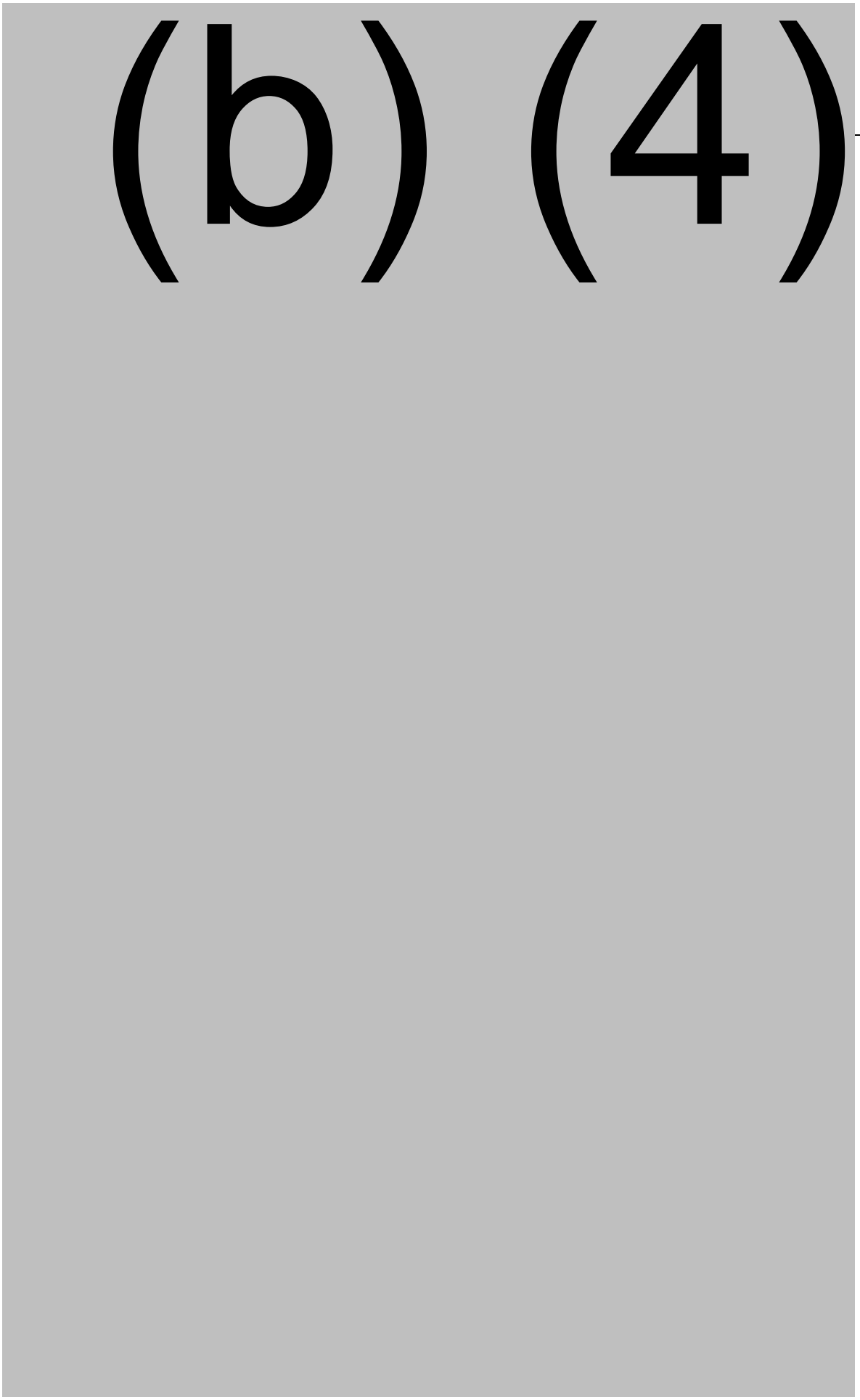
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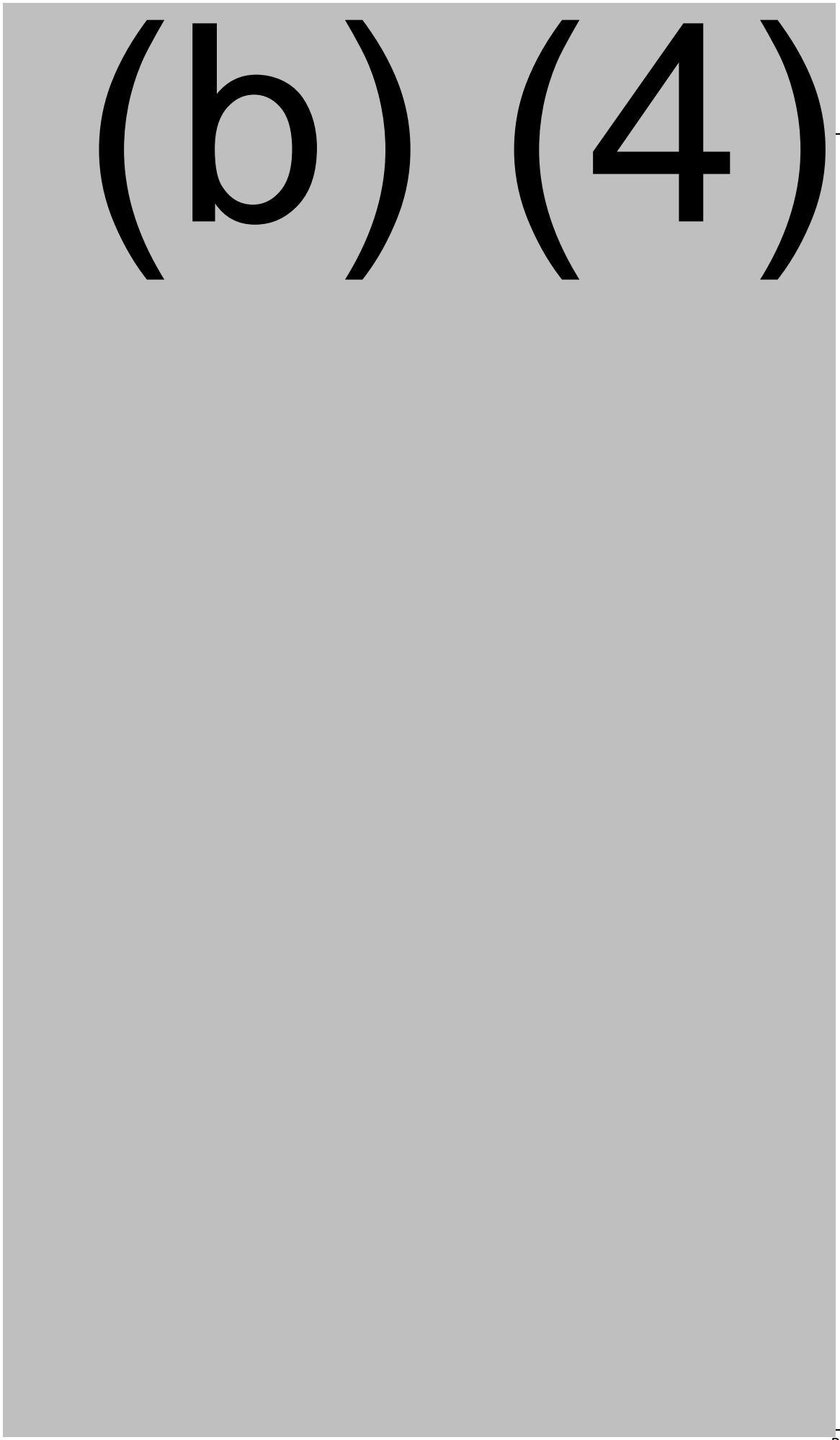
Table 14. Apparent ileal digestibility of nutrients raw data

PEN	TRT	BLOCK	Dry matter (%)	Organic matter (%)	Crude protein (%)	Ether extract (%)	Gross energy (%)	AIDE (kcal/kg)
(b) (4)								

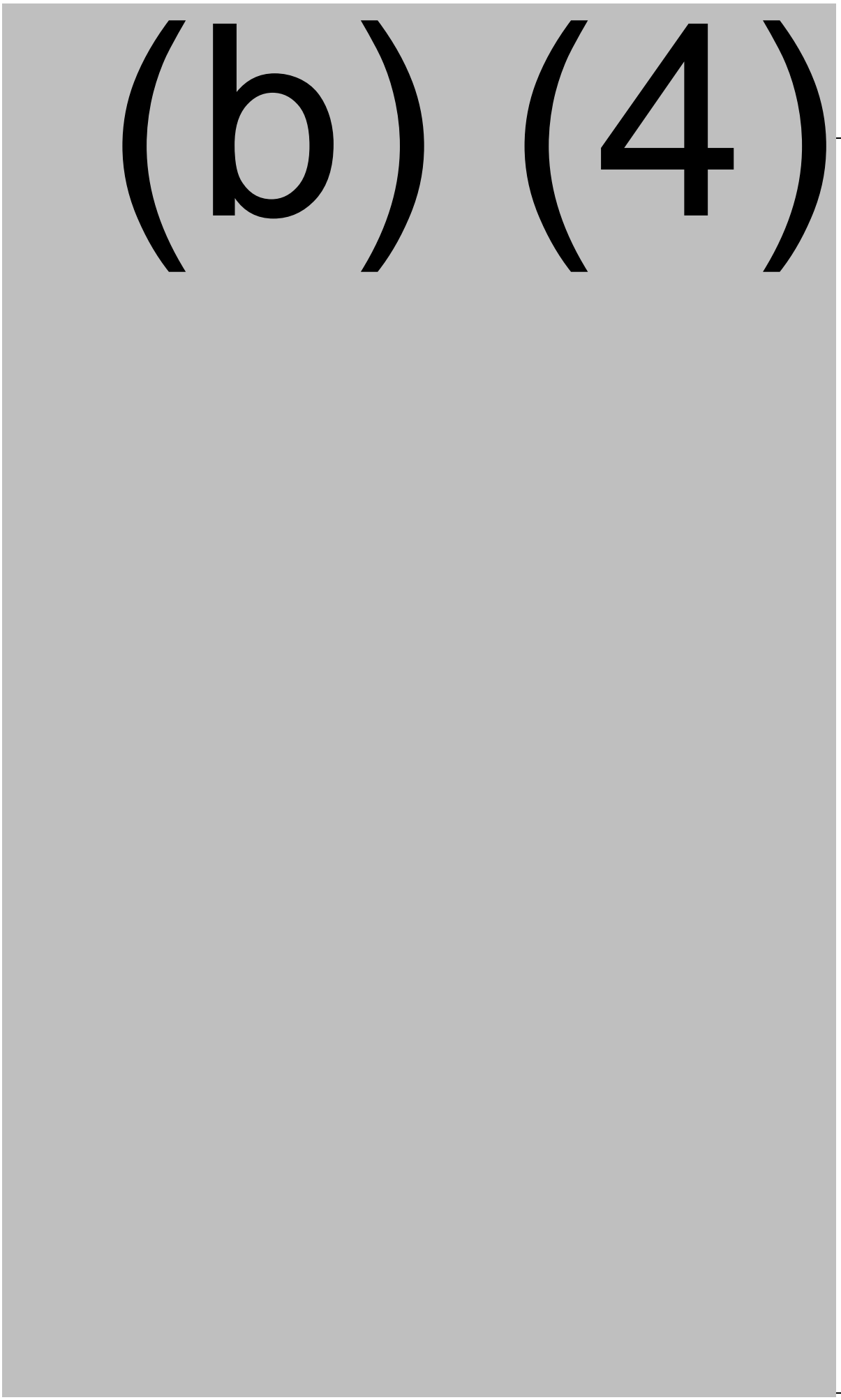
ANNEX IV- STATISTICAL CODES, LOG AND COMPLETE OUTPUTS


(b) (4)

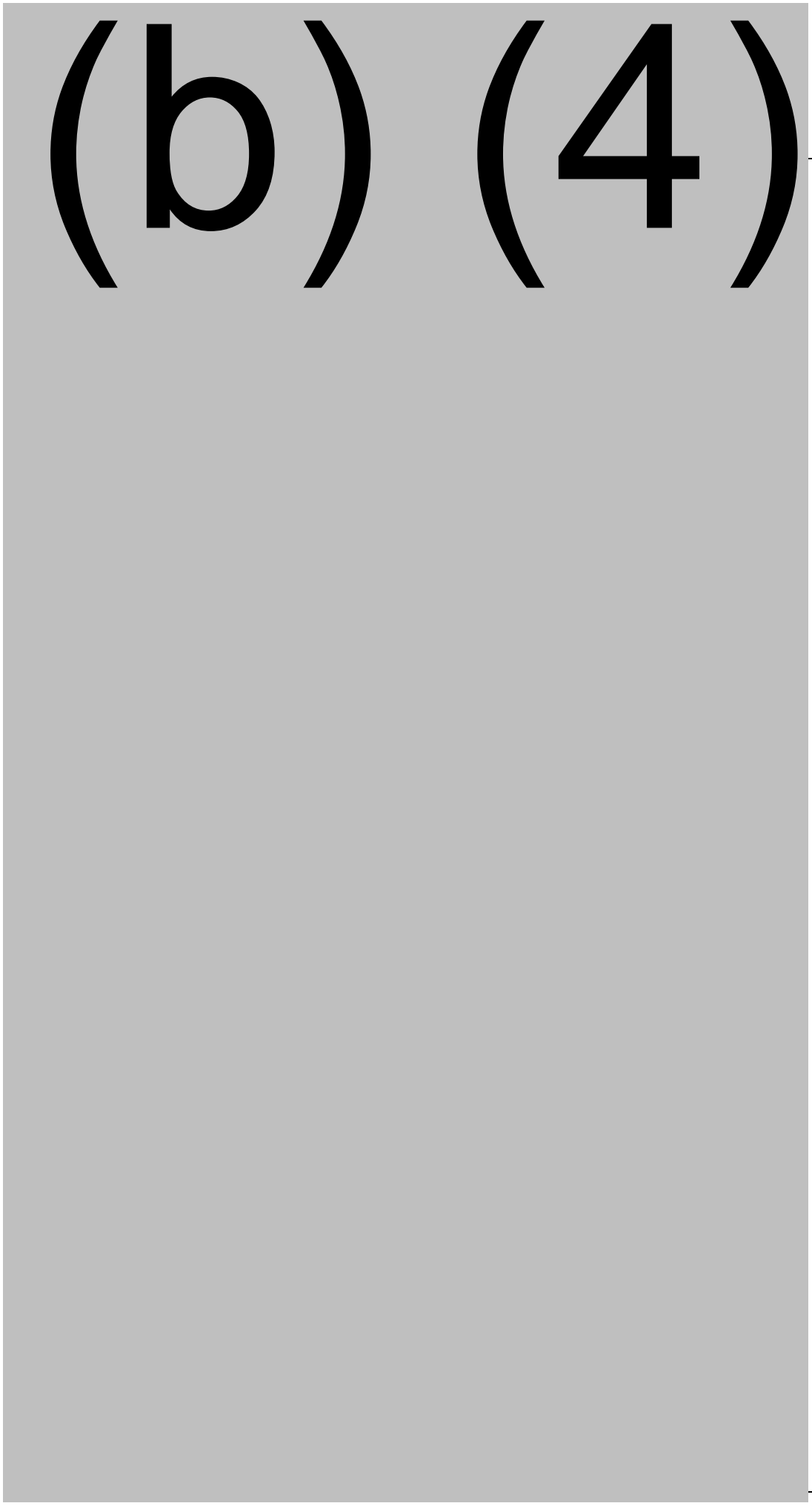

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(b) (4)

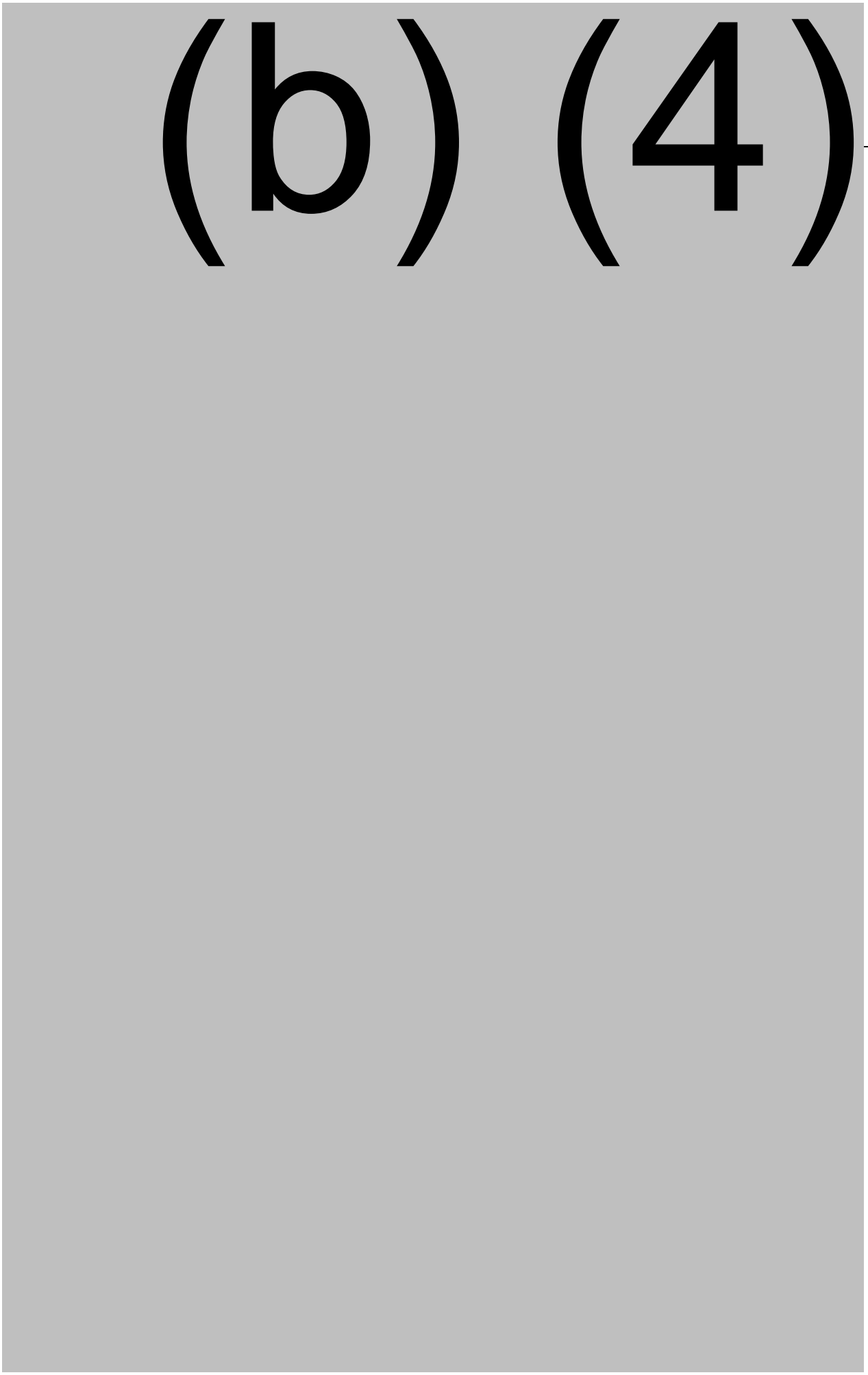


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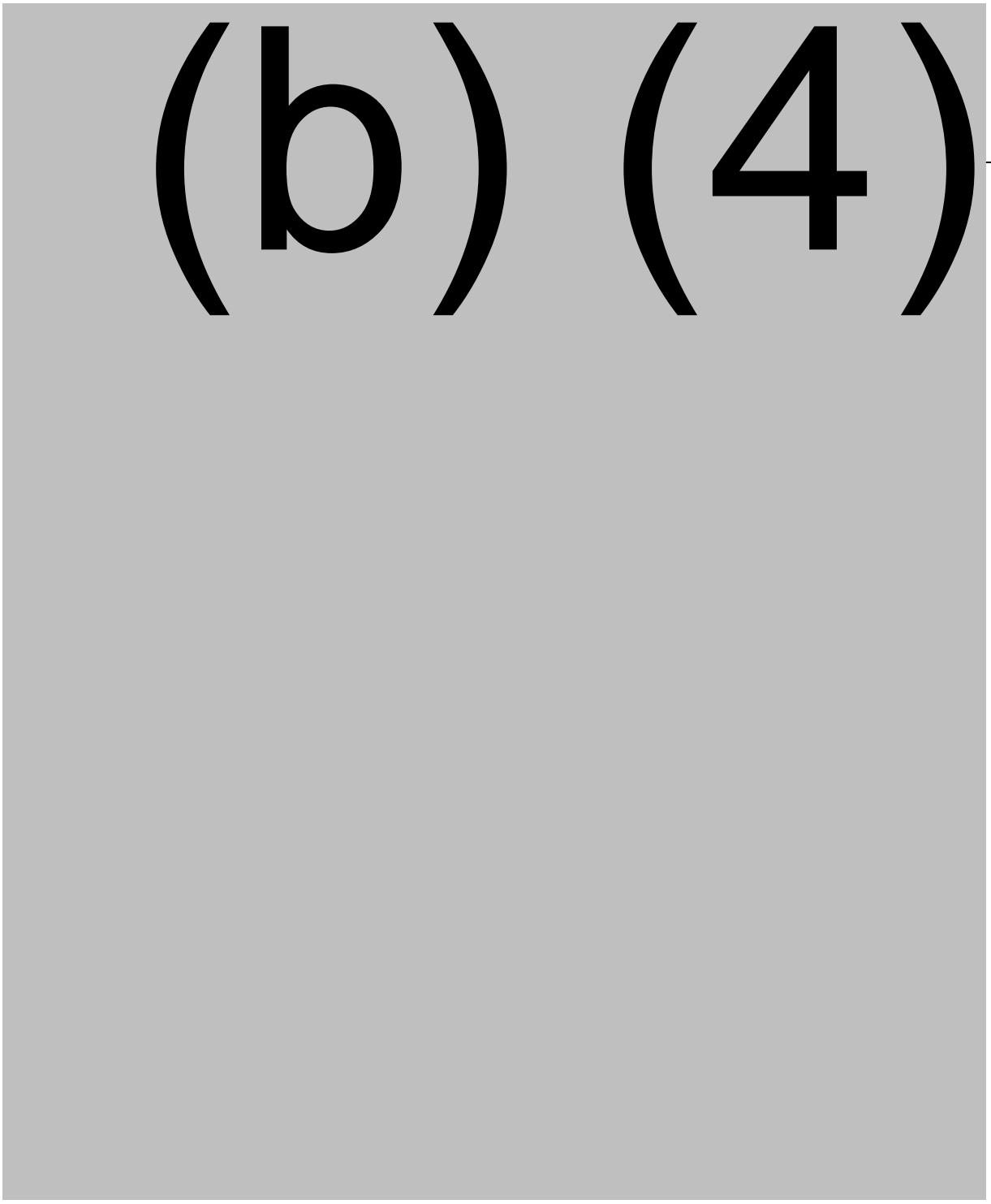


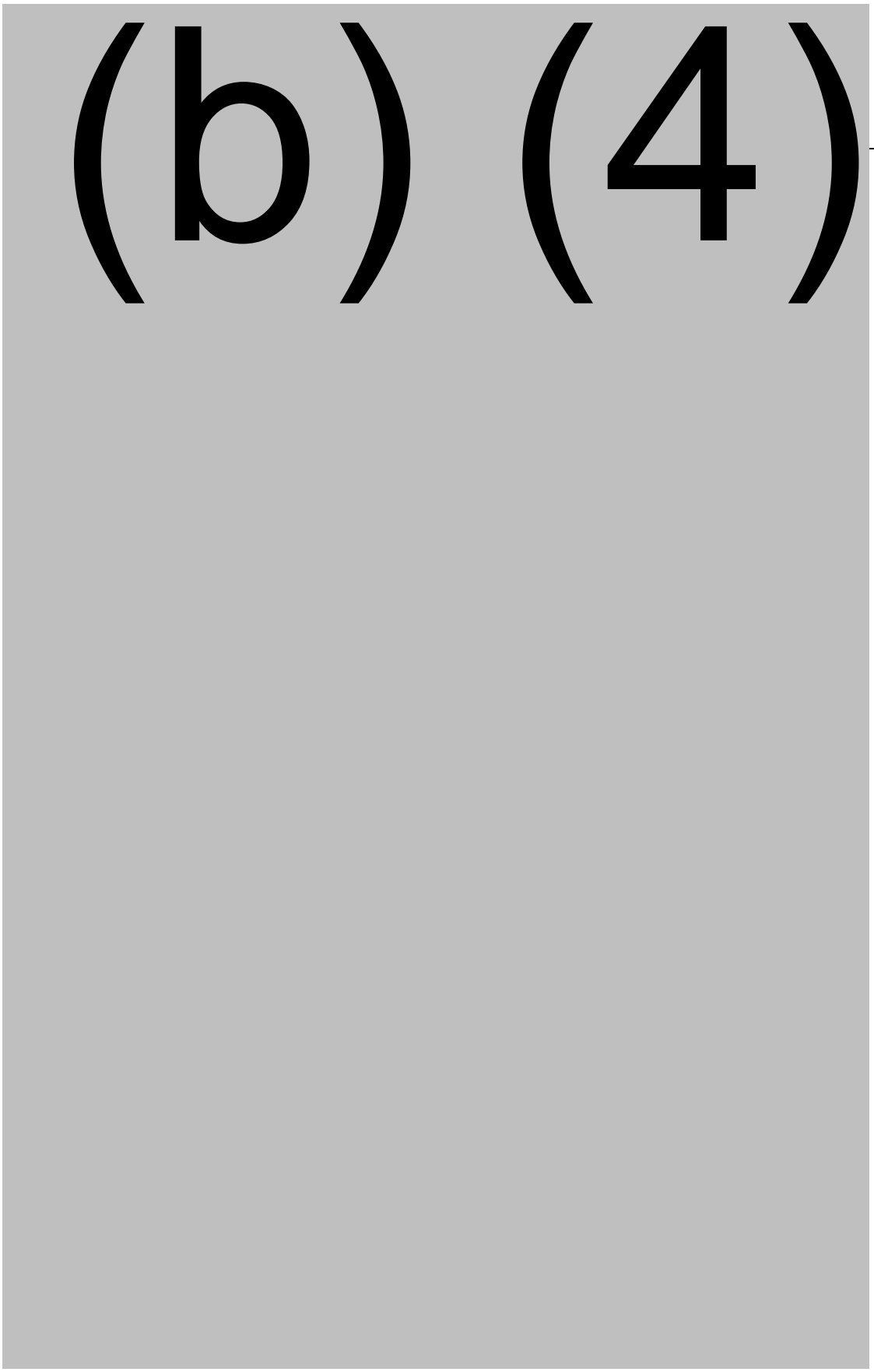
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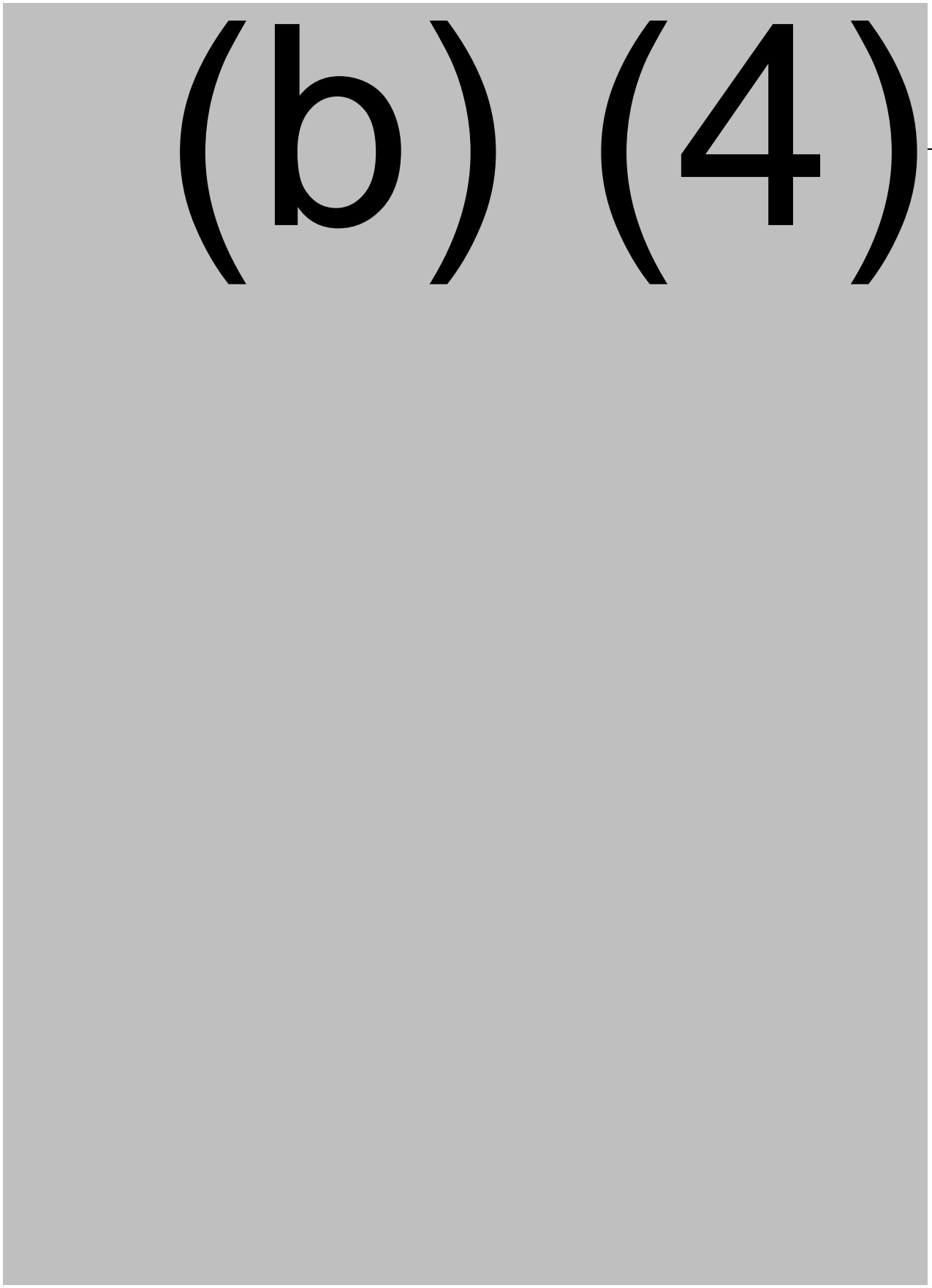
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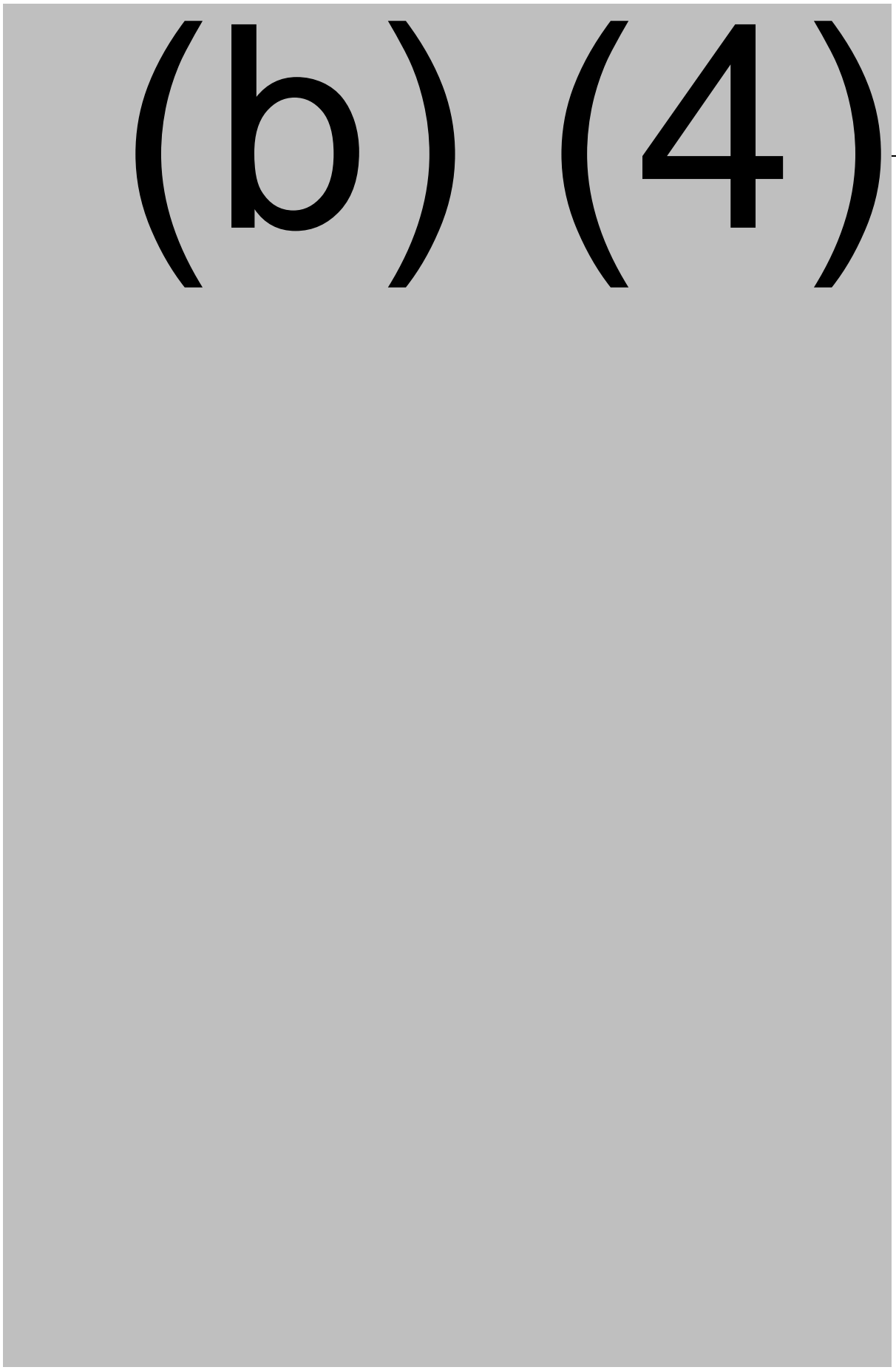

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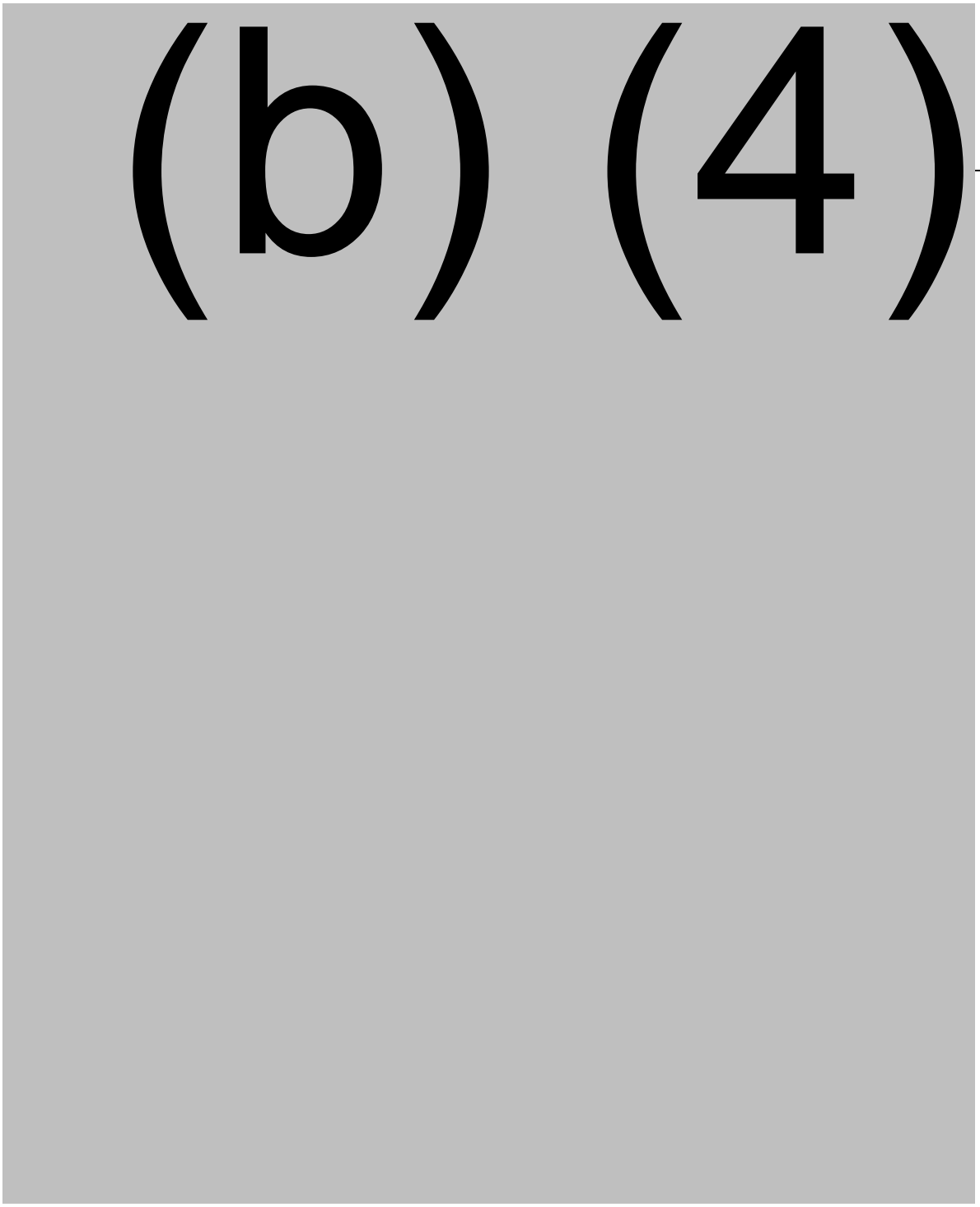

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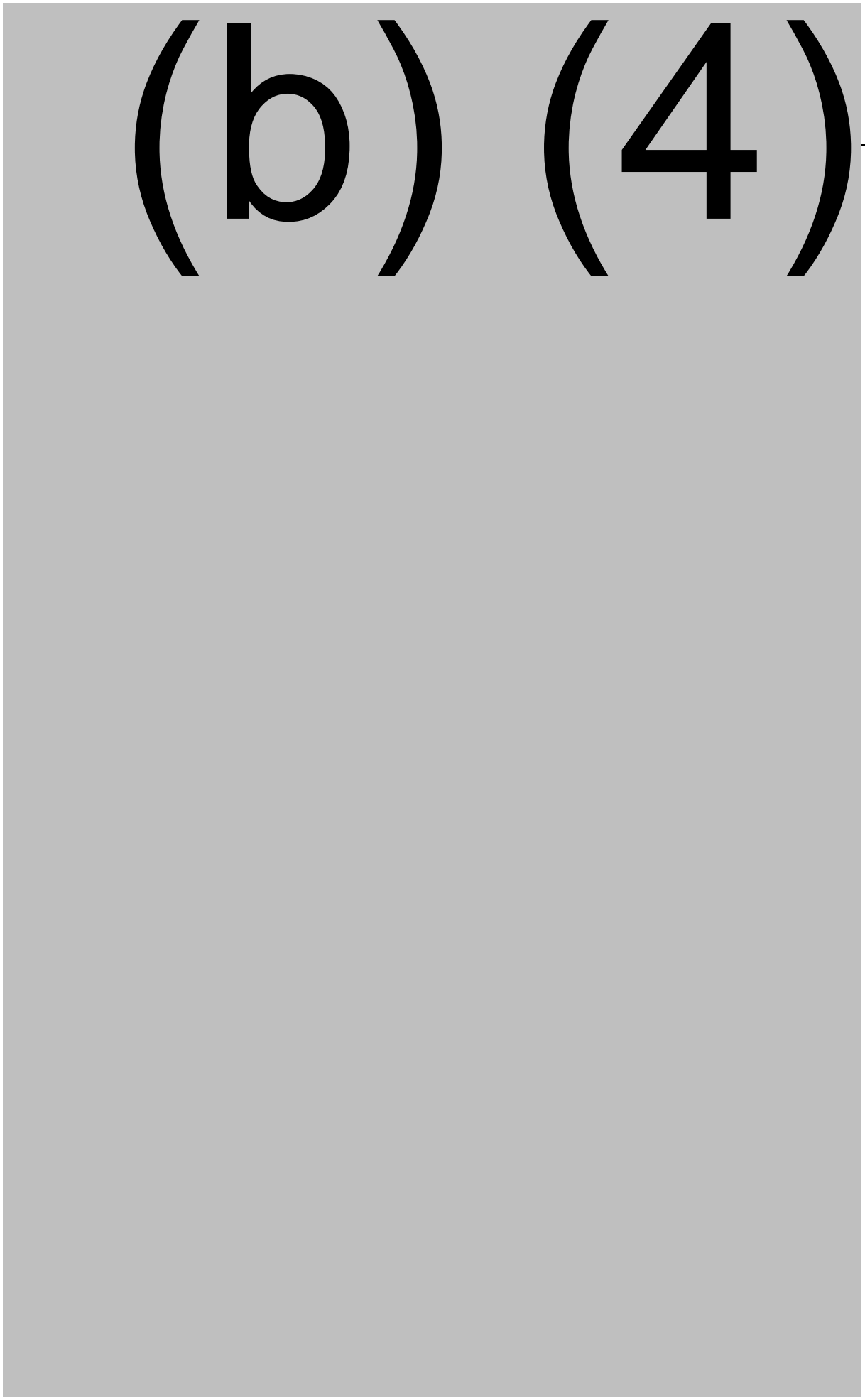

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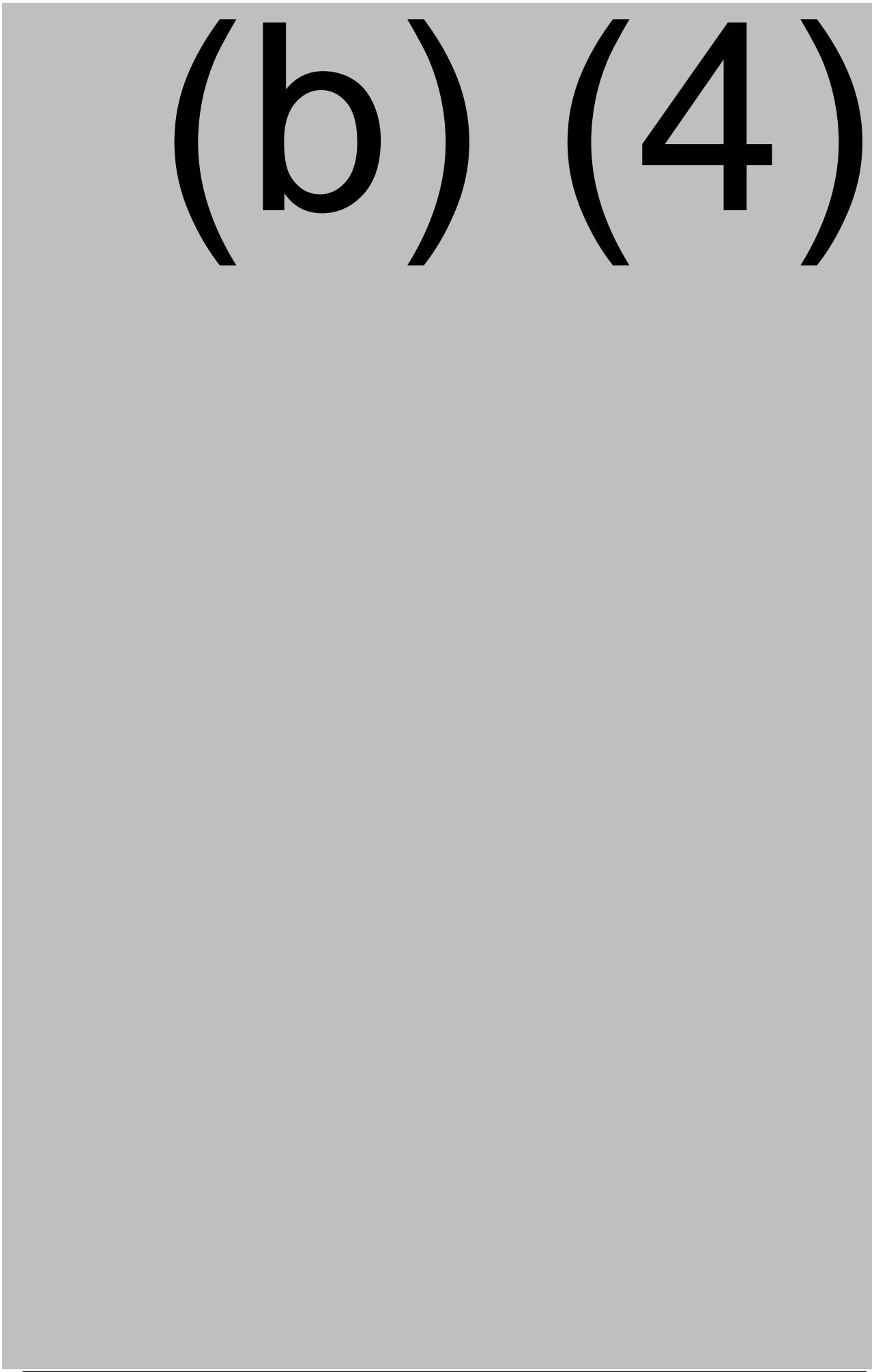

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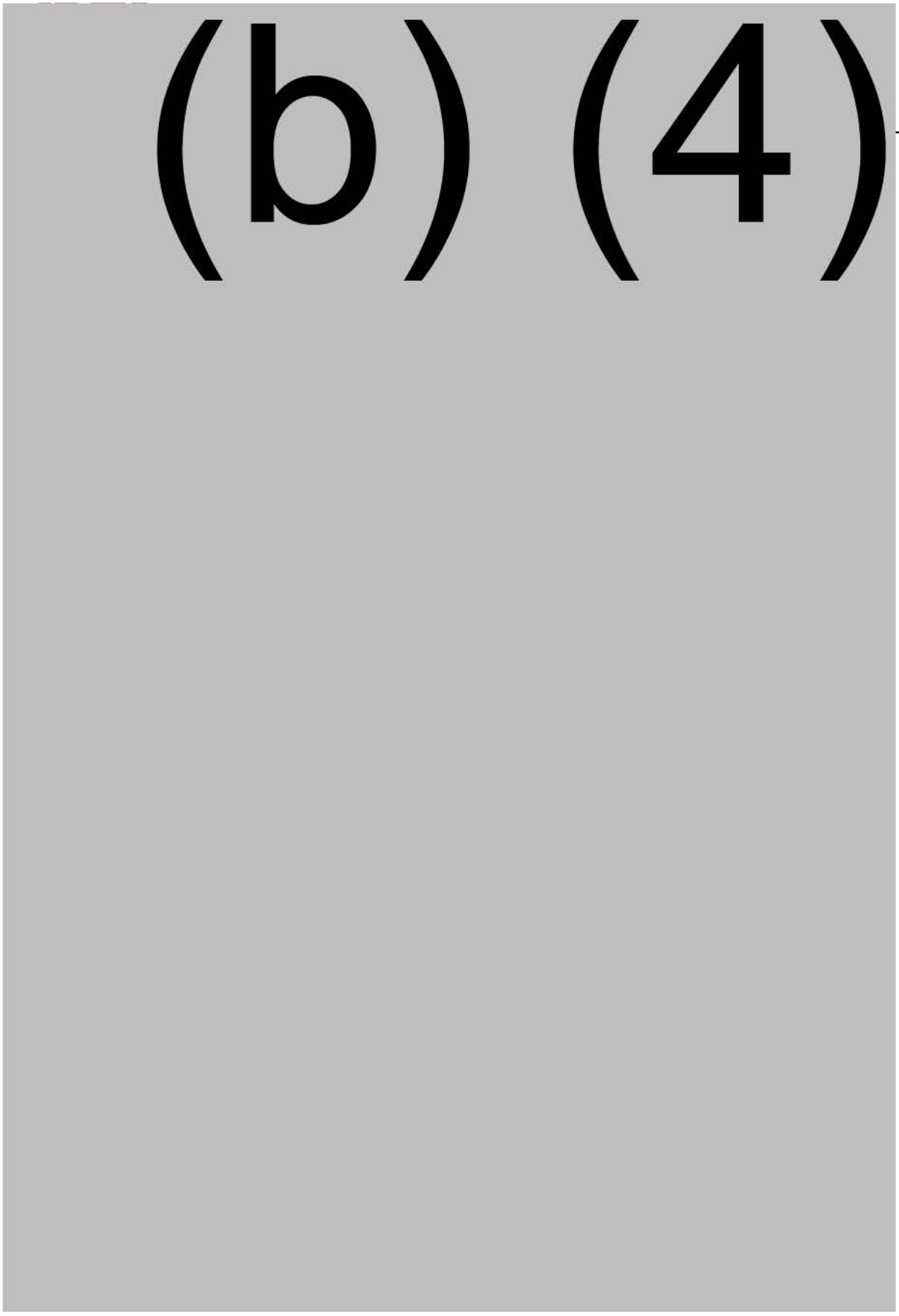

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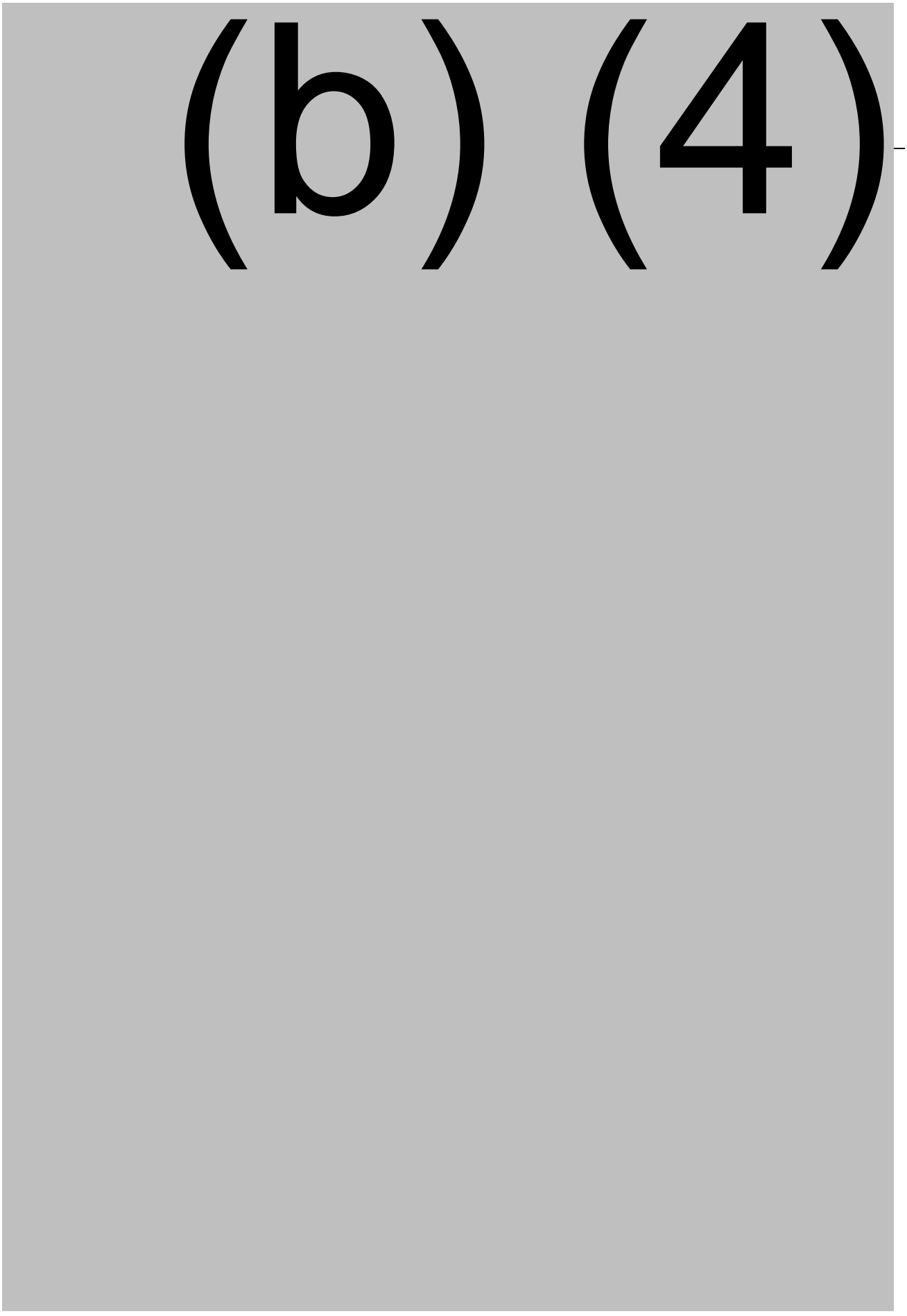

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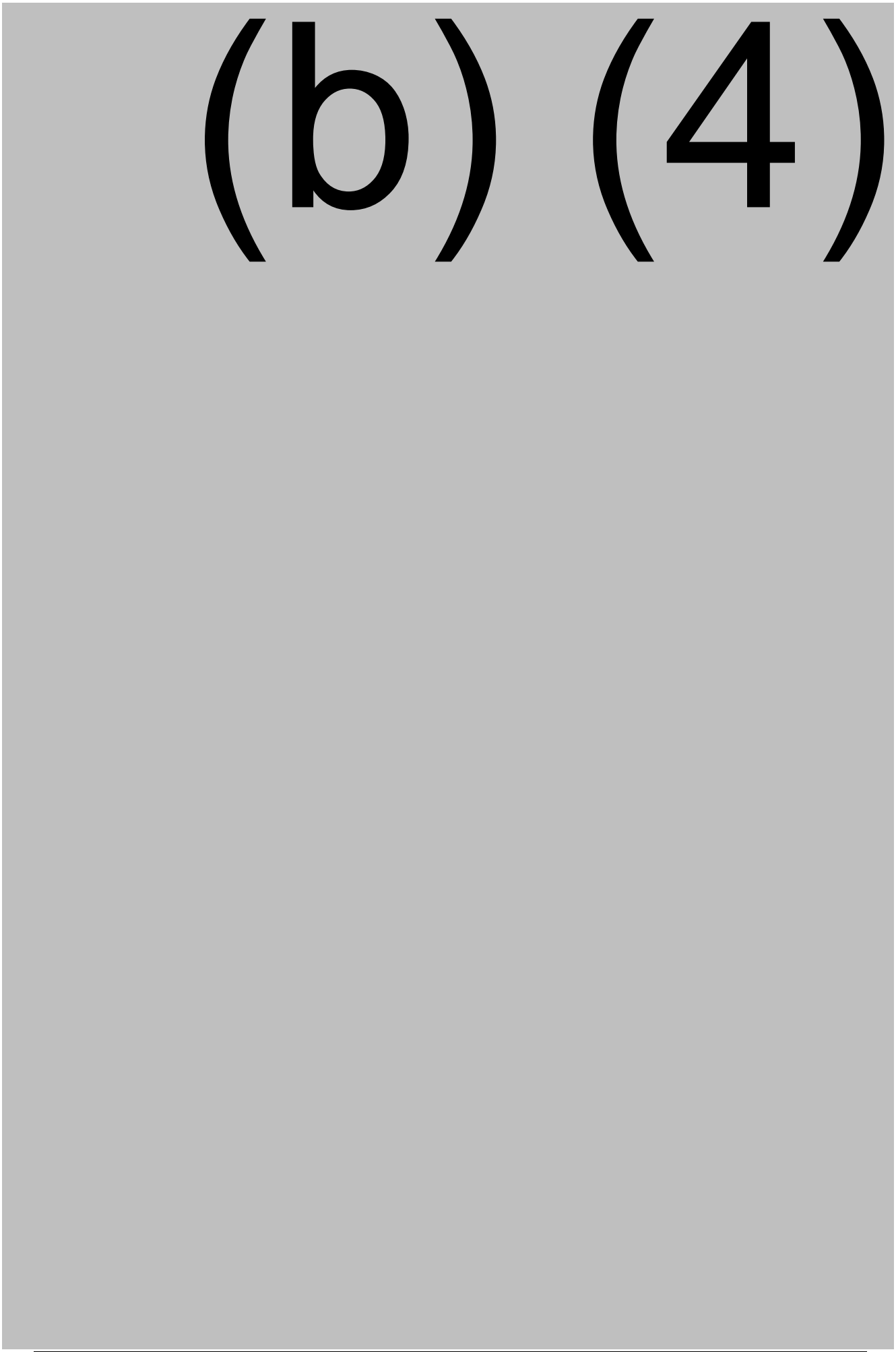

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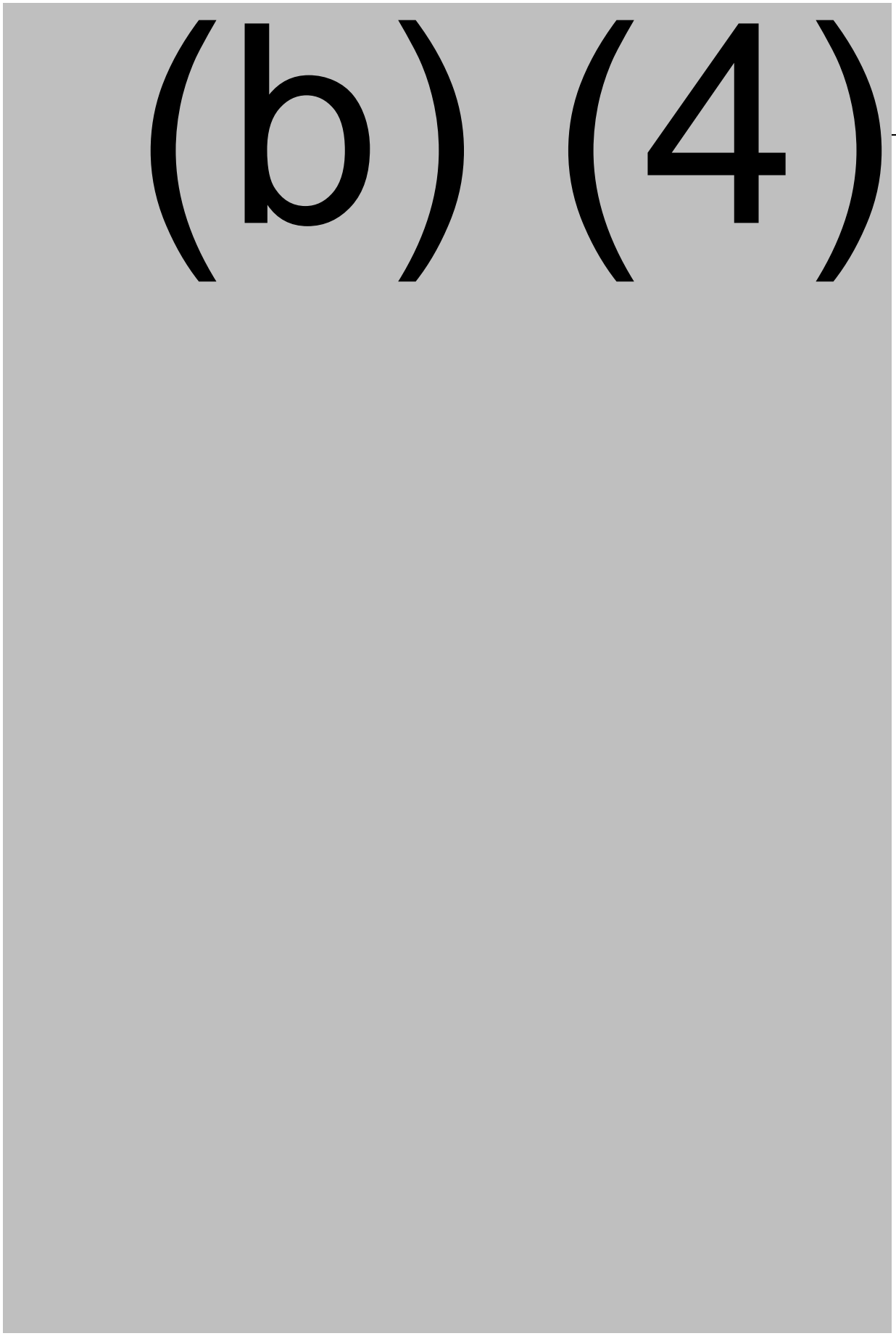

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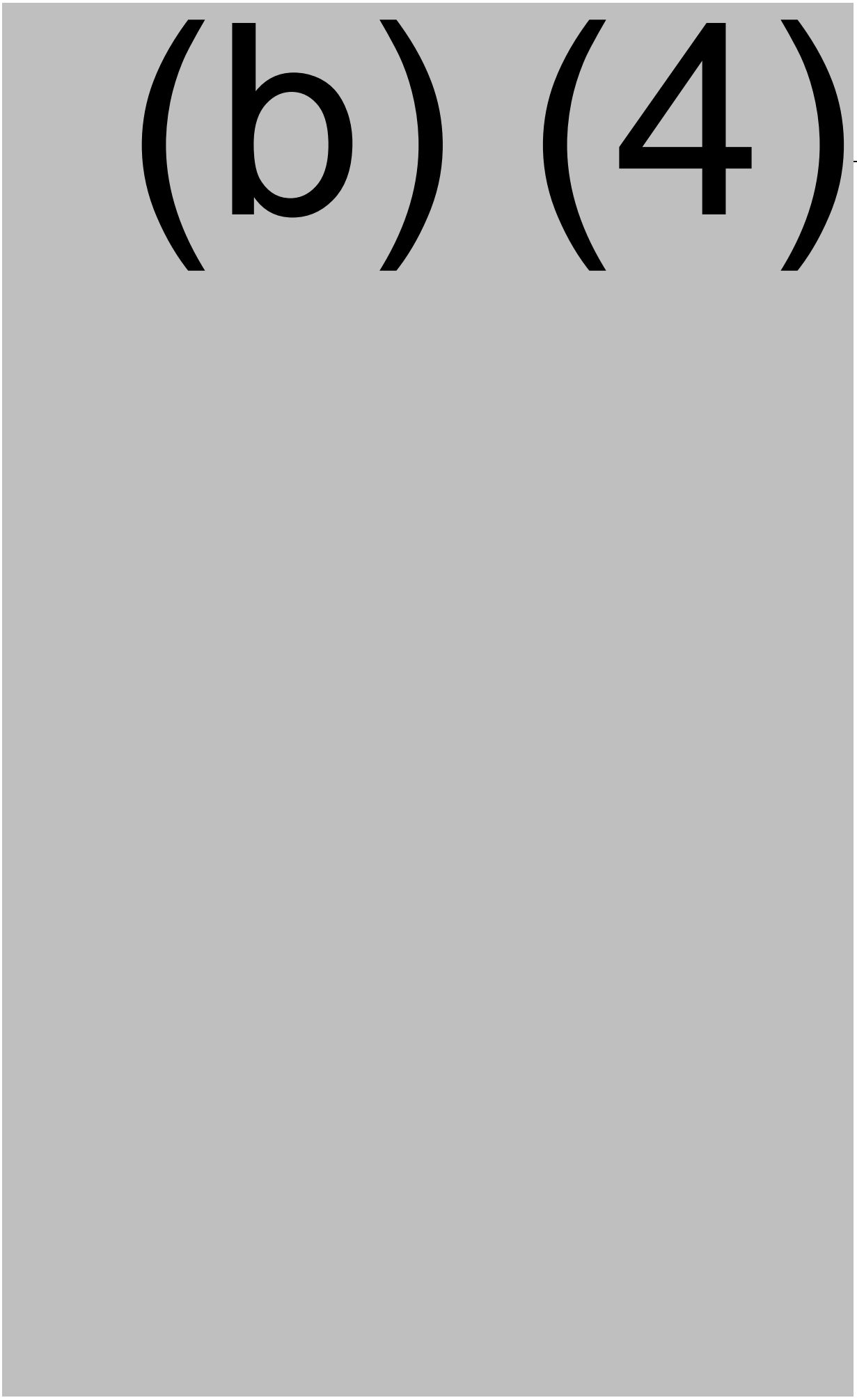

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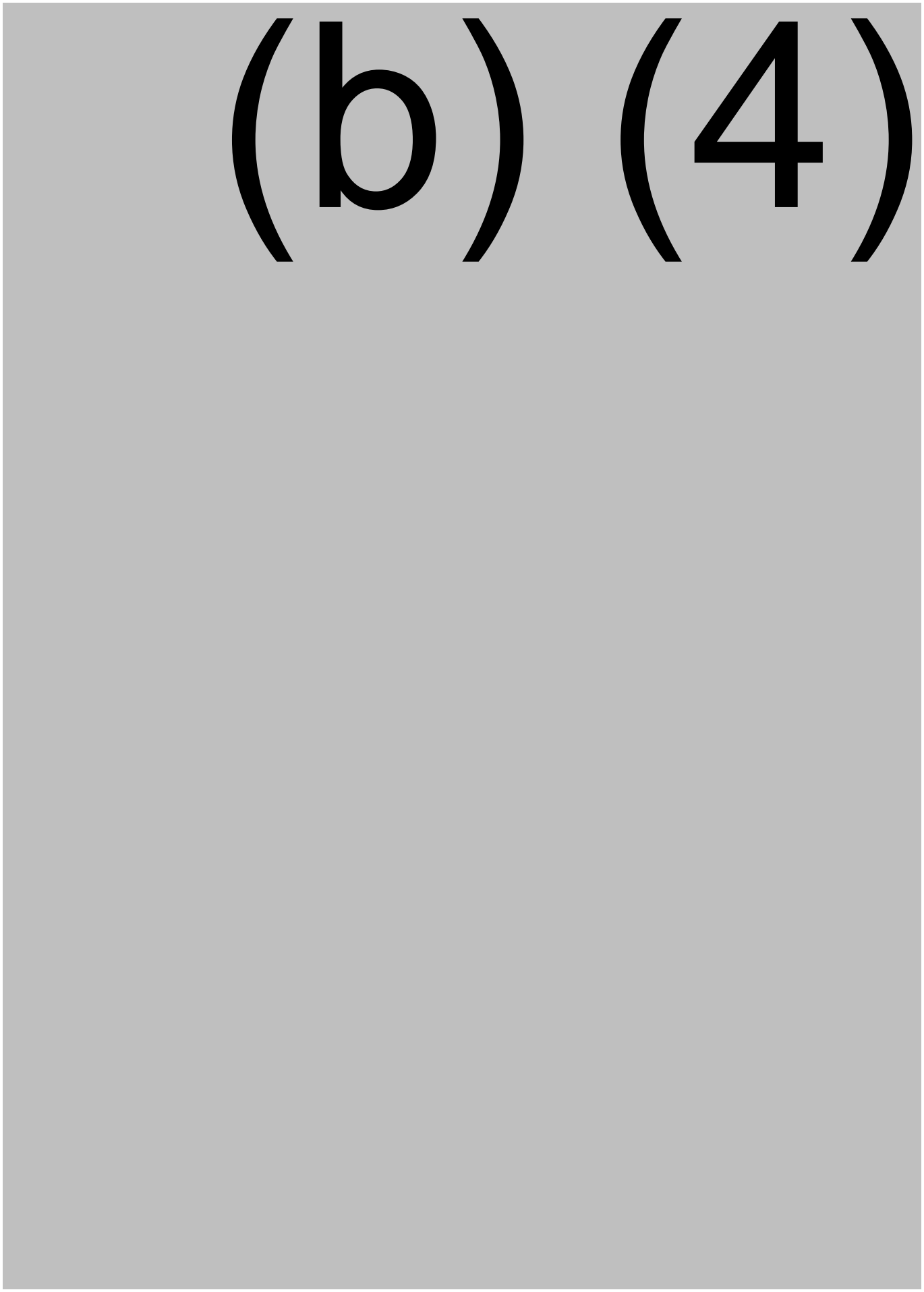

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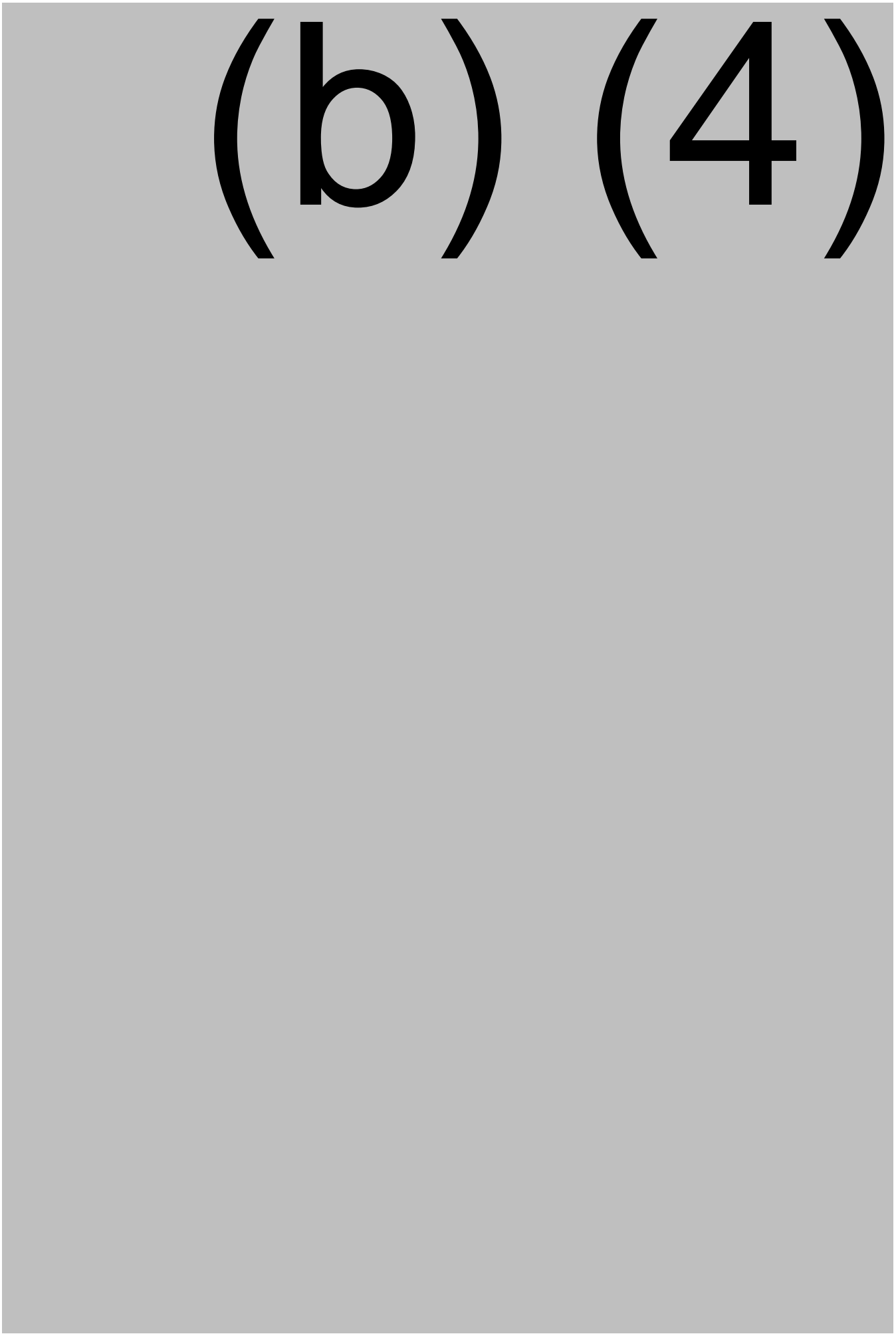

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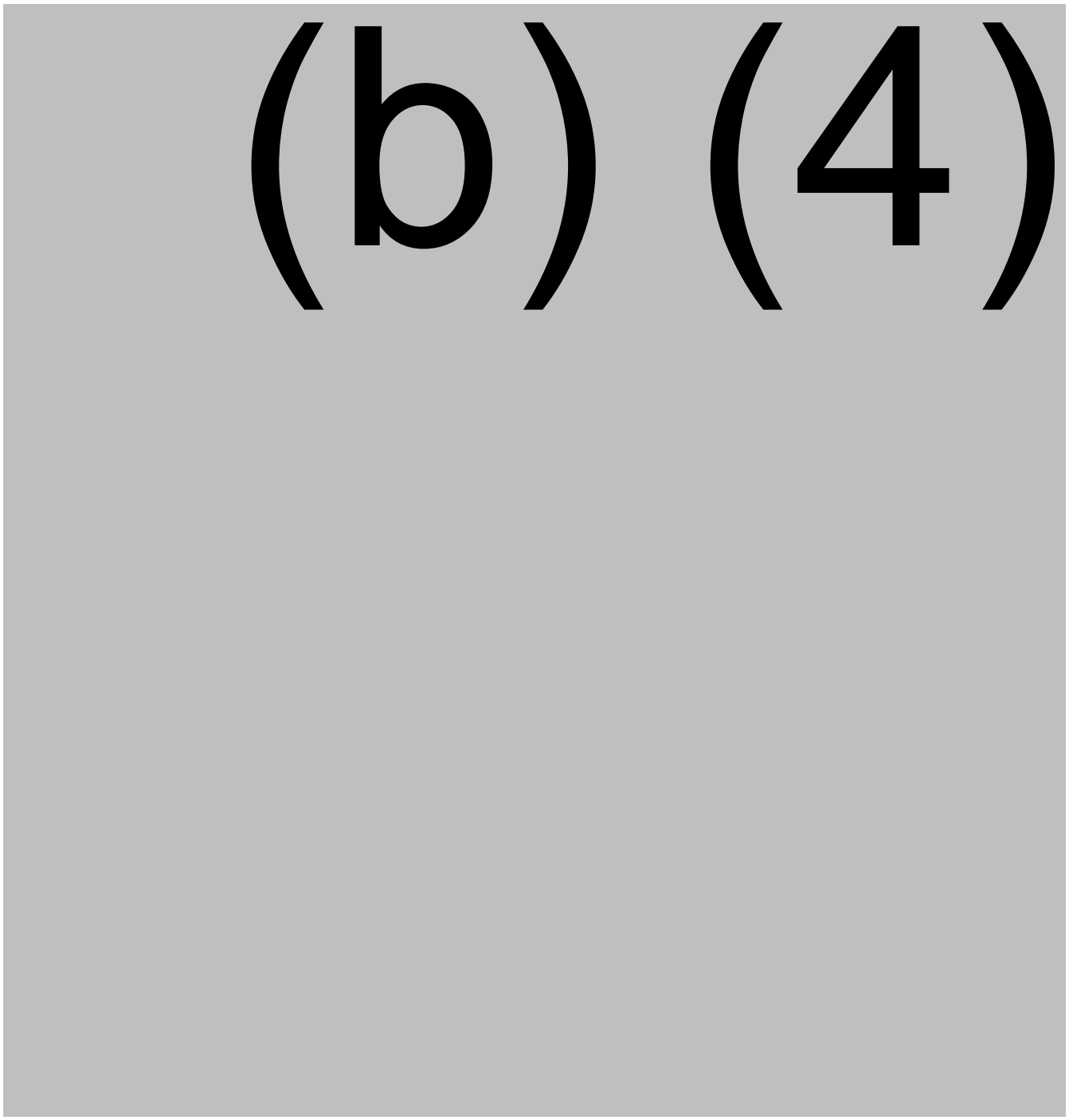

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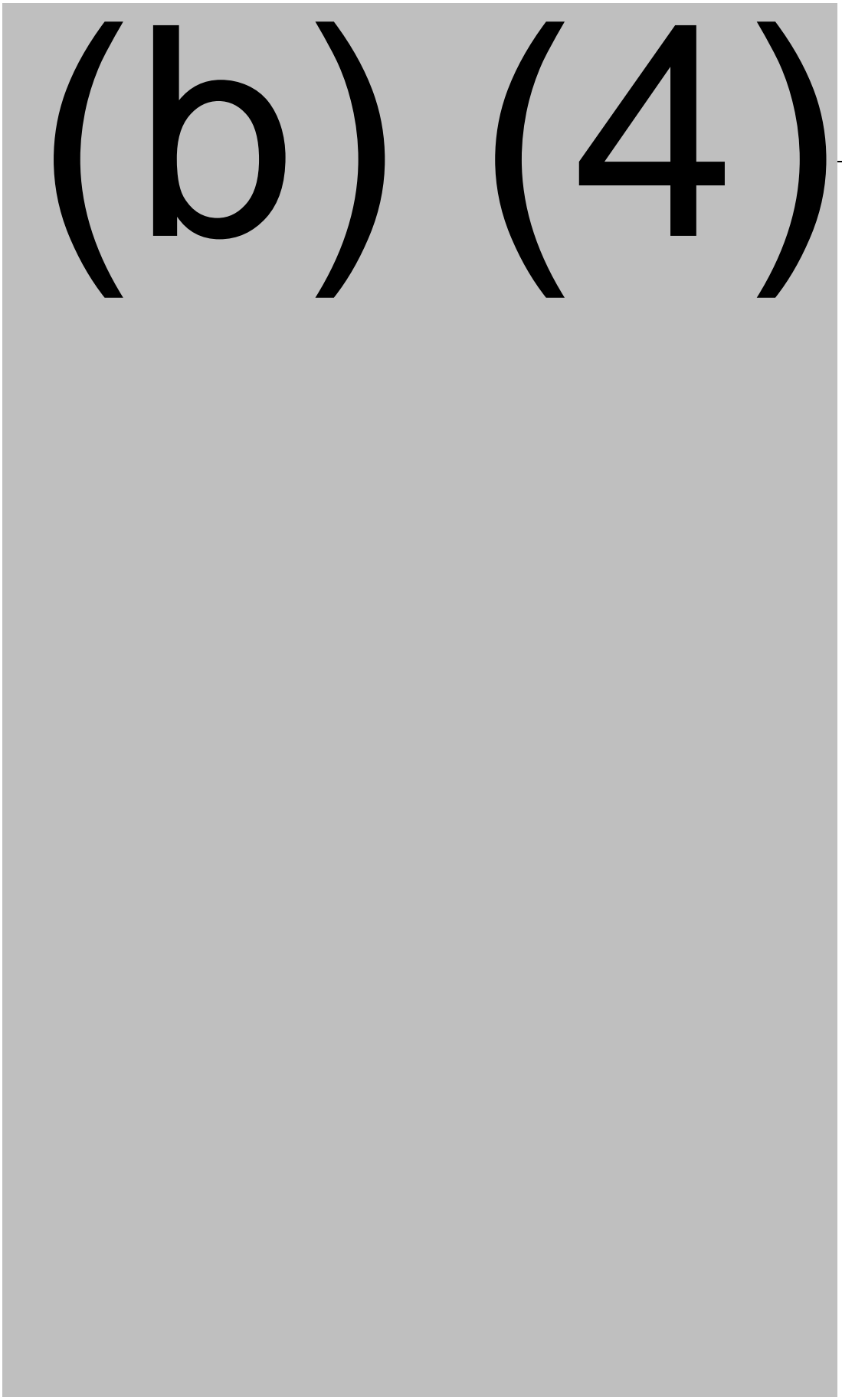

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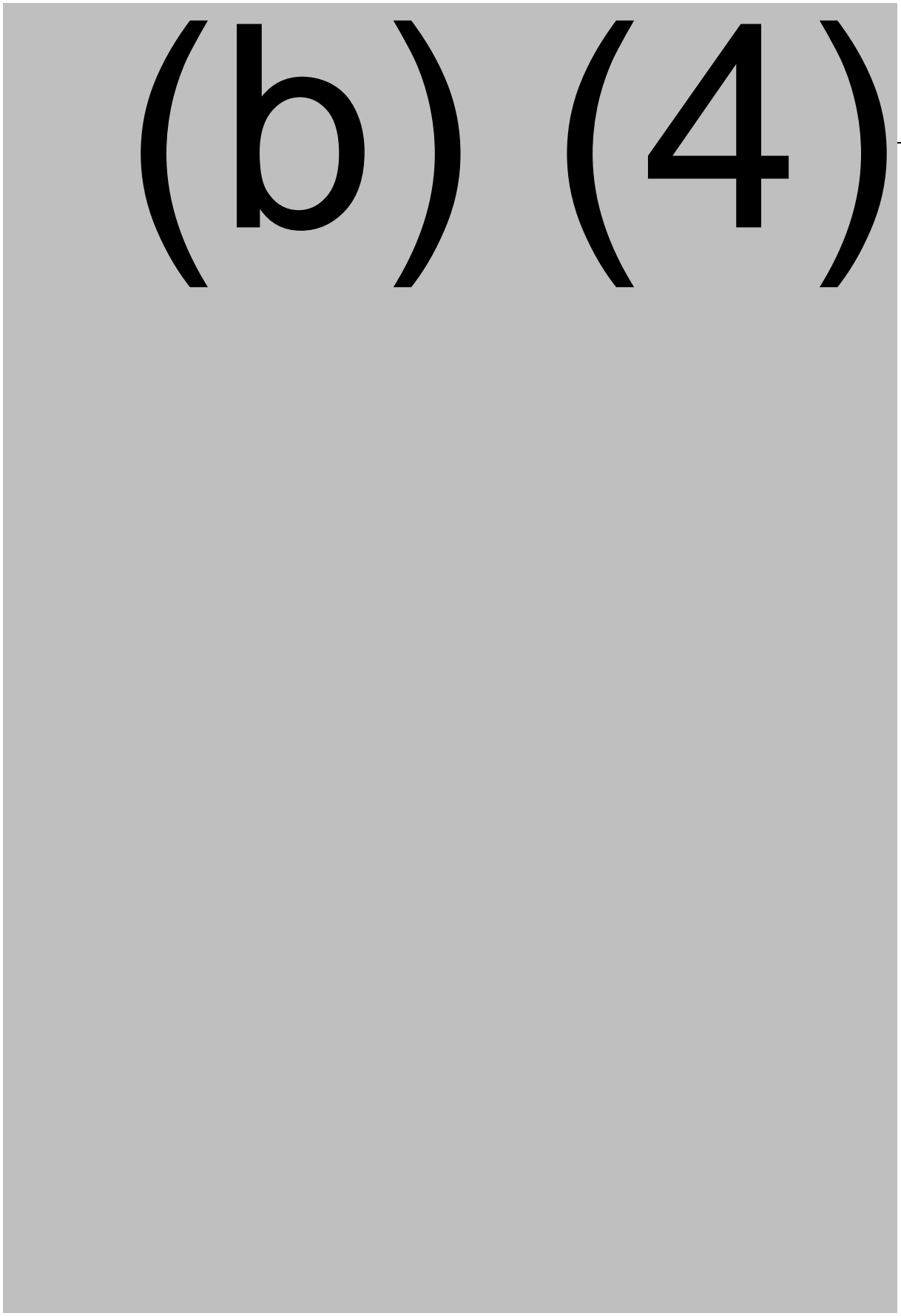

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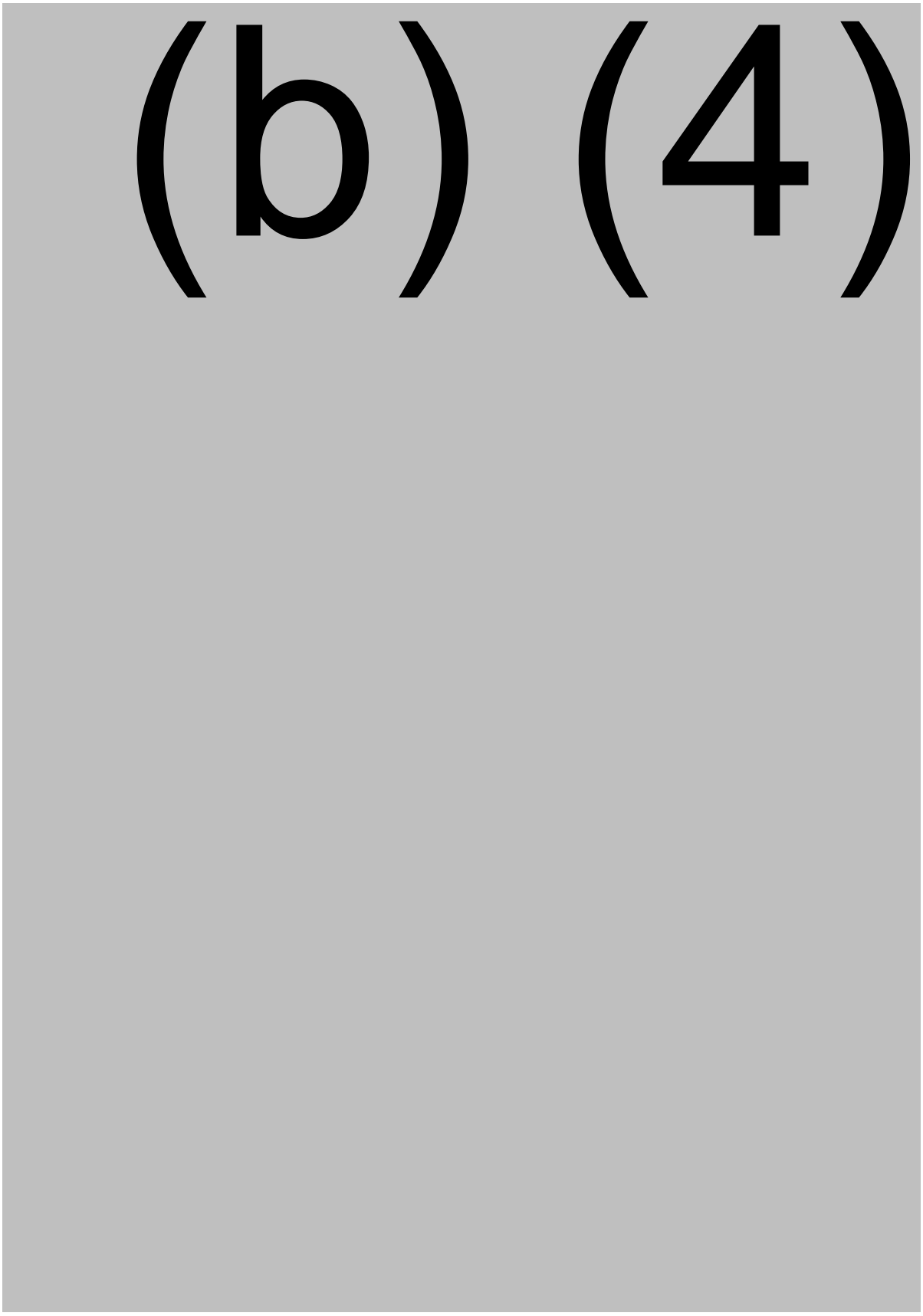

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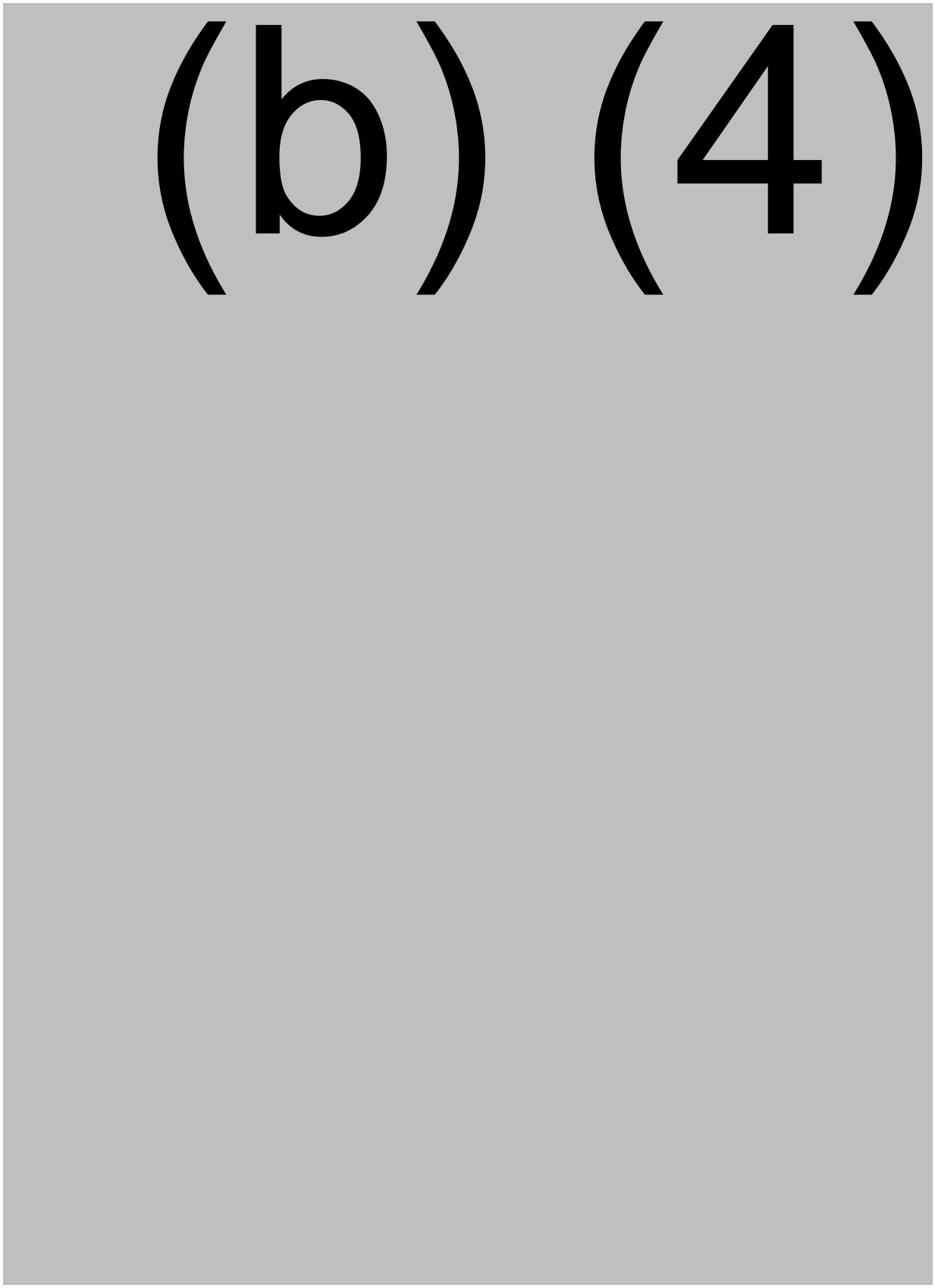

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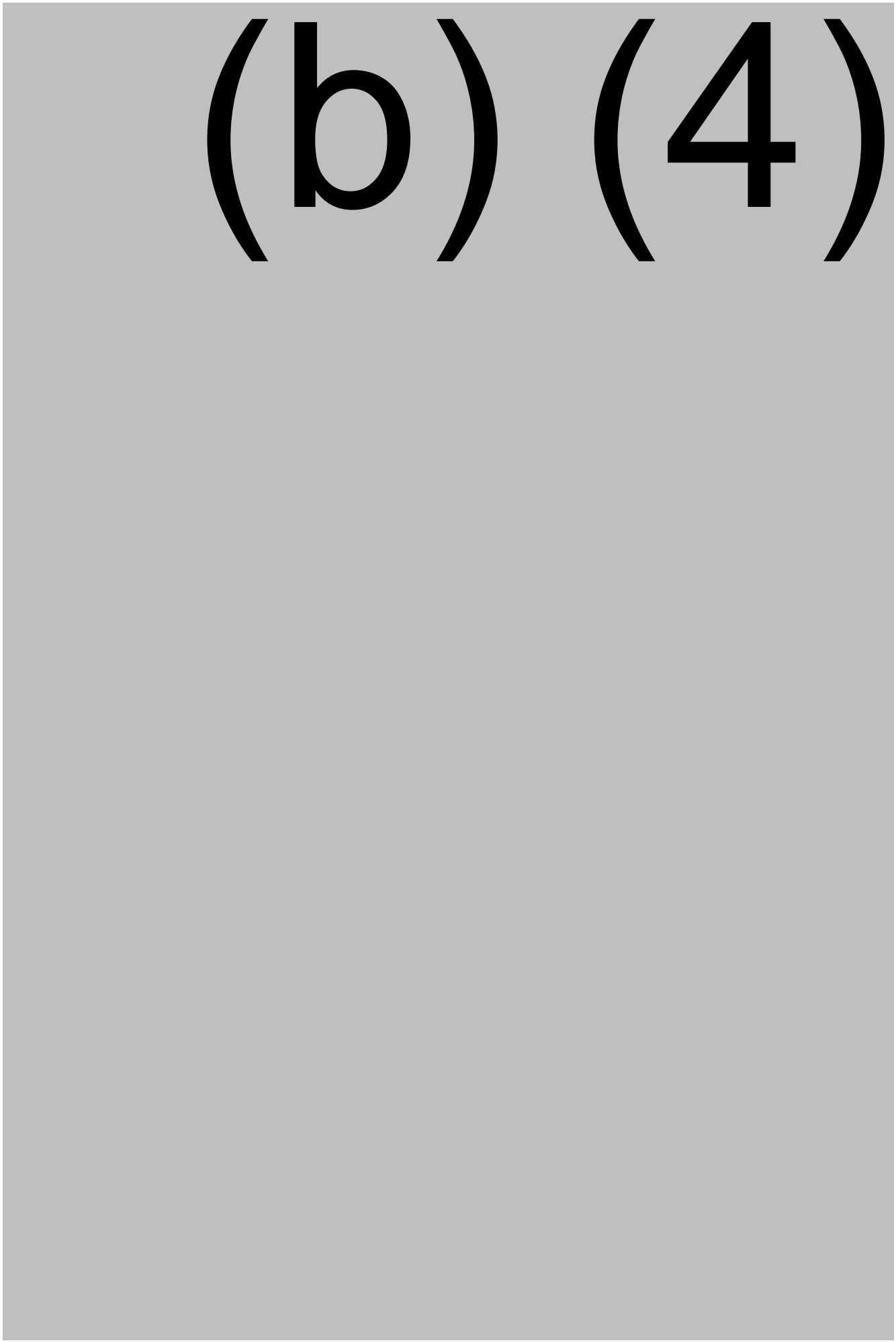

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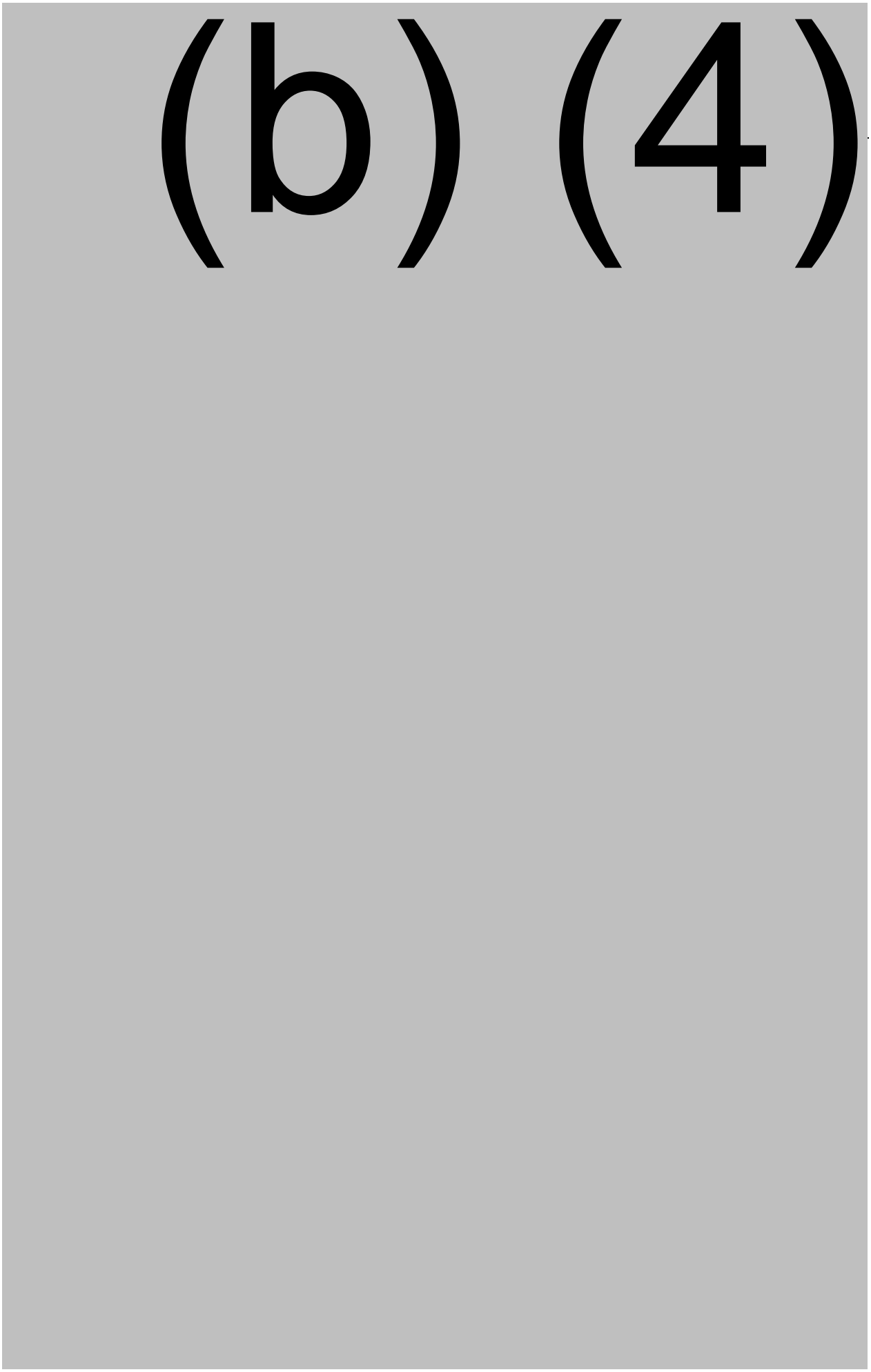
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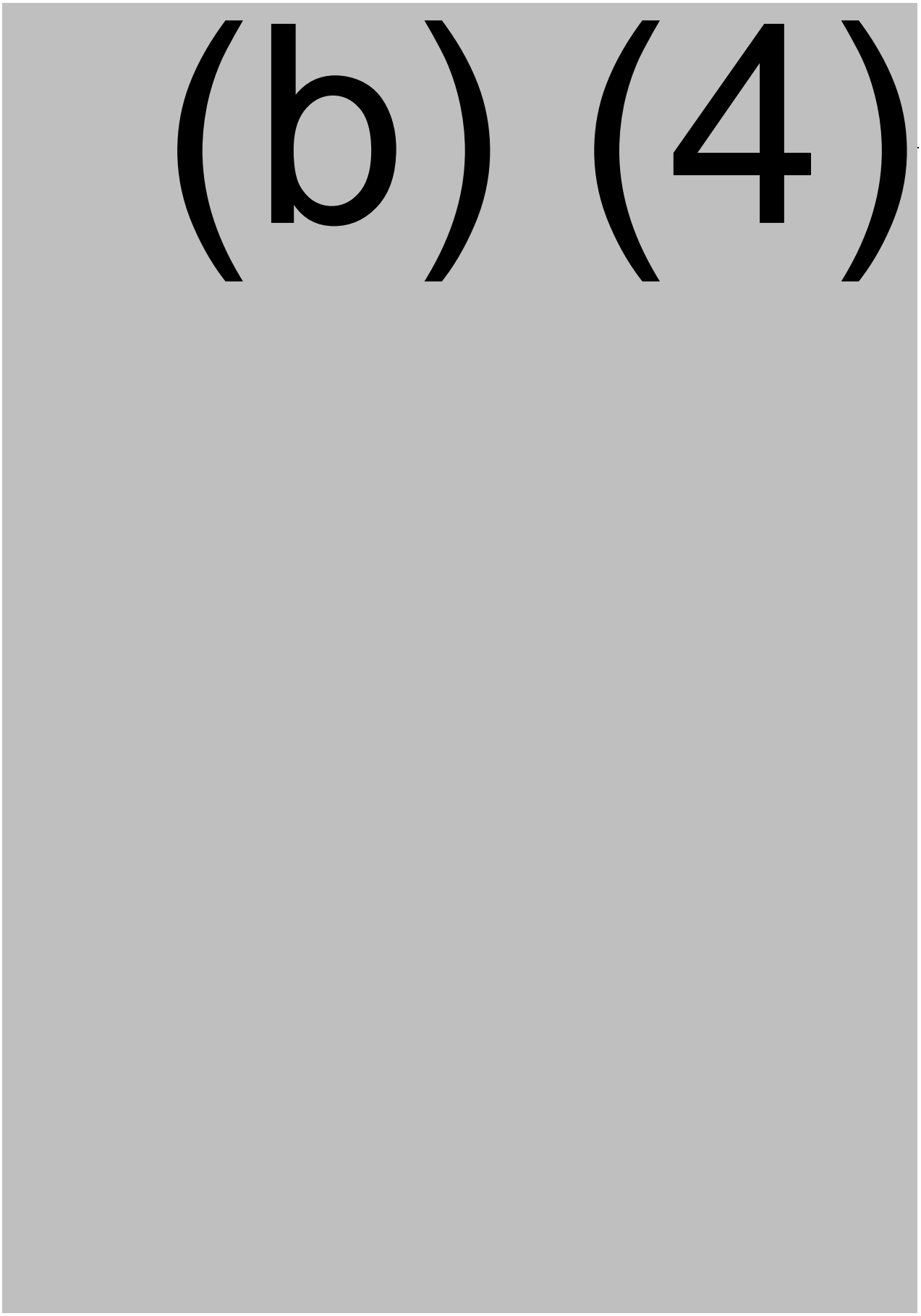


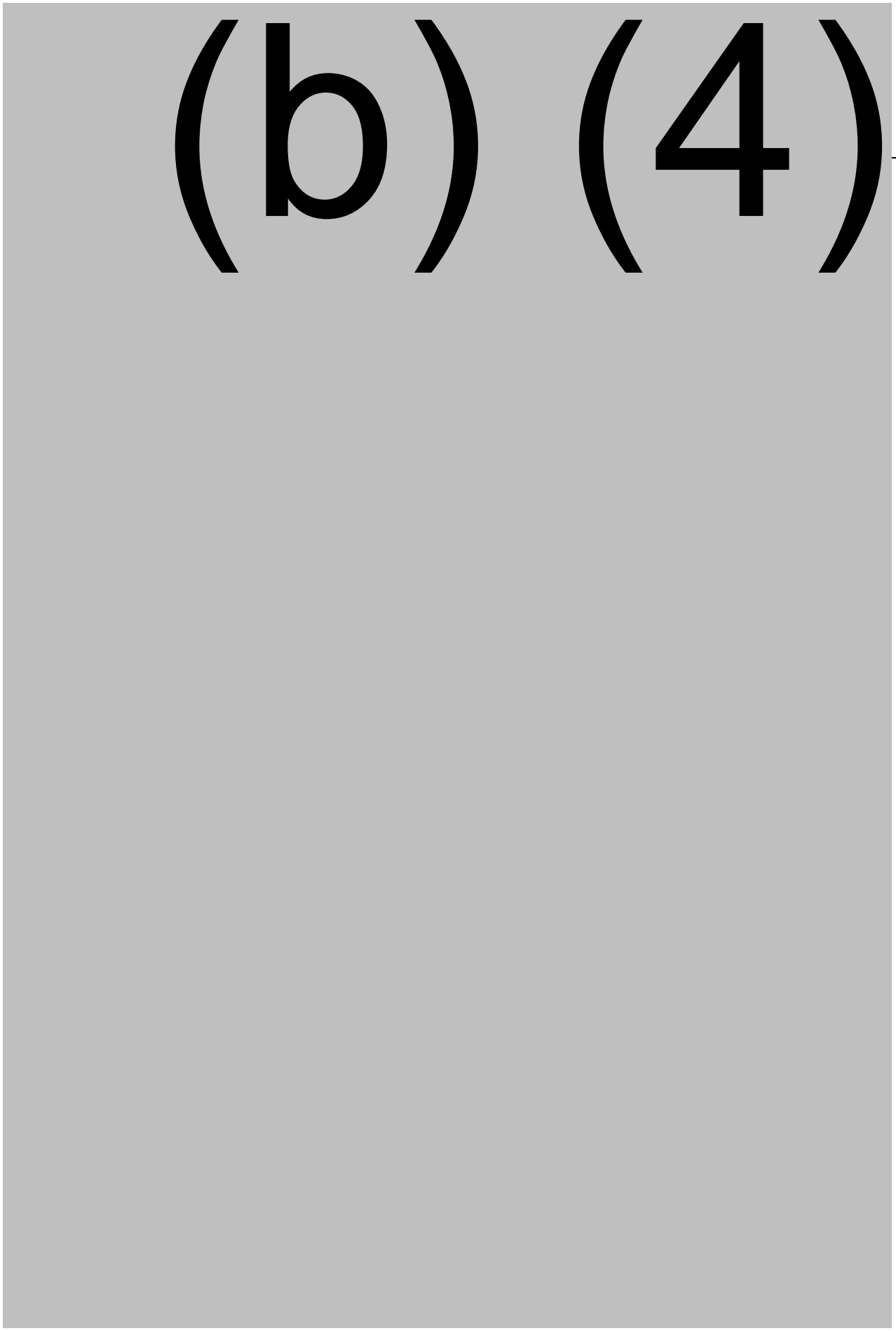
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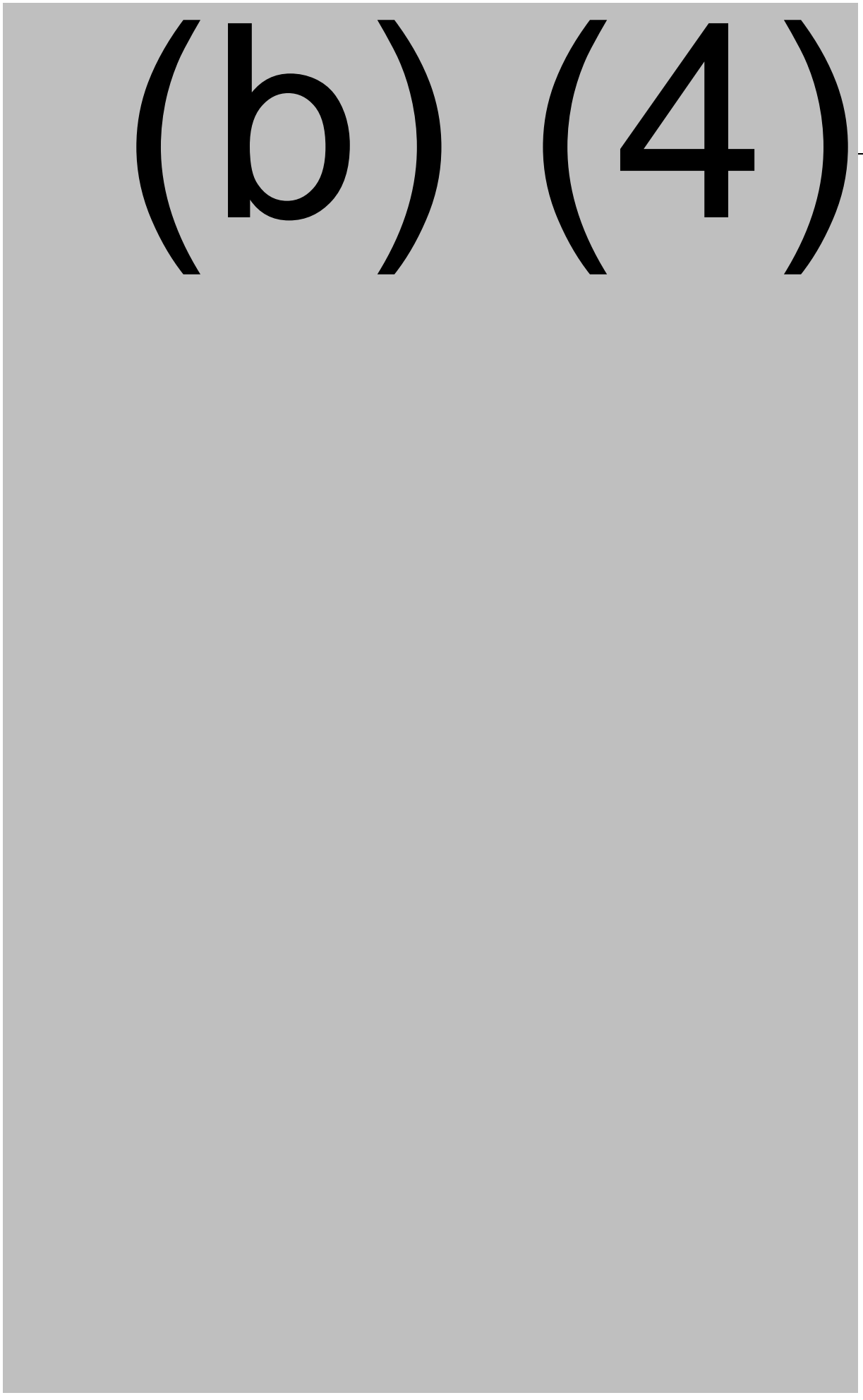


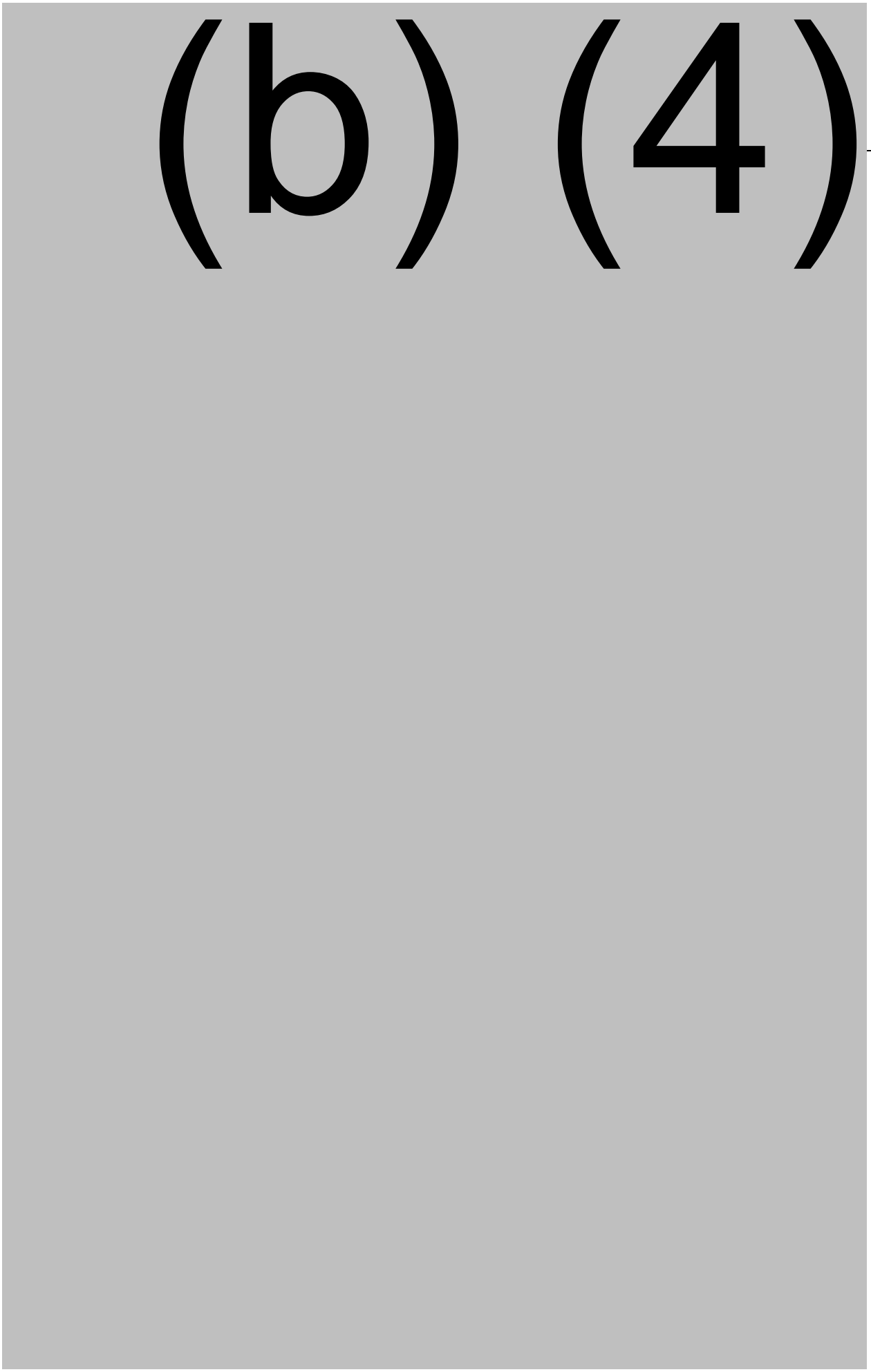

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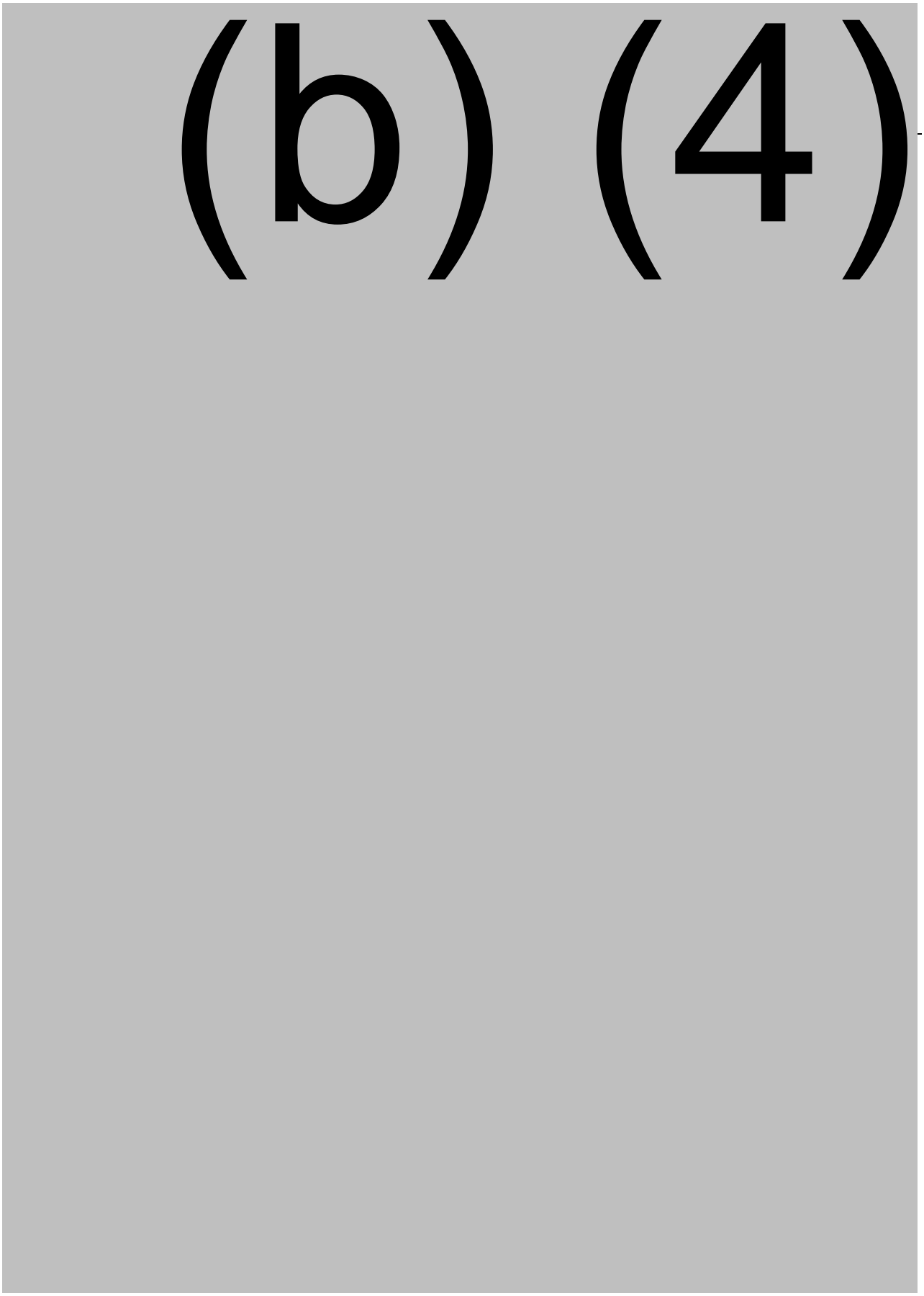

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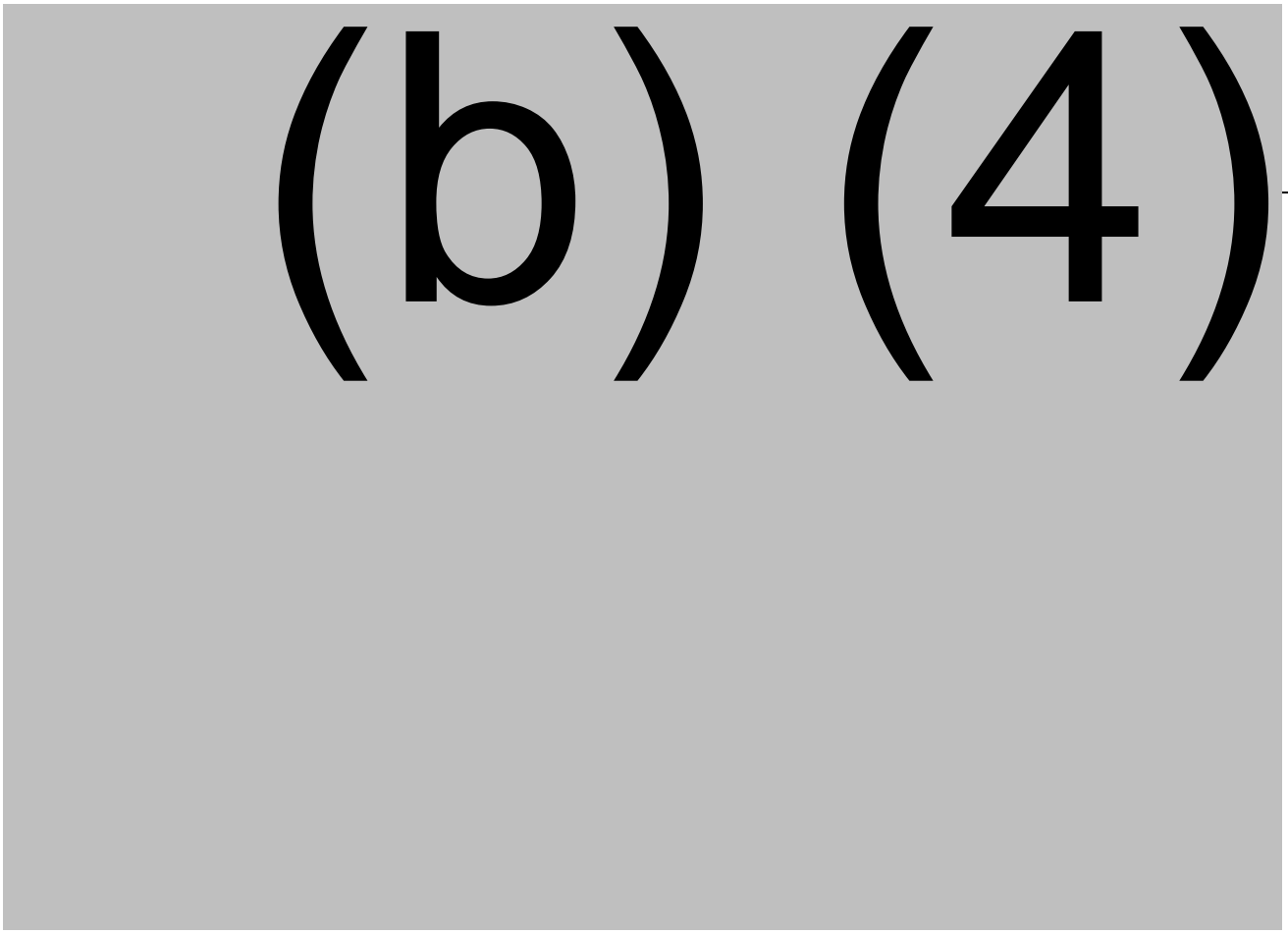

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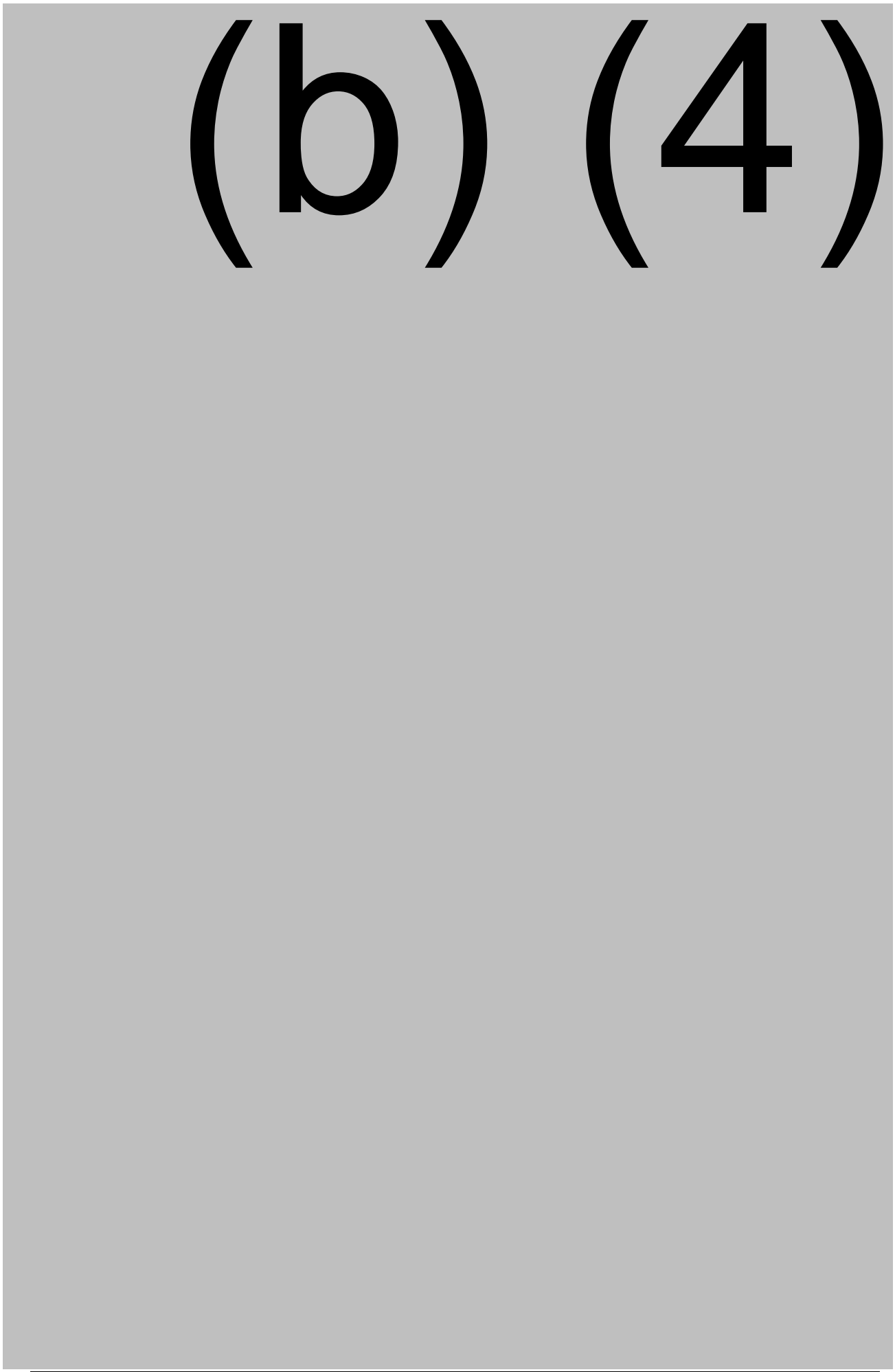

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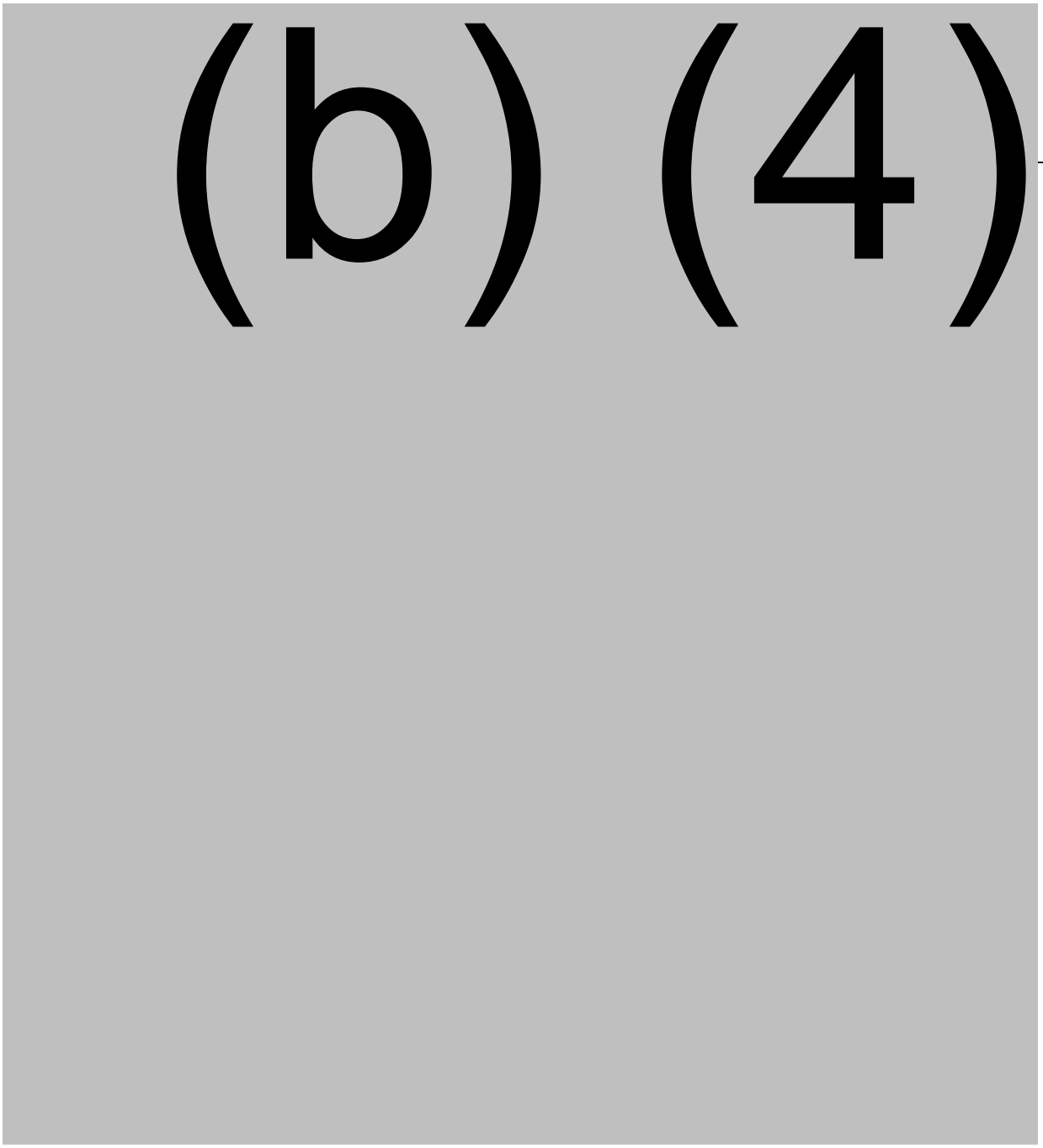

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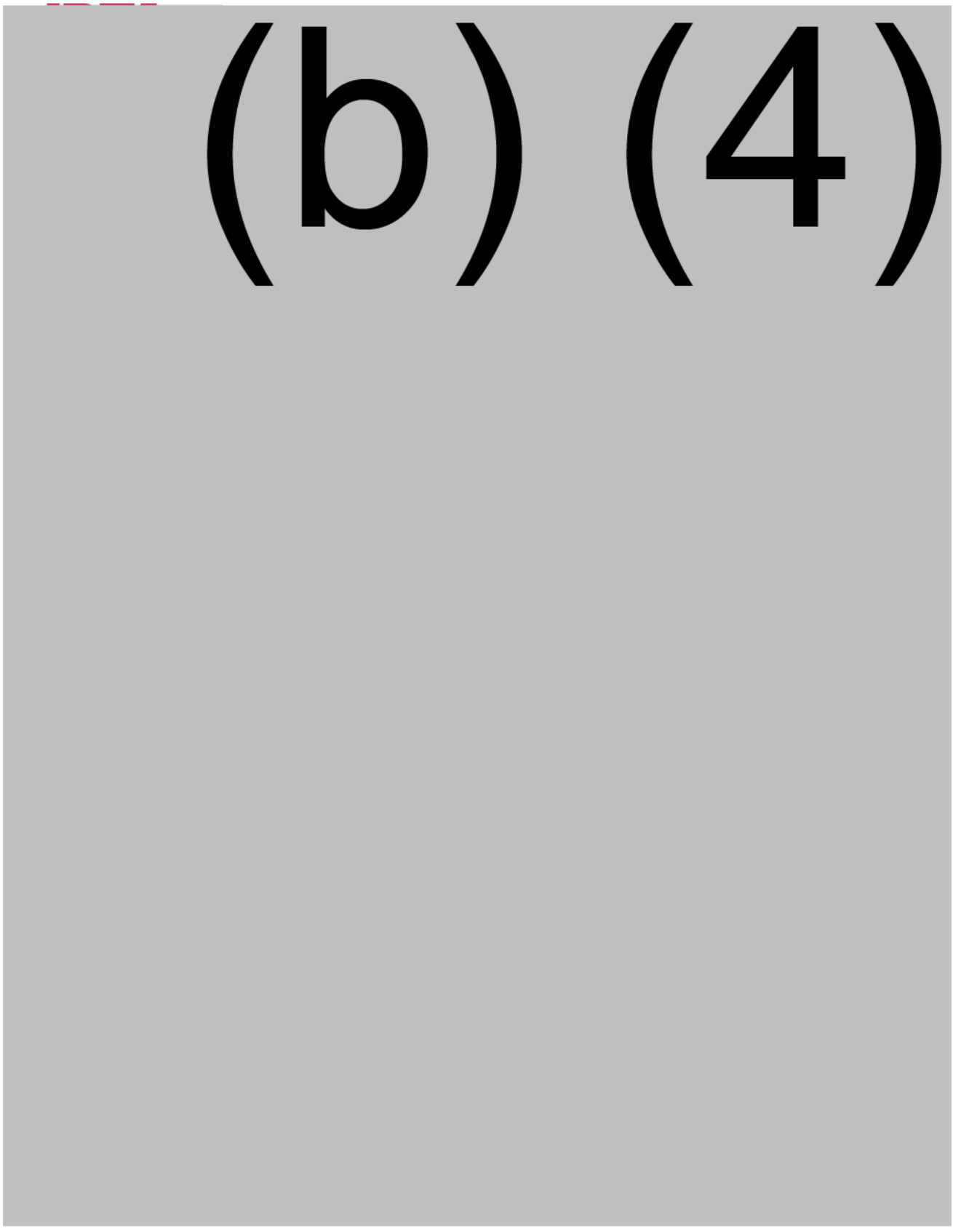

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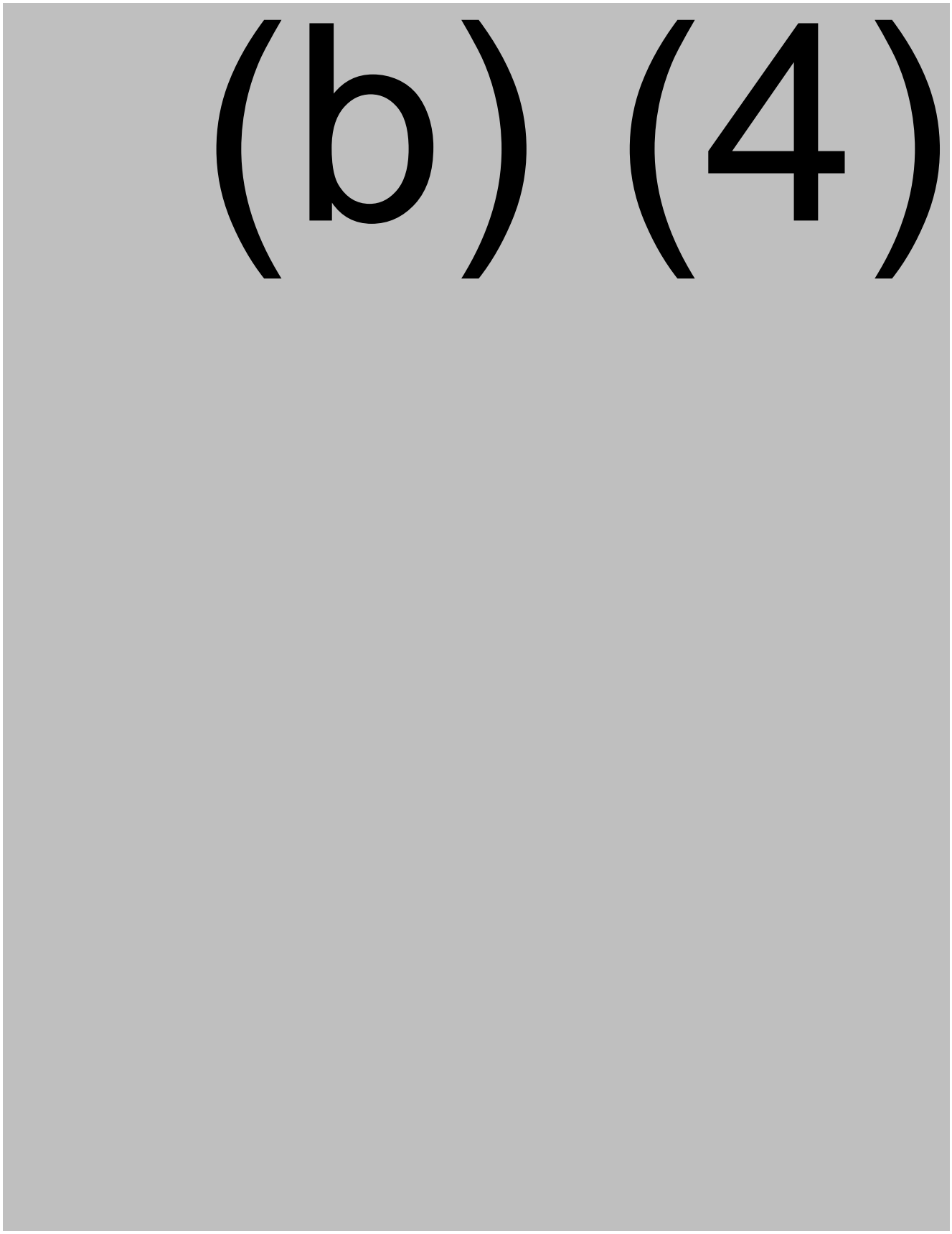

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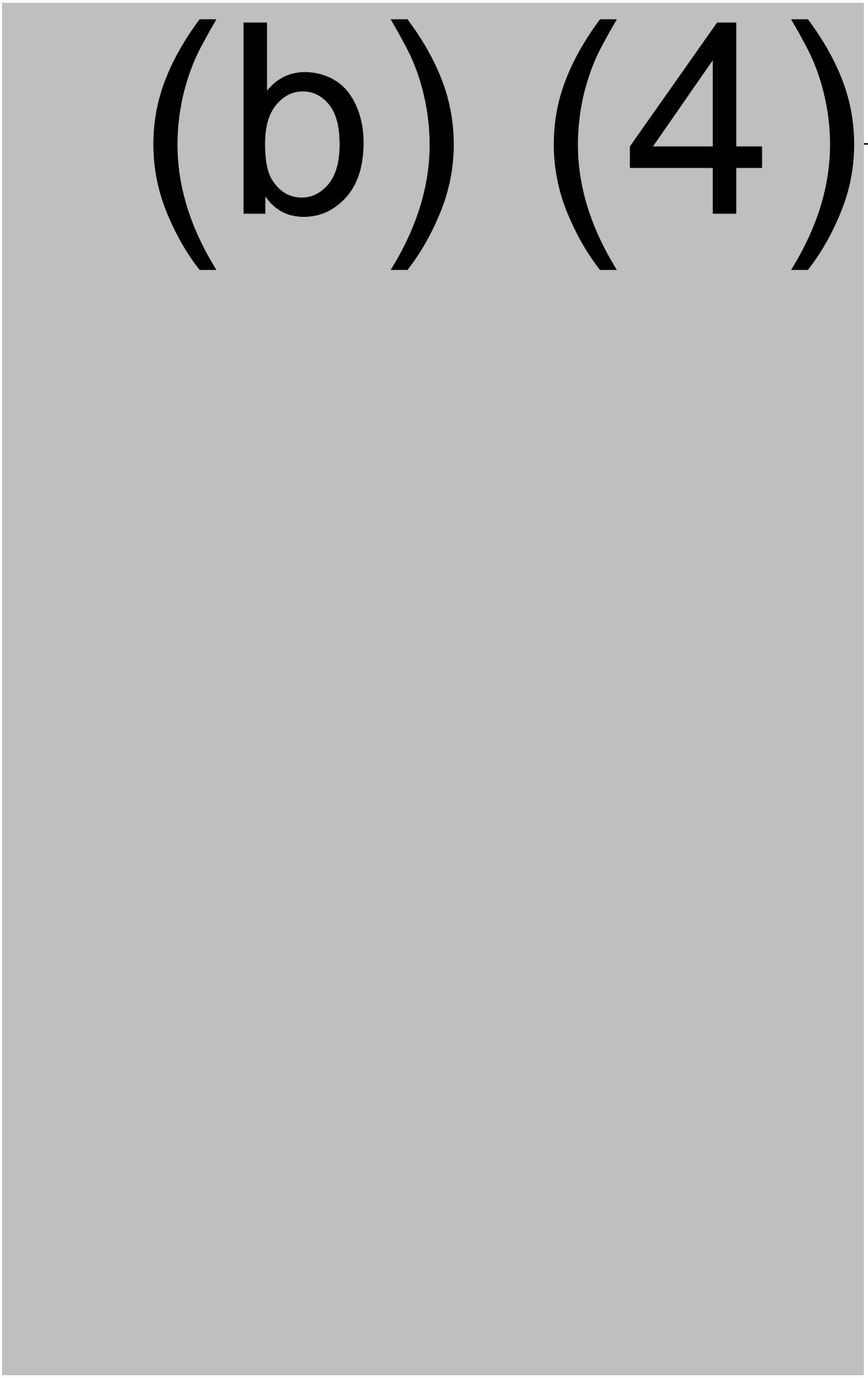

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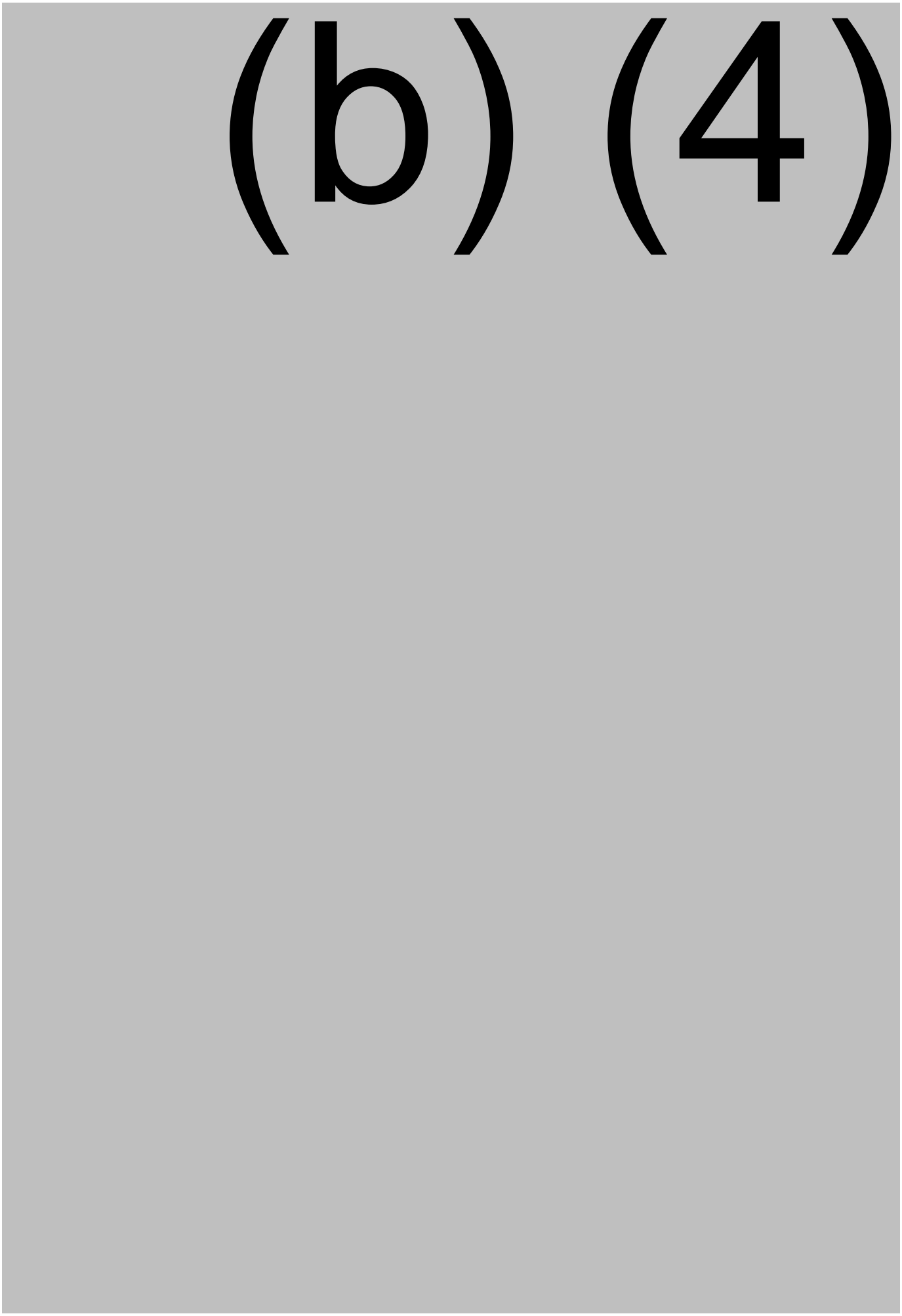

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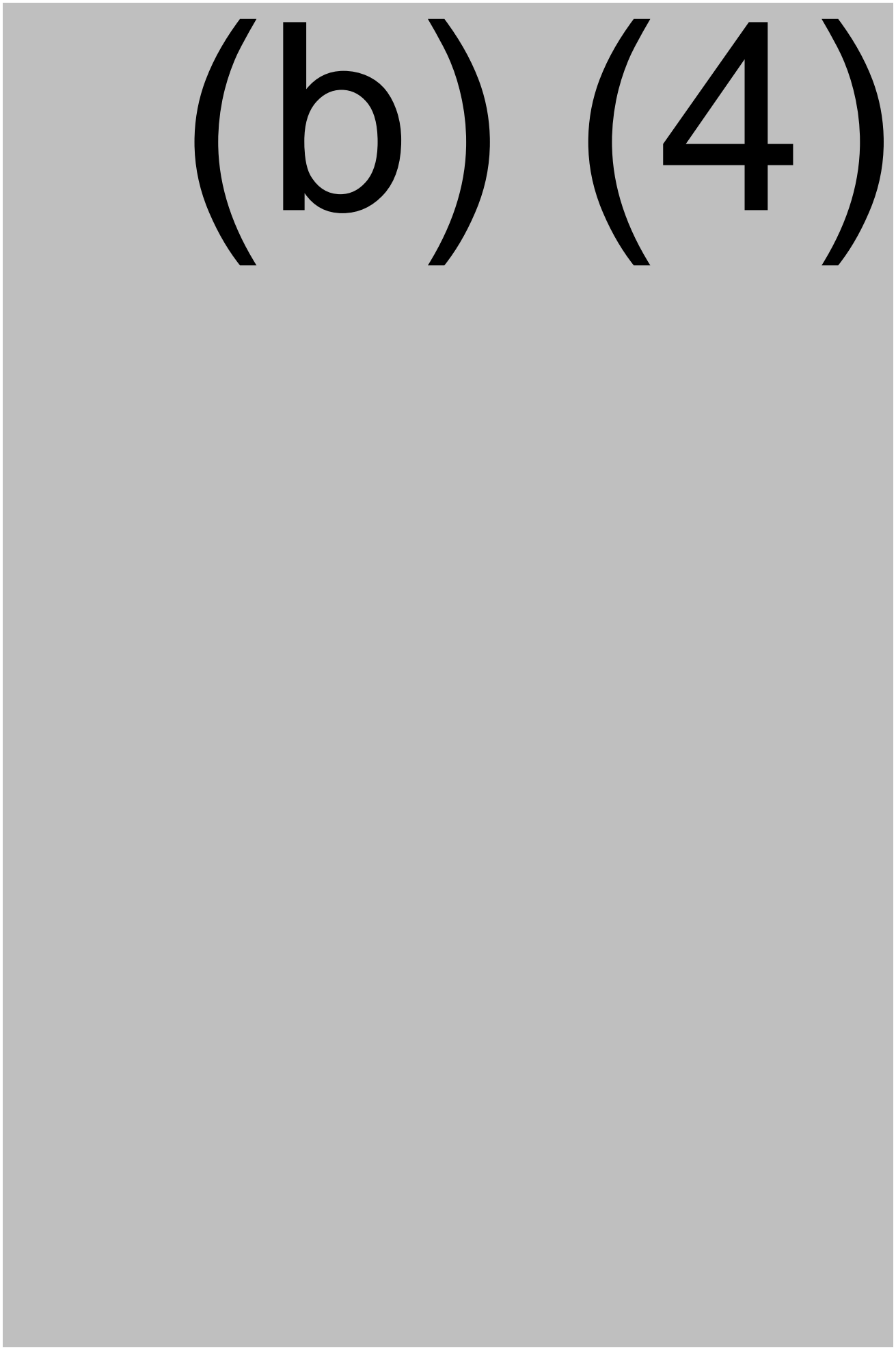

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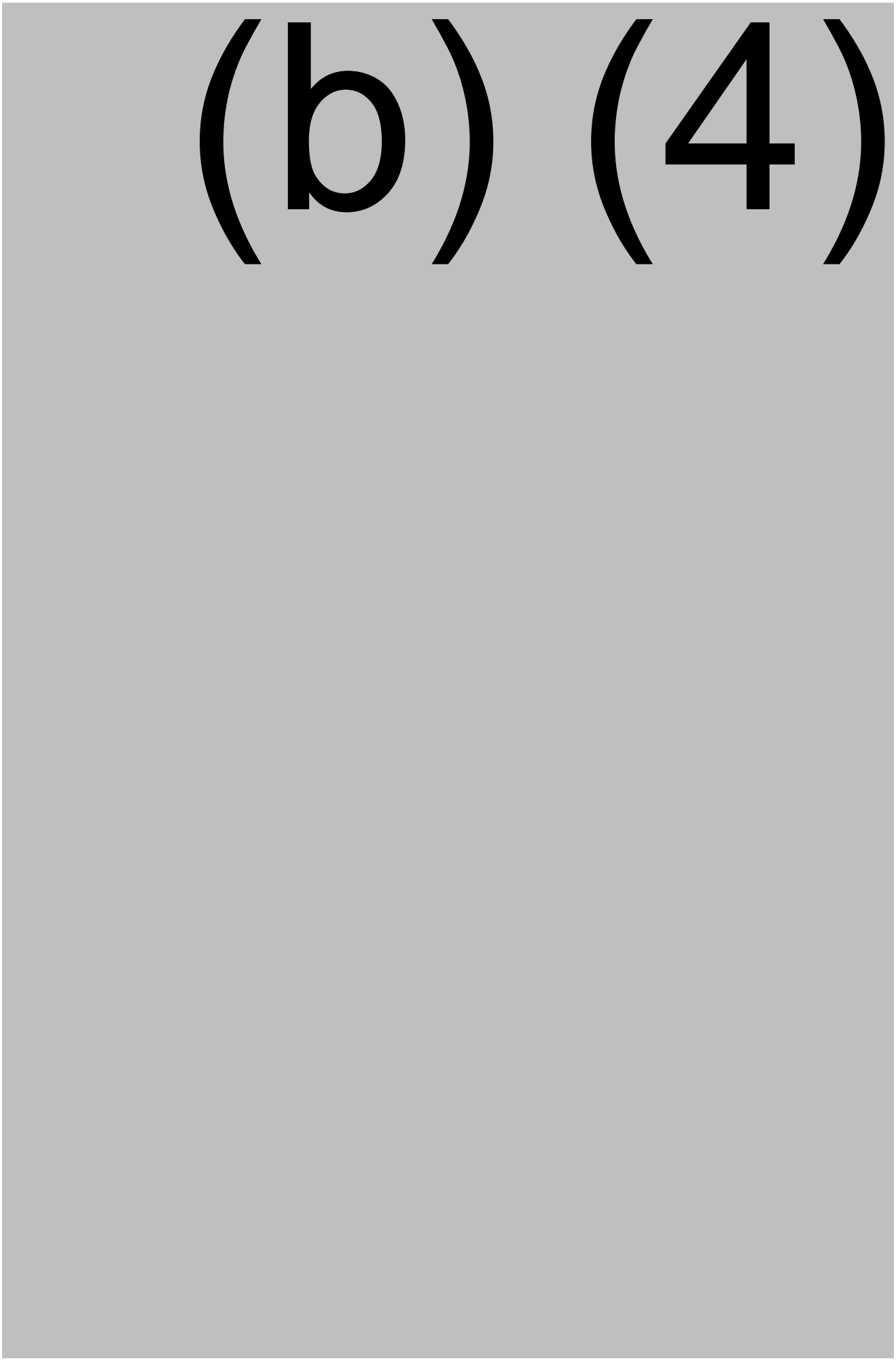

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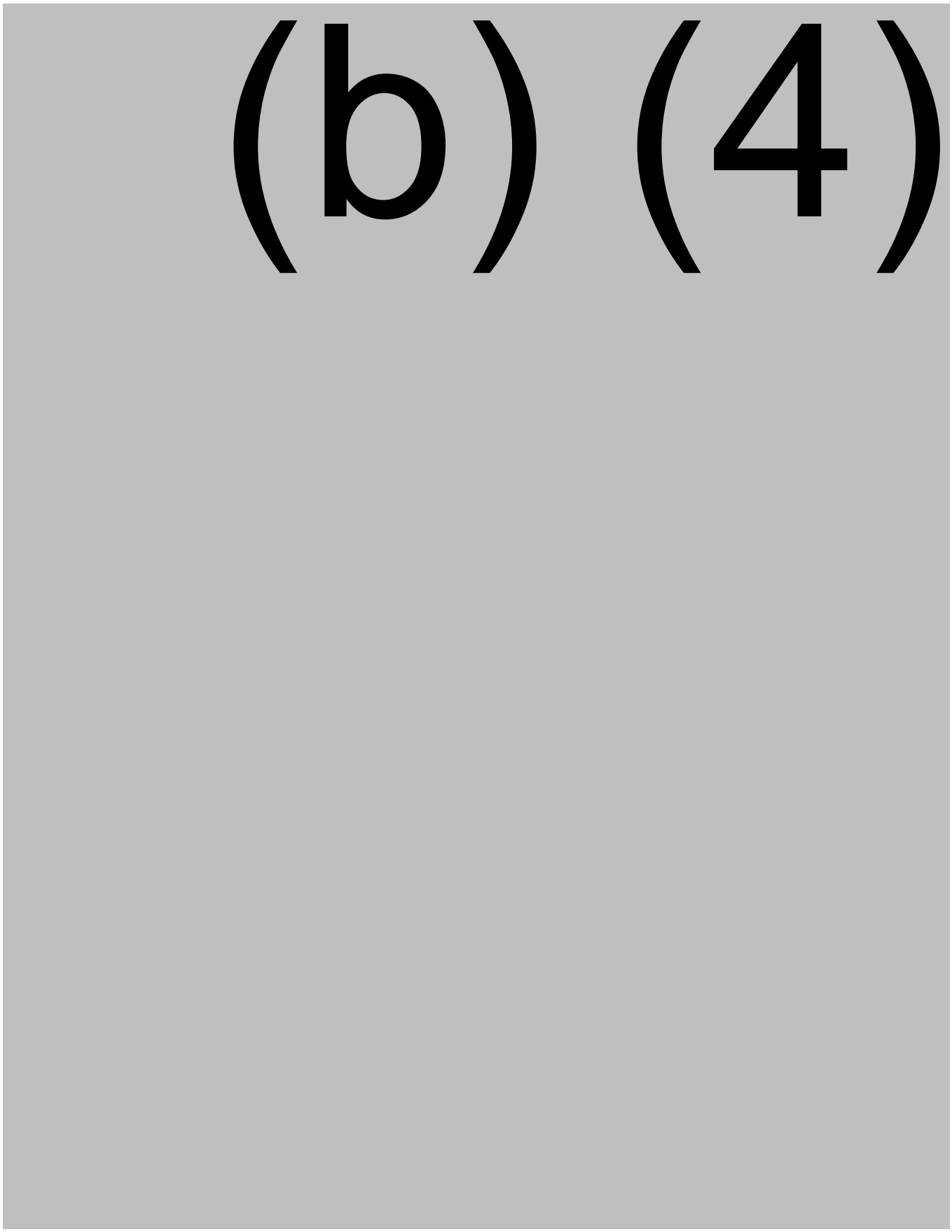

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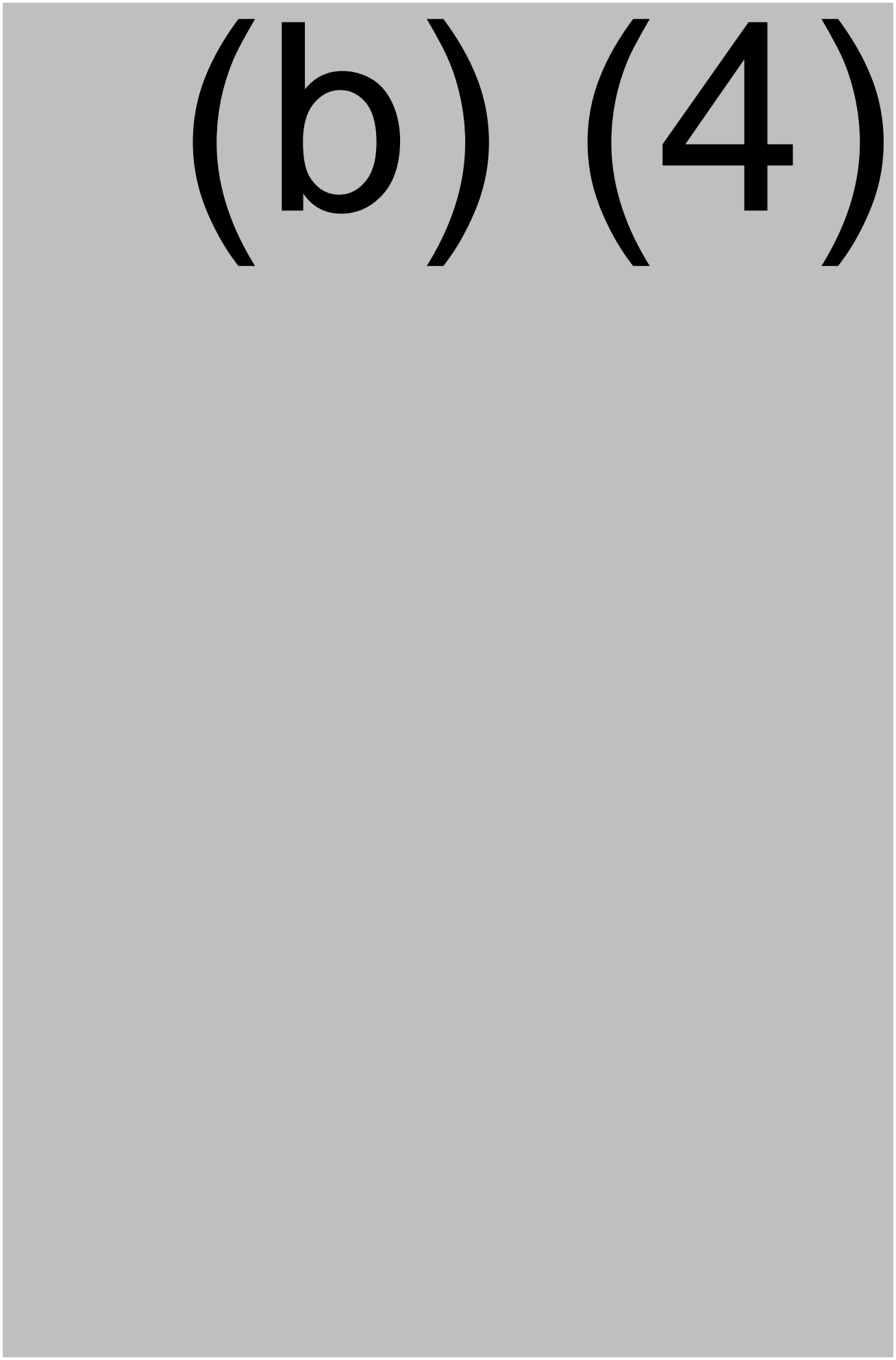

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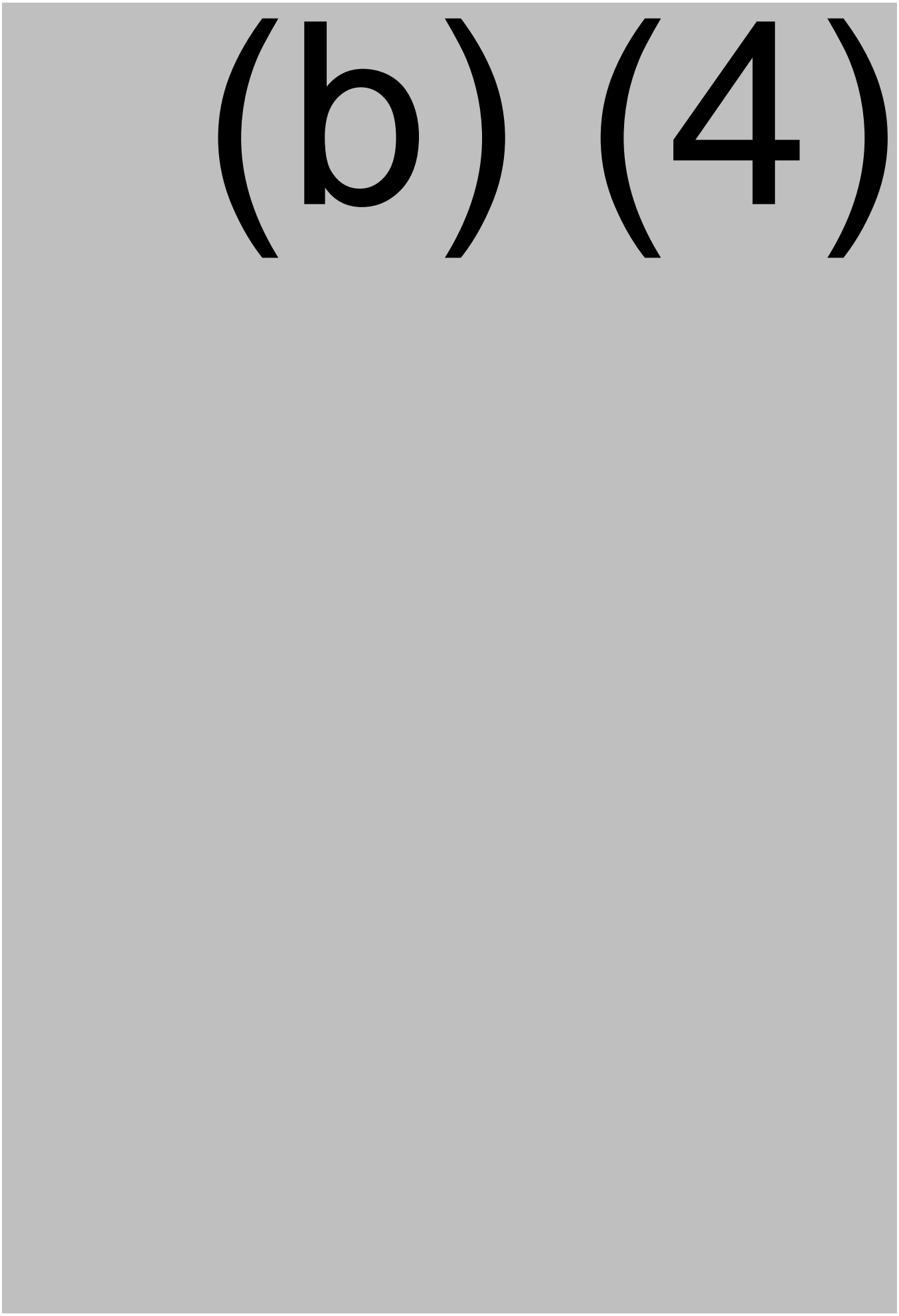

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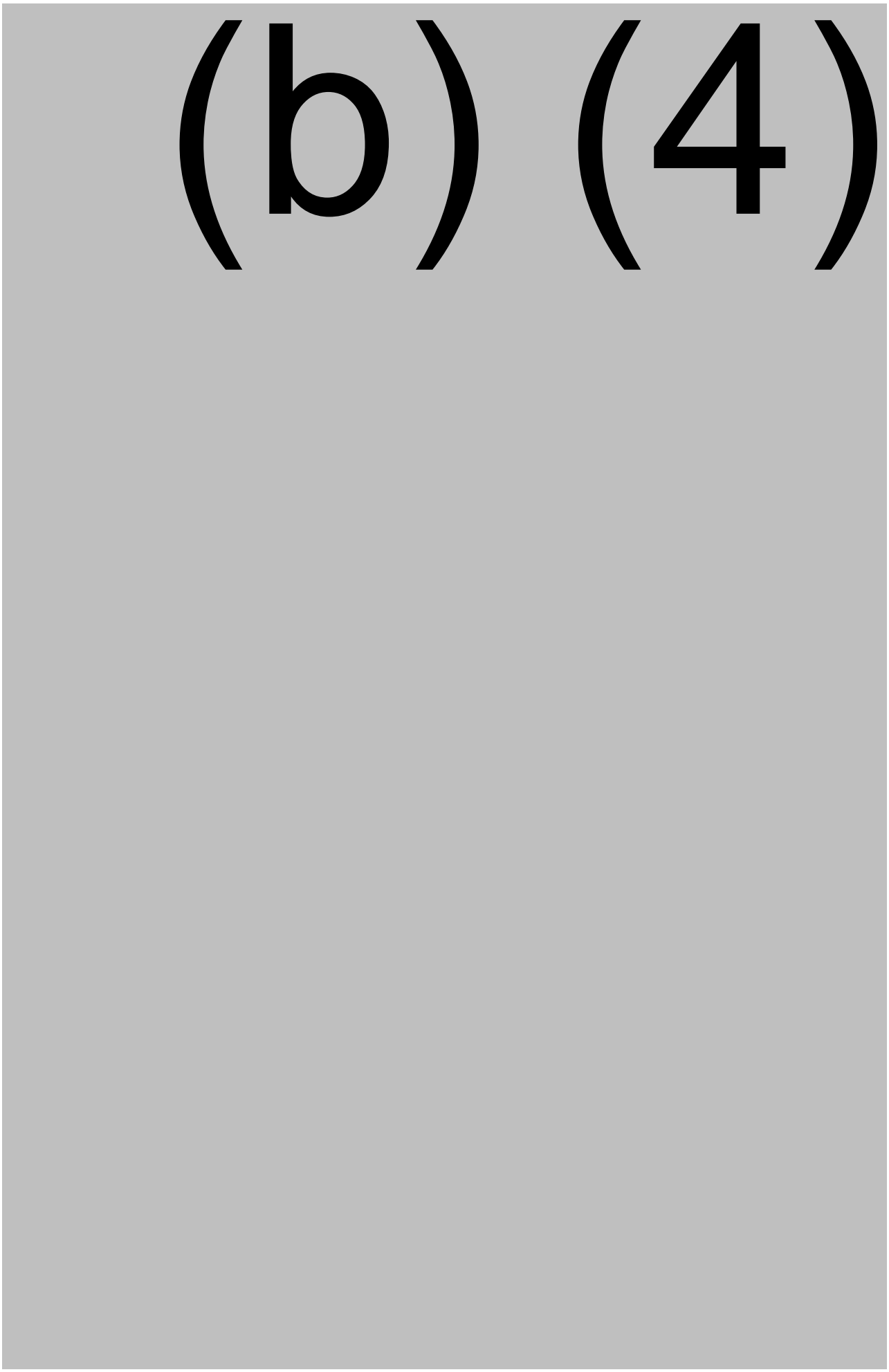

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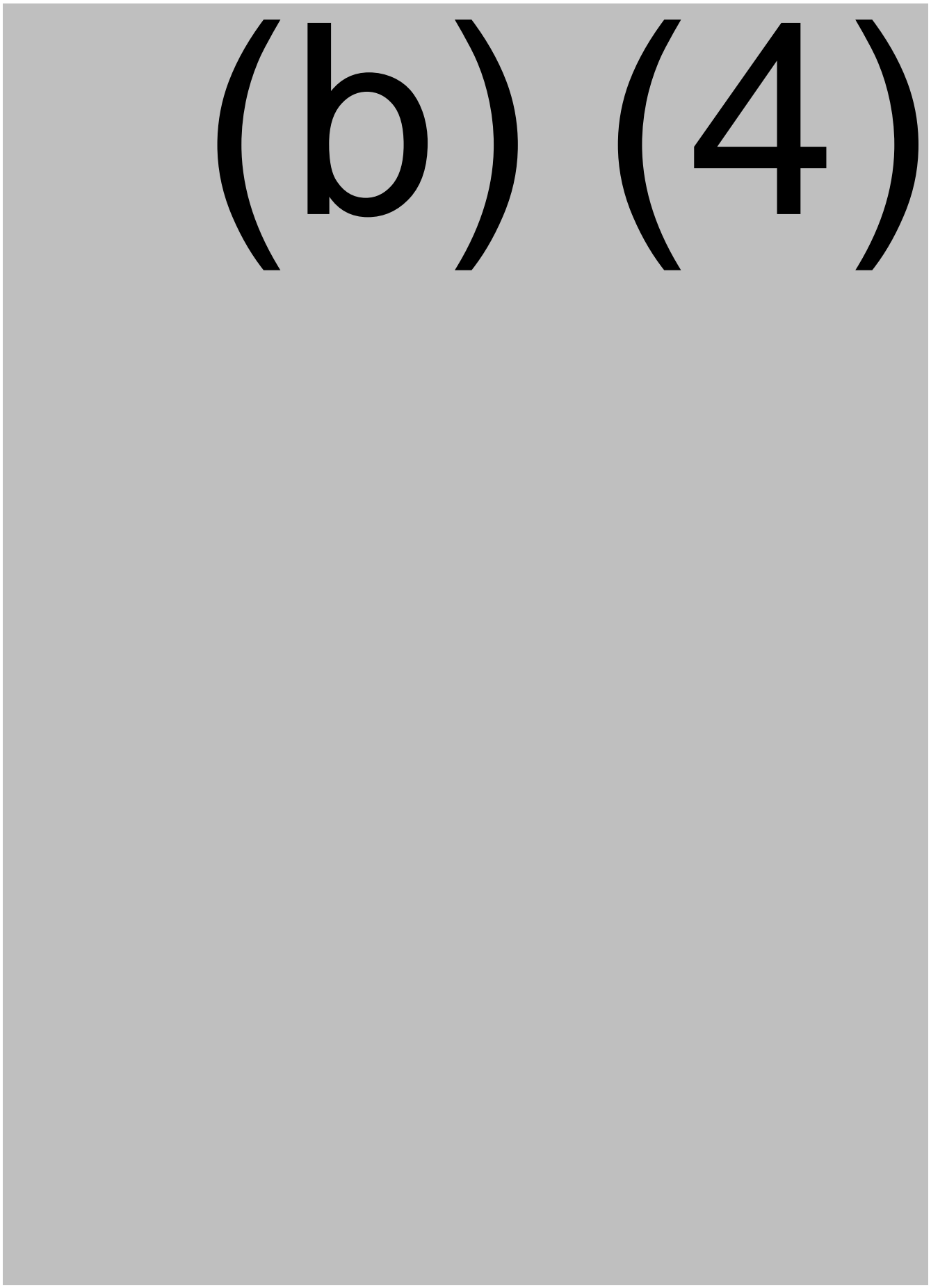

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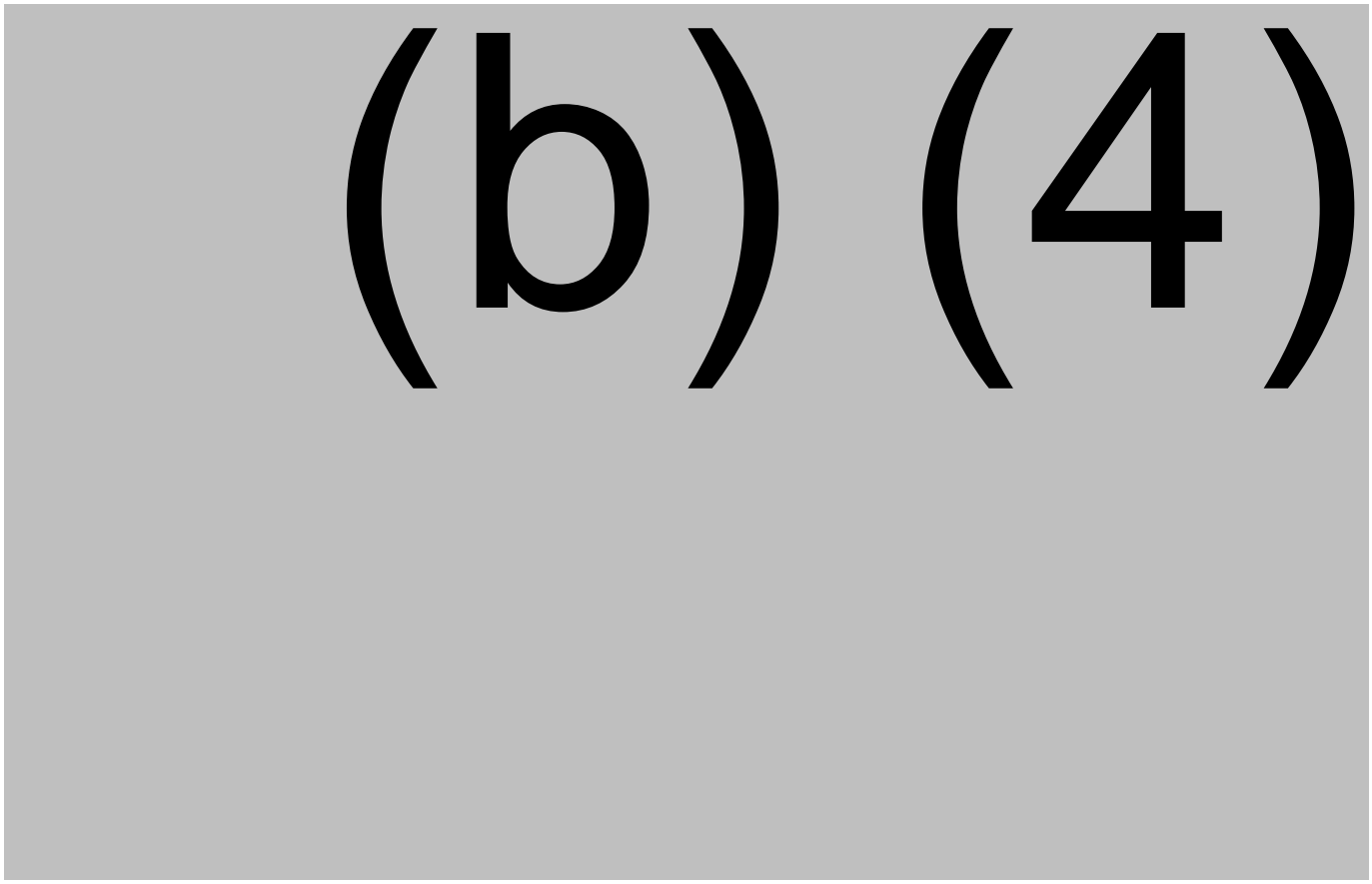

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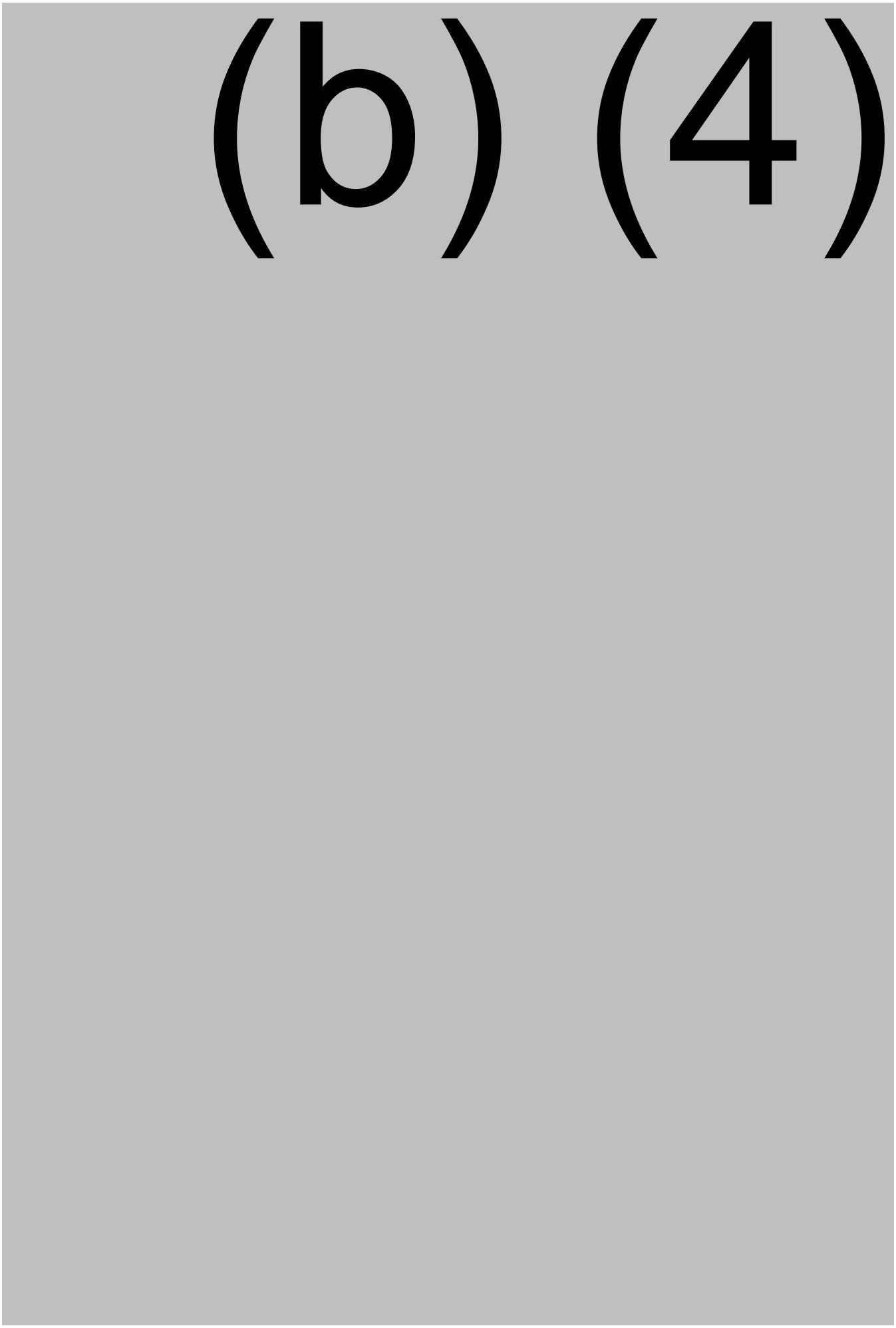

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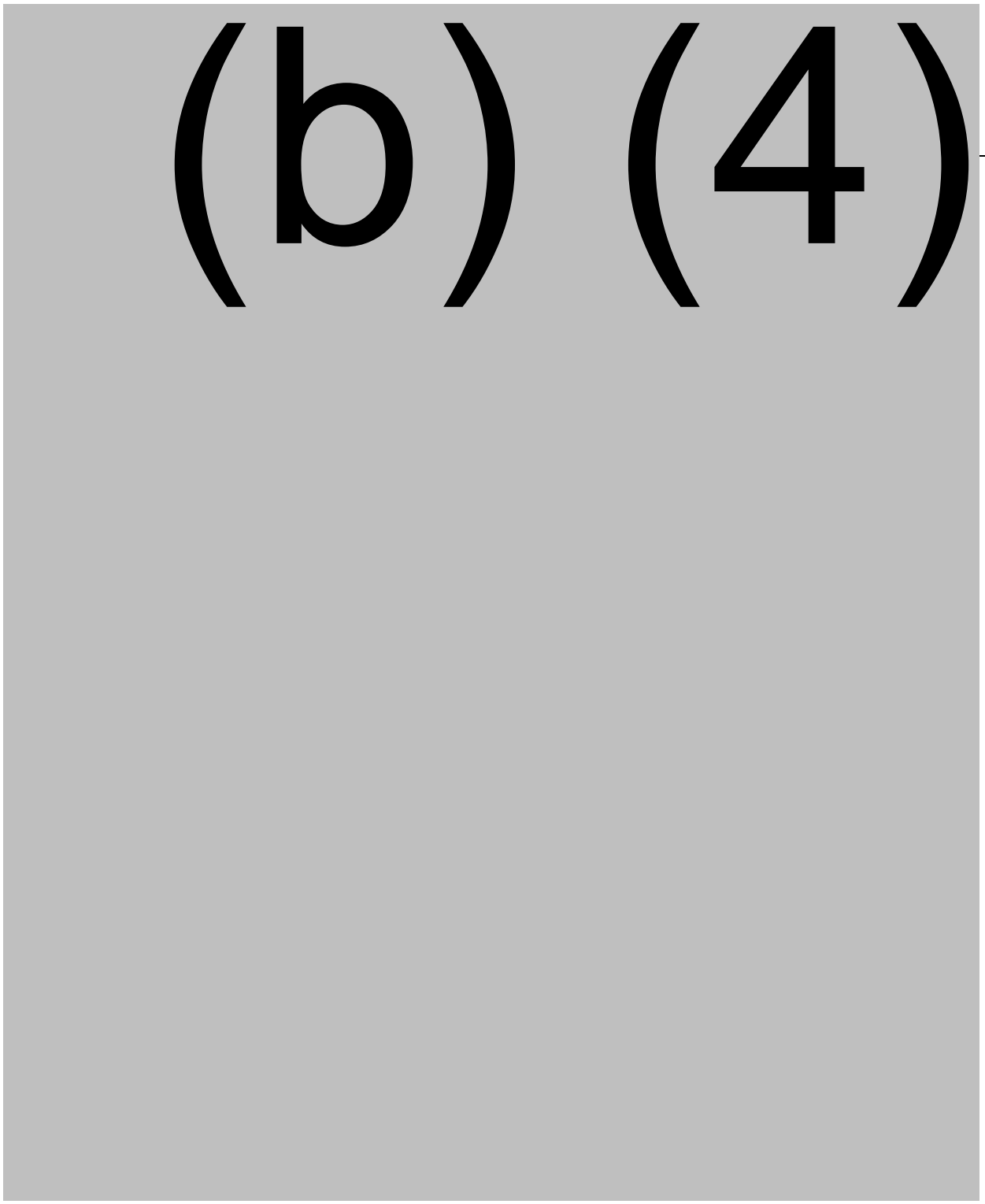

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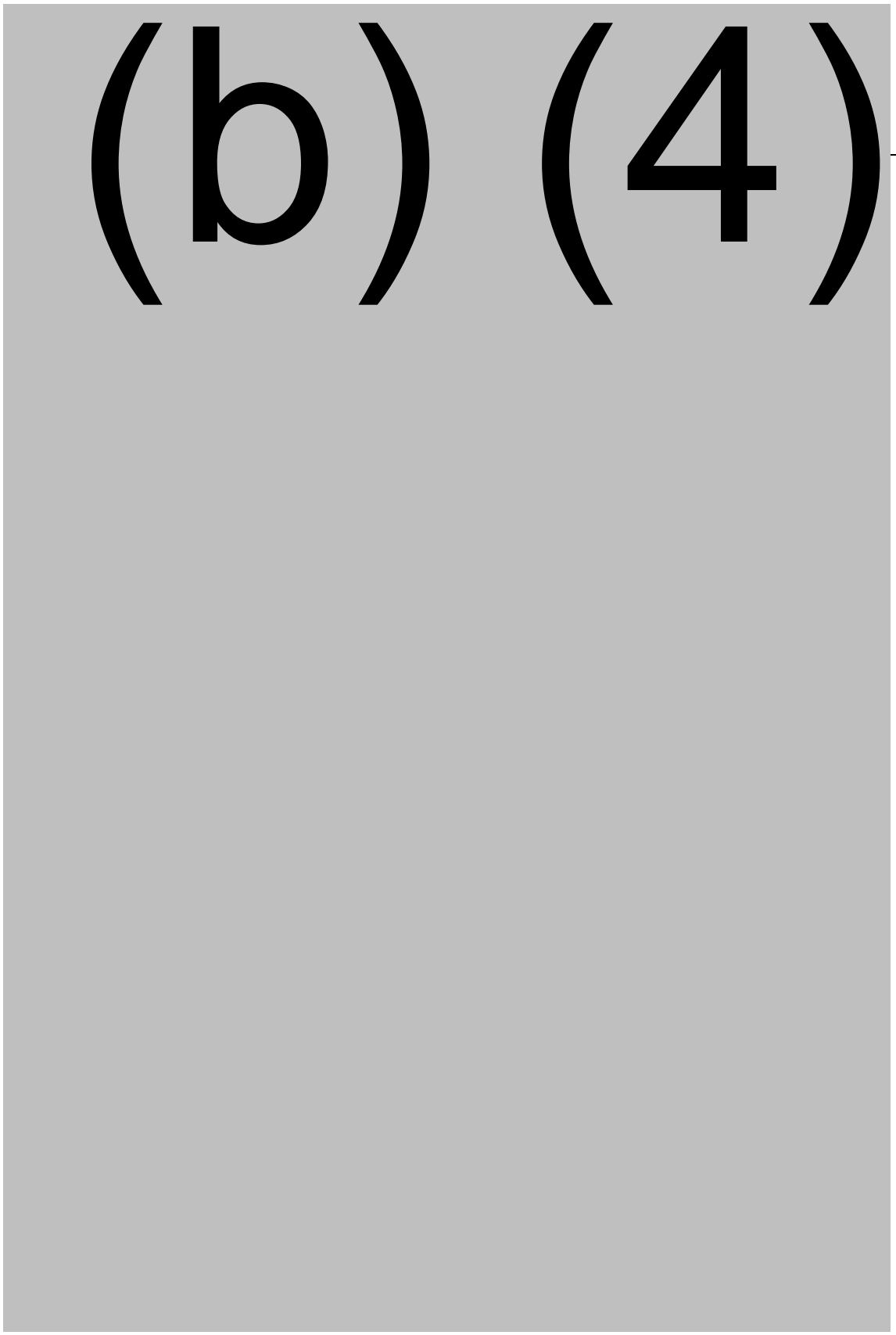

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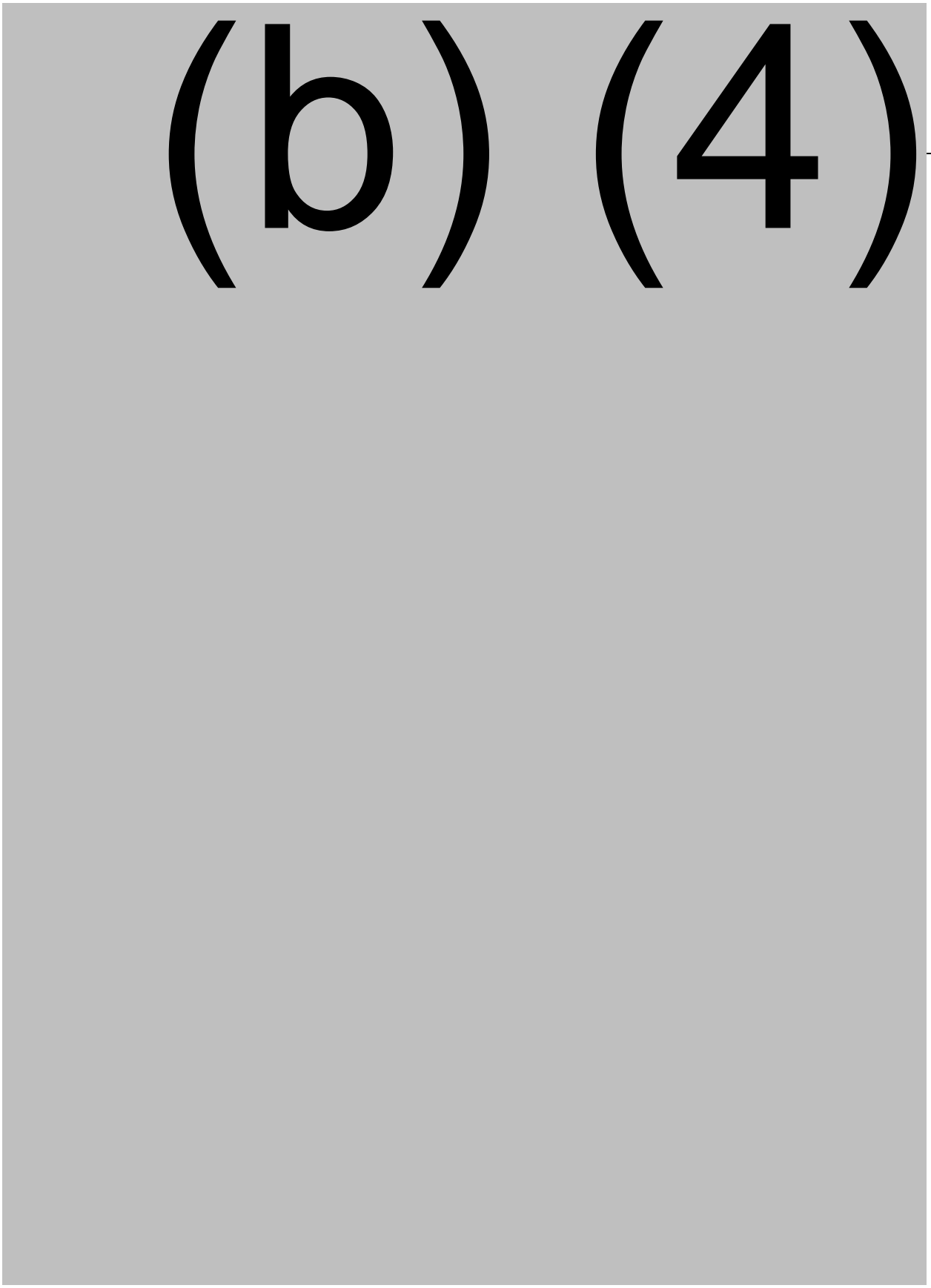

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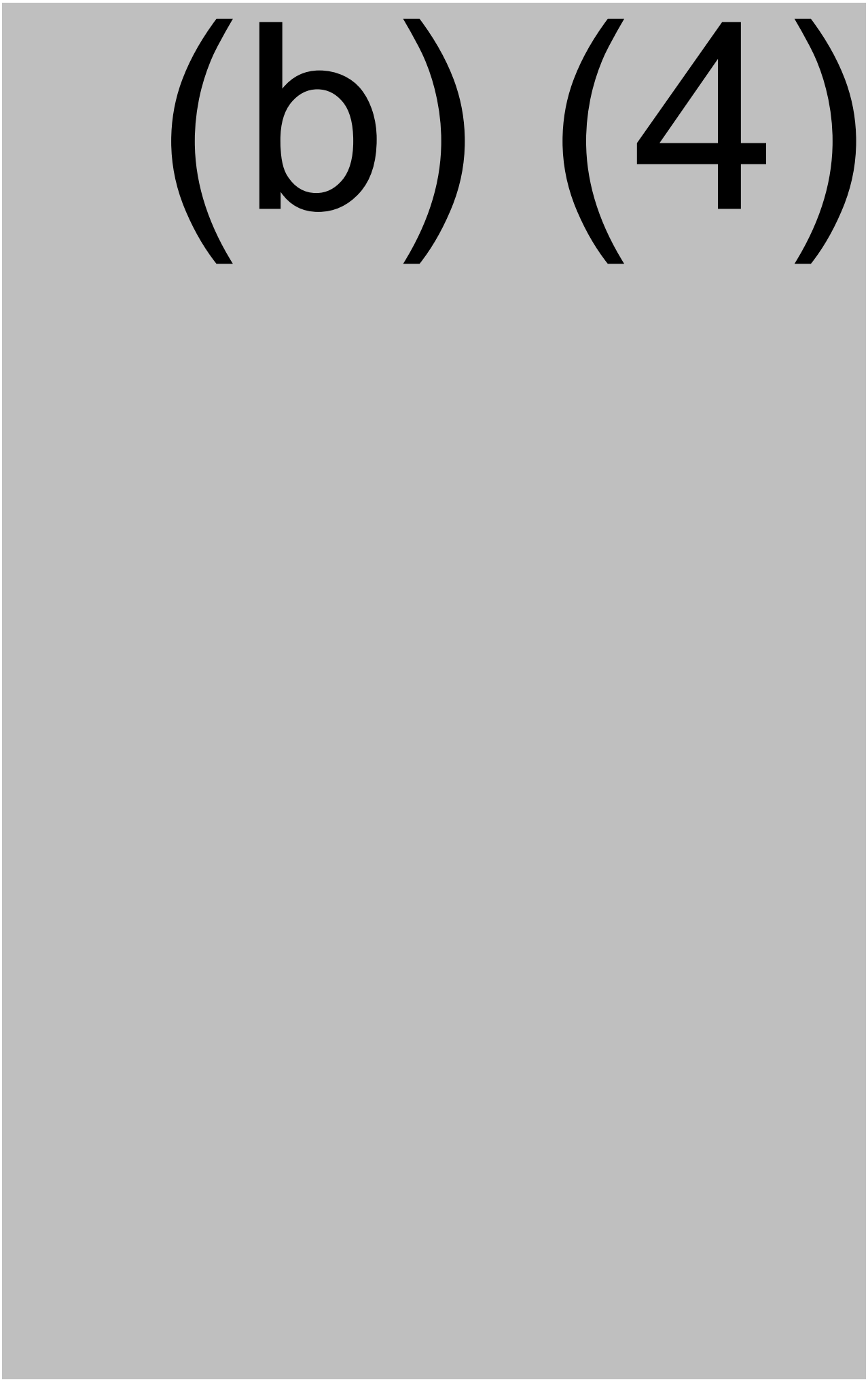

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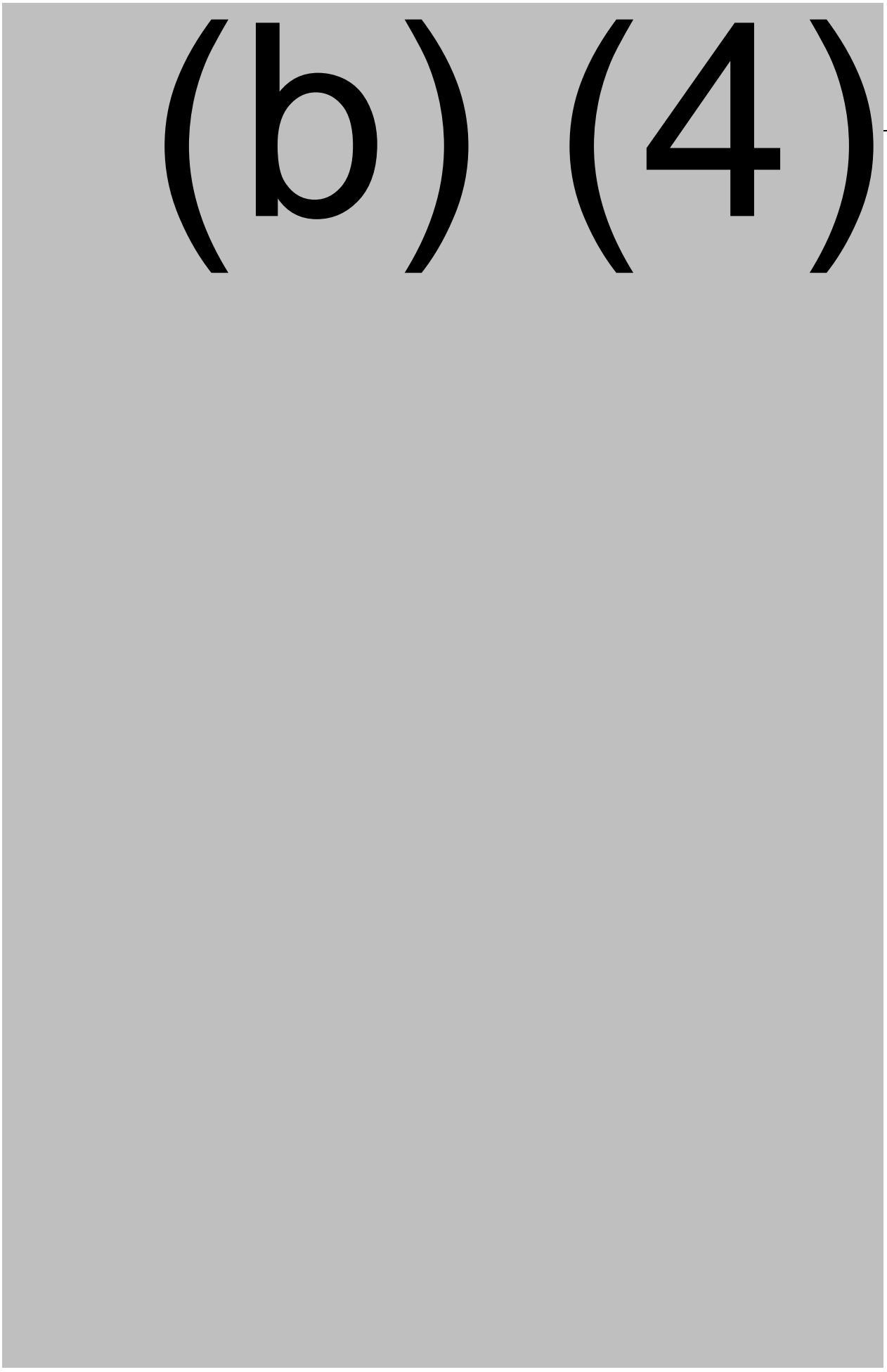

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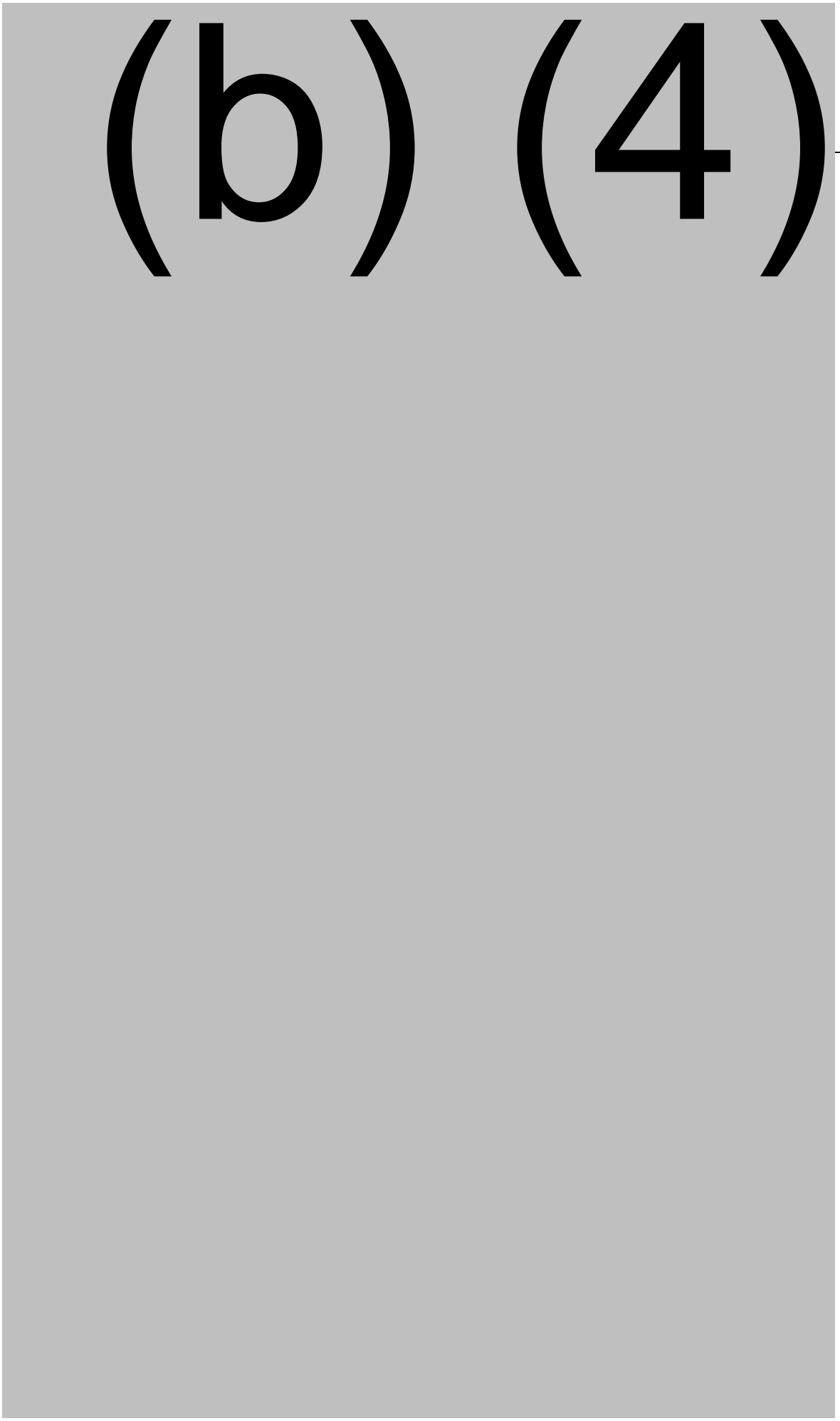

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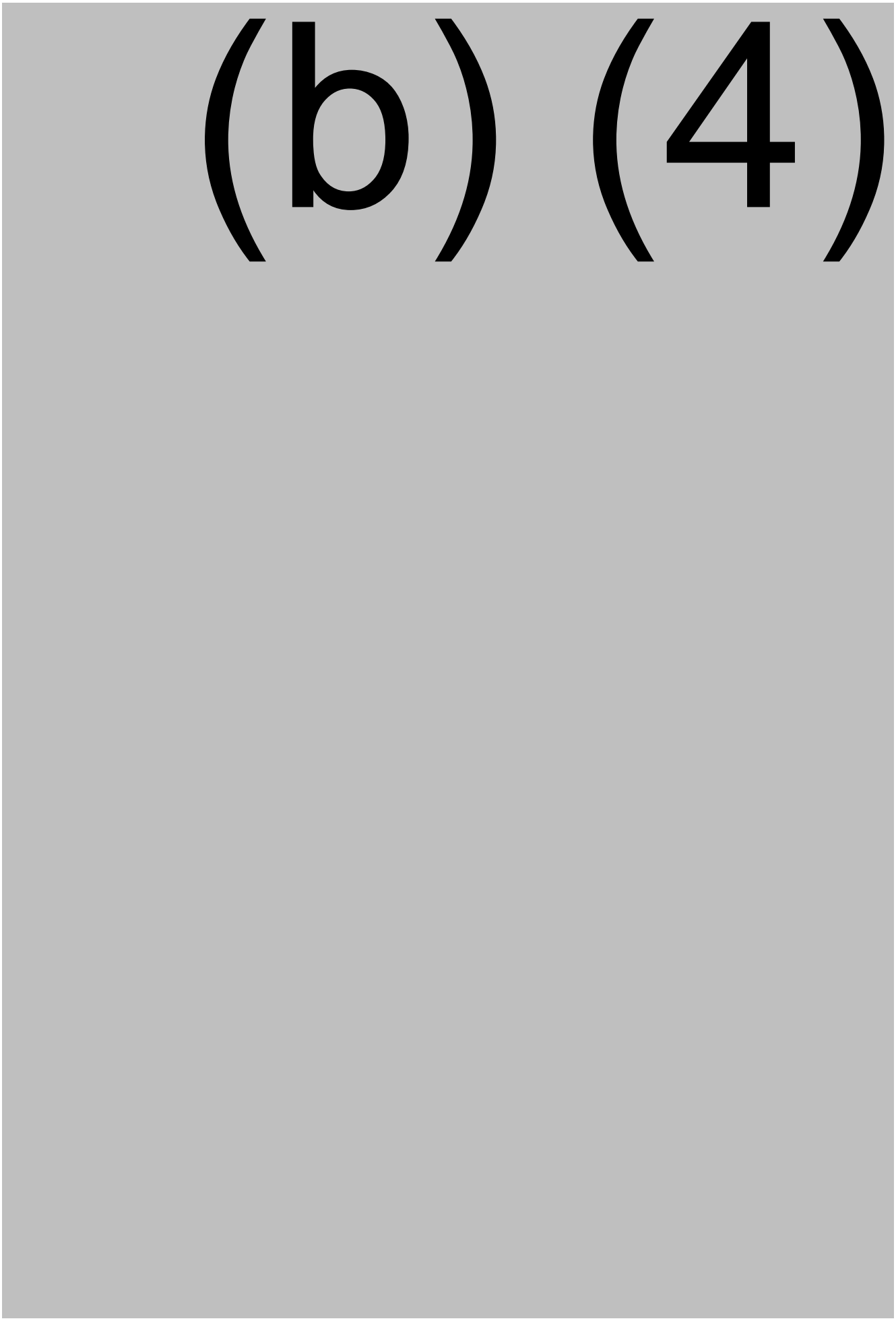

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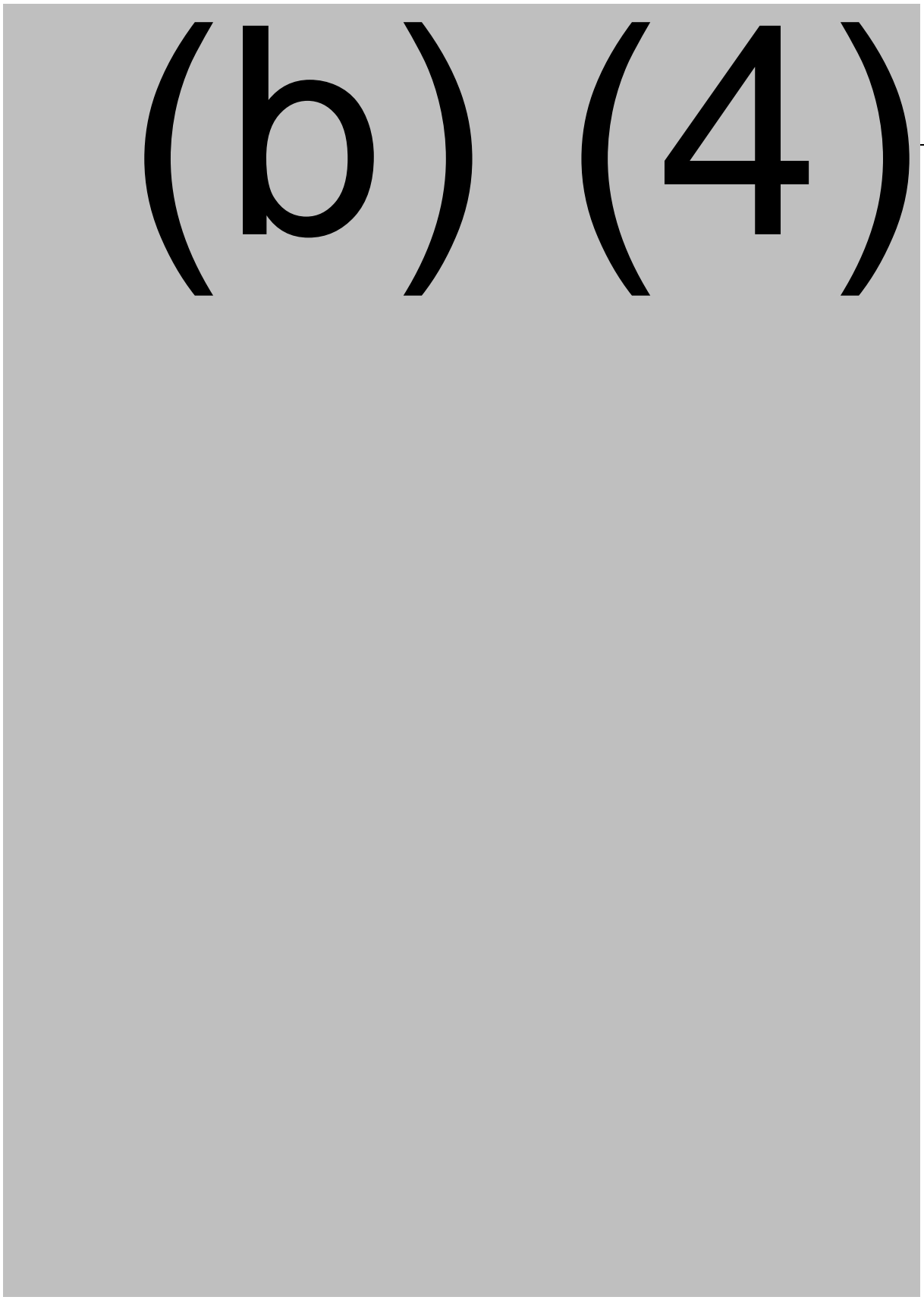

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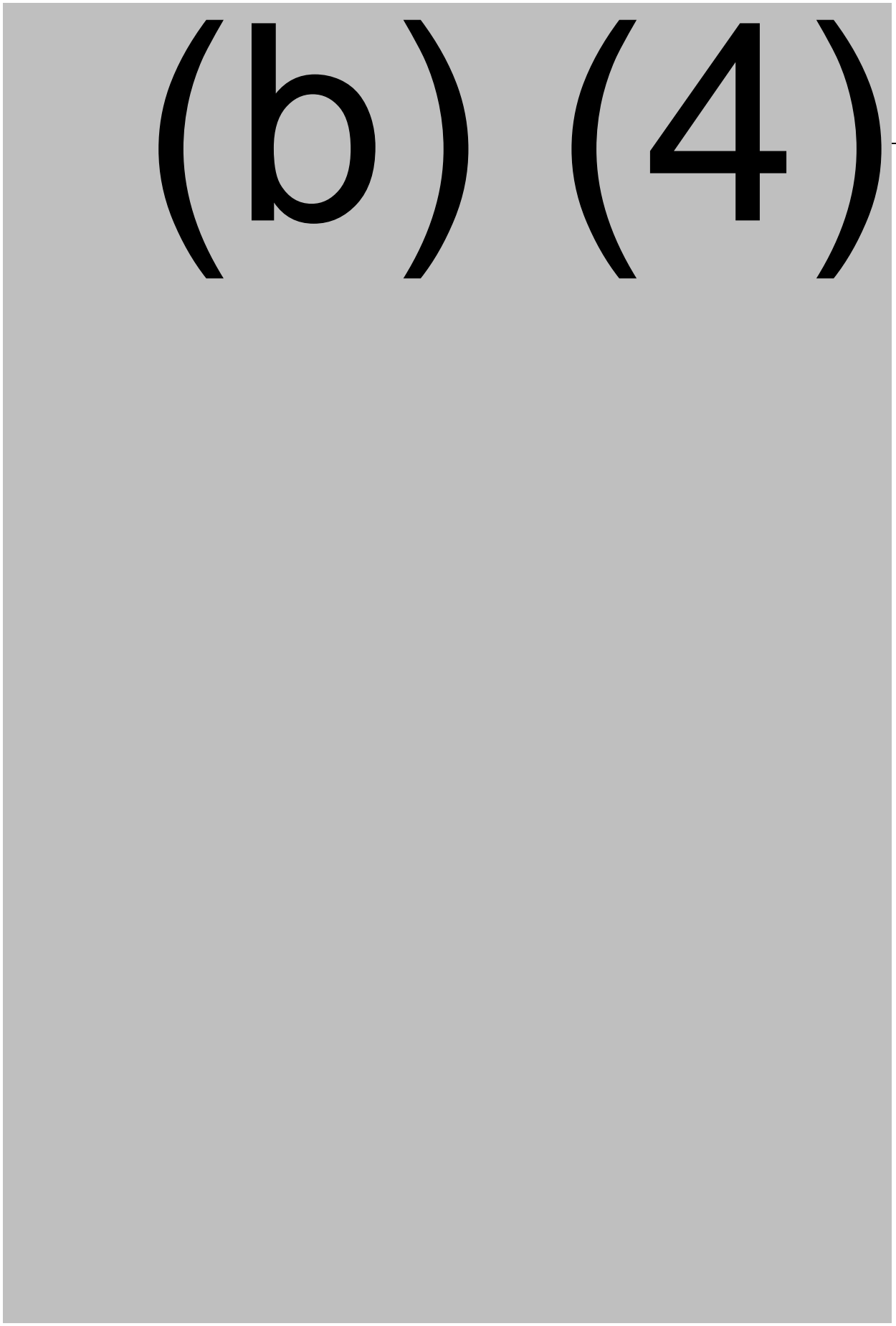

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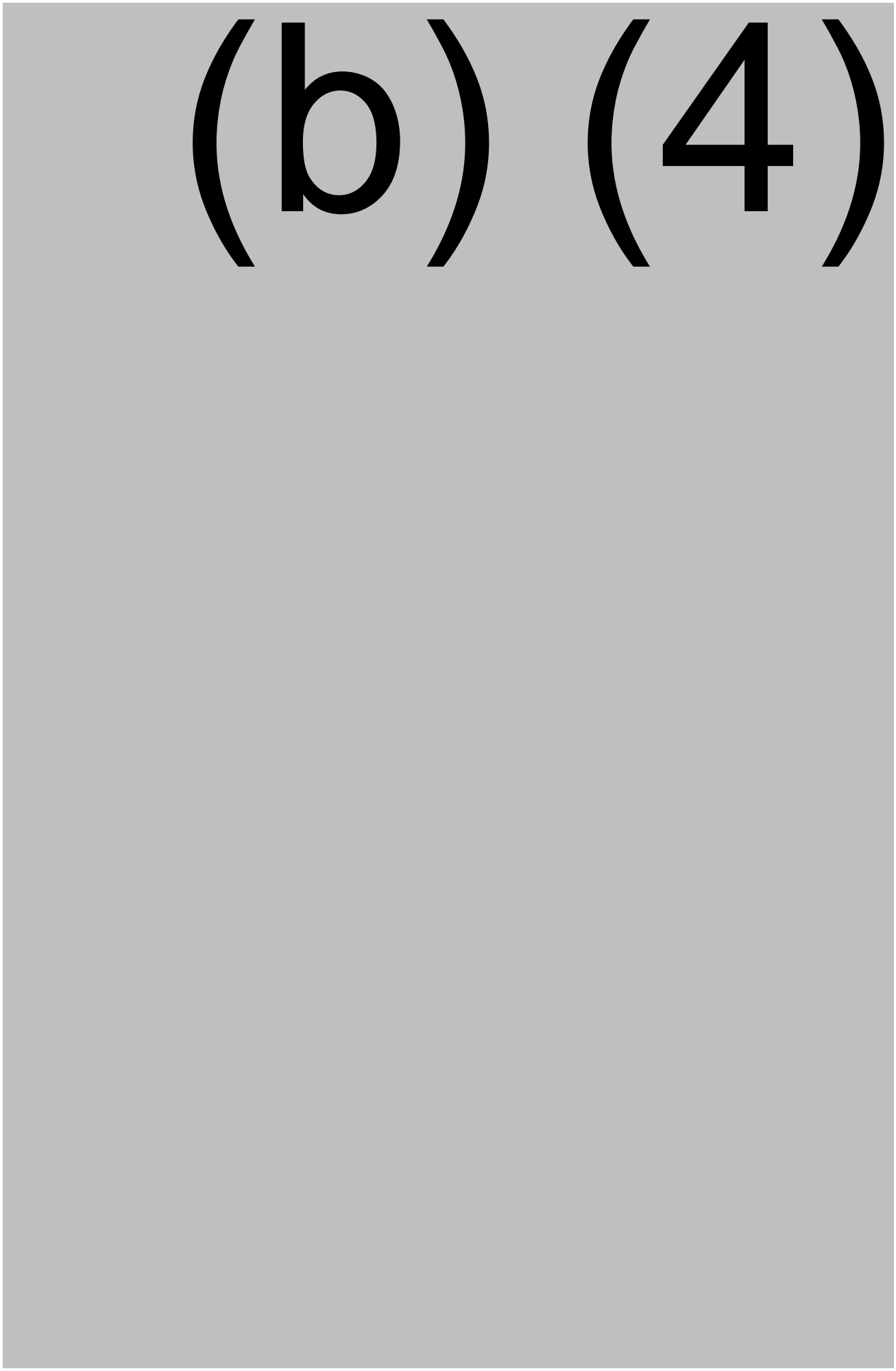

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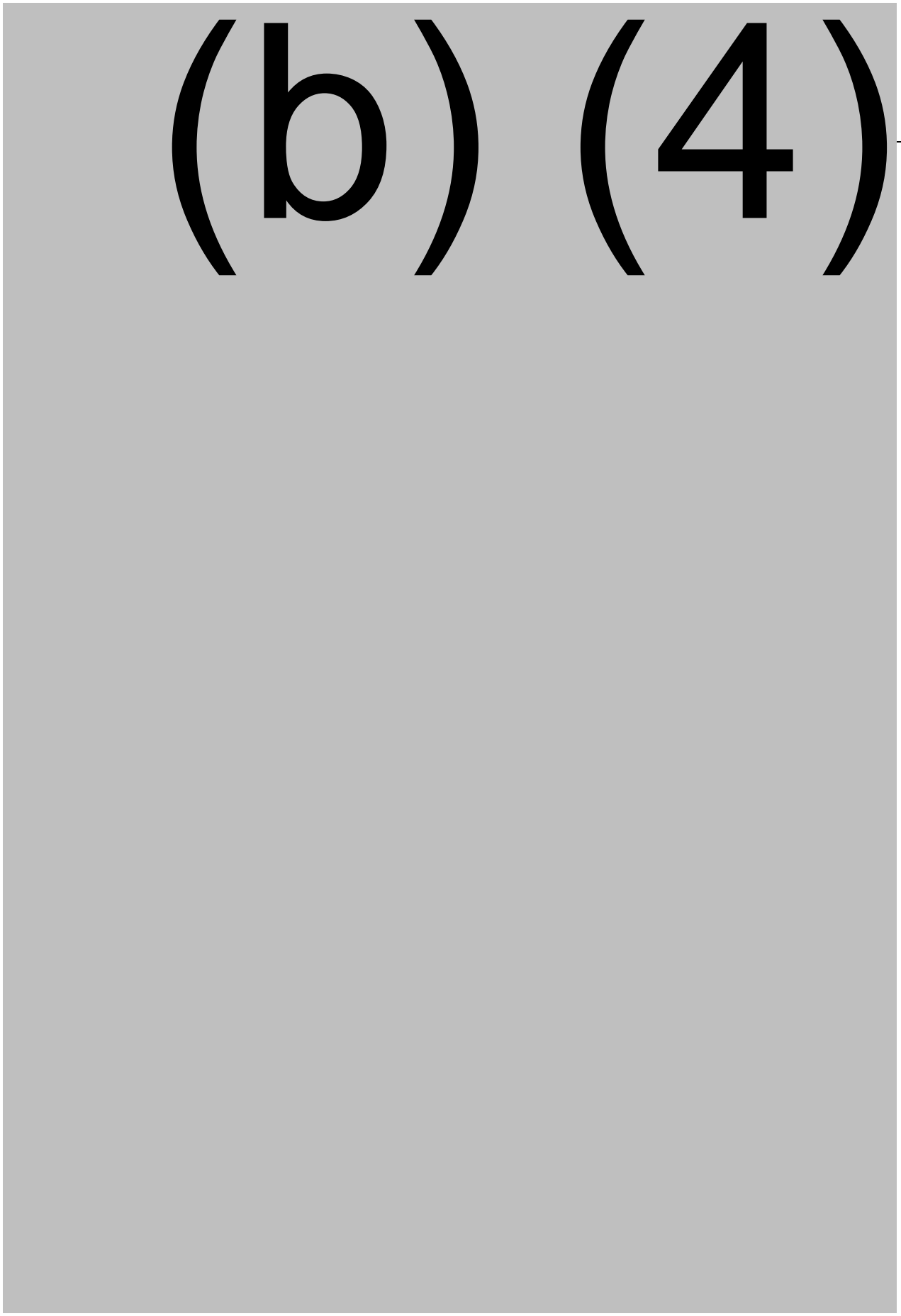

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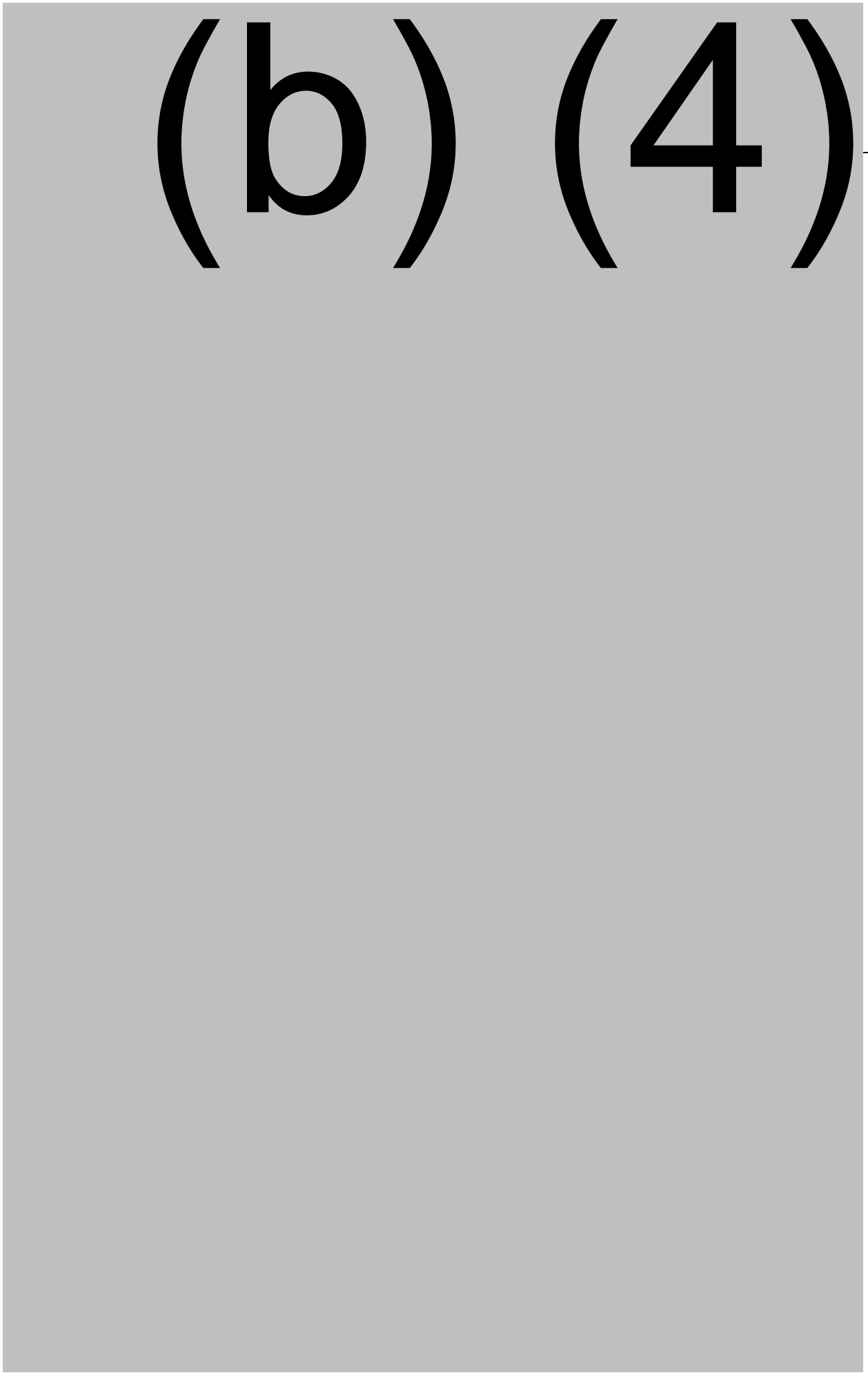

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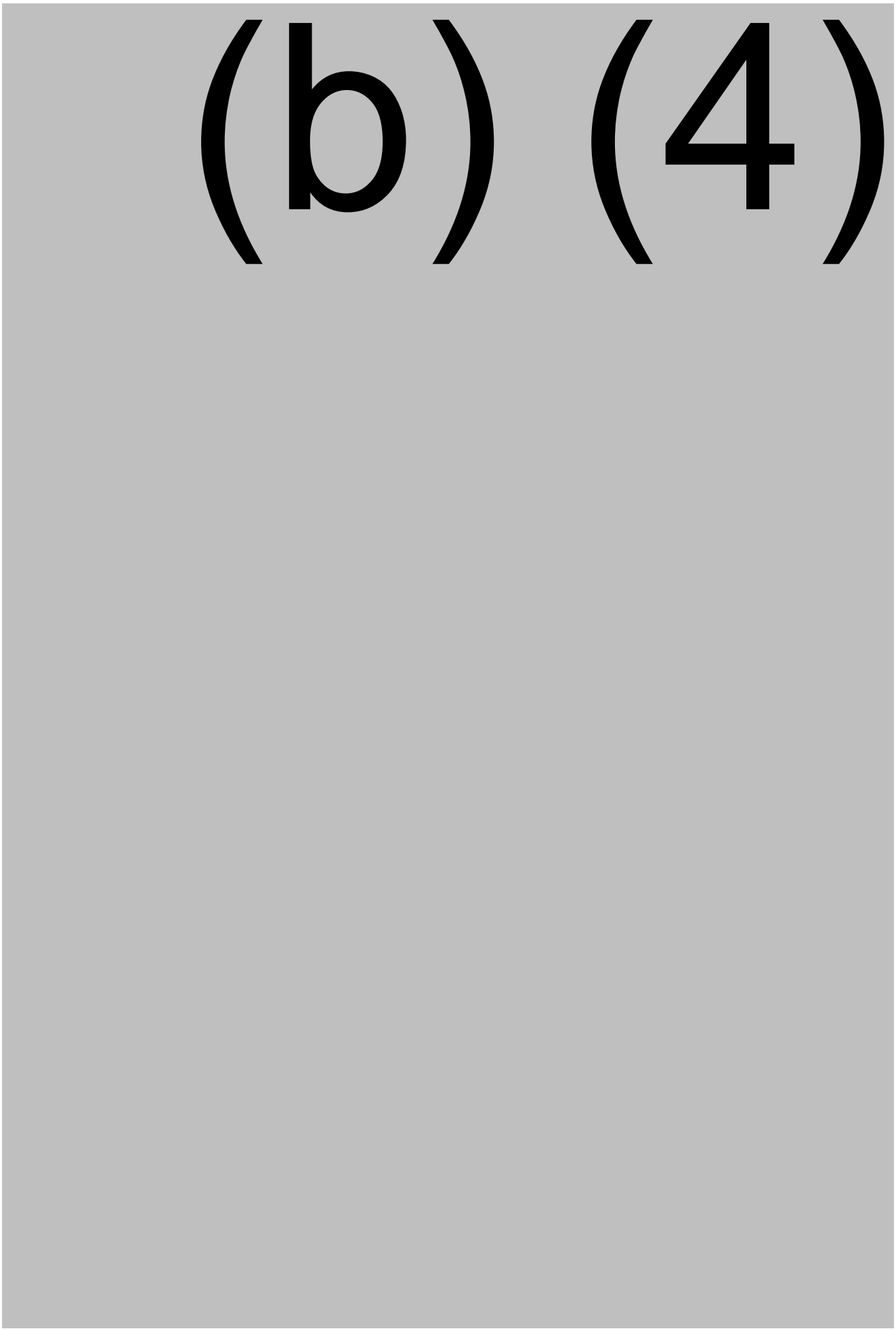

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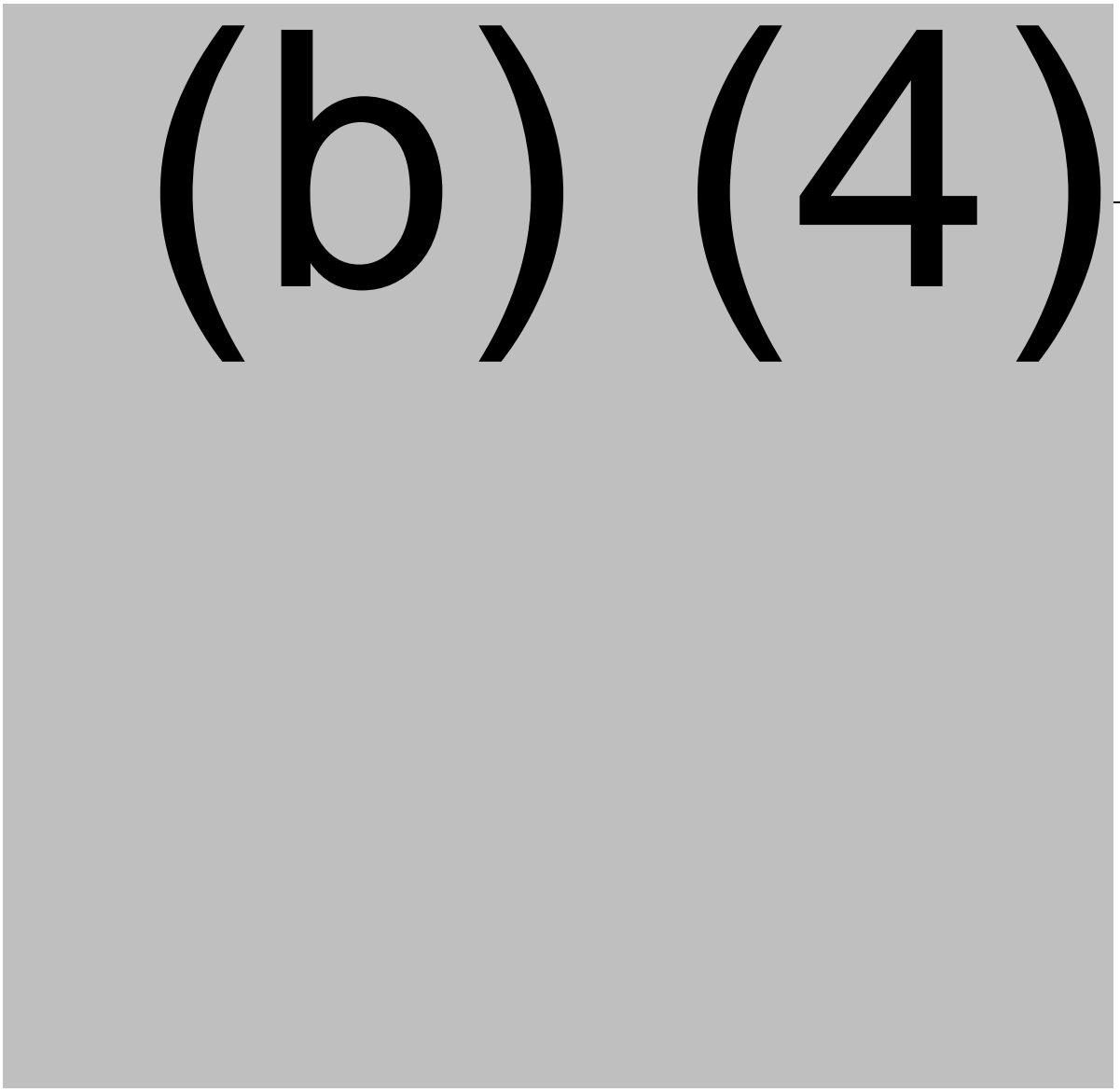

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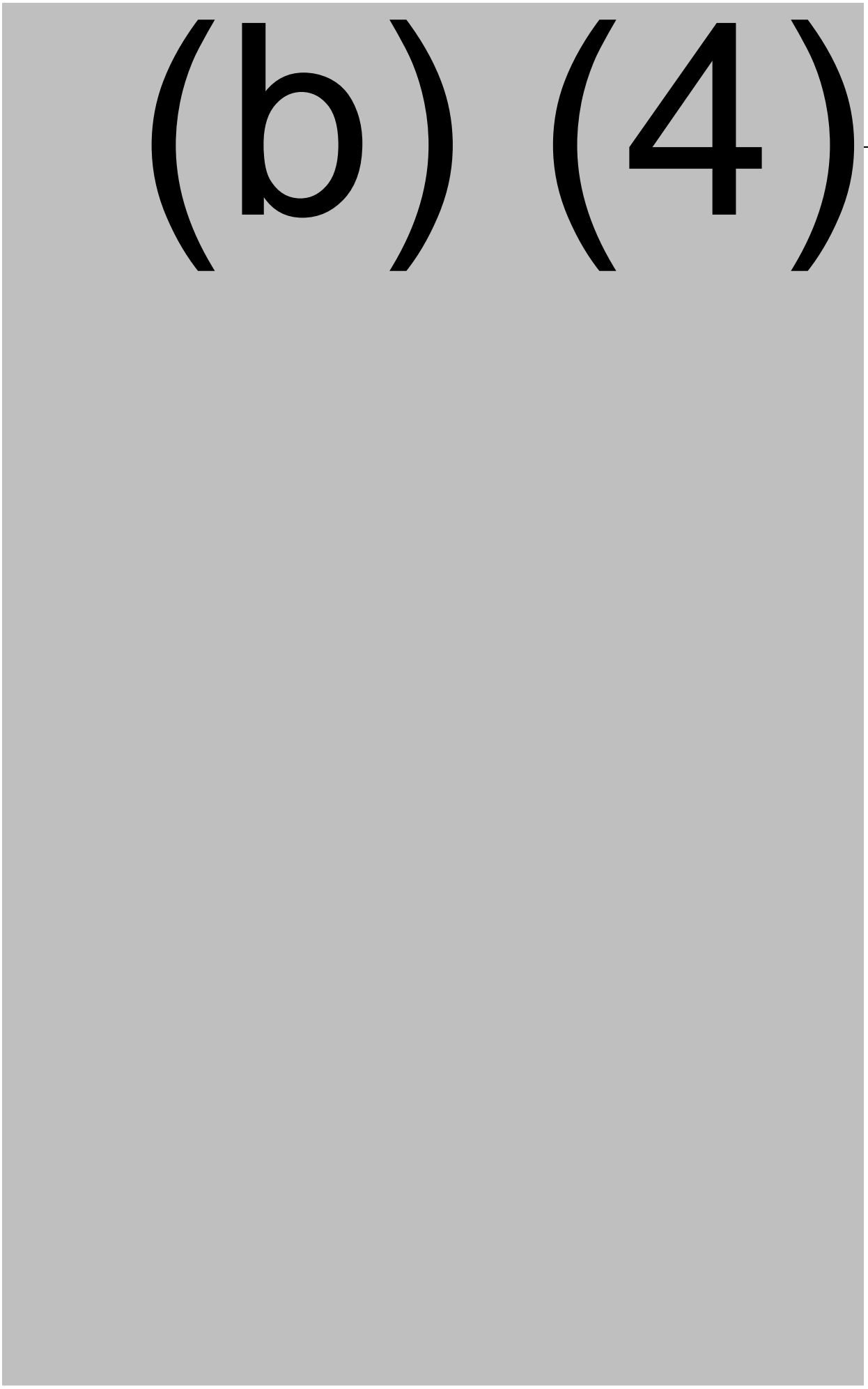

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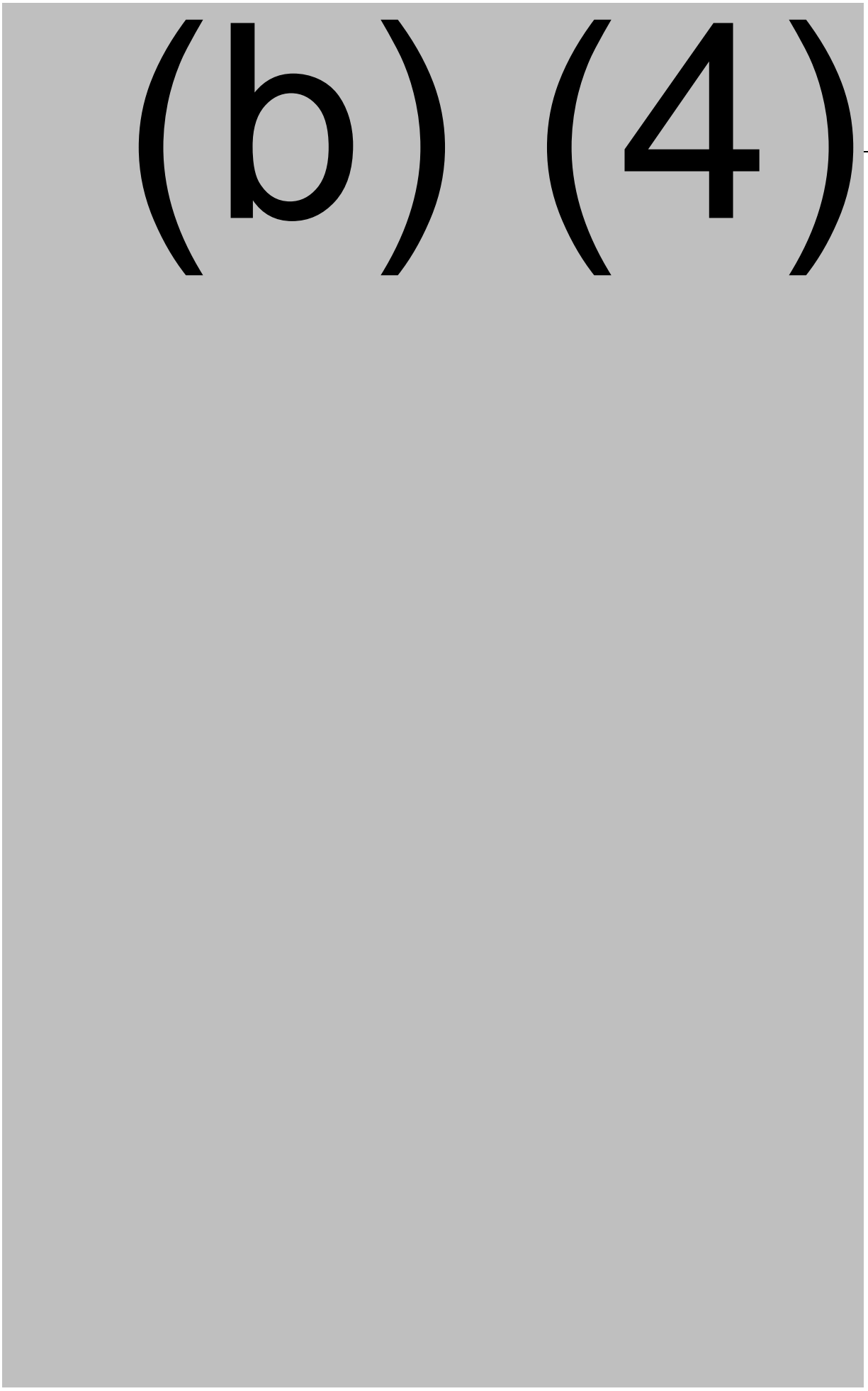

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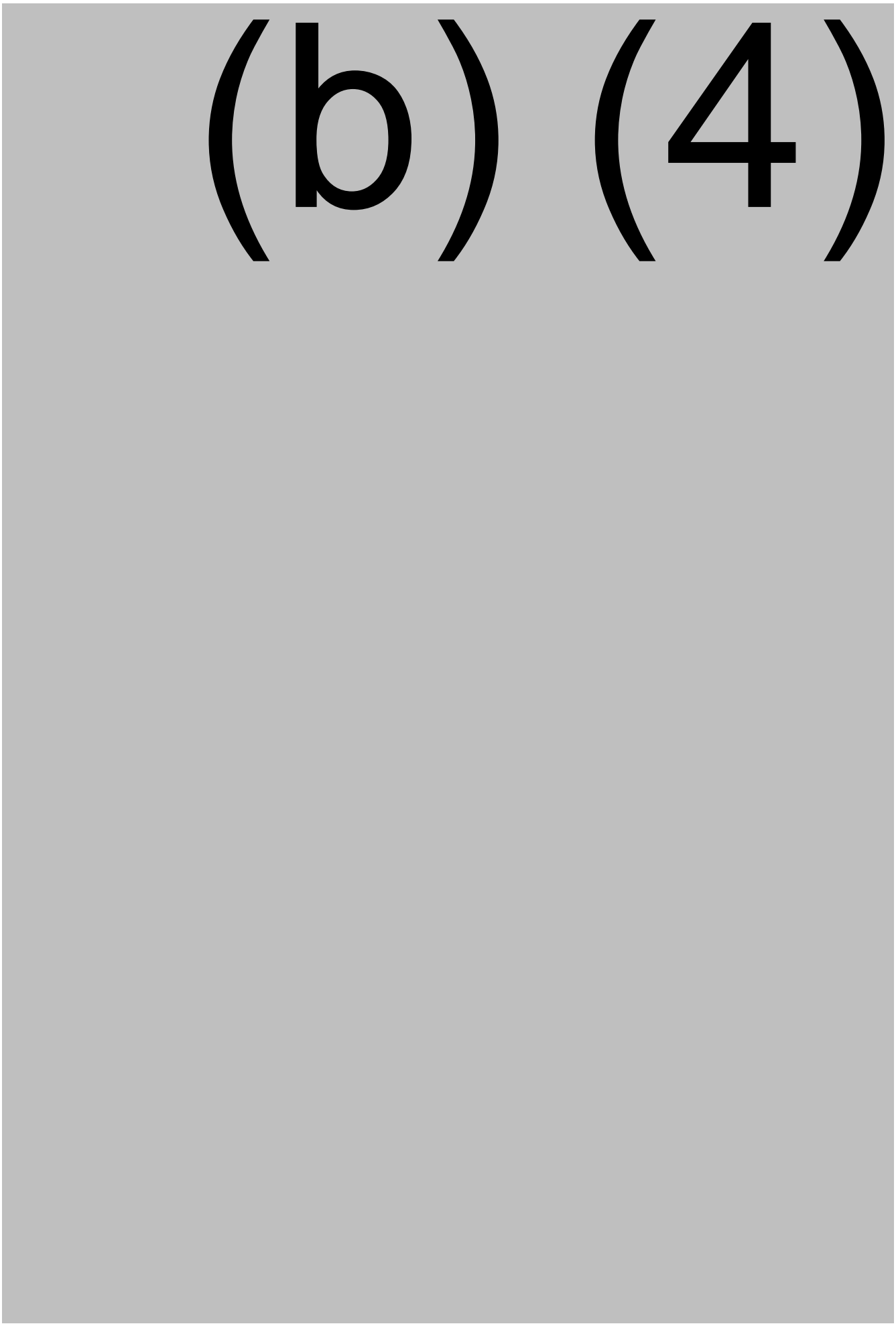

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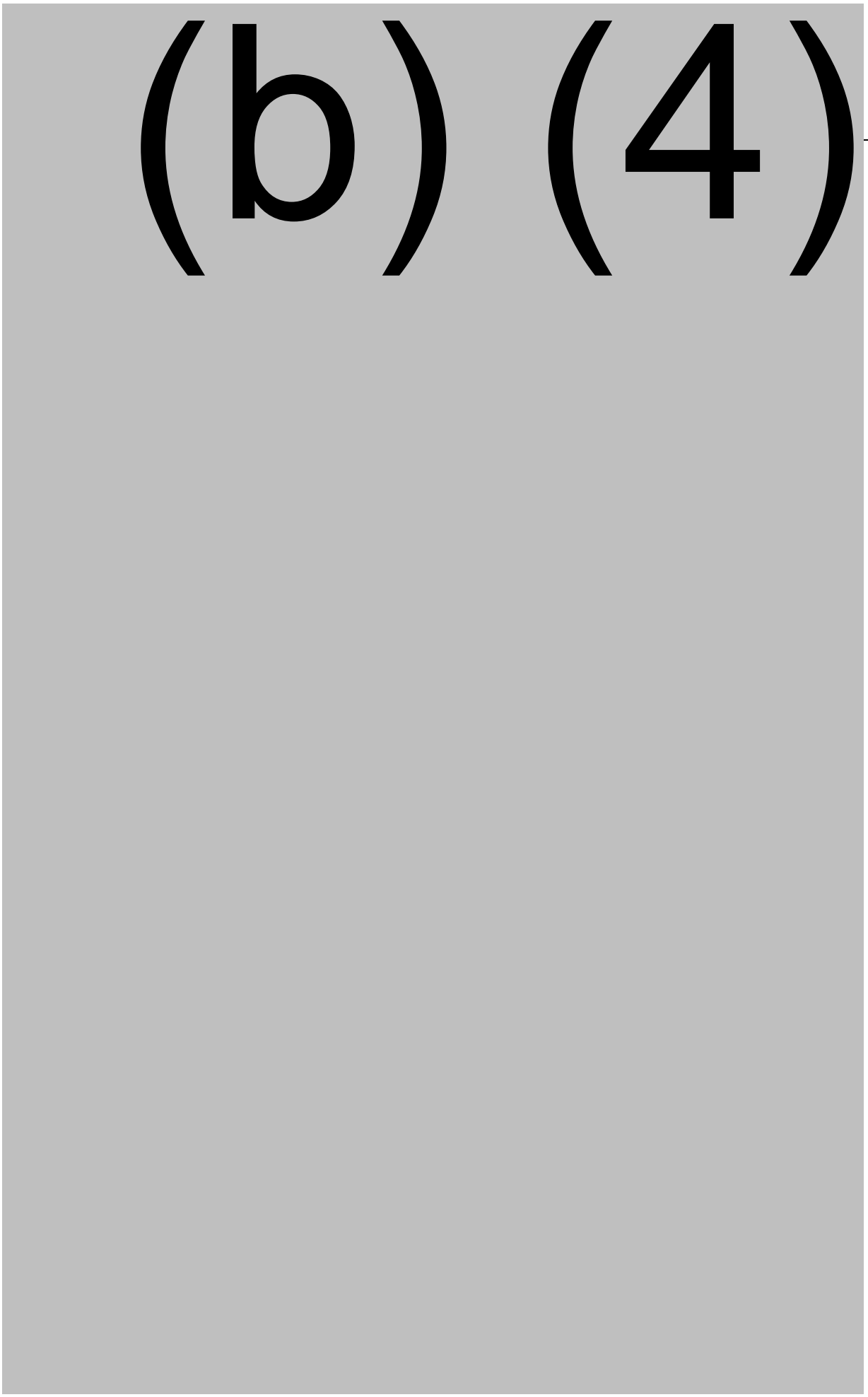

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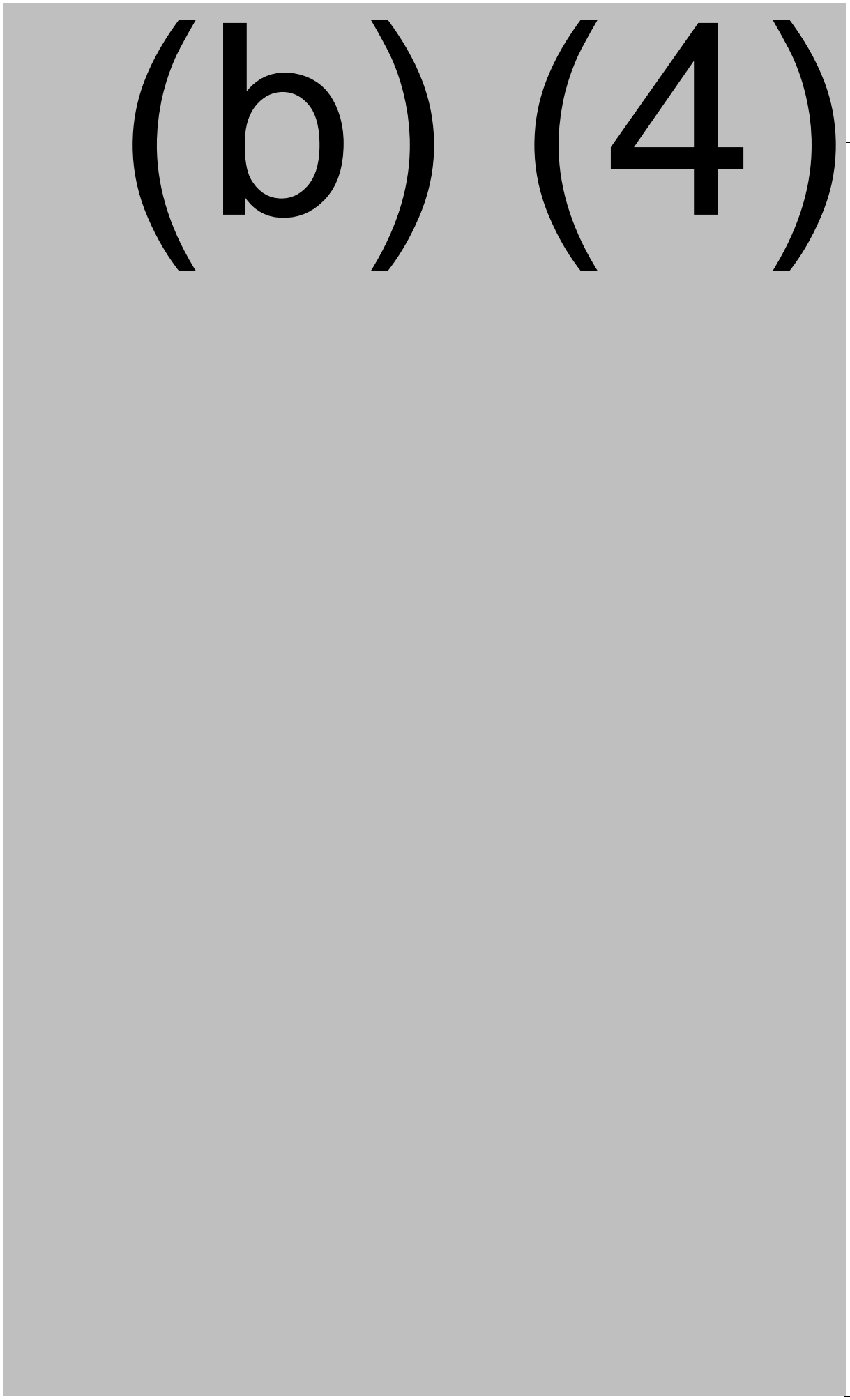

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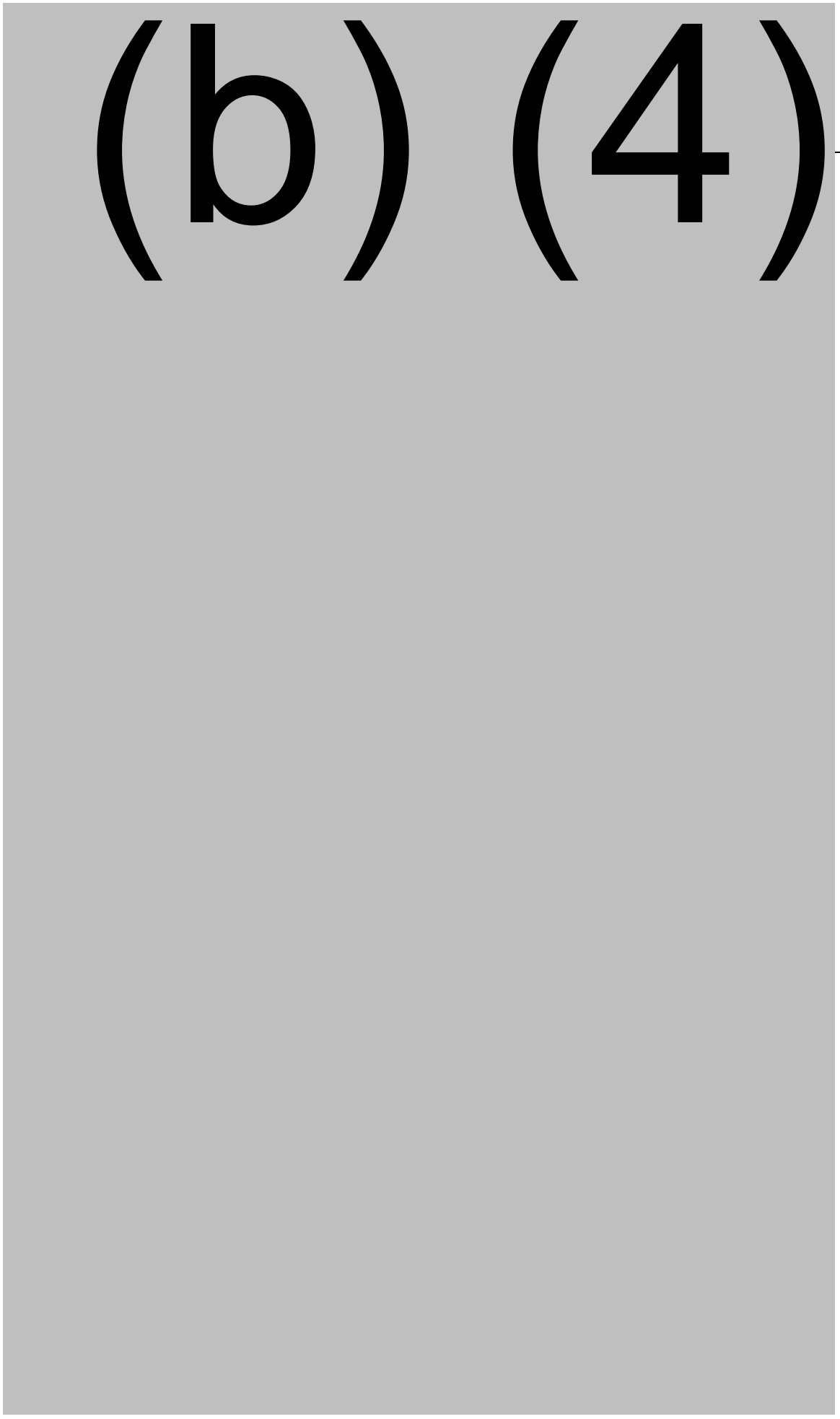

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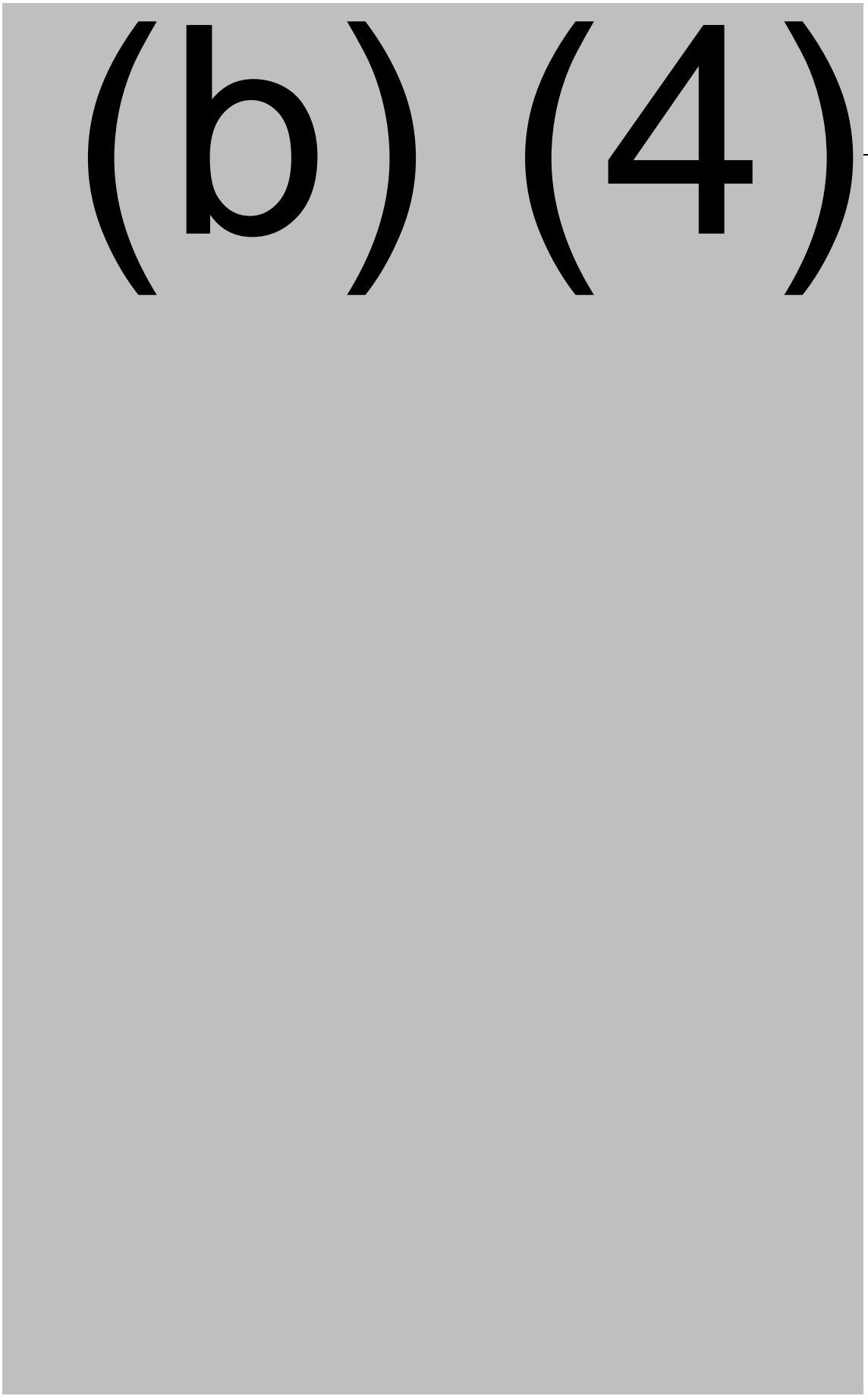

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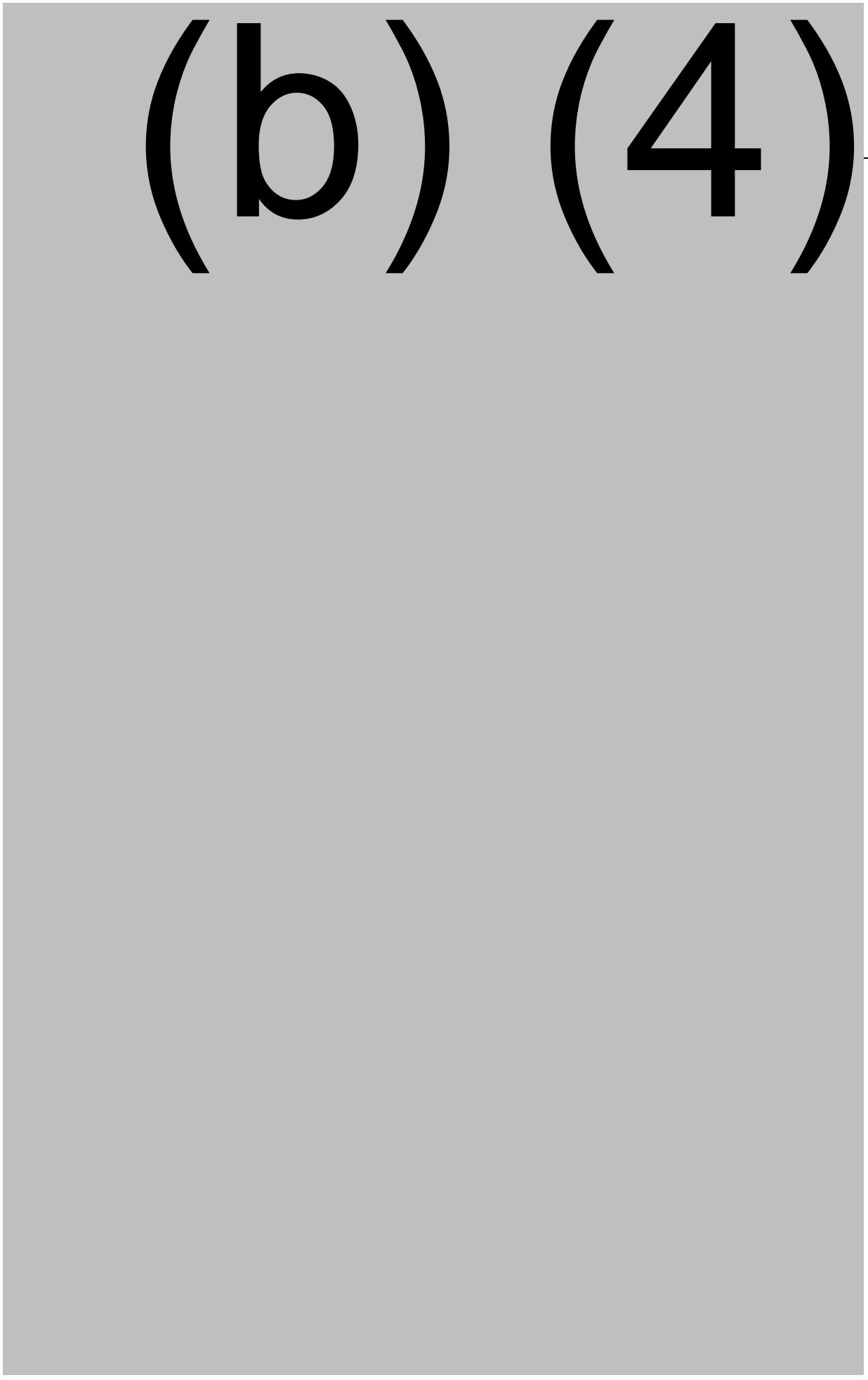

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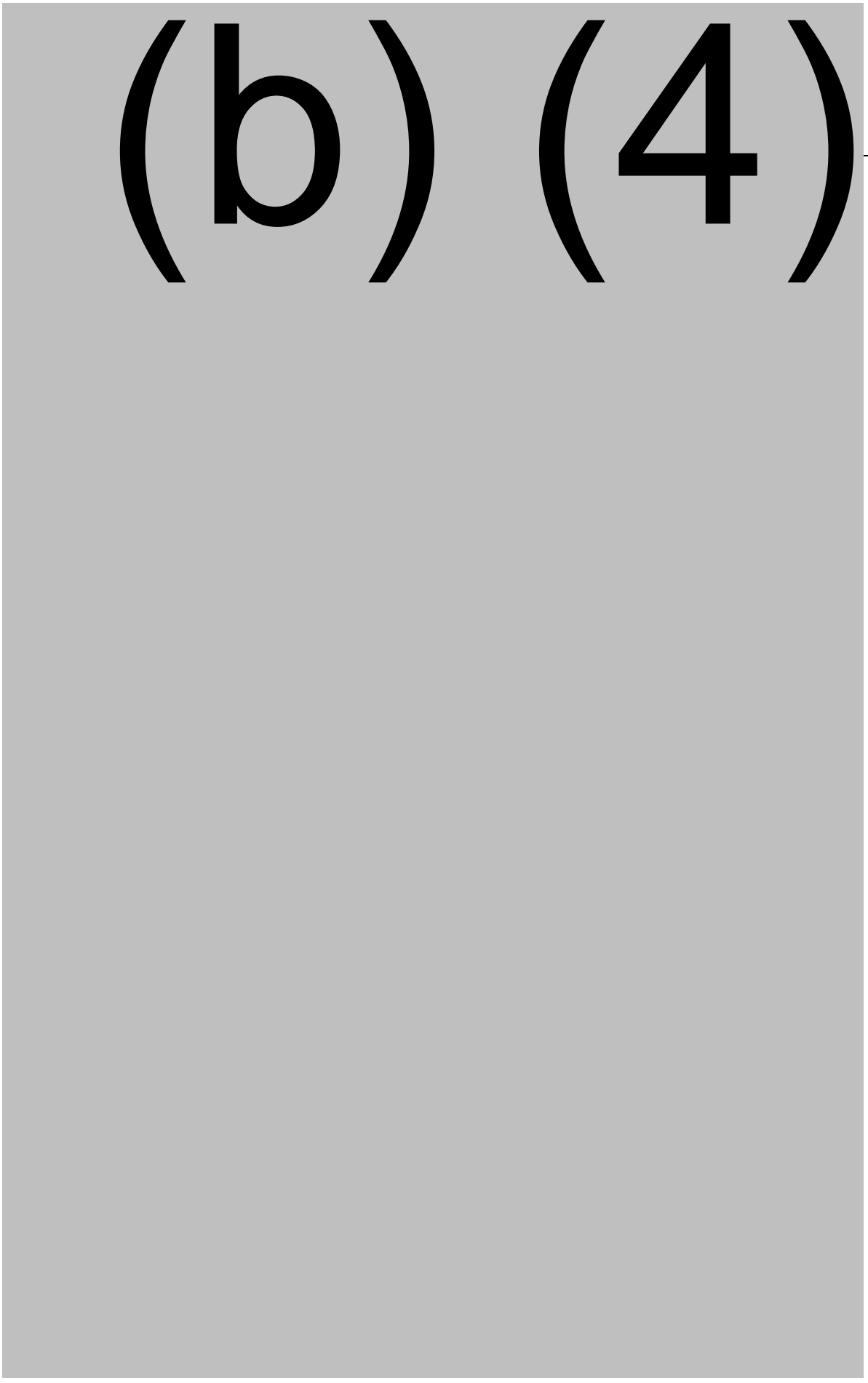


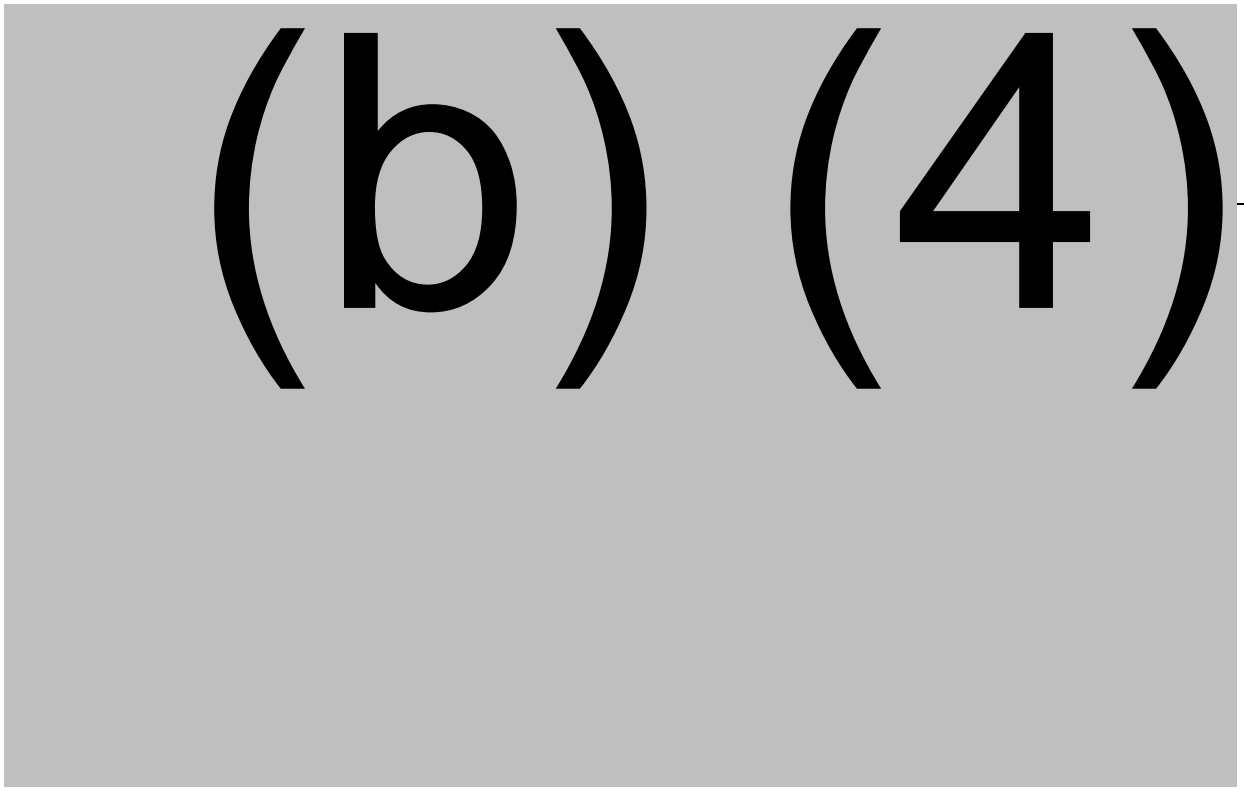
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Appendix 23: Literature Searches

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Review of Scientific Literature

The initial literature review was conducted following the principles of Section 3 of the Guidance on the assessment of the safety of feed additives for the target species – Extensive literature search for studies with target animals (EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) *et al.*, 2017) and its additional search instructions (European Food Safety Authority (EFSA), 2015), internal ID (b) (4) An update of the search was performed covering the period from August 2020 until September 2023, internal ID (b) (4)

A scoping review was performed for the host organism *Thermothelomyces thermophilus*. The objective of this scoping review was to survey the evidence base for *T. thermophilus*, in order to identify any specific issues related to toxicology, pathology or environmental safety that might require in-depth examination. The review question was formulated to conform to PECO/PICO structure (Population, Exposure/Intervention, Comparators, Outcome), and to address data requirements. It was modeled after the review question examples provided in the "Technical Manual for Performing Electronic Literature Searches in Food and Feed Safety" (Glanville, J. *et al.*, 2014).

Question: Are there any studies published during the reporting period that describe adverse effects on humans, animals, plants, or the environment, of *Thermothelomyces thermophilus* or compounds produced by *Thermothelomyces thermophilus*?

Key elements:
Population: Humans; animals (including vertebrates and invertebrates); plants; environment
Exposure: *Thermothelomyces thermophilus*; compounds produced by *Thermothelomyces thermophilus*
Comparators: N/A
Outcome: Adverse effects

A set of broad literature searches was performed using several bibliographic databases covering scientific literature from January 1, 2000 to June 19, 2020. Additional sources of information, such as web pages of food safety, agriculture, and biotechnology-related authorities were manually searched for the same time window, along with the bibliographies of relevant reviews. The references identified were evaluated for potential relevance to the scoping review question, according to pre-defined criteria.

These literature searches identified a total of 225 unique publications, which were subjected to rapid assessment to exclude obviously irrelevant publications based on title and abstract (stage 1 evaluation) (see Annex 1). A total of 21 publications were progressed for detailed assessment based on full text (stage 2 evaluation) (see Annex 1). In addition, manual searches in web pages from relevant Regulatory Authorities identified 6 additional documents that describe the regulatory evaluation of *T. thermophilus* by different authorities. No additional publications were identified from the manual search of bibliographic listings in relevant reviews.

The 21 publications that progressed to Stage 2 were evaluated in detail, based on full text, for potential relevance, following pre-established criteria. Eleven of these were excluded during Stage 2. Of the remaining 10 publications, eight report adverse effects and *T. thermophilus*. Most

of them refer to exceptional single patient cases and there is no evidence of repetition.

An update of the search in 2023 evaluated a total of 200 publications, of which 8 progressed to Stage 2 (Annex 2). The manual search found 1 article in the database of FDA/CVM, the "GRAS-Response-Letter-AGRN-57" confirming BASF's request to cease evaluation of the AGRN for the mannanase 19287 enzyme preparation. Two articles are directly related to the safety evaluation of the notified substance ((EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) *et al.*, 2023; Kern, A. *et al.*, 2020)); two articles describe the isolation of *Myceliothermophin*-derived substances and their structural identifications. Only one of the four remaining articles show a direct clinical case of a fatal case of pneumonia involving T. thermophilus. Similar to cases identified in the original search, the infection with a wild-type T. thermophilus occurred in an immunocompromised patient.

In summary, we conclude that the literature search and its update did not identify any publications that would change our evaluation of *T. thermophilus* as a safe host for enzyme production. This conclusion agrees with assessments by Regulatory bodies identified in the manual searches of web pages.

References

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Bampidis, V., Azimonti, G., Bastos, M.d.L. et al. *Safety and efficacy of a feed additive consisting of endo-1,4-β-d-mannanase produced by Thermothelomyces thermophilus DSM 33149 (Natupulse® TS/TS L) for chickens and turkeys for fattening, minor poultry species for fattening and ornamental birds (BASF SE)*. EFSA Journal 21 (2), p. e07873 (2023).

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Rychen, G., Aquilina, G., Azimonti, G. et al. *Guidance on the assessment of the safety of feed additives for the target species*. EFSA Journal 15 (10), p. e05021 (2017).

European Food Safety Authority (EFSA). *Tools for critically appraising different study designs, systematic review and literature searches*. EFSA Supporting Publications 12 (7), p. 836E (2015).

Glanville, J., Varley, D., Brazier, H., Arber, M. et al. *Inventory of Sources of Scientific Evidence Relevant to EFSA's Risk Assessments and Information Sessions on Literature Searching Techniques (CFT/EFSA/SAS/2011/03 Inventory Report)*. EFSA Supporting Publications 11 (6), p. 593E (2014).

Kern, A., Shanahan, D., Buesen, R. and Geiger, D. *Safety evaluation of a β-mannanase enzyme preparation produced with Thermothelomyces thermophilus expressing a protein-engineered β-mannanase gene*. PLoS ONE 15 (12), p. e0243647 (2020).

Annex 1: 88046224 *Thermothelomyces* 2020 Search Results

Annex 1 - Part A: 2020 Search List

Title	Author	Company/Institute	PATWWW/AutoDoc	Publ. Date	Not relevant	Abstracts	IDFromSource
A simple approach to mediate genome editing in the filamentous fungus Trichoderma reesei by CRISPR/Cas9-coupled in vivo gRNA transcription.	Wu Chuan Chen Yumeng Qiu Yifei Niu Xiao Chen Jiehui Yao Hong Wang Wei Ma Yushu Zhu Ningjian	State Key Lab of Bioreactor Engineering, New World Institute of Biotechnology, East China University of Science and Technology, 130 Meilong Road, P.O.B. 311, Shanghai, 200237, China. wadexp@ecust.edu.cn; myushu@ecust.edu.cn The First Affiliated Middle School of East China Normal University, Shanghai, 200086, China.	Biotechnology letters, (2020 Jul) Vol. 42, No. 7, pp. 1203-1210. Electronic Publication Date: 16 Apr 2020 Journal code: 8008051. E-ISSN: 1573-6776. L-ISSN: 0141-5492.	20200101	other	OBJECTIVE: To simplify CRISPR/Cas9 genome editing in the industrial filamentous fungus Trichoderma reesei based on in vivo guide RNA (gRNA) transcription. RESULTS: Two putative RNA polymerase III U6 snRNA genes were identified in the genome of T. reesei QM6a by BLASTN using Myceliophthora . thermophila U6 snRNA gene as the template. The regions approximately 500 bp upstream of two U6 genes were efficient promoters for the in vivo expression of gRNA. The CRISPR system consisting of Cas9 and in vivo synthesized gRNA under control of the T. reesei U6 snRNA promoters was sufficient to cause a frameshift mutation in the ura5 gene via non-homologous end-joining-mediated events. CONCLUSIONS: We report a simple gene editing method using a CRISPR/Cas9-coupled in vivo gRNA transcription system in T. reesei.	MEDLINE/2022362887
Endophytic fungi from the medicinal herb Euphorbia geniculata as a potential source for bioactive metabolites.	Kamel Noha M Abdel-Motaal Fatma F El-Zayat Soad A	Botany Department, Faculty of Science, Aswan University, Aswan, Egypt. fatma.fakhry@aswu.edu.eg	Archives of microbiology, (2020 Mar) Vol. 202, No. 2, pp. 247-255. Electronic Publication Date: 10 Oct 2019 Journal code: 0410427. E-ISSN: 1432-072X. L-ISSN: 0302-8933.	20200101	other	Many researchers proved that plant endophytes manage successful issues to synthesize active chemicals within plant cells. These bioactive compounds might support a range of plant defense mechanism against many pathogenic microorganisms. In this study, a total of 22 isolates representing 21 fungal species belonging to 15 fungal genera in addition to one variety were isolated and identified for the first time from Euphorbia geniculate plants. The genus Aspergillus was the most common fungus isolated from the studied plant. The fungus Isaria feline was recorded in both leaves and stem, while Aspergillus flavus, A. ochraceus, A. terreus var. terreus, Emercilla nidulans var. acristata, Macrophomina phaseolina colonized both stem and root. The isolated fungi showed antagonistic activities against six strains of plant pathogenic fungi viz., Eupenicillium brefeldianum, Penicillium echinulatum, Alternaria phragmospora, Fusarium oxysporum, Fusarium verticilloid, and Alternaria alternata in dual culture assay. The highest antagonistic activity fungal species (Aspergillus flavus, A. fumigatus, and Fusarium lateritium) and the lowest (Cladosprium herbarum, F. culomrum, and Sporotrichum thermophile) showed twining in their secondary metabolites especially terpens and alkaloids with that of their host E. geniculata. Three concentrations of (0.5, 1.0, and 2.0 mg/ml) of these secondary metabolites extracted by ethyl acetate and n-butanol from the above six endophytic fungal species were tested against three pathogenic fungi isolated from infected tomato plant (E. brefeldianum-EBT-1, P. echinulatum-PET-2, and A. phragmospora-APT-3), whereas these pathogens showed promising sensitivity to these fungal secondary metabolites. In conclusion, this is the first report on the isolation of endophytic fungi from E. geniculata and evaluation of their antifungal activity.	MEDLINE/2021668740
Metabolic adaptation of fungal strains in response to contamination by polychlorinated biphenyls.	Perigon Sophie Massier Martin Germain Joaquim Binet Marie-Noelle Mouhamadou Bello Legay Nicolas	Laboratoire d'Ecologie Alpine, UMR 5553 CNRS/USMB Universite Grenoble Alpes, 38058, Grenoble CEDEX 9, France. bello.mouhamadou@univ-grenoble-alpes.fr Ecole de la Nature et du Paysage, INSA Centre Val de Loire, 9 Rue Chocolaterie, 41000, Blois, France. CNRS, CITERES, UMR 7324, 37200, Tours, France.	Environmental science and pollution research international, (2019 May) Vol. 26, No. 15, pp. 14943-14950. Electronic Publication Date: 27 Mar 2019 Journal code: 9441769. E-ISSN: 1614-7499. L-ISSN: 0944-1344.	20190101	other	Polychlorinated biphenyls (PCBs) represent a large group of recalcitrant environmental pollutants. Up to now, many studies have focused on bioremediation of PCBs by fungal strains; however, the mechanisms of adaptation of these strains towards PCBs remain unknown despite their importance in developing effective bioremediation processes. We studied five species, each consisting of two strains isolated either from PCB-polluted or PCB-unpolluted substrates (control strains). We investigated their responses to PCB contamination by studying their tolerance to PCBs, their ability to reduce these pollutants, and their expression level of Laccase genes. In Thermothelomyces thermophila , Thermothelomyces heterothallica, Thermoascus crustaceus, and Fusarium solani, all the studied strains showed a similar tolerance and PCB degradation regardless of their origin. In Schizophyllum commune, while both strains showed similar resistance to PCBs, i.e., PCBs and their degradation products presented no toxicity for these strains, the rate of PCB degradation of the strain from a PCB-polluted environment was significantly slightly higher. The PCB degradation did not correlate with the expression level of genes encoding Laccases. These results demonstrate that the tolerance and PCB degradation by the fungal strains, which did not involve Laccase genes, required different adaptation systems which seem to be constitutive or rapidly inducible by PCB according to the fungal species.	MEDLINE/2020988928

Physical and chemical mutagens improved Sporotrichum thermophile , strain ST20 for enhanced Phytase activity.	Mehmood Asim Baneen Umal Wasim Sajid Muhammad Hussain Abrar Saleem Shahzad Yousafi Qudsia Rashid Hamid Riaz Hassan Ihsan Awais Jamil Farrukh Zaheer Ahmad Sajjad Yasar Zahid Nageena Shahzad Anjam Muhammad Arshad Muhammad Mirza Zeenat Karim Sajjad Rasool Mahmood	Department of Biosciences, COMSATS University Islamabad, Sahiwal, Pakistan. Institute of Molecular Biology and Biotechnology, The University of Lahore, Defence Road, Lahore, Pakistan. Department of Biotechnology, COMSATS University Islamabad, Abbottabad Campus, Pakistan. Institute of Molecular Biology and Biotechnology (IMBB), Bahauddin Zakariya University, Multan, Pakistan. Department of Biotechnology, University of Okara, Pakistan. King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia. Center of Excellence in Genomic Medicine Research (CEGMR), King Abdulaziz University, Jeddah, Saudi Arabia.	Saudi journal of biological sciences, (2019 Nov) Vol. 26, No. 7, pp. 1485-1491. Electronic Publication Date: 11 Jul 2019 Journal code: 101543796. ISSN: 1319-562X. L-ISSN: 1319-562X. Report No.: PMC-PMC6864213.	20190101	other	Objective: Phosphorous is an essential micronutrient of plants and involved in critical biological functions. In nature, phosphorous is mostly present in immobilized inorganic mineral and in the fixed organic form including phytic acid and phosphoesteric compounds. However, the bioavailability of bound phosphorous could be enhanced by the use of phosphate solubilizing microorganisms such as bacteria and fungi. The phytases are widespread in an environment and have been isolated from different sources comprising bacteria and fungi. Methodology: In current studies, we show the successful use of gamma rays and EMS (Ethyl Methane Sulphonate) mutagenesis for enhanced activity of phytases in a fungal strain Sporotrichum thermophile . Results: We report an improved strain ST2 that could produce a clear halo zone around the colony, up to 24 mm. The maximum enzymatic activity was found of 382 U/mL on pH 5.5. However, the phytase activity was improved to 387 U/ml at 45 .degree.C. We also report that the mutants produced through EMS showed the greater potential for phytase production. Conclusion: The current study highlights the potential of EMS mutagenesis for strain improvement over physical mutagens . .COPYRGT. 2019 King Saud University.	MEDLINE/2021826986
Peptides with antioxidant properties identified from casein, whey, and egg albumin hydrolysates generated by two novel fungal proteases.	Neto Youssef A A Hamin Cabral Hamilton Rosa Jose Cesar	a School of Pharmaceutical Science of Ribeirao Preto , University of Sao Paulo , Sao Paulo , Brazil. b Department of Molecular and Cell Biology and Pathogenic Bioagents, Protein Chemistry Center, School of Medicine of Ribeirao Preto , University of Sao Paulo , Sao Paulo , Brazil.	Preparative biochemistry + biotechnology, (2019) Vol. 49, No. 7, pp. 639-648. Electronic Publication Date: 25 May 2019 Journal code: 9607037. E-ISSN: 1532-2297. L-ISSN: 1082-6068.	20190101	other	There are many diseases linked to oxidative stress, including cancer . Importantly, endogenous antioxidants are insufficient to protect against this process. Peptides derived from food proteins produced by hydrolysis have been investigated as exogenous antioxidants. The present study aimed to identify novel peptides with antioxidant potential produced from egg and milk proteins hydrolysis with two new fungal proteases isolated from Eupenicillium javanicum and Myceliophthora thermophila . The degree of hydrolysis at several time points was calculated and correlated to DPPH scavenging and metal chelating assays, all hydrolysates presented antioxidant activity. Casein hydrolyzed by the M. thermophila protease showed the best antioxidant activity. The identified sequences showed that the proportions of amino acids that influence antioxidant activity support the antioxidant assay. Our data reveal the conditions necessary for the successful generation of antioxidant peptides using two novel fungal proteases. This opens a potential new avenue for the design and manufacture of antioxidant molecules.	MEDLINE/2021200464
Practical guidance for the implementation of the CRISPR genome editing tool in filamentous fungi.	Kwon Min Jin Schutze Tabea Meyer Vera Spohner Sebastian Haefner Stefan	1Chair of Applied and Molecular Microbiology, Institute of Biotechnology, Technische Universitat Berlin, 10263 Berlin, Germany. 2BASF SE, Carl-Bosch-Strasse 38, 67056 Ludwigshafen, Germany.	Fungal biology and biotechnology, (2019) Vol. 6, pp. 15. Electronic Publication Date: 17 Oct 2019 Journal code: 101655873. E-ISSN: 2054-3085. L-ISSN: 2054-3085. Report No.: PMC-PMC6796461.	20190101	other	Background: Within the last years, numerous reports described successful application of the CRISPR nucleases Cas9 and Cpf1 for genome editing in filamentous fungi. However, still a lot of efforts are invested to develop and improve protocols for the fungus and genes of interest with respect to applicability, scalability and targeting efficiencies. These efforts are often hampered by the fact that-although many different protocols are available-none have systematically analysed and compared different CRISPR nucleases and different application procedures thereof for the efficiency of single- and multiplex-targeting approaches in the same fungus. Results: We present here data for successful genome editing in the cell factory Thermothelomyces thermophilus , formerly known as Myceliophthora thermophila , using the three different nucleases SpCas9, FnCpf1, AsCpf1 guided to four different gene targets of our interest. These included a polyketide synthase (pks4.2), an alkaline protease (alp1), a SNARE protein (snc1) and a potential transcription factor (ptf1). For all four genes, guide RNAs were developed which enabled successful single-targeting and multiplex-targeting. CRISPR nucleases were either delivered to T. thermophilus on plasmids or preassembled with in vitro transcribed gRNA to form ribonucleoproteins (RNPs). We also evaluated the efficiency of single oligonucleotides for site-directed mutagenesis . Finally, we were able to scale down the transformation protocol to microtiter plate format which generated high numbers of positive transformants and will thus pave the way for future high-throughput investigations. Conclusion: We provide here the first comprehensive analysis and evaluation of different CRISPR approaches for a filamentous fungus. All approaches followed enabled successful genome editing in T. thermophilus; however, with different success rates. In addition, we show that the success rate depends on the respective nuclease and on the targeted gene locus. We finally present a practical guidance for experimental considerations aiming to guide the reader for successful implementation of CRISPR technology for other fungi. .COPYRGT. The Author(s) 2019.	MEDLINE/2021707588

Decolorization and detoxification of textile wastewaters by recombinant Myceliophthora thermophila and Trametes trogii laccases.	Herkommerova Klara Dostal Jiri Pichova Iva	1Laboratory of Viral and Microbial Proteins, Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic. 2Department of Membrane Transport, Institute of Physiology of the Czech Academy of Science, Prague, Czech Republic.	3 Biotech, (2018 Dec) Vol. 8, No. 12, pp. 505. Electronic Publication Date: 30 Nov 2018 Journal code: 101565857. ISSN: 2190-572X. L-ISSN: 2190-5738. Report No.: PMC-PMC6269326.	20180101	other	Laccases are multi-copper oxidoreductases with broad biotechnological applications. Here, we report detailed biochemical characterization of purified recombinant laccases originating from Myceliophthora thermophila (MtL) and Trametes trogii (TtL). We identified optimal conditions for decolorization of commercial dyes and textile wastewater samples. We also tested the toxicity of decolorized wastewater samples using human peripheral blood mononuclear cells. MtL and TtL were expressed in Saccharomyces cerevisiae, and secreted enzymes were purified by consecutive hydrophobic and gel chromatography. The molecular masses of TtL (~ 65 kDa) and MtL (> 100 kDa) suggested glycosylation of the recombinant enzymes. Deglycosylation of MtL and TtL led to 25% and 10% decreases in activity, respectively. In a thermal stability assay, TtL retained 61% and MtL 86% of the initial activity at 40 .degree.C. While TtL retained roughly 50% activity at 60 .degree.C, MtL lost stability at temperatures higher than 40 .degree.C. MtL and TtL preferred syringaldazine as a substrate, and the catalytic efficiencies for ABTS oxidation were 7.5 times lower than for syringaldazine oxidation. In the presence of the mediator HBT, purified TtL almost completely decolorized dyes within 30 min and substantially decolorized wastewater samples from a textile factory (up to 74%) within 20 h. However, products of TtL-catalyzed decolorization were more toxic than MtL-decolorized products, which were almost completely detoxified .	MEDLINE/2020626312
Fine-Tuned Enzymatic Hydrolysis of Organosolv Pretreated Forest Materials for the Efficient Production of Cellobiose.	Karnaouri Anthi Topakas Evangelos Matsakas Leonidas Rova Ulrika Christakopoulos Paul	Biochemical Process Engineering, Chemical Engineering, Department of Civil, Environmental and Natural Resources Engineering, Lulea University of Technology, Lulea, Sweden. Biotechnology Laboratory, Department of Synthesis and Development of Industrial Processes, School of Chemical Engineering, National Technical University of Athens, Athens, Greece.	Frontiers in chemistry, (2018) Vol. 6, pp. 128. Electronic Publication Date: 19 Apr 2018 Journal code: 101627988. ISSN: 2296-2646. L-ISSN: 2296-2646. Report No.: PMC-PMC5917092.	20180101	other	Non-digestible oligosaccharides (NDOs) are likely prebiotic candidates that have been related to the prevention of intestinal infections and other disorders for both humans and animals. Lignocellulosic biomass is the largest carbon source in the biosphere, therefore cello-oligosacharides (COS), especially cellobiose, are potentially the most widely available choice of NDOs. Production of COS and cellobiose with enzymes offers numerous benefits over acid-catalyzed processes, as it is milder, environmentally friendly and produces fewer by-products. Cellobiohydrolases (CBHs) and a class of endoglucanases (EGs), namely processive EGs, are key enzymes for the production of COS, as they have higher preference toward glycosidic bonds near the end of cellulose chains and are able to release soluble products. In this work, we describe the heterologous expression and characterization of two CBHs from the filamentous fungus Thermothelomyces thermophila , as well as their synergism with proccessive EGs for cellobiose release from organosolv pretreated spruce and birch. The properties, inhibition kinetics and substrate specific activities for each enzyme are described in detail. The results show that a combination of EGs belonging to Glycosyl hydrolase families 5, 6, and 9, with a CBHI and CBHII in appropriate proportions, can enhance the production of COS from forest materials, underpinning the potential of these biocatalysts in the production of NDOs.	MEDLINE/2019797097
Laccase catalyzed elimination of morphine from aqueous systems.	Huber Daniela Pellis Alessandro Vielnascher Robert Daxbacher Andreas Bleymaier Klaus Greimel Katrin J Guebitz Georg M	Institute of Environmental Biotechnology, University of Natural Resources and Life Sciences, Vienna, Konrad Lorenz Strasse 20, 3430 Tulln, Austria. ACIB - Austrian Centre of Industrial Biotechnology, Konrad Lorenz Strasse 20, 3430 Tulln, Austria. Institute of Environmental Biotechnology, University of Natural Resources and Life Sciences, Vienna, Konrad Lorenz Strasse 20, 3430 Tulln, Austria, ACIB - Austrian Centre of Industrial Biotechnology, Konrad Lorenz Strasse 20, 3430 Tulln, Austria. katrin.greimel@acib.at Institute of Environmental Biotechnology, University of Natural Resources and Life Sciences, Vienna, Konrad Lorenz Strasse 20, 3430 Tulln, Austria, ACIB - Austrian Centre of Industrial Biotechnology, Konrad Lorenz Strasse 20, 3430 Tulln, Austria.	New biotechnology, (2018 May 25) Vol. 42, pp. 19-25. Electronic Publication Date: 6 Jan 2018 Journal code: 101465345. E-ISSN: 1876-4347. L-ISSN: 1871-6784.	20180101	other	Pharmaceuticals contaminate the environment for several reasons, including metabolic excretion after intake, industrial waste and improper disposal. The narcotic drug morphine is commonly utilized for chronic pain management, and the distribution of morphine in aqueous systems and in waste waters is of high concern. Here, the removal of morphine by a laccase from Myceliophthora thermophila both in its free form as well as immobilized on Accurel MP1000 beads was investigated. Complete morphine elimination was achieved within 30 min for the free and the immobilized enzyme (70% bound protein) for concentrations between 1 and 1,000 mg L(-1) according to LC-TOF mass spectrometry analysis. Higher morphine concentrations up to 60 g L(-1) were also tested and total elimination was achieved within 6 h. Therefore, laccases are ideal candidates for removing morphine from aqueous systems. Copyright .COPYRGT. 2018 Elsevier B.V. All rights reserved.	MEDLINE/2019390911

Versatile Fungal Polyphenol Oxidase with Chlorophenol Bioremediation Potential: Characterization and Protein Engineering.	Nikolaivits Efstratios Karagiannaki Ioanna Chalima Angelina Topakas Evangelos Dimarogona Maria Fishman Ayelet	Biotechnology Laboratory, School of Chemical Engineering, National Technical University of Athens, Athens, Greece. vtopakas@chemeng.ntua.gr Department of Chemical Engineering, University of Patras, Patras, Greece. Department of Biotechnology and Food Engineering, Technion-Israel Institute of Technology, Haifa, Israel. Biochemical and Chemical Process Engineering, Division of Sustainable Process Engineering, Department of Civil, Environmental and Natural Resources Engineering, Lulea University of Technology, Lulea, Sweden. vtopakas@chemeng.ntua.gr	Applied and environmental microbiology, (20181201) Vol. 84, No. 23. Electronic Publication Date: 15 Nov 2018 Journal code: 7605801. E-ISSN: 1098-5336. L-ISSN: 0099-2240. Report No.: PMC-PMC6238066.	20180101	other	Polyphenol oxidases (PPOs) have been mostly associated with the undesirable postharvest browning in fruits and vegetables and have implications in human melanogenesis. Nonetheless, they are considered useful biocatalysts in the food, pharmaceutical, and cosmetic industries. The aim of the present work was to characterize a novel PPO and explore its potential as a bioremediation agent. A gene encoding an extracellular tyrosinase-like enzyme was amplified from the genome of Thermothelomyces thermophila and expressed in Pichia pastoris The recombinant enzyme (TtPPO) was purified and biochemically characterized. Its production reached 40 mg/liter, and it appeared to be a glycosylated and N-terminally processed protein. TtPPO showed broad substrate specificity, as it could oxidize 28/30 compounds tested, including polyphenols, substituted phenols, catechols, and methoxyphenols. Its optimum temperature was 65.degree.C, with a half-life of 18.3 h at 50.degree.C, while its optimum pH was 7.5. The homology model of TtPPO was constructed, and site-directed mutagenesis was performed in order to increase its activity on mono- and dichlorophenols (di-CPs). The G292N/Y296V variant of TtPPO 5.3-fold increased activity on 3,5-dichlorophenol (3,5-diCP) compared to the wild type.IMPORTANCE A novel fungal PPO was heterologously expressed and biochemically characterized. Construction of single and double mutants led to the generation of variants with altered specificity against CPs. Through this work, knowledge is gained regarding the effect of mutations on the substrate specificity of PPOs. This work also demonstrates that more potent biocatalysts for the bioremediation of harmful CPs can be developed by applying site-directed mutagenesis . Copyright .COPYRGT. 2018 Nikolaivits et al.	MEDLINE/2020339223
Functional characterization of a lytic polysaccharide monooxygenase from the thermophilic fungus Myceliophthora thermophila .	Kadowaki Marco A S T Leite Ana E Kumagai Patricia S Polikarpov Igor Varnai Aniko Jameson John-Kristian Eijsink Vincent G H Costa-Filho Antonio J Prade Rolf A	Department of Physics and Interdisciplinary Science, Sao Carlos Institute of Physics, University of Sao Paulo, Sao Carlos, Sao Paulo, Brazil. Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences (NMBU), .ANG.As, Norway. Department of Physics, Faculdade de Filosofia, Ciencias e Letras de Ribeirao Preto, University of Sao Paulo, Ribeirao Preto, Sao Paulo, Brazil. Departments of Biochemistry + Molecular Biology and Microbiology + Molecular Genetics, Oklahoma State University, Stillwater, OK, United States.	PloS one, (2018) Vol. 13, No. 8, pp. e0202148. Electronic Publication Date: 20 Aug 2018 Journal code: 101285081. E-ISSN: 1932-6203. L-ISSN: 1932-6203. Report No.: PMC-PMC6101365.	20180101	other	Thermophilic fungi are a promising source of thermostable enzymes able to hydrolytically or oxidatively degrade plant cell wall components. Among these enzymes are lytic polysaccharide monooxygenases (LPMOs), enzymes capable of enhancing biomass hydrolysis through an oxidative mechanism. Myceliophthora thermophila (synonym Sporotrichum thermophile), an Ascomycete fungus, expresses and secretes over a dozen different LPMOs. In this study, we report the overexpression and biochemical study of a previously uncharacterized LPMO (MtLPMO9J) from M. thermophila M77 in Aspergillus nidulans. MtLPMO9J is a single-domain LPMO and has 63% sequence similarity with the catalytic domain of NcLPMO9C from Neurospora crassa. Biochemical characterization of MtLPMO9J revealed that it performs C4-oxidation and is active against cellulose, soluble cello-oligosaccharides and xyloglucan. Moreover, biophysical studies showed that MtLPMO9J is structurally stable at pH above 5 and at temperatures up to 50.degree.C. Importantly, LC-MS analysis of the peptides after tryptic digestion of the recombinantly produced protein revealed not only the correct processing of the signal peptide and methylation of the N-terminal histidine, but also partial autoxidation of the catalytic center. This shows that redox conditions need to be controlled, not only during LPMO reactions but also during protein production, to protect LPMOs from oxidative damage.	MEDLINE/2020198541
Optimized synthesis of novel prenyl ferulate performed by feruloyl esterases from Myceliophthora thermophila in microemulsions.	Antonopoulou Io Rova Ulrika Christakopoulos Paul Leonov Laura Jutten Peter Cerullo Gabriella Faraco Vincenza Papadopoulou Adamantia Kletsas Dimitris Ralli Marianna	Division of Chemical Engineering, Department of Civil, Environmental and Natural Resources Engineering, Lulea University of Technology, 97187, Lulea, Sweden. paul.christakopoulos@ltu.se DuPont Industrial Biosciences, Nieuwe Kanaal 7-S, 6709 PA, Wageningen, The Netherlands. Taros Chemicals GmbH + Co. KG, Emil Figge Str 76a, 44227, Dortmund, Germany. Department of Chemical Sciences, University of Naples 'Federico II', Via Cintia 4, 80126, Naples, Italy. Institute of Biosciences and Applications NCSR 'Demokritos,' Laboratory of Cell Proliferation and Aging, T. Patriarchou Grigoriou + Neapoleos, 15310, Athens, Greece. Korres Natural Products, 57 Km National Road, 32011, Lamia, Athens, Greece.	Applied microbiology and biotechnology, (2017 Apr) Vol. 101, No. 8, pp. 3213-3226. Electronic Publication Date: 11 Jan 2017 Journal code: 8406612. E-ISSN: 1432-0614. L-ISSN: 0175-7598. Report No.: PMC-PMC5380708.	20170101	other	Five feruloyl esterases (FAEs; EC 3.1.1.73), FaeA1, FaeA2, FaeB1, and FaeB2 from Myceliophthora thermophila C1 and MtFae1a from M. thermophila ATCC 42464, were tested for their ability to catalyze the transesterification of vinyl ferulate (VFA) with prenol in detergentless microemulsions. Reaction conditions were optimized investigating parameters such as the medium composition, the substrate concentration, the enzyme load, the pH, the temperature, and agitation. FaeB2 offered the highest transesterification yield (71.5 .+-. 0.2%) after 24 h of incubation at 30 .degree.C using 60 mM VFA, 1 M prenol, and 0.02 mg FAE/mL in a mixture comprising of 53.4:43.4:3.2 v/v/v n-hexane:t-butanol:100 mM MOPS-NaOH, pH 6.0. At these conditions, the competitive side hydrolysis of VFA was 4.7-fold minimized. The ability of prenyl ferulate (PFA) and its corresponding ferulic acid (FA) to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals was significant and similar (IC50 423.39 .mu.M for PFA, 329.9 .mu.M for FA). PFA was not cytotoxic at 0.8-100 .mu.M (IC50 220.23 .mu.M) and reduced intracellular reactive oxygen species (ROS) in human skin fibroblasts at concentrations ranging between 4 and 20 .mu.M as determined with the dichloro-dihydro-fluorescein diacetate (DCFH-DA) assay.	MEDLINE/2018148406

N-Linked glycans are an important component of the processive machinery of cellobiohydrolases.	Gusakov Alexander V Dotsenko Anna S Rozhkova Aleksandra M Sinitsyn Arkady P	Department of Chemistry, M. V. Lomonosov Moscow State University, Vorobyovy Gory 1/11, Moscow 119991, Russia, Federal Research Centre 'Fundamentals of Biotechnology', Russian Academy of Sciences, Leninsky Pr. 33, Moscow 119071, Russia. avgusakov@enzyme.chem.msu.ru Department of Chemistry, M. V. Lomonosov Moscow State University, Vorobyovy Gory 1/11, Moscow 119991, Russia, Federal Research Centre 'Fundamentals of Biotechnology', Russian Academy of Sciences, Leninsky Pr. 33, Moscow 119071, Russia.	Biochimie, (2017 Jan) Vol. 132, pp. 102-108. Electronic Publication Date: 14 Nov 2016 Journal code: 1264604. E-ISSN: 1638-6183. L-ISSN: 0300-9084.	20170101	other	Cellobiohydrolases (CBHs), belonging to glycoside hydrolase families 6 and 7 (GH6 and GH7), are the major components of cellulase systems of filamentous fungi involved in biodegradation of cellulose in nature. Previous studies demonstrated that N-linked glycans in the catalytic domains of GH7 CBHs significantly affect the enzyme activity against cellulosic substrates. The influence of N-linked glycans on the activity and processivity of recombinant GH6 CBH II from Penicillium verruculosum (PvCel6A) was studied using site-directed mutagenesis of the respective Asn residues. Depending on the position of N-glycans on the surface of a protein globule, they affected the enzyme activity against cellulose either negatively or positively. The decrease or increase in the degree of processivity of recombinant forms of PvCel6A generally correlated with activity changes against Avicel. The mechanism of the N-glycan influence seems to be universal for GH6 and GH7 CBHs. The observed effects for CBHs from both families are explained in terms of a mechanistic model that also makes clear our previously published data on the highly active CBH IIb from Myceliophthora thermophila (MtCel6B). This study, together with data of other researchers, strongly suggests that the N-linked glycans in the catalytic domains of GH6 and GH7 CBHs are involved in processive catalytic machinery of these enzymes. Data obtained should be taken into account during development of new and more effective biocatalysts by protein engineering techniques. Copyright .COPYRGT. 2016 Elsevier B.V. and Societe Francaise de Biochimie et Biologie Moleculaire (SFBBM). All rights reserved.	MEDLINE/2017673071
Bioprocess for the production of recombinant HAP phytase of the thermophilic mold Sporotrichum thermophile and its structural and biochemical characteristics.	Maurya Anay Kumar Parashar Deepak Satyanarayana T	Department of Microbiology, University of Delhi South Campus, New Delhi 110021, India. Department of Microbiology, University of Delhi South Campus, New Delhi 110021, India. tsnarayana@gmail.com	International journal of biological macromolecules, (2017 Jan) Vol. 94, No. Pt A, pp. 36-44. Electronic Publication Date: 30 Sep 2016 Journal code: 7909578. E-ISSN: 1879-0003. L-ISSN: 0141-8130.	20170101	other	Thermophilc mold Sporotrichum thermophile secretes an acidstable and thermostable phytase, which finds application as a food and feed additive because of its adequate thermostability, acid stability, protease insensitivity and broad substrate spectrum. Low extracellular phytase production by the mold is a major bottleneck for its application on a commercial scale. We have successfully overcome this problem by constitutive secretary expression of codon optimized rStPhy under glyceraldehyde phosphate dehydrogenase (GAP) promoter in Pichia pastoris. A .apprx.41-fold improvement in rStPhy production has been achieved. Circular Dichroism (CD) spectra revealed that rStPhy is composed of 26.65% .alpha.-helices, 5.26% .beta.-sheets and 68.09% random coils at pH 5.0 and 60.degree.C, the optima for the enzyme activity. The melting temperature (Tm) of the enzyme is .apprx.73.degree.C. The 3D structure of rStPhy displayed characteristic signature sequences (RHGXRXP and HD) of HAP phytase. The catalytically important amino acids (Arg74, His75, Arg78, His368 and Asp369) were identified by docking and site directed mutagenesis . Fluorescence quenching by N-bromosuccinimide (NBS) and CsCl exposed tryptophan residues surrounded by negative charges, which play a key role in maintaining structural integrity of rStPhy. Copyright A.COPYRGT. 2016 Elsevier B.V. All rights reserved.	MEDLINE/2017463828
Development of a genome-editing CRISPR/Cas9 system in thermophilic fungal Myceliophthora species and its application to hyper-cellulase production strain engineering.	Liu Qian Gao Ranran Li Jingen Lin Liangcai Zhao Junqi Sun Wenliang Tian Chaoguang	Key Laboratory of Systems Microbial Biotechnology, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin, 300308 China.	Biotechnology for biofuels, (2017) Vol. 10, pp. 1. Electronic Publication Date: 3 Jan 2017 Journal code: 101316935. ISSN: 1754-6834. L-ISSN: 1754-6834. Report No.: PMC-PMC5209885.	20170101	other	BACKGROUND: Over the past 3 years, the CRISPR/Cas9 system has revolutionized the field of genome engineering. However, its application has not yet been validated in thermophilic fungi. Myceliophthora thermophila , an important thermophilic biomass-degrading fungus, has attracted industrial interest for the production of efficient thermostable enzymes. Genetic manipulation of Myceliophthora is crucial for metabolic engineering and to unravel the mechanism of lignocellulose deconstruction. The lack of a powerful, versatile genome-editing tool has impeded the broader exploitation of M. thermophila in biotechnology. RESULTS: In this study, a CRISPR/Cas9 system for efficient multiplexed genome engineering was successfully developed in the thermophilic species M. thermophila and M. heterothallica. This CRISPR/Cas9 system could efficiently mutate the imported amdS gene in the genome via NHEJ-mediated events. As a proof of principle, the genes of the cellulase production pathway, including cre-1, res-1, gh1-1, and alp-1, were chosen as editing targets. Simultaneous multigene disruptions of up to four of these different loci were accomplished with neomycin selection marker integration via a single transformation using the CRISPR/Cas9 system. Using this genome-engineering tool, multiple strains exhibiting pronounced hyper-cellulase production were generated, in which the extracellular secreted protein and lignocellulase activities were significantly increased (up to 5- and 13-fold, respectively) compared with the parental strain. CONCLUSIONS: A genome-wide engineering system for thermophilic fungi was established based on CRISPR/Cas9. Successful expansion of this system without modification to M. heterothallica indicates it has wide adaptability and flexibility for use in other Myceliophthora species. This system could greatly accelerate strain engineering of thermophilic fungi for production of industrial enzymes, such as cellulases as shown in this study and possibly bio-based fuels and chemicals in the future.	MEDLINE/2018123695

Efficacy and safety of itraconazole use in infants.	Chen Shuang Sun Kai-Yi Feng Xiao-Wei Ran Xin Lama Jebina Ran Yu-Ping	Department of Dermatovenereology, West China Hospital, Sichuan University, Chengdu, 610041, China. ranyuping@vip.sina.com	World journal of pediatrics : WJP, (2016 Nov) Vol. 12, No. 4, pp. 399-407. Electronic Publication Date: 10 Jun 2016 Journal code: 101278599. E-ISSN: 1867-0687.	20160101	other	BACKGROUND: Itraconazole has been used to treat fungal infections , in particular invasive fungal infections in infants or neonates in many countries. DATA SOURCES: Literature search was conducted through Ovid EMBASE, PubMed, ISI Web of Science, CNKI and Google scholarship using the following key words: 'pediatric' or 'infant' or 'neonate' and 'fungal infection ' in combination with 'itraconazole'. Based on the literature and our clinical experience, we outline the administration of itraconazole in infants in order to develop evidence-based pharmacotherapy. RESULTS: Of 45 articles on the use of itraconazole in infancy, 13 are related to superficial fungal infections including tinea capitis, sporotrichosis, mucosal fungal infections and opportunistic infections . The other 32 articles are related to systemic fungal infections including candidiasis, aspergillosis, histoplasmosis, zygomycosis, trichosporonosis and opportunistic infections as caused by Myceliophthora thermophila . CONCLUSION: Itraconazole is safe and effective at a dose of 5 mg/kg per day in a short duration of therapy for superficial fungal infections and 10 mg/kg per day for systemic fungal infections in infants. With a good compliance, it is cost-effective in treating infantile fungal infections . The profiles of adverse events induced by itraconazole in infants are similar to those in adults and children.	MEDLINE/2017088222
Effect of mutations on the thermostability of Aspergillus aculeatus .beta.-1,4-galactanase.	Torpenholt Sos Olsson Mats H M Jensen Jan H Lo Leggio Leila De Maria Leonardo Christensen Lars H Skjot Michael Westh Peter	Department of Chemistry, University of Copenhagen, Universitetsparken 5, 2100 Copenhagen, Denmark. Novozymes A/S, Smormosevej 25, 2880 Bagsvaerd, Denmark. NSM, Research Unit for Functional Biomaterials, University of Roskilde, Universitetsvej 1, 4000 Roskilde, Denmark.	Computational and structural biotechnology journal, (2015) Vol. 13, pp. 256-64. Electronic Publication Date: 9 Apr 2015 Journal code: 101585369. ISSN: 2001-0370. L-ISSN: 2001-0370. Report No.: PMC-PMC4412966.	20150101	other	New variants of .beta.-1,4-galactanase from the mesophilic organism Aspergillus aculeatus were designed using the structure of .beta.-1,4-galactanase from the thermophile organism Myceliophthora thermophila as a template. Some of the variants were generated using PROPKA 3.0, a validated pKa prediction tool, to test its usefulness as an enzyme design tool. The PROPKA designed variants were D182N and S185D/Q188T, G104D/A156R. Variants Y295F and G306A were designed by a consensus approach, as a complementary and validated design method. D58N was a stabilizing mutation predicted by both methods. The predictions were experimentally validated by measurements of the melting temperature (Tm) by differential scanning calorimetry. We found that the Tm is elevated by 1.1 .degree.C for G306A, slightly increased (in the range of 0.34 to 0.65 .degree.C) for D182N, D58N, Y295F and unchanged or decreased for S185D/Q188T and G104D/A156R. The Tm changes were in the range predicted by PROPKA. Given the experimental errors, only the D58N and G306A show significant increase in thermodynamic stability. Given the practical importance of kinetic stability, the kinetics of the irreversible enzyme inactivation process were also investigated for the wild-type and three variants and found to be biphasic. The half-lives of thermal inactivation were approximately doubled in G306A, unchanged for D182N and, disappointingly, a lot lower for D58N. In conclusion, this study tests a new method for estimating Tm changes for mutants, adds to the available data on the effect of substitutions on protein thermostability and identifies an interesting thermostabilizing mutation , which may be beneficial also in other galactanases.	MEDLINE/2015808193
Laccase-catalysed oxidation of ferulic acid and ethyl ferulate in aqueous medium: a green procedure for the synthesis of new compounds.	Aljawish Abdulhadi Chevalot Isabelle Jasniewski Jordane Paris Cedric Scher Joel Muniglia Lionel	Laboratoire d'Ingenierie des Biomolecules (LIBio), Universite de Lorraine, 2 avenue de la Foret de Haye, TSA 40602, 54518 Vandoeuvre-les-Nancy, France.	Food chemistry, (2014 Feb 15) Vol. 145, pp. 1046-54. Electronic Publication Date: 31 Jul 2013 Journal code: 7702639. E-ISSN: 1873-7072. L-ISSN: 0308-8146.	20140101	other	The enzymatic oxidation of ferulic acid (FA) and ethyl ferulate (EF) with Myceliophthora thermophila laccase, as biocatalyst, was performed in aqueous medium using an eco-friendly procedure to synthesize new active molecules. First, the commercial laccase was ultrafiltrated allowing for the elimination of phenolic contaminants and increasing the specific activity by a factor of 2. Then, kinetic parameters of this laccase were determined for both substrates (FA, EF), indicating a higher substrate affinity for ethyl ferulate. Additionally, enzymatic oxidation led to the synthesis of a FA-major product, exhibiting a molecular mass of 386 g/mol and a EF-major product with a molecular mass of 442 g/mol. Structural analyses by mass spectrometry allowed the identification of dimeric derivatives. The optical properties of the oxidation products showed the increase of red and yellow colours, with FA-products compared to EF-products. Additionally, enzymatic oxidation led to a decrease of antioxidant and cytotoxic activities compared to initial substrates. Consequently, this enzymatic procedure in aqueous medium could provide new compounds presenting optical, antioxidant and cytotoxic interest. Copyright .COPYRGT. 2013 Elsevier Ltd. All rights reserved.	MEDLINE/2014078007
Autochthonous ascomycetes in depollution of polychlorinated biphenyls contaminated soil and sediment.	Sage Lucile Perigon Sophie Faure Mathieu Geremia Roberto A Gaignaire Carole Abdelghafour Mohamed Mehu Jacques Mouhamadou Bello	Laboratoire d'Ecologie Alpine LECA, UMR 5553 CNRS Universite Joseph Fourier Grenoble 1, BP 53, 38041 Grenoble Cedex 9, France. INSA de Lyon - PROVADEMSE, 40 Avenue des Arts, 69100 Villeurbanne, France. Laboratoire d'Ecologie Alpine LECA, UMR 5553 CNRS Universite Joseph Fourier Grenoble 1, BP 53, 38041 Grenoble Cedex 9, France. bello.mouhamadou@ujf-grenoble.fr	Chemosphere, (2014 Sep) Vol. 110, pp. 62-9. Electronic Publication Date: 22 Apr 2014 Journal code: 0320657. E-ISSN: 1879-1298. L-ISSN: 0045-6535.	20140101	other	We investigated the capacity of a consortium of ascomycetous strains, Doratomyces nanus, Doratomyces purpureofuscus, Doratomyces verrucisporus, Myceliophthora thermophila , Phoma eupyrena and Thermoascus crustaceus in the mycoremediation of historically contaminated soil and sediment by polychlorinated biphenyls (PCBs). Analyses of 15 PCB concentrations in three mesocosms containing soil from which the fungal strains had previously been isolated, revealed significant PCB depletions of 16.9% for the 6 indicator PCBs (i-PCBs) and 18.7% for the total 15 PCBs analyzed after 6months treatment. The degradation rate did not statistically vary whether the soil had been treated with non-inoculated straw or colonized straw or without straw and inoculated with the consortium of the six strains. Concerning the sediment, we evidenced significant depletions of 31.8% for the 6 i-PCBs and 33.3% for the 15 PCB congeners. The PCB depletions affected most of the 15 PCBs analyzed without preference for lower chlorinated congeners. Bioaugmented strains were evidenced in different mesocosms, but their reintroduction, after six months treatment, did not improve the rate of PCB degradation, suggesting that the biodegradation could affect the bioavailable PCB fraction. Our results demonstrate that the ascomycetous strains potentially adapted to PCBs may be propitious to the remediation of PCB contaminated sites. Copyright .COPYRGT. 2014 Elsevier Ltd. All rights reserved.	MEDLINE/2014781611

Potential of autochthonous fungal strains isolated from contaminated soils for degradation of polychlorinated biphenyls.	Mouhamadou Bello Faure Mathieu Sage Lucile Marcais Johanna Souard Florence Geremia Roberto A	Laboratoire d'Ecologie Alpine, UMR 5553 CNRS, Universite Joseph Fourier, Grenoble 1, Grenoble Cedex 9, France. bello.mouhamadou@ujf-grenoble.fr	Fungal biology, (2013 Apr) Vol. 117, No. 4, pp. 268-74. Electronic Publication Date: 14 Mar 2013 Journal code: 101524465. ISSN: 1878-6146.	20130101	other	Up to now, most studies on polychlorinated biphenyl (PCB) bioremediation have examined the ability of model fungal strains to biodegrade PCBs. Yet, there is limited information concerning the potential of autochthonous filamentous fungal strains in the biodegradation of PCBs and their possible use in the environmental technologies. In this study, we investigated the capacity of autochthonous fungal strains in the biodegradation of PCBs by isolating 24 taxa from former industrial sites highly contaminated by PCBs. Microscopic and molecular analyses using the internal transcribed spacer (ITS) region revealed that the fungal strains belonged to the phyla Ascomycota (19 strains) and Zygomycota (five strains). The chromatography gas analysis revealed evidence of degradation of seven PCB congeners. With the exception of Circinella muscae which presented no degradation potential, the other fungal strains exhibited a rate of biodegradation ranging from 29 to 85 % after 7 d of incubation in liquid medium. Among these strains, Doratomyces nanus, Doratomyces purpureofuscus, Doratomyces verrucisporus, Myceliophthora thermophila , Phoma eupyrena, and Thermoascus crustaceus showed remarkable degradation ability (>70 %) regardless of the number of chlorine substituents on the biphenyl nucleus and a high tolerance towards PCBs. To our knowledge, this is the first study that demonstrates the ability of PCB degradation by these species and indicates the potential effectiveness of some autochthonous fungal strains in bioremediation systems. Copyright .COPYRGT. 2013 The British Mycological Society. Published by Elsevier Ltd. All rights reserved.	MEDLINE/2013591161
Functional expression of a thermophilic glucuronyl esterase from Sporotrichum thermophile : identification of the nucleophilic serine.	Topakas Evangelos Moukouli Maria Dimarogona Maria Vafiadi Christina Christakopoulos Paul	BIOtechMASS Unit, Biotechnology Laboratory, School of Chemical Engineering, National Technical University of Athens, 5 Iroon Polytechniou Str., Zografou Campus, Athens 15780, Greece.	Applied microbiology and biotechnology, (2010 Aug) Vol. 87, No. 5, pp. 1765-72. Electronic Publication Date: 16 May 2010 Journal code: 8406612. E-ISSN: 1432-0614. L-ISSN: 0175-7598.	20100101	other	A glucuronyl esterase (GE) from the thermophilic fungus Sporotrichum thermophile , belonging to the carbohydrate esterase family 15 (CE-15), was functionally expressed in the methylotrophic yeast Pichia pastoris. The putative GE gene ge2 from the genomic DNA was successfully cloned in frame with the sequence for the Saccharomyces cerevisiae alpha-factor secretion signal under the transcriptional control of the alcohol oxidase (AOX1) promoter and integrated in P. pastoris X-33 to confirm that the encoded enzyme StGE2 exhibits esterase activity. The enzyme was active on substrates containing glucuronic acid methyl ester, showing optimal activity at pH 7.0 and 55 degrees C. The esterase displayed broad pH range stability between 4-10 and temperature stability up to 50 degrees C, rendering StGE2 a strong candidate for future biotechnological applications that require robust biocatalysts. ClustalW alignment of StGE2 with characterized GEs and selected homologous sequences, members of CE-15 family, revealed a novel consensus sequence G-C-S-R-X-G that features the characteristic serine residue involved in the generally conserved catalytic mechanism of the esterase family. The putative serine has been mutated , and the corresponding enzyme has been expressed in P. pastoris to prove that the candidate nucleophilic residue is responsible for catalyzing the enzymatic reaction.	MEDLINE/2010667396
Derivatization of the azole 1-aminobenzotriazole using laccase of Pycnoporus cinnabarinus and Myceliophthora thermophila : influence of methanol on the reaction and biological evaluation of the derivatives.	Hahn Veronika Mikolasch Annett Wende Kristian Bartrow Hannelore Lindequist Ulrike Schauer Frieder	Institute of Microbiology, Ernst-Moritz-Arndt-University Greifswald, F.-L.-Jahnstr. 15, 17487 Greifswald, Germany. veronikahahn@gmx.at	Biotechnology and applied biochemistry, (2010 May 27) Vol. 56, No. 2, pp. 43-8. Electronic Publication Date: 27 May 2010 Journal code: 8609465. E-ISSN: 1470-8744. L-ISSN: 0885-4513.	20100101	other	The laccases of Pycnoporus cinnabarinus and Myceliophthora thermophila are extracellular enzymes with high protein stability. They were used for the 'one pot' synthesis of azole derivatives from 1-aminobenzotriazole together with the p-dihydroxylated laccase substrates 2,5-dihydroxybenzoic acid methyl ester and 2,5-dihydroxybenzoic acid ethyl ester. The reactions yielded heteromolecular dimers (in yields of up to 34%). Methanol was used as the co-solvent to determine the influence of solvent concentration on the course of reaction. The resulting products were isolated, structurally characterized and tested for their antibacterial, antifungal and cytotoxic activities. The products showed low antimicrobial activity and low cytotoxicity compared with commercial available standard compounds but these variables exceeded those of the initial reactants used for the synthesis. In addition to the synthesis of heteromolecular dimers, oligomers were formed and structurally characterized by LC/MS (liquid chromatography/MS).	MEDLINE/2010508966
Synthesis of model morpholine derivatives with biological activities by laccase-catalysed reactions.	Hahn Veronika Mikolasch Annett Wende Kristian Bartrow Hannelore Lindequist Ulrike Schauer Frieder	Institute of Microbiology, Ernst-Moritz-Arndt-University Greifswald, Greifswald, Germany. veronikahahn@gmx.at	Biotechnology and applied biochemistry, (2009 Nov 13) Vol. 54, No. 4, pp. 187-95. Electronic Publication Date: 13 Nov 2009 Journal code: 8609465. E-ISSN: 1470-8744. L-ISSN: 0885-4513.	20090101	other	The efficient enzyme-catalysed reaction of morpholines as model structures for bioactive compounds with para-dihydroxylated aromatic systems was carried out using the oxidoreductase laccase and atmospheric oxygen to produce eight novel morpholine-substituted aromatics. The laccase of Myceliophthora thermophila was used for cross-linking morpholines containing primary or secondary amino groups with para-dihydroxylated laccase substrates. We demonstrate that not only primary amino groups, but also secondary amino groups, are able to couple with para-dihydroxylated aromatic systems in laccase-catalysed reactions. The resulting model products (yields up to 80%) were isolated, structurally characterized and tested for their antibacterial, antifungal and cytotoxic activities. Four of the eight products showed low to moderate growth inhibition against several Gram-positive and -negative bacterial strains and against the yeasts Candida maltosa and Candida albicans. The antibacterial and antifungal activities were determined by an agar disc diffusion test and a modified method according to the EUCAST discussion document E.Dis 7.1 [Rodriguez-Tudela et al. (2003) Clin. Microbiol. Infect . 9, i-viii] for the evaluation of MIC (minimal inhibitory concentration). Differences in cytotoxicity against the human urinary bladder carcinoma cell line 5637 are discussed.	MEDLINE/2009757366

Combinatorial saturation mutagenesis of the Myceliophthora thermophila laccase T2 mutant: the connection between the C-terminal plug and the conserved (509)VSG(511) tripeptide.	Zumarraga Miren Vaz Dominguez Cristina Camarero Susana Shleev Sergey Polaina Julio Martinez-Arias Arturo Ferrer Manuel De Lacey Antonio L Fernandez Victor M Ballesteros Antonio Plou Francisco J Alcalde Miguel	Department of Biocatalysis, Institute of Catalysis, Consejo Superior de Investigaciones Cientificas, Cantoblanco, Madrid, Spain.	Combinatorial chemistry + high throughput screening, (2008 Dec) Vol. 11, No. 10, pp. 807-16. Journal code: 9810948. ISSN: 1386-2073. L-ISSN: 1386-2073.	20080101	other	A mutant laccase from the Ascomycete Myceliophthora thermophila has been submitted to iterative cycles of combinatorial saturation mutagenesis through in vivo overlap extension in Saccharomyces cerevisiae. Over 180,000 clones were explored, among which the S510G mutant revealed a direct interaction between the conserved (509)VSG(511) tripeptide, located in the neighborhood of the T1 site, and the C-terminal plug. The K(m)(O)(2) value of the mutant increased 1.5-fold, and the electron transfer pathway between the reducing substrate and the T1 copper ion was altered, improving the catalytic efficiency towards non-phenolic and phenolic substrates by about 3- and 8-fold. Although the geometry at the T1 site was perturbed by the mutation , paradoxically the laccase redox potential was not significantly altered. Together, the results obtained in this study suggest that the (509)VSG(511) tripeptide may play a hitherto unrecognized role in regulating the traffic of oxygen through the C-terminal plug, the latter blocking access to the T2/T3 copper cluster in the native enzyme.	MEDLINE/2009028204
Altering the laccase functionality by in vivo assembly of mutant libraries with different mutational spectra.	Zumarraga Miren Camarero Susana Shleev Sergey Martinez-Arias Arturo Ballesteros Antonio Plou Francisco J Alcalde Miguel	Instituto de Catalisis y Petroleoquimica, CSIC, Cantoblanco, Madrid 28049, Spain.	Proteins, (2008 Apr) Vol. 71, No. 1, pp. 250-60. Journal code: 8700181. E-ISSN: 1097-0134. L-ISSN: 0887-3585.	20080101	other	The generation of diversity for directed protein evolution experiments shows an important bottleneck in the in vitro random mutagenesis protocols. Most of them are biased towards specific changes that eventually confer a predicted and conservative mutational spectrum, limiting the exploration of the vast protein space. The current work describes a simple methodology to in vivo recombine mutant libraries with different nucleotide bias created by in vitro methods. This in vivo assembly was based on the accurate physiology of Saccharomyces cerevisiae, which as host, provided its high homologous recombination frequency to shuffle the libraries in a nonmutagenic way. The fungal thermophilic laccase from Myceliophthora thermophila expressed in S. cerevisiae was submitted to this protocol under the selective pressure of high concentrations of organic solvents. Mutant 2E9 with approximately 3-fold better kinetics than parent type showed two consecutive amino acid changes (G614D -GGC/GAC- and E615K -GAG/AAG-) because of the in vivo shuffling of the mutant libraries. Both mutations are located in the C-terminal tail that is specifically processed at the Golgi during the maturation of the protein by the Kex2 protease. Notoriously, the oxygen consumption at the T2/T3 trinuclear copper cluster was altered and the catalytic copper at the T1 site was perturbed showing differences in its redox potential and geometry. The change in the isoelectric point of C-terminal extension upon mutations seems to affect the folding of the protein at the posttranslational processing steps providing new insights in the significance of the C-terminal tail for the functionality of the ascomycete laccases. (c) 2007 Wiley-Liss, Inc.	MEDLINE/2008141145
Novel cephalosporins synthesized by amination of 2,5-dihydroxybenzoic acid derivatives using fungal laccases II.	Mikolasch Annett Niedermeyer Timo Horst Johannes Lalk Michael Witt Sabine Seefeldt Simone Hammer Elke Schauer Frieder Gesell Salazar Manuela Hessel Susanne Julich Wolf-Dieter Lindequist Ulrike	Institute of Microbiology, Ernst-Moritz-Arndt-University Greifswald, Greifswald, Germany. annett.mikolasch@gmx.de	Chemical + pharmaceutical bulletin, (2007 Mar) Vol. 55, No. 3, pp. 412-6. Journal code: 0377775. ISSN: 0009-2363. L-ISSN: 0009-2363.	20070101	other	Sixteen novel cephalosporins were synthesized by amination of 2,5-dihydroxybenzoic acid derivatives with the aminocephalosporins cefadroxil, cefalexin, cefaclor, and the structurally related carbacephem loracarbef using laccases from Trametes sp. or Myceliophthora thermophila . All products inhibited the growth of several Gram positive bacterial strains in the agar diffusion assay, among them methicillin-resistant Staphylococcus aureus and vancomycin-resistant enterococci. The products protected mice against an infection with Staphylococcus aureus lethal to the control animals. Cytotoxicity and acute toxicity of the new compounds were negligible. The results show the usefulness of laccase for the synthesis of potential new antibiotics. The biological activity of the new compounds stimulates intensified pharmacological tests.	MEDLINE/2007130009
Combinatorial saturation mutagenesis by in vivo overlap extension for the engineering of fungal laccases.	Alcalde Miguel Zumarraga Miren Polaina Julio Ballesteros Antonio Plou Francisco J	Departamento de Biocatalisis, Instituto de Catalisis y Petroleoquimica, CSIC, Cantoblanco 28049, Madrid, Spain. malcalde@icp.csic.es	Combinatorial chemistry + high throughput screening, (2006 Dec) Vol. 9, No. 10, pp. 719-27. Journal code: 9810948. ISSN: 1386-2073. L-ISSN: 1386-2073.	20060101	other	Combinatorial saturation mutagenesis -CSM- is a valuable tool for improving enzymatic properties from hot-spot residues discovered by directed enzyme evolution or performing semi-rational studies. CSM coupled to a reliable high-throughput screening assay -coefficient of variance below 10%- has been used to enhance turnover rates in the fungal laccase variant T2 from Myceliophthora thermophila . The influence of the highly conserved pentapeptide 509-513 on the redox potential of blue-copper containing enzymes is well described. We focused combinatorial saturation mutagenesis in residues Ser510 and Leu513. Libraries were constructed in Saccharomyces cerevisiae by in vivo overlap extension -IVOE- of the PCR products. This methodology provides a simple manner to build CSM libraries avoiding extra PCR reactions, by-products formation and in vitro ligation steps. After exploring more than 1,700 clones, mutant (7E1) with approximately 3-fold higher kinetics than parent type was found. 7E1 showed one synonymous mutation (L513L, CGT/TTG) and one beneficial mutation S510G (TCG/GGG) that can not be achieved by conventional error-prone PCR techniques. Mutation S510G seems to affect the C-terminal plug, which modulates the transit of water and oxygen to the trinuclear copper cluster.	MEDLINE/2006731282

Screening mutant libraries of fungal laccases in the presence of organic solvents.	Alcalde Miguel Bulter Thomas Zumarraga Miren Garcia-Arellano Humberto Mencia Mario Plou Francisco J Ballesteros Antonio	Departamento de Biocatalisis, Instituto de Catalisis y Petroleoquimica, CSIC, Madrid, Spain. malcalde@icp.csic.es	Journal of biomolecular screening, (2005 Sep) Vol. 10, No. 6, pp. 624-31. Electronic Publication Date: 15 Aug 2005 Journal code: 9612112. ISSN: 1087-0571. L-ISSN: 1087-0571.	20050101	other	Reliable screening methods are being demanded by biocatalysts' engineers, especially when some features such as activity or stability are targets to improve under non-natural conditions (i.e., in the presence of organic solvents). The current work describes a protocol for the design of a fungal laccase-expressed in Saccharomyces cerevisiae-highly active in organic cosolvents. A high-throughput screening assay based on ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)) oxidation was validated. The stability of the ABTS radical cation was not significantly altered in the presence of acetonitrile, ethanol, or DMSO. With a coefficient of variance below 10% and a sensitivity limit of 15 pg laccase/microL, the assay was reproducible and sensitive. The expression system of Myceliophthora thermophila laccase variant T2 in S. cerevisiae was highly dependent on the presence of Cu2+. Copper concentration was limited up to 10 microM CuSO4 where expression levels (approximately 14-18 mg/L) were acceptable without compromising the reliability of the assay. A mutant library was created by error-prone PCR with 1.1 to 3.5 mutations per kb. After only 1 generation of directed evolution, mutant 6C9 displayed about 3.5-fold higher activities than parent type in the presence of 20% acetonitrile or 30% ethanol. The method provided here should be generally useful to improve the activity of other redox enzymes in mixtures of water/cosolvents.	MEDLINE/2005492200
Spectroscopic characterization of the Leu513His variant of fungal laccase: effect of increased axial ligand interaction on the geometric and electronic structure of the type 1 Cu site.	Palmer Amy E Szilagyi Robert K Cherry Joel R Jones Aubrey Xu Feng Solomon Edward I	Department of Chemistry, Stanford University, Stanford, California 94305 and Novozymes Biotech, Davis, California 95616, USA.	Inorganic chemistry, (2003 Jun 30) Vol. 42, No. 13, pp. 4006-17. Journal code: 0366543. ISSN: 0020-1669. L-ISSN: 0020-1669.	20030101	other	A variety of spectroscopic techniques, combined with density functional calculations, are used to describe the electronic structure of the Leu513His variant of the type 1 Cu site in Myceliophthora thermophila laccase. This mutation changes the type 1 Cu from a blue to a green site. Electron paramagnetic resonance (EPR), optical absorption, circular dichroism, and magnetic circular dichroism (MCD) spectroscopies reveal that, relative to the trigonal planar blue type 1 Cu site in wild-type fungal laccase, the covalency and the ligand field strength at the Leu513His green type 1 Cu center decrease. Additionally, there is a significant reorientation of the d(x)()()2(-)(y)()()2( )singly occupied MO, such that the overlap with the Cys sulfur valence orbital changes from pi to sigma. A density functional study in which internal coordinates are systematically altered reveals that these changes are due to the increased strength of the axial ligand (none to His), leading to a tetragonal distortion and elongation of the equatorial Cu-ligand bonds. These calculations provide insight into the experimental differences in the EPR parameters, charge-transfer absorption spectrum, and ligand-field MCD spectrum between the axial-His variant and blue Cu centers (plastocyanin and the type 1 site in fungal laccase). There are also significant differences between the green site in the Leu513His variant and other naturally occurring, green type 1 Cu sites such as in nitrite reductase, which have short axial Cu-S(Met) bonds. The large difference in EPR parameters between these green type 1 sites derives from a change in ligand field excitation energies observed by MCD, which reflects a decrease in ligand field strength. This is associated with different steric interactions of a His vs an axial Met ligand in a tetragonally distorted type 1 site. Changes in the electronic structure of the Cu site correlate with the difference in reactivity of the green His variant relative to blue wild-type fungal laccase.	MEDLINE/2003291879
Functional expression of a fungal laccase in Saccharomyces cerevisiae by directed evolution.	Bulter Thomas Alcalde Miguel Sieber Volker Meinhold Peter Schlachtbauer Christian Arnold Frances H	Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125,USA.	Applied and environmental microbiology, (2003 Feb) Vol. 69, No. 2, pp. 987-95. Journal code: 7605801. ISSN: 0099-2240. L-ISSN: 0099-2240. Report No.: PMC-PMC143632.	20030101	other	Laccase from Myceliophthora thermophila (MtL) was expressed in functional form in Saccharomyces cerevisiae. Directed evolution improved expression eightfold to the highest yet reported for a laccase in yeast (18 mg/liter). Together with a 22-fold increase in k(cat), the total activity was enhanced 170-fold. Specific activities of MtL mutants toward 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) and syringaldazine indicate that substrate specificity was not changed by the introduced mutations . The most effective mutation (10-fold increase in total activity) introduced a Kex2 protease recognition site at the C-terminal processing site of the protein, adjusting the protein sequence to the different protease specificities of the heterologous host. The C terminus is shown to be important for laccase activity, since removing it by a truncation of the gene reduces activity sixfold. Mutations accumulated during nine generations of evolution for higher activity decreased enzyme stability. Screening for improved stability in one generation produced a mutant more stable than the heterologous wild type and retaining the improved activity. The molecular mass of MtL expressed in S. cerevisiae is 30% higher than that of the same enzyme expressed in M. thermophila (110 kDa versus 85 kDa). Hyperglycosylation, corresponding to a 120-monomer glycan on one N-glycosylation site, is responsible for this increase. This S. cerevisiae expression system makes MtL available for functional tailoring by directed evolution.	MEDLINE/2003060302
Colorimetric assays for biodegradation of polycyclic aromatic hydrocarbons by fungal laccases.	Alcalde Miguel Bulter Thomas Arnold Frances H	Division of Chemistry and Chemical Engineering 210-41, California Institute of Technology, Pasadena, CA, USA.	Journal of biomolecular screening, (2002 Dec) Vol. 7, No. 6, pp. 547-53. Journal code: 9612112. ISSN: 1087-0571. L-ISSN: 1087-0571.	20020101	other	Polycyclic aromatic hydrocarbons (PAHs) are highly toxic organic pollutants widely distributed in terrestrial and aquatic environments. In the present work, 2 colorimetric assays for laccase-catalyzed degradation of PAHs were developed based on studies of the oxidation of 12 aromatic hydrocarbons by fungal laccases from Trametes versicolor and Myceliophthora thermophila . Using a sodium borohydride water-soluble solution, the authors could reduce the single product of laccase-catalyzed anthracene biooxidation into the orange-colored 9,10-anthrahydroquinone, which is quantifiable spectrophotometrically. An assay using polymeric dye (Poly R-478) as a surrogate substrate for lignin degradation by laccase in the presence of mediator is also presented. The decolorization of Poly R-478 was correlated to the oxidation of PAHs mediated by laccases. This demonstrates that a ligninolytic indicator such as Poly R-478 can be used to screen for PAH-degrading laccases; it will also be useful in screening mutant libraries in directed evolution experiments. Poly R-478 is stable and readily soluble. It has a high extinction coefficient and low toxicity toward white rot fungi, yeast, and bacteria, which allow its application in a solid-phase assay format.	MEDLINE/2003522260

Toxicological studies on Laccase from Myceliophthora thermophila expressed in Aspergillus oryzae.	Brinch D S Pedersen P B	Novozymes A/S, Krogshoejvej 36, DK-2880 Bagsvaerd, Denmark. dsbr@novozymes.com	Regulatory toxicology and pharmacology : RTP, (2002 Jun) Vol. 35, No. 3, pp. 296-307. Journal code: 8214983. ISSN: 0273-2300. L-ISSN: 0273-2300.	20020101	other	The bioindustrially produced enzyme laccase can be used in different technical and food applications to facilitate processes. It can be added to different oral care products such as mouthwash, toothpaste, mints, and gums to prevent halitosis. Laccase, produced by submerged fermentation of Aspergillus oryzae, containing a gene originating from Myceliophthora thermophila , was subject to a series of toxicological tests to document its safety in use. It was not found to be mutagenic in the Salmonella typhimurium reverse mutation assay, nor did it cause chromosomal aberrations in cultured human lymphocytes. No evidence of inhalation toxicity or skin and eye irritation was found. There was no evidence of possible skin sensitization in a human skin sensitization test when Laccase was tested at 10% (w/v): thus Laccase would appear to have a low skin sensitization potential. Oral administration to rats of up to 10.0 mL/kg body wt/day (equivalent to a total organic solids dosage of 1.72 g/kg body wt/day) for 13 weeks did not cause any adverse effect . Copyright 2002 Elsevier Science (USA)	MEDLINE/2002444952
Biochemical and structural insights into a thermostable cellobiohydrolase from Myceliophthora thermophila .	Kadowaki Marco A S Higasi Paula de Godoy Mariana O Polikarpov Igor Prade Rolf A	Sao Carlos Institute of Physics, University of Sao Paulo, Brazil. Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK, USA.	The FEBS journal, (20180200) Vol. 285, No. 3, pp. 559-579. Electronic Publication Date: 1 Jan 2018 Journal code: 101229646. E-ISSN: 1742-4658. L-ISSN: 1742-464X.	20180101	other	Cellobiohydrolases hydrolyze cellulose, a linear polymer with glucose monomers linked exclusively by .beta.-1,4 glycosidic linkages. The widespread hydrogen bonding network tethers individual cellulose polymers forming crystalline cellulose, which prevent the access of hydrolytic enzymes and water molecules. The most abundant enzyme secreted by Myceliophthora thermophila M77 in response to the presence of biomass is the cellobiohydrolase MtCel7A, which is composed by a GH7-catalytic domain (CD), a linker, and a CBM1-type carbohydrate-binding module. GH7 cellobiohydrolases have been studied before, and structural models have been proposed. However, currently available GH7 crystal structures only define separate catalytic domains and/or cellulose-binding modules and do not include the full-length structures that are involved in shaping the catalytic mode of operation. In this study, we determined the 3D structure of catalytic domain using X-ray crystallography and retrieved the full-length enzyme envelope via small-angle X-ray scattering (SAXS) technique. The SAXS data reveal a tadpole-like molecular shape with a rigid linker connecting the CD and CBM. Our biochemical studies show that MtCel7A has higher catalytic efficiency and thermostability as well as lower processivity when compared to the well-studied TrCel7A from Trichoderma reesei. Based on a comparison of the crystallographic structures of CDs and their molecular dynamic simulations, we demonstrate that MtCel7A has considerably higher flexibility than TrCel7A. In particular, loops that cover the active site are more flexible and undergo higher conformational fluctuations, which might account for decreased processivity and enhanced enzymatic efficiency. Our statistical coupling analysis suggests co-evolution of amino acid clusters comprising the catalytic site of MtCel7A, which correlate with the steps in the catalytic cycle of the enzyme. DATABASE: The atomic coordinates and structural factors of MtCel7A have been deposited in the Protein Data Bank with accession number 5W11. .COPYRGT. 2017 Federation of European Biochemical Societies.	MEDLINE/2019297053
Screening of candidate substrates and coupling ions of transporters by thermostability shift assays.	Majd Homa King Martin S Palmer Shane M Smith Anthony C Kunji Edmund Rs Elbourne Liam Dh Paulsen Ian T Sharples David Henderson Peter Jf	Medical Research Council Mitochondrial Biology Unit, University of Cambridge, Cambridge, United Kingdom. Department of Molecular Sciences, Macquarie University, Sydney, Australia. Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, United Kingdom. School of Biomedical Sciences, University of Leeds, Leeds, United Kingdom.	eLife, (20181015) Vol. 7. Electronic Publication Date: 15 Oct 2018 Journal code: 101579614. E-ISSN: 2050-084X. L-ISSN: 2050-084X. Report No.: PMC-PMC6211832.	20180101	other	Substrates of most transport proteins have not been identified, limiting our understanding of their role in physiology and disease . Traditional identification methods use transport assays with radioactive compounds, but they are technically challenging and many compounds are unavailable in radioactive form or are prohibitively expensive, precluding large-scale trials. Here, we present a high-throughput screening method that can identify candidate substrates from libraries of unlabeled compounds. The assay is based on the principle that transport proteins recognize substrates through specific interactions, which lead to enhanced stabilization of the transporter population in thermostability shift assays. Representatives of three different transporter (super)families were tested, which differ in structure as well as transport and ion coupling mechanisms. In each case, the substrates were identified correctly from a large set of chemically related compounds, including stereo-isoforms. In some cases, stabilization by substrate binding was enhanced further by ions, providing testable hypotheses on energy coupling mechanisms. .COPYRGT. 2018, Majd et al.	MEDLINE/2020392828
Designing a cocktail containing redox enzymes to improve hemicellulosic hydrolysate fermentability by microorganisms.	Tramontina, Robson Brenelli, Livia Beatriz Sousa, Amanda Alves, Rafael Zetty Arenas, Ana Maria Nascimento, Viviane Marcos Rabelo, Sarita Candida Freitas, Sindelia Ruller, Roberto Squina, Fabio Marcio [Reprint Author]	Rod Raposo Tavares,Km 92-5, BR-18023000 Sorocaba, Brazil fabio.squina@prof.uniso.br	Enzyme and Microbial Technology, (APR 2020) Vol. 135, pp. Article No.: 109490. http://www.journals.elsevier.com/enzyme-and-microbial-? technology/#description. CODEN: EMTED2. ISSN: 0141-0229. E-ISSN: 1879-0909.	20200101	other	Bioproducts production using monomeric sugars derived from lignocellulosic biomass presents several challenges, such as to require a physicochemical pretreatment to improve its conversion yields. Hydrothermal lignocellulose pretreatment has several advantages and results in solid and liquid streams. The former is called hemicellulosic hydrolysate (HH), which contains inhibitory phenolic compounds and sugar degradation products that hinder microbial fermentation products from pentose sugars. Here, we developed and applied a novel enzyme process to detoxify HH. Initially, the design of experiments with different redox activities enzymes was carried out. The enzyme mixture containing the peroxidase (from Armoracia rusticana) together with superoxide dismutase (from Coptotermes gestroi) are the most effective to detoxify HH derived from sugarcane bagasse. Butanol fermentation by the bacteria Clostridium saccharoperbutylacetonicum and ethanol production by the yeast Scheffersomyces stipitis increased by 24.0 x and 2.4 x , respectively, relative to the untreated hemicellulosic hydrolysates. Detoxified HH was analyzed by chromatographic and spectrometric methods elucidating the mechanisms of phenolic compound modifications by enzymatic treatment. The enzyme mixture degraded and reduced the hydroxyphenyl- and feruloyl-derived units and polymerized the lignin fragments. This strategy uses biocatalysts under environmentally friendly conditions and could be applied in the fuel, food, and chemical industries.	BIOSIS/2020:362279

Anti-inflammatory and anti-oxidant properties of laccase-synthesized phenolic-O-carboxymethyl chitosan hydrogels.	Huber, Daniela Grzelak, Adrianna Baumann, Martina Borth, Nicole Schleining, Gerhard Nyanhongo, Gibson S. [Reprint Author] Guebitz, Georg M.	Univ Nat Resources and Life Sci Vienna, Inst Environm Biotechnol, Konrad Lorenz Str 20, A-3430 Tulln, Austria g.nyanhongo@boku.ac.at	New Biotechnology, (JAN 25 2018) Vol. 40, No. Part B, pp. 236-244. http://www.journals.elsevier.com/new-biotechnology/#description. ISSN: 1871-6784. E-ISSN: 1876-4347.	20180101	other	A bioactive O-carboxymethyl chitosan (CMCS) hydrogel crosslinked with natural phenolics with potential application in wound dressings was synthesized using a laccase from Myceliophthora thermophila (MTL). The highest degree of cross-linking (49.7%) was achieved with catechol. All the phenolic-CMCS hydrogels synthesized showed excellent anti-oxidant properties with a free radical scavenging activity up to 4-fold higher than in the absence of the phenolics, as quantified by the di(phenyl)-(2,4,6-trinitrophenyl) iminoazanium (DPPH) assay. In addition, the hydrogels produced showed an anti-inflammatory effect as evidenced by the inhibition of enzymes [myeloperoxidase (MPO), matrix-metalloproteinase-1 (MMP-1) and human neutrophil elastase (HNE)] over-expressed in chronic wounds. Sinapyl-CMCS hydrogels showed an MMP-1 inhibition of 37%. Further, the phenolic-CMCS hydrogels did not affect the viability of the NIH 3T3 mouse fibroblast cell line and were also able to slowly release human fibroblast growth factor 2, reaching 48.3% over a period of 28 days. This study thus shows the possibility of synthesizing multifunctional bioactive chitosan based hydrogels with anti-oxidant and anti-inflammatory properties using natural occurring phenolics as crosslinkers.	BIOSIS/2018:267138
Optimization of enzymatic synthesis of L-arabinose ferulate catalyzed by feruloyl esterases from Myceliophthora thermophila in detergentless microemulsions and assessment of its antioxidant and cytotoxicity activities.	Antonopoulou, Io Papadopoulou, Adamantia Iancu, Laura Cerullo, Gabriella Ralli, Marianna Juetten, Peter Piechot, Alexander Faraco, Vincenza Kletsas, Dimitris Rova, Ulrika Christakopoulos, Paul [Reprint Author]	Lulea Univ Technol, Dept Civil Environm and Nat Resources Engn, Div Chem Engn, Biochem Proc Engn, S-97187 Lulea, Sweden paul.christakopoulos@ltu.se	Process Biochemistry, (FEB 2018) Vol. 65, pp. 100-108. http://www.journals.elsevier.com/process-biochemistry/#description. ISSN: 1359-5113. E-ISSN: 1873-3298.	20180101	other	The feruloyl esterases FaeA1, FaeA2, FaeB1, FaeB2 from Myceliophthora thermophila Cl and MtFae1a from M. thermophila ATCC 42464 were used as biocatalysts for the transesterification of vinyl ferulate (VFA) with L-arabinose in detergentless microemulsions. The effect of parameters such as the microemulsion composition, the substrate concentration, the enzyme load, the pH, the temperature and the agitation was investigated. FaeA1 offered the highest transesterification yield (52.2 +/- 4.3%) after 8 h of incubation at 50 degrees C using 80 mM VFA, 55 mM L-arabinose and 0.02 mg FAE mL(-1) in a mixture comprising of 19.8: 74.7: 5.5 v/v/v n-hexane: t-butanol: 100 mM MOPS-NaOH pH 8.0. The ability of L-arabinose ferulate (AFA) to scavenge 2,2-diphenyl-1-picrylhy-drazyl (DPPH) radicals was significant (IC50 386.5 mu M). AFA was not cytotoxic even at high concentrations (1 mM) however was found to be pro-oxidant at concentrations higher than 20 mu M when the antioxidant activity was determined with the dichloro-dihydro-fluorescein diacetate (DCFH-DA) assay in human skin fibroblasts.	BIOSIS/2018:286487
Statistical optimization and mutagenesis for high level of phytase production by Rhizopus oligosporus MTCC 556 under solid state fermentation.	Suresh, S. [Reprint Author] Radha, K. V.	Anna Univ, AC Tech, Dept Chem Engn, Madras 600025, Tamil Nadu, India sureshbiochem14@gmail.com	Journal of Environmental Biology, (MAR 2016) Vol. 37, No. 2, pp. 253-259. http://www.jeb.co.in. CODEN: JEBIDP. ISSN: 0254-8704. E-ISSN: 0254-8704.	20160101	other	The present study deals with production of phytase from Rhizopus oligosporus MTCC 556 by solid state fermentation (SSF) using different (ADT27, IR20, PAIYUR1, KG, and RASI) rice bran varieties, in which ADT27 rice bran yield maximum of 6.2 U gds(-1) phytase. Statistical optimization was employed by Central Composite Design (CCD); the results showed that 3.0 g dextrose, 2.5 g ammonium nitrate, substrate size of 80 mesh, 10 mg calcium chloride was 116 hr at optimal for phytase production by SSF, with maximum of 23.14 U gds(-1). Phytase production improved by 4 fold (31.3 U/gds) due to chemical mutagenesis (mutant Rhizopus oligosporus MTCC 1116) in optimized media composition. Partially purified phytase showed approximately 90 kDa of molecular mass and was optimally active at 5.5 pH and 50 degrees C temperature. Substrate specificity exhibited in sodium phytic acid and phytase activity was stimulated by Zn2+ and Ca2+.	BIOSIS/2016:340570
Formation of a cytoplasmic salt bridge network in the matrix state is a fundamental step in the transport mechanism of the mitochondrial ADP/ATP carrier.	King, Martin S. Kerr, Matthew Crichton, Paul G. Springett, Roger Kunji, Edmund R. S. [Reprint Author]	MRC, Mitochondrial Biol Unit, Cambridge Biomed Campus,Wellcome Trust, Cambridge CB2 0XY, UK ek@mrc-mbu.cam.ac.uk	Biochimica et Biophysica Acta, (JAN 2016) Vol. 1857, No. 1, pp. 14-22. http://www.journals.elsevier.com/bba-bioenergetics/#description. ISSN: 0005-2728. E-ISSN: 1879-2650.	20160101	other	Mitochondrial ADP/ATP carriers catalyze the equimolar exchange of ADP and ATP across the mitochondrial inner membrane. Structurally, they consist of three homologous domains with a single substrate binding site. They alternate between a cytoplasmic and matrix state in which the binding site is accessible to these compartments for binding of ADP or ATP. It has been proposed that cycling between states occurs by disruption and formation of a matrix and cytoplasmic salt bridge network in an alternating way, but formation of the latter has not been shown experimentally. Here, we show that state-dependent formation of the cytoplasmic salt bridge network can be demonstrated by measuring the effect of mutations on the thermal stability of detergent-solubilized carriers locked in a specific state. For this purpose, mutations were made to increase or decrease the overall interaction energy of the cytoplasmic network. When locked in the cytoplasmic state by the inhibitor carboxyatractyloside, the thermostabilities of the mutant and wild-type carriers were similar, but when locked in the matrix state by the inhibitor bongkrekic acid, they correlated with the predicted interaction energy of the cytoplasmic network, demonstrating its formation. Changing the interaction energy of the cytoplasmic network also had a profound effect on the kinetics of transport, indicating that formation of the network is a key step in the transport cycle. These results are consistent with a unique alternating access mechanism that involves the simultaneous rotation of the three domains around a central translocation pathway. (C) 2015 The Authors. Published by Elsevier B.V.	BIOSIS/2016:139606

Enzymatic synthesis of poly(catechin)-antibiotic conjugates: an antimicrobial approach for indwelling catheters.	Goncalves, Idalina Abreu, Ana S. Matama, Teresa Ribeiro, Artur Gomes, Andreia C. Silva, Carla [Reprint Author] Cavaco-Paulo, Artur	Univ Minho, European Inst Excellence Tissue Engn and Regenerat, 3Bs Res Grp, AvePk, P-4806909 Taipas, Guimaraes, Portugal cmpsilva@ceb.uminho.pt; artur@deb.uminho.pt	Applied Microbiology and Biotechnology, (JAN 2015) Vol. 99, No. 2, pp. 637-651. http://www.springerlink.com/content/100457/. CODEN: AMBIDG. ISSN: 0175-7598. E-ISSN: 1432-0614.	20150101	other	Biofilm formation in urinary indwelling catheters is one of the most critical issues that patients face. Catheters were coated with poly(catechin)-antibiotic conjugates with enhanced antimicrobial properties. Catechin was conjugated with two antibiotics, namely trimethoprim (TMP) and sulfamethoxazole (SMZ) via activation with N,N'-disuccinimidyl carbonate (DSC) and subsequent coupling to molecules containing alpha-amine moieties. Silicone and polyurethane catheters were functionalized in situ through laccase oxidation of catechin-antibiotic conjugates. Four antimicrobial coatings were produced, namely with poly(catechin), poly(catechin)-TMP, poly(catechin)-SMZ and poly(catechin)-TMP-SMZ. The bacterial adhesion reduction was tested on the functionalized devices using gram-negative and gram-positive strains. The most significant reduction in adhesion was observed with poly(catechin)-TMP (gram-negative -85 % and gram-positive -87 %) and with poly(catechin)-TMP-SMZ (gram-negative -85 % and gram-positive -91 %). The cytotoxicity to mammalian cells was tested by indirect contact for 5 days and revealed that all the tested coatings supported more than 90 % of viable cells. A promising approach for the increase of the indwelling catheter lifespan was developed aiming to reduce catheter-associated chronic infections .	BIOSIS/2016:333394
Assessing the use of xylanase and laccases in biobleaching stages of a TCF sequence for flax pulp.	Fillat, Amanda Blanca Roncero, M. Vidal, Teresa [Reprint Author]	Univ Politecn Cataluna, Text and Paper Engn Dept, ETSEIAT, Colom 11, E-08222 Terrassa, Spain tvidal@etp.upc.edu	Journal of Chemical Technology and Biotechnology, (DEC 2011) Vol. 86, No. 12, pp. 1501-1507. CODEN: JCTBED. ISSN: 0268-2575. E-ISSN: 0197-4660.	20110101	other	BACKGROUND: In this study, two different laccases were used in combination with various mediators as biobleaching agents in a TCF sequence for flax fibre and pulp. Thus, non-commercial laccase from Pycnoporus cinnabarinus (PcL) was used with the synthetic mediator violuric acid (VA) or the natural mediator syringaldehyde (SA), and commercial laccase from Myceliophthora thermophila (MtL) was used with the natural mediator methyl syringate (MS). Each laccase-mediator treatment was used in the following TCF bleaching sequence: LQPo (Q denoting a chelating stage and Po a pressurized alkaline peroxide treatment). Also, a xylanase pretreatment was carried out for first time here to assess its effect on flax fibre. Pulp (kappa number, viscosity and brightness) and effluent properties (COD, colour, residual activity and toxicity) were determined after each bleaching stage. The hexenuronic acid (HexA) content of the pulp was also determined to assess its integrity.RESULTS: The best L stage was PcL + VA, which provided a final brightness and kappa number of 81% ISO and 1.3, respectively. Using a xylanase pretreatment was found to efficiently remove HexA and enhance delignification by laccase (final kappa number 0.7).CONCLUSIONS: These results warrant upscaling any of these biobleaching sequences for flax pulp as they provide environmentally sustainable flax fibre with a high cellulose content and brightness above 80% ISO. (C) 2011 Society of Chemical Industry	BIOSIS/2012:64395
Oxidative dehalogenation of chlorinated hydroxybiphenyls by laccases of white-rot fungi.	Kordon, Korinna Mikolasch, Annett [Reprint Author] Schauer, Frieder	Ernst Moritz Arndt Univ Greifswald, Inst Microbiol, Friedrich Ludwig Jahn Str 15, D-17487 Greifswald, Germany annett.mikolasch@uni-greifswald.de	International Biodeterioration + Biodegradation, (JUN 2010) Vol. 64, No. 3, pp. 203-209. CODEN: IBBIES. ISSN: 0964-8305.	20100101	other	Chlorinated biphenyls have been widely used in industry because of their advantageous chemical and physical properties. But the transformation products of chlorinated biphenyls derived from hydroxylation, the chlorinated hydroxybiphenyls, have oestrogenic, immunosuppressive, and carcinogenic activities. Because of the risks to humans and animals, the ability to transform chlorinated hydroxybiphenyls is a major goal of environmental research. We here investigated transformations of the chlorinated hydroxybiphenyls 5-chloro-2-hydroxybiphenyl, 3,5-dichloro-2-hydroxybiphenyl, and 2',5,5'-trichloro-2-hydroxybiphenyl by laccases from Pycnoporus cinnabarinus and Myceliophthora thermophila , because very little is known about the transformation pathways of the named substances by filamentous fungi though these organisms play important roles in soil ecosystems and in contaminated sludges and wastewater. Oxidative dechlorination and formation of quinoid and hydroquinoid derivatives were observed in all three cases. Furthermore, dimer formation was detected. Therefore, laccase leads not only to dechlorination of the initial chlorinated hydroxybiphenyls but also to dechlorinated di- and oligomeric products. In addition 4-chloro-2-hydroxydibenzofuran was found and purified as a product of the laccase reaction on 3,5-dichloro-2-hydroxybiphenyl. (c) 2010 Elsevier Ltd. All rights reserved.	BIOSIS/2010:327299
Biodegradation of indole at high concentration by persolvent fermentation with the thermophilic fungus Sporotrichum thermophile .	Katapodis, P. Moukouli, M. Christakopoulos, P. [Reprint Author]	Natl Tech Univ Athens, Sch Chem Engn, Biotechnol Lab, 5 Iroon Polytechniou Str,Zografou Campus, Athens 15700, Greece hristako@orfeas.chemeng.ntua.gr	International Biodeterioration + Biodegradation, (DEC 2007) Vol. 60, No. 4, pp. 267-272. http://www.journals.elsevier.com/international-? biodeterioration-and-biodegradation/#description. CODEN: IBBIES. ISSN: 0964-8305. E-ISSN: 1879-0208.	20070101	other	Indole and its derivatives form a class of toxic recalcitrant environmental pollulants. Sporotrichum thermophile was grown in a persolvent fermentation system containing a large amount of indole. The medium contained up to 20% by volume soybean oil and up to 2 g L-1 indole. Most of the indole was partitioned in the organic solvent layer. When the organism was grown in the medium containing indole at 1 g L-1, indole was totally consumed after 6 days. Under a fed-batch fermentation process where daily batches of indole (1 g L-1) supplemented the microbial culture for 4 days, the biodegradation level was 3.0 g L-1. These values make this process promising and worthy of further investigation for the microbial degradation of other toxic compounds. (C) 2007 Elsevier Ltd. All rights reserved.	BIOSIS/2008:251783

Bioremediation of polycyclic aromatic hydrocarbons by fungal laccases engineered by directed evolution.	Zumarraga, Miren Plou, Francisco J. Garcia-Arellano, Humberto Ballesteros, Antonio Alcalde, Miguel [Reprint Author]	CSIC, Inst Catalisis and Petroleoquim, E-28049 Madrid, Spain malcalde@icp.csic.es	Biocatalysis and Biotransformation, (2007) Vol. 25, No. 2-4, pp. 219-228. http://www.tandfonline.com/toc/ibab20/. ISSN: 1024-2422. E-ISSN: 1029-2446.	20070101	other	Fungal laccases are useful for several remarkable transformations, such as bioremediation of polycyclic aromatic hydrocarbons (PAHs), synthesis of phenolic-based resins, oxidation of lignin derivatives and others. Most of these substrates are barely water-soluble, and although polar organic co-solvents may be added to enhance their solubility, transformation rates dramatically decrease due to the negative effect of organic solvents on the protein structure. Laccase from Myceliophthora thermophila variant T2 (MtLT2) has been submitted to laboratory evolution in Saccharomyces cerevisiae with the aim of improving activity and stability in organic co-solvents. Some 4500 clones created by random mutagenesis were screened in two rounds of directed evolution. Libraries were explored under increasing concentrations of acetonitrile and ethanol, and several mutants with improved features were purified and further characterised. Turnover rates of MtLT2 in 30% (v/v) acetonitrile and 50% (v/v) ethanol were increased up to 6.5- and 7.5-fold, respectively. The best variants showed similar rates in 20% (v/v) acetonitrile or 30% (v/v) ethanol as the parent type in aqueous media. Mutant laccases were also tested for the oxidation of anthracene in the presence of 20% (v/v) acetonitrile.	BIOSIS/2008:9846
Laccase-catalyzed oxidation of naphthol in the presence of soluble polymers.	Kulys, Juozas [Reprint Author] Vidziunaite, Regina Schneider, Palle	Institute of Biochemistry, Mokslininku 12, 2600, Vilnius, Lithuania jkulys@bchi.lt	Enzyme and Microbial Technology, (3 March 2003) Vol. 32, No. 3-4, pp. 455-463. print. CODEN: EMTED2. ISSN: 0141-0229.	20030101	other	The oxidation of 1-naphthol and 2-naphthol was studied in presence of recombinant laccases (benzenediol:oxygen oxidoreductase, EC 1.10.3.2) from Polyporus pinsitus (rPpL), Myceliophthora thermophila (rMtL), Coprinus cinereus (rCcL) and Rhizoctonia solani (rRsL). During 1-naphthol oxidation a violet colored insoluble product was formed. The product of 2-naphthol oxidation was a white insoluble precipitate. Almost two moles of 1-naphthol was oxidized by one mole of oxygen. Kinetic measurements of oxygen consumption showed that laccases have been inactivated during naphthols oxidation. Water soluble polymers and albumins changed oxidation profile. The polymers investigated were divided into three groups. The first group includes polymers which increase total oxygen consumption without changing an initial rate: polyvinyl alcohol, ficoll, a copolymer of acrylamide, N-vinylpyrrolidone and ethyl acrylate, hydroxyethyl cellulose, dextrans 500, 110 and 20, polyvinylpyrrolidone, polyethylene glycol and albumins. These polymers intermediately bound hydrophobic naphtholic radicals preventing inactivation of laccases. Cationic polymers such as diethylaminoethyl-dextran, poly-L-lysine and protasan that inhibited the initial oxidation rate, but did not change total oxygen consumption, were attributed to the second group. The inhibition was associated with complexation of positively charged polymers with negatively charged laccases since isoelectric points of rPpL and of rMtL were 3.5 and 4.2, respectively. The polymers such as alginic acid, polyacrylic acid and heparin were attributed to the third group. These polymers carried negative charges and formed random coil in solution due to electrostatic repulsion. They did not interact with laccases and did not combine intermediates. Therefore, they practically did not change total oxygen consumption and initial rate of naphthol oxidation.	BIOSIS/2003:159735
Mutants of myceliophthora laccase with enhanced activity.	Feng, X. [Inventor] Berka, R. M. [Inventor] Wahleithner, J. A. [Inventor]	NOVO NORDISK BIOTECH, INC.	US5770419	19980623	other		BIOSIS/2002:110657
Method of producing proteins in filamentous fungi with decreased CLR1 activity.	Haefner, Stefan [Inventor] Anonymous Thywissen, Andreas [Inventor] Hartmann, Holger [Inventor] Boehmer, Nico [Inventor]	BASF SE	US10597429	20200324	other	The present invention relates to a method of producing a recombinant polypeptide in a filamentous fungus which is genetically modified to decrease or eliminate the activity of cellulase regulator 1 (CLR1) and to express the recombinant polypeptide. The method further relates to a filamentous fungus Myceliophthora thermophila , which is genetically modified to decrease or eliminate the activity of CLR1 and to the use of this filamentous fungus in the production of a recombinant polypeptide.	BIOSIS/2020:352278
Improving cellulases production by Myceliophthora thermophila through disruption of protease genes.	Li, Xiaolin [Reprint Author] Liu, Qian Sun, Wenliang He, Qun [Reprint Author] Tian, Chaoguang [Reprint Author]	China Agr Univ, Coll Biol Sci, State Key Lab Agrobiotechnol, Beijing 100193, Peoples R China qunhe@cau.edu.cn; tian_cg@tib.cas.cn	Biotechnology Letters, (FEB 2020) Vol. 42, No. 2. http://www.springerlink.com/content/100138/. CODEN: BILED3. ISSN: 0141-5492. E-ISSN: 1573-6776.	20200101	other	Objective To identify main protease genes for the proteolytic degradation of cellulases in M. thermophila and generate a lower-proteases fungal host that can be used for further metabolic engineering to increase cellulase production and heterologous protein expression. Results Systematic transcriptomic analysis were conducted on the expression of proteases genes in M. thermophila genome and five highly expressed genes encoding extracellular proteases were selected for mutation analyses. A series of single- and multi-gene mutants of these five selected genes was constructed using the CRISPR-Cas9 technique. Compared with WT, the Delta Mtalp1 and the quintuple mutant showed significantly lower protease activity (decreased 52.7% and 58.4%, respectively) and at least double enhanced cellulase production. Conclusions The results indicated that Mtalp1 is a critical protease gene in cellulase degradation in M. thermophila and disruption of protease genes showed significantly decreased protease activity and obviously enhanced cellulase production in the fermentation broth of Delta Mtalp1 and the quintuple mutant.	BIOSIS/2020:232448

New Tetramic Acids Comprising of Decalin and Pyridones From Chaetomium olivaceum SD-80A With Antimicrobial Activity.	Wang, Xinzhu [Reprint Author] Zhao, Liya Liu, Chao Qi, Jun Zhao, Peipei Liu, Zhaoming Li, Chunlei Hu, Yingying Yin, Xin Liu, Xin Liao, Zhixin [Reprint Author] Zhang, Lixin [Reprint Author] Xia, Xuekui [Reprint Author]	Southeast Univ, Dept Pharmaceut Engn, Sch Chem and Chem Engn, Nanjing, Peoples R China zxliao@seu.edu.cn; lxzhang@ecust.edu.cn; xiaxk@sdas.org	Frontiers in Microbiology, (JAN 15 2020) Vol. 10, pp. Article No.: 2958. http://www.frontiersin.org/microbiology/. ISSN: 1664-302X. E-ISSN: 1664-302X.	20200101	other	Cycloaddition reactions such as intramolecular Diels-Alder (IMDA) are extremely important in constructing multicyclic scaffolds with diverse bioactivities. Using MycB as a biomarker, three new polyketides - Chaetolivacines A (1), B (3), and C (4) - with one known compound Myceliothermophin E (2) comprising of decalin and 4-hydroxy-2-pyridones were obtained from the culture of Chaetomium olivaceum SD-80A under the guidance of gene mining. The structures of these compounds were established using detailed 1D, 2D NMR, and high-resolution electron spray ionization mass spectroscopy (HRESIMS) analysis. The relative and absolute configurations of the compounds 1, 3, and 4 were elucidated by NOESY and ECD. The biosynthesis pathways of these compounds were proposed, which involves in three key genes ChaA [polyketide synthase-non-ribosomal peptide synthetases (PKS-NRPS)], ChaB, and ChaC. Compounds 1-4 were tested for their antimicrobial activities, and compounds 2 and 3 showed moderate bioactivity against Staphylococcus aureus (SA) and methicillin-resistant S. aureus (MRSA) with MIC values of 15.8 and 27.1 mu M. The results showed that configuration of C-21 in 3 and 4 is important for anti-SA and anti-MRSA activities. This study reveals the significant potential of the genus Chaetomium in producing new PKS-NRPS, therefore increasing the speed in the mining for new sources of antimicrobial agents.	BIOSIS/2020:207820
Discovery of New Extremophilic Enzymes from Diverse Fungal Communities.	Berde, Chanda Parulekar [Reprint Author] Berde, Vikrant Balkrishna Sheela, G. Mohana Veerabramhachari, Pallaval	Gogate Jogalekar Coll, Dept Microbiol, Ratnagiri, Maharashtra, India	Yadav, AN [Editor]; Mishra, S [Editor]; Singh, S [Editor]; Gupta, A [Editor]. (2019) pp. 505-535. Recent Advancement in White Biotechnology Through Fungi, Vol 1: Diversity and Enzymes Perspectives. Publisher: SPRINGER INTERNATIONAL PUBLISHING AG, GEWERBESTRASSE 11, CHAM, CH-6330, SWITZERLAND. Series: Fungal Biology. ISSN: 2198-7777. ISBN: 978-3-030-10479-5(H), 978-3-030-10480-1(P).	20190101	other	Extremophiles are microorganisms that are found in environments of extreme temperature (-2-15, 60-110 degrees C), ionic strength (2-5 M NaCl), or pH (9). Extremophiles are a source of enzymes (extremozymes) with extreme stability, and the application of these enzymes as biocatalysts has attracted attention because they are stable and active under conditions at which the normal enzymes do not work. These microorganisms are capable of surviving under extreme conditions in non-conventional environments, and their enzymes are adapted to these conditions. The properties of their enzymes have been optimized for these conditions. Extremophiles, particularly those from the Archaea, have novel metabolic pathways; hence, they serve as a source of enzymes with novel activities and applications. Among eukaryotes, fungi are the most versatile and ecologically successful phylogenetic lineage. With the exception of hyperthermophily, they adapt well to extreme environments. Fungi are found to live in acidic and metal-enriched waters from mining regions, alkaline conditions, hot and cold deserts, and the deep ocean and in hypersaline regions such as the Dead Sea. Extremophilic enzymes from fungi have been sought for because of the increasing industrial demands for biocatalysts that can cope with industrial process needs. Extremozymes have a great economic potential in many industrial processes, including agricultural, chemical, and pharmaceutical applications.	BIOSIS/2019:831187
Production of D-glucuronic acid from myo-inositol using Escherichia coli whole-cell biocatalyst overexpressing a novel myo-inositol oxygenase from Thermothelomyces thermophile .	Teng, Fei [Reprint Author] You, Ran Hu, Meirong Liu, Weifeng Wang, Lei [Reprint Author] Tao, Yong [Reprint Author]	Chinese Acad Sci, Inst Microbiol, CAS Key Lab Microbial Physiol and Metab Engn, Beijing, Peoples R China wl8893@163.com; taoyong@im.ac.cn	Enzyme and Microbial Technology, (AUG 2019) Vol. 127, pp. 70-74. http://www.journals.elsevier.com/enzyme-and-microbial-? technology/#description. CODEN: EMTED2. ISSN: 0141-0229. E-ISSN: 1879-0909.	20190101	other	D-glucuronic acid (GlcUA) is an important intermediate with numerous applications in the food, cosmetics, and pharmaceutical industries. Its biological production routes which employ myo-inositol oxygenase (MIOX) as the key enzyme are attractive. In this study, five diverse MIOX-encoding genes, from Cryptococcus neoformans, Chaetomium thermophilum, Arabidopsis thaliana, Thermothelomyces thermophila , and Mus musculus were over-expressed in Escherichia coli, respectively. A novel MIOX from Thermothelomyces thermophila (TtMIOX) exhibited high specific activity, and efficiently converted myo-inositol to GlcUA. Meanwhile, the degradation of GlcUA was inhibited by inactivation of uxaC from the Escherichia coil genome. Finally, the BW Delta uxaC whole-cell bio-catalyst harboring TtMIOX resulted in the production of 106 g/L GlcUA within 12h in a 1-L bioreactor, corresponding to a conversion of 91% and productivity of 8.83 g/L/h. This study provides a feasible method for the industrial production of GlcUA.	BIOSIS/2019:507133
Fungal Genomics: Methods and Protocols, Second Edition.	DeVries, RP [Editor] Tsang, A [Editor] Grigoriev, IV [Editor]		DeVries, RP [Editor]; Tsang, A [Editor]; Grigoriev, IV [Editor]. Methods in Molecular Biology, (2018) Fungal Genomics: Methods and Protocols, Second Edition. Publisher: HUMANA PRESS INC, 999 RIVERVIEW DR, STE 208, TOTOWA, NJ 07512-1165 USA. Series: Methods in Molecular Biology. ISSN: 1064-3745. ISBN: 978-1-4939-7803-8(H), 978-1-4939-7804-5(P).	20180101	other	(b) (4) .	BIOSIS/2018:863116
Transcriptional analysis of Myceliophthora thermophila on soluble starch and role of regulator AmyR on polysaccharide degradation.	Xu, Guanbao Li, Jingen Liu, Qian Sun, Wenliang Jiang, Min Tian, Chaoguang [Reprint Author]	Chinese Acad Sci, Tianjin Inst Ind Biotechnol, Key Lab Syst Microbial Biotechnol, Tianjin 300308, Peoples R China tian_cg@tib.cas.cn	Bioresource Technology, (OCT 2018) Vol. 265, pp. 558-562. http://www.journals.elsevier.com/bioresource-technology/#description. CODEN: BIRTEB. ISSN: 0960-8524. E-ISSN: 1873-2976.	20180101	other	Thermophilic fungus Myceliophthora thermophila has great capacity for biomass degradation and is an attractive option for use as cell factory to produce chemicals directly from renewable polysaccharides, such as starch, rather than monomer glucose. To date, there has been no transcriptomic analysis of this thermophilic fungus on starch. This study determined the transcriptomic profile of M. thermophila responding to soluble starch and a 342-gene set was identified as a +quot;starch regulon+quot;, including the major amylolytic enzyme (Mycth_72393). Its overexpression led to increased amylase activities on starch by 35%. Furthermore, overexpressing the key amylolytic enzyme regulator AmyR in M. thermophila significantly increased amylase activity by 30%. Deletion of amyR by the CRISPR/Cas9 system led to the relief of carbon catabolite repression and 3-fold increased lignocellulase activities on cellulose. This study will accelerate rational fungal strain engineering for biochemical production from biomass substrates such as raw corn starch and even crop straw.	BIOSIS/2018:724615

Cryo-EM structure of human ATR-ATRIP complex.	Rao, Qinhui [Reprint Author] Liu, Mengjie Tian, Yuan Wu, Zihan Hao, Yuhan Song, Lei Qin, Zhaoyu Ding, Chen Wang, Hong-Wei [Reprint Author] Wang, Jiawei [Reprint Author] Xu, Yanhui [Reprint Author]	Fudan Univ, Shanghai Canc Ctr, Inst Biomed Sci, Shanghai Med Coll, Shanghai 200032, Peoples R China hongweiwang@tsinghua.edu.cn; jwwang@tsinghua.edu.cn; xuyh@fudan.edu.cn	Cell Research, (FEB 2018) Vol. 28, No. 2, pp. 143-156. http://www.nature.com/cmi/index.html. ISSN: 1001-0602. E-ISSN: 1748-7838.	20180101	other	ATR (ataxia telangiectasia-mutated and Rad3-related) protein kinase and ATRIP (ATR-interacting protein) form a complex and play a critical role in response to replication stress and DNA damage. Here, we determined the cryo-electron microscopy (EM) structure of the human ATR-ATRIP complex at 4.7 angstrom resolution and built an atomic model of the C-terminal catalytic core of ATR (residues 1 521-2 644) at 3.9 angstrom resolution. The complex adopts a hollow +quot;heart+quot; shape, consisting of two ATR monomers in distinct conformations. The EM map for ATRIP reveals 14 HEAT repeats in an extended +quot;S+quot; shape. The conformational flexibility of ATR allows ATRIP to properly lock the N-termini of the two ATR monomers to favor ATR-ATRIP complex formation and functional diversity. The isolated +quot;head-head+quot; and +quot;tail-tail+quot; each adopts a pseudo 2-fold symmetry. The catalytic pockets face outward and substrate access is not restricted by inhibitory elements. Our studies provide a structural basis for understanding the assembly of the ATR-ATRIP complex and a framework for characterizing ATR-mediated DNA repair pathways.	BIOSIS/2018:241376
Inhibition of enzymatic hydrolysis of pretreated corn stover and sugar cane straw by laccases.	Rocha-Martin, Javier [Reprint Author] Martinez-Bernal, Claudio Zamorano, Laura S. Manuel Reyes-Sosa, Francisco Diez Garcia, Bruno [Reprint Author]	Abengoa Res, Dept Biotechnol, Campus Palmas Altas,C Energia Solar 1, Seville 41014, Spain javier.rocha@csic.es; bdiez321@yahoo.es	Process Biochemistry, (APR 2018) Vol. 67, pp. 88-91. http://www.journals.elsevier.com/process-biochemistry/#description. ISSN: 1359-5113. E-ISSN: 1873-3298.	20180101	other	Laccase detoxification has been proposed to enhance the enzymatic hydrolysis of lignocellulosics by reducing the effect of inhibitors formed during the pretreatment step. Herein, we investigated the effect of the laccase treatment on the enzymatic hydrolysis of two lignocellulosic materials pretreated by diluted acid/steam explosion through two strategies. Simultaneous laccase treatment and enzymatic hydrolysis of pretreated corn stover (PCS) and sugar cane straw (PSCS) led to a significant reduction of the concentration of glucose released from 67.7 down to 39.2 g/Kg and from 69 down to 53.5 g/Kg. Laccase treatment prior to hydrolysis of both substrates showed the same inhibitory effect. After hydrolysis of PCS and PSCS, the glucose concentration decreased by approximately 44 and 21% compared to the control assays, respectively. Therefore, these results do not support the use of laccases to detoxify pretreated lignocellulosic materials for improving the bioethanol production.	BIOSIS/2018:500880
Side-by-side biochemical comparison of two lytic polysaccharide monooxygenases from the white-rot fungus Heterobasidion irregulare on their activity against crystalline cellulose and glucomannan.	Liu, Bing Krishnaswamyreddy, Sumitha Muraleedharan, Madhu Nair Olson, Ake Broberg, Anders Stahlberg, Jerry Sandgren, Mats [Reprint Author]	Swedish Univ Agr Sci, Dept Mol Sci, Uppsala, Sweden mats.sandgren@slu.se	PLoS One, (SEP 5 2018) Vol. 13, No. 9, pp. Article No.: e0203430. http://journals.plos.org/plosone/. ISSN: 1932-6203. E-ISSN: 1932-6203.	20180101	other	Our comparative studies reveal that the two lytic polysaccharide monooxygenases HiLP-MO9B and HiLPMO9I from the white-rot conifer pathogen Heterobasidion irregulare display clear difference with respect to their activity against crystalline cellulose and glucomannan. HiLPMO9I produced very little soluble sugar on bacterial microcrystalline cellulose (BMCC). In contrast, HiLPMO9B was much more active against BMCC and even released more soluble sugar than the H. irregulare cellobiohydrolase I, HiCel7A. Furthermore, HiLPMO9B was shown to cooperate with and stimulate the activity of HiCel7A, both when the BMCC was first pretreated with HiLPMO9B, as well as when HiLPMO9B and HiCel7A were added together. No such stimulation was shown by HiLPMO9I. On the other hand, HiLPMO9I was shown to degrade glucomannan, using a C4-oxidizing mechanism, whereas no oxidative cleavage activity of glucomannan was detected for HiLPMO9B. Structural modeling and comparison with other glucomannan-active LPMOs suggest that conserved sugar-interacting residues on the L2, L3 and LC loops may be essential for glucomannan binding, where 4 out of 7 residues are shared by HiLPMO9I, but only one is found in HiLPMO9B. The difference shown between these two H. irregulare LPMOs may reflect distinct biological roles of these enzymes within deconstruction of different plant cell wall polysaccharides during fungal colonization of softwood.	BIOSIS/2018:876617
Distinct Substrate Specificities and Electron-Donating Systems of Fungal Lytic Polysaccharide Monooxygenases.	Frommhagen, Matthias Westphal, Adrie H. van Berkel, Willem J. H. [Reprint Author] Kabel, Mirjam A.	Wageningen Univ and Res, Biochem Lab, Wageningen, Netherlands willem.vanberkel@wur.nl	Frontiers in Microbiology, (MAY 29 2018) Vol. 9, pp. Article No.: 1080. http://www.frontiersin.org/microbiology/. ISSN: 1664-302X. E-ISSN: 1664-302X.	20180101	other	Lytic polysaccharide monooxygenases (LPMOs) are powerful enzymes that oxidatively cleave glycosidic bonds in polysaccharides. The ability of these copper enzymes to boost the degradation of lignocellulose has greatly stimulated research efforts and biocatalytic applications within the biorefinery field. Initially found as oxidizing recalcitrant substrates, such as chitin and cellulose, it is now clear that LPMOs cleave a broad range of oligo- and poly-saccharides and make use of various electron-donating systems. Herein, substrate specificities and electron-donating systems of fungal LPMOs are summarized. A closer look at LPMOs as part of the fungal enzyme machinery might provide insights into their role in fungal growth and plant-pathogen interactions to further stimulate the search for novel LPMO applications.	BIOSIS/2018:564617
Nuclear Pore Complex: From Structural View to Chemical Tools.	Wong, Richard W. [Reprint Author]	Kanazawa Univ, Inst Frontier Sci Initiat, Innovat Integrated Biores Core, Cell Bion Res Unit, Kakuma Machi, Kanazawa, Ishikawa 9201192, Japan rwong@staff.kanazawa-u.ac.jp	Chemistry + Biology (Cambridge), (OCT 22 2015) Vol. 22, No. 10, pp. 1285-1287. http://www.cell.com/chemistry-biology/home. ISSN: 1074-5521. E-ISSN: 1879-1301.	20150101	other	Nuclear pore complexes (NPCs) are the macromolecular turnstiles between the cytoplasm and the nucleus that control the trafficking of proteins, RNAs and viruses. The giant NPC structures are extremely complex. Here, I highlight several recent findings on NPC architectures, and briefly discuss how chemical biologists might use this information to design synthetic devices and improve strategies for nuclear drug delivery.	BIOSIS/2016:104223
A new thermophilic species of Myceliophthora from China.	Zhang, Yu Wu, Wen-Ping Hu, Dian-Ming Su, Yuan-Ying Cai, Lei [Reprint Author]	Chinese Acad Sci, Inst Microbiol, State Key Lab Mycol, Beijing 100101, Peoples R China mrcailei@gmail.com	Mycological Progress, (FEB 2014) Vol. 13, No. 1, pp. 165-170. http://www.springerlink.com/content/120015/. ISSN: 1617-416X. E-ISSN: 1861-8952.	20140101	other	(b) (4)	BIOSIS/2014:235444

Transcriptome and exoproteome analysis of utilization of plant-derived biomass by Myceliophthora themiophila.	Kolbusz, Magdalena Anna [Reprint Author] Di Falco, Marcos Ishmael, Nadeeza Marqueteau, Sandrine Moisan, Marie-Claude Baptista, Cassio da Silva Powlowski, Justin Tsang, Adrian	Concordia Univ, Ctr Struct and Funct Genom, L GE-120-11,7141 Sherbrooke St West, Montreal, PQ H4B 1R6, Canada magdalena.kolbusz@concordia.ca; marcos.difalco@concordia.ca; nadeeza.ishmael@concordia.ca; sandrine.marqueteau@concordia.ca; marie-claude.moisan@concordia.ca; Cassio.Baptista@nrc-cnrc.gc.ca; justin.powlowski@concordia.ca; adrian.tsang@concordia.ca	Fungal Genetics and Biology, (NOV 2014) Vol. 72, No. Sp. Iss. SI, pp. 10-20. http://www.journals.elsevier.com/fungal-genetics-and-? biology/#description. ISSN: 1087-1845. E-ISSN: 1096-0937.	20140101	other	Myceliophthora thermophila is a thermophilic fungus whose genome encodes a wide range of carbohydrate-active enzymes (CAZymes) involved in plant biomass degradation. Such enzymes have potential applications in turning different kinds of lignocellulosic feedstock into sugar precursors for biofuels and chemicals. The present study examined and compared the transcriptomes and exoproteomes of M. thermophila during cultivation on different types of complex biomass to gain insight into how its secreted enzymatic machinery varies with different sources of lignocellulose. In the transcriptome analysis three monocot (barley, oat, triticale) and three dicot (alfalfa, canola, flax) plants were used whereas in the proteome analysis additional substrates, i.e. wood and corn stover pulps, were included. A core set of 59 genes encoding CAZymes was up-regulated in response to both monocot and dicot straws, including nine polysaccharide monooxygenases and GH10, but not GH11, xylanases. Genes encoding additional xylanolytic enzymes were up-regulated during growth on monocot straws, while genes encoding additional pectinolytic enzymes were up-regulated in response to dicot biomass. Exoproteome analysis was generally consistent with the conclusions drawn from transcriptome analysis, but additional CAZymes that accumulated to high levels were identified. Despite the wide variety of biomass sources tested some CAZy family members were not expressed under any condition. The results of this study provide a comprehensive view from both transcriptome and exoproteome levels, of how M. thermophila responds to a wide range of biomass sources using its genomic resources. (b) (4)	BIOSIS/2015:19863
The structure of a novel glucuronoyl esterase from Myceliophthora thermophila gives new insights into its role as a potential biocatalyst.	Charavgi, Maria-Despoina Dimarogona, Maria Topakas, Evangelos Christakopoulos, Paul Chrysina, Evangelia D. [Reprint Author]	Natl Hellen Res Fdn, Inst Biol Med Chem and Biotechnol, 48 Vassileos Constantinou Ave, Athens 11635, Greece echrysina@eie.gr	Acta Crystallographica Section D Biological Crystallography, (JAN 2013) Vol. 69, No. Part 1, pp. 63-73. http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1399-0047. ISSN: 0907-4449. E-ISSN: 1399-0047.	20130101	other	The increasing demand for the development of efficient biocatalysts is a consequence of their broad industrial applications. Typical difficulties that are encountered during their exploitation in a variety of processes are interconnected with factors such as temperature, pH, product inhibitors etc. To eliminate these, research has been directed towards the identification of new enzymes that would comply with the required standards. To this end, the recently discovered glucuronoyl esterases (GEs) are an enigmatic family within the carbohydrate esterase (CE) family. Structures of the thermophilic StGE2 esterase from Myceliophthora thermophila (synonym Sporotrichum thermophile), a member of the CE15 family, and its S213A mutant were determined at 1.55 and 1.9 angstrom resolution, respectively. The first crystal structure of the S213A mutant in complex with a substrate analogue, methyl 4-O-methyl-beta-D-glucopyranuronate, was determined at 2.35 angstrom resolution. All of the three-dimensional protein structures have an alpha/beta-hydrolase fold with a three-layer alpha beta alpha-sandwich architecture and a Rossmann topology and comprise one molecule per asymmetric unit. These are the first crystal structures of a thermophilic GE both in an unliganded form and bound to a substrate analogue, thus unravelling the organization of the catalytic triad residues and their neighbours lining the active site. The knowledge derived offers novel insights into the key structural elements that drive the hydrolysis of glucuronic acid esters.	BIOSIS/2013:161279
Stabilization of Candida antarctica lipase B in hydrophilic organic solvent by rational design of hydrogen bond.	Park, Hyun June [Reprint Author] Joo, Jeong Chan Park, Kyungmoon Yoo, Young Je	Seoul Natl Univ, Grad Program Bioengn, Seoul 151742, South Korea yjyoo@snu.ac.kr	Biotechnology and Bioprocess Engineering, (AUG 2012) Vol. 17, No. 4, pp. 722-728. ISSN: 1226-8372. E-ISSN: 1976-3816.	20120101	other	Enzymatic reactions conducted in organic solvents have many advantages. However, organic solvent molecules may replace water molecules at the protein surface and penetrate into the enzyme, which could lead to the denaturation of the enzyme or changes in its reaction kinetics and substrate specificity. Thus, it is important to enhance the stability of enzymes in organic solvents. To date, there has been no efficient rational approach developed to enhance enzyme stability in hydrophilic solvents. We developed a rational approach to enzyme design. The design rules were established by investigating stable mutants from previous studies of directed evolution. Candida antarctica lipase B (CalB) was used as a target enzyme due to its versatile applications in organic solvents. The N97Q, N264Q, and D265E mutants of CalB showed higher organic solvent stability than the wild type.	BIOSIS/2012:634830
Enzymatic surface functionalisation of lignocellulosic materials with tannins for enhancing antibacterial properties.	Widsten, Petri [Reprint Author] Heathcote, Carol Kandelbauer, Andreas Guebitz, Georg Nyanhongo, Gibson S. Prasetyo, Endry Nugroho Kudanga, Tukayi	Scion, Te Papa Tipu Innovat Pk,49 Sala St,Private Bag 30, Rotorua, New Zealand petri.widsten@scionresearch.com	Process Biochemistry, (JUL 2010) Vol. 45, No. 7, pp. 1072-1081. http://www.journals.elsevier.com/process-biochemistry/#description. ISSN: 1359-5113. E-ISSN: 1873-3298.	20100101	other	Grafting natural antibacterial phenols onto lignocellulosic materials is an environmentally friendly way of imparting antibacterial properties to the substrates. In the present investigation, wood veneer and pulp were treated with tannins in the presence or absence of laccase. Treatments with hydrolysable tannins significantly improved the antibacterial resistance of veneers and paper made from tannin-treated pulp against a Gram-positive bacterium (Staphylococcus aureus) while a more modest protective effect was observed against a Gram-negative bacterium (Escherichia coli). Condensed tannin improved the antibacterial resistance against S. aureus, albeit less than hydrolysable tannin, but had little effect on E. coli. A cationic condensed tannin derivative bearing a quaternary amino group provided far better resistance to pulp against S. aureus and E. coli than the corresponding unmodified condensed tannin. These findings agree with the minimal inhibitory concentrations (MICs) of the tannins and their reactivities toward laccase as determined by O-2 consumption measurements. Due to a better retention of tannins via covalent bonding, treatments with laccase usually resulted in greater antibacterial effects than those without laccase. LC-MS investigations with monomeric tannin and lignin model compounds showed that covalent bonding of tannin to lignin via radical coupling occurred in the presence of laccase. (C) 2010 Elsevier Ltd. All rights reserved.	BIOSIS/2010:433995

Thermotolerant and thermostable laccases.	Hilden, Kristiina [Reprint Author] Hakala, Terhi K. Lundell, Taina	Univ Nottingham, Sch Biol, Univ Pk, Nottingham NG7 2RD, UK kristiina.s.hilden@helsinki.fi	Biotechnology Letters, (AUG 2009) Vol. 31, No. 8, pp. 1117-1128. http://www.springerlink.com/content/100138/. CODEN: BILED3. ISSN: 0141-5492. E-ISSN: 1573-6776.	20090101	other	Laccases are phenol-oxidizing, usually four-copper containing metalloenzymes. For industrial and biotechnological purposes, laccases were among the first fungal oxidoreductases providing larger-scale applications such as removal of polyphenols in wine and beverages, conversion of toxic compounds and textile dyes in waste waters, and in bleaching and removal of lignin from wood and non-wood fibres. In order to facilitate novel and more efficient bio-catalytic process applications, there is a need for laccases with improved biochemical properties, such as thermostability and thermotolerance. This review gives a current overview on the sources and characteristics of such laccases, with particular emphasis on the fungal enzymes.	BIOSIS/2009:470478
In vitro evolution of a fungal laccase in high concentrations of organic cosolvents.	Zumarraga, Miren Bulter, Thomas Shleev, Sergey Polaina, Julio Martinez-Arias, Arturo Plow, Francisco J. Ballesteros, Antonio Alcalde, Miguel [Reprint Author]	CSIC, Inst Catalysis, Dept Biocatalysis, Plaza Murillo 2, E-28049 Madrid, Spain malcalde@icp.csic.es	Chemistry + Biology (Cambridge), (SEP 2007) Vol. 14, No. 9, pp. 1052-1064. http://www.cell.com/chemistry-biology/home. ISSN: 1074-5521. E-ISSN: 1879-1301.	20070101	other	Fungal laccases are remarkable green catalysts that have a broad substrate specificity and many potential applications in bioremediation, lignocellulose processing, organic synthesis, and more. However, most of these transformations must be carried out at high concentrations of organic cosolvents in which laccases undergo unfolding, thereby losing their activity. We have tailored a thermostable laccase that tolerates high concentrations of cosolvents, the genetic product of five rounds of directed evolution expressed in Saccharomyces cerevisiae. This evolved laccase-R2 variant-was capable of resisting a wide array of cosolvents at concentrations as high as 50% (v/v). Intrinsic laccase features such as the redox potential and the geometry of catalytic coppers varied slightly during the course of the molecular evolution. Some mutations at the protein surface stabilized the laccase by allowing additional electrostatic and hydrogen bonding to occur.	BIOSIS/2008:22608
Preserving boiled eggs with a sterilization system employing microbial laccase and wood vinegar.	Sakaguchi, Hiromichi Uyama, Naoto Uyama, Hiroshi [Reprint Author]	Novozymes Japan, Applicat Technol R and D, Chiba, Japan hiro@novozymes.com	Animal Science Journal, (DEC 2007) Vol. 78, No. 6, pp. 668-671. ISSN: 1344-3941. E-ISSN: 1740-0929.	20070101	other	A combination of a microbial laccase and wood vinegar was used to treat the shell surface of boiled eggs. The laccase enhanced the sterilization effect of phenolic compounds contained in the wood vinegar and resulted in prevention of microbial infection of the boiled eggs after storage for 21 days at 40 degrees C and 75% relative humidity. Significant phenoxy radicals, formed by the catalytic reaction of the laccase, could be detected in the shell of the boiled eggs.	BIOSIS/2008:94519
Taxonomic studies on thermophilic fungi Deuteromycetes.	Wang Dong-mei [Reprint Author] Wang Feng-xiang Gao Hong	Laiwu Vocat Technol Inst, Laiwu 271100, Peoples R China mwdmj75@yahoo.com.cn	Journal of Fungal Research, (MAR 2007) Vol. 5, No. 1, pp. 10-13. ISSN: 1672-3538.	20070101	other	(b) (4)	BIOSIS/2007:484458
Amperometric biosensors based on recombinant laccases for phenols determination.	Kulys, Juozas [Reprint Author] Vidziunaite, Regina	Institute of Biochemistry, Mokslininku 12, 2600, Vilnius, Lithuania jkulys@bchi.lt	Biosensors + Bioelectronics, (March 2003) Vol. 18, No. 2-3, pp. 319-325. print. CODEN: BBIOE4. ISSN: 0956-5663.	20030101	other	Polyporus pinsitus (rPpL), and Myceliophthora thermophila (rMtL) were developed. The enzymes were immobilized using bovine serum albumin and glutaraldehyde. At pH 5.5 and -0.1 V, the calibration graphs of GE based biosensors were hyperbolic if pyrocatechol was used. The concentration of substrate that results in 50% of steady-state response (EC50) was 0.7 mM and sensitivity (S) was 3.8 mA/M. The sensitivity increased up to 4 A/M if larger amount of rPpL was used. The sensitivity of biosensors changed little during 9 days of exploitation, but decreased at longer time. The PGE based biosensors were mounted into the flow-through cell and calibrated under kinetic regime. EC50 of the biosensors containing rPpL varied from 0.6 to 4.0 mM and sensitivity varied from 0.11 to 1.9 mA/M. The response of biosensor containing thermostable laccase rMtL was less, but response saturated at larger pyrocatechol concentration. The sensitivity changed little during 6 days. Both type of biosensors responded also to 1-naphthol, o-phenylenediamine, guaiacol, o-anizidine, benzidine. The experiments demonstrate recombinant laccases application to biosensor engineering and their use to phenol and related compound determination under steady-state and flow-through regimes.	BIOSIS/2003:177611
Highly Efficient Degradation of Xylan into Xylose by a Single Enzyme	Basit, Abdul Miao, Ting Liu, Junquan Wen, Jiaqi Song, Lina Zheng, Fengzhen Lou, Huiqiang Jiang, Wei	State Key Laboratory of Agro-Biotechnology, College of Biological Sciences, China Agricultural University, Beijing, 100193, Peop. Rep. China	ACS Sustainable Chemistry + Engineering (2019), 7(13), 11360-11368 CODEN: ASCECG; ISSN: 2168-0485	20190101	other	Here, we report highly efficient degrdn. of xylan into xylose by a single multifunctional xylanolytic enzyme from the filamentous fungus Thermothelomyces thermophila (termed Ttxy43). Ttxy43 shows three different enzyme activities toward carbohydrates, .beta.-xylosidase (80.8 U/mg), endoxylanase (105.42 U/mg), and .alpha.-L-arabinofuranosidase enzyme activities (15.81 U/mg). Anal. of the catalytic mode of action of Ttxy43 for birchwood-xylan (BWX) reveals that endoxylanase initially degrades xylan to unbranched xylooligosaccharides (XOSs) (xylobiose, xylotriose, xylotetraose) as intermediates, which are then quickly hydrolyzed into single xylose by .beta.-xylosidase. Site-directed mutagenesis studies indicate that Ttxy43 residues Asp134 and Glu228 are essential catalytic sites, while Glu176, Asp38, and Asp85 play an accessory role. More importantly, Ttxy43 displays higher degrdn. efficiency in comparison with a com. .beta.-xylosidase and endoxylanase 'cocktail'. These findings elucidate an efficient integrated degrdn. mechanism of xylan under industrial reaction conditions, which provides a novel strategy to design techniques for biomass energy prodn.	CAPLUS/2019:1108588

Exploring the biocatalytic potential of a self-sufficient cytochrome P450 from Thermothelomyces thermophila	Fuerst, Maximilian J. L. J. Kerschbaumer, Bianca Rinnofner, Claudia Migglautsch, Anna K. Winkler, Margit Fraaije, Marco W.	Molecular Enzymology Group, University of Groningen, Groningen, 9747 AG, Neth.	Advanced Synthesis + Catalysis (2019), 361(11), 2487-2496 CODEN: ASCAF7; ISSN: 1615-4150	20190101	other	Among nature's arsenal of oxidative enzymes, cytochrome P450s (CYPs) catalyze the most challenging reactions, the hydroxylations of non-activated C-H bonds. Human CYPs are studied in drug development due to their physiol. role at the forefront of metabolic detoxification , but their challenging handling makes them unsuitable for application. CYPs have a great potential for biocatalysis, but often lack appropriate features such as high and sol. expression, self-sufficient internal electron transport, high stability, and an engineerable substrate scope. We have probed these characteristics for a recently described CYP that originates from the thermophilic fungus Thermothelomyces thermophila (CYP505A30), a homolog of the well-known P 450-BM3 from Bacillus megaterium. CYP505A30 is a natural monooxygenase-reductase fusion, is well expressed, and moderately tolerant towards temp. and solvent exposure. Although overall comparable, we found the stability of the enzyme's domains to be inverse to P 450-BM3, with a more stable reductase compared to the heme domain. After anal. of a homol. model, we created mutants of the enzyme based on literature data for P 450-BM3. We then probed the enzyme variants in bioconversions using a panel of active pharmaceutical ingredients, and activities were detected for a no. of structurally diverse compds. Ibuprofen was biooxidized in a preparative scale whole cell bioconversion to 1-, 2- and 3-hydroxyibuprofen.	CAPLUS/2019:515339
Electrochemical degradation of the recalcitrant compound 4-Nitrophenol, using lacasa enzyme	Lozano-Camargo, Maria Luisa Galicia, Laura Rodriguez, Juan Manuel Granados Guzman, Enrique Caneda	Depto. Ingenieria Ambiental, Tecnologico de Estudios Superiores del Oriente del Estado de Mexico, Los Reyes la Paz, 56400, Mex.	ECS Transactions (2018), 84(1, XXXII National Congress of the Mexican Society of Electrochemistry/10th Meeting of the ECS Mexican Section, 2017), 29-34 CODEN: ECSTF8; ISSN: 1938-5862	20180101	other	(b) (4)	CAPLUS/2019:228014
Bioethanol production from Naoh + H2O2 pretreated Populus deltoides wood using cocktail of inhouse and commercial enzymes under four different modes of separate hydrolysis and fermentation	Sharma, Nisha Sharma, Nivedita	PhD Scholar in Microbiology Laboratory Department of Basis Sciences Dr. Y S Parmar, University of Horticulture and Forestry, Solan, Himachal Pradesh, 173230, India	World Journal of Pharmaceutical Research (2016), 5(2), 661-676 CODEN: WJPRAP; ISSN: 2277-7105 URL: http://www.wjpr.net/download/article/1454147357.pdf	20160101	other	In the present study the bioconversion of alk. hydrogen peroxide pretreated P. deltoides wood by using cocktail of inhouse enzymes from Bacillus stratosphericus N12 (M), Myceliophthora thermophila SH1 and Bacillus altitudinis Kd1 (M) under submerged as well as solid state fermn. was done which was compared with com. enzymes. Comparative study of bioethanol prodn. from P. deltoides wood was carried out using different methods of non-detoxification /detoxification , enzymic hydrolysis and fermn. In method I and II supernatant of alk. hydrogen peroxide pretreated P. deltoides wood was pooled together with enzyme saccharified residue fraction, while in method III and IV supernatant after pretreatment was discarded and only enzymic hydrolyzate contg. simple sugars was used for the fermn. Different ethanologenic yeasts and bacteria were employed under monoculture and coculture combinations for fermn. The highest ethanol yield of 18.47 g/l was obtained in method IV as compared to others and 21.41g/l in case of com. enzymes. The co-culture of Saccharomyces cerevisiae II + Pichia stipitis was found best combination of ethanologens in both cases i.e. in inhouse enzyme saccharified hydrolyzate as well as by using com. enzymes.	CAPLUS/2016:2071116
Evolved alkaline fungal laccase secreted by Saccharomyces cerevisiae as useful tool for the synthesis of C-N heteropolymeric dye	Vicente, Ana I. Vina-Gonzalez, Javier Santos-Moriano, Paloma Marquez-Alvarez, Carlos Ballesteros, Antonio O. Alcalde, Miguel	Department of Biocatalysis, Institute of Catalysis, CSIC, Madrid, 28049, Spain	Journal of Molecular Catalysis B: Enzymatic (2016), 134(Part B), 323-330 CODEN: JMCEF8; ISSN: 1381-1177	20160101	other	Enzymic prodn. of C-N heteropolymeric dyes at alk. pHs is an attractive process for the textile industry. In this work, we have designed a fungal laccase by directed evolution so that it may be used at alk. pHs for the synthesis of C-N heteropolymeric dyes (C-N polydye) from catechol and 2,5-diaminobenzenesulfonic acid (2,5-DABSA). Firstly, several medium- and high-redox potential fungal laccases from previous lab. evolution campaigns were benchmarked for the synthesis of the C-N polydye at pH 8.0, choosing an alk. laccase mutant from Myceliophthora thermophila as the departure point for further engineering. Mutant libraries were then constructed, expressed in Saccharomyces cerevisiae and screened using a high-throughput colorimetric assay for the detection of the C-N polydye. By combining directed and focused mol. evolution, a novel, strongly expressed alk. laccase variant was identified. This laccase was secreted at 37 mg/L and its catalytic efficiency for the oxidn. of catechol and 2,5-DABSA at pH 8.0 was enhanced 3.5-fold relative to that of the wild-type, promoting the synthesis of the C-N polydye at basic pHs. While the improved expression was mostly the result of accumulating mutations that favor the yeast's codon usage together with the recovery of a secretion mutation , the enhanced C-N polydye synthetic activity of the mutant laccase was dependent on the alk. mutations it inherited. Readily secreted, this laccase mutant would appear to be a valuable platform for org. synthesis at basic pHs.	CAPLUS/2016:1689105
Fungal dry retting - an ecofriendly and water saving technology for retting of jute	Banik, Shyamal	National Institute of Research on Jute and Allied Fibre Technology, Kolkata, 700 040, India	Indian Journal of Fibre + Textile Research (2016), 41(2), 212-216 CODEN: IJFRET; ISSN: 0971-0426	20160101	other	Ecofriendly and water saving retting technol. of jute has been developed, using pectinolytic fungi by dry fermn. procedure to overcome the shortcomings of conventional retting of jute. Four pectinolytic fungi have been used for these fungal dry retting of jute, viz. Aspergillus tamarii, A. flavus, A. niger and Sporotrichum thermophile . This fungal dry retting is found to be an aerobic process unlike conventional water retting, and hence is environment pollution free, faster, water saving and able to produce good quality jute fiber with strong and unbroken full length jute stick as desired by the jute farmers. In field trial, the av. fiber strength is 27.7 g tex-1, fiber fineness 2.8 tex and fiber grade between TD-4 and TD-5. These fungi have no adverse effect on the succeeding crop rice. Eight pound regular yarn is prepd. from these fibers having normal textile properties. It is expected that this new concept of waterless retting technique by pectinolytic fungi may solve the present water crisis for retting of jute and other allied bast fibers.	CAPLUS/2016:1909245

Cost effective production of cellulase and xylanase enzymes by Myceliophthora thermophila SH1 using lignocellulosic forest waste and bioconversion of alkaline hydrogen peroxide pretreated P. deltoides wood to bioethanol under SHF	Sharma, Nisha Sharma, Nivedita	Microbiology Research Laboratory, Department of Basic Science, Dr Y S Parmar University of Horticulture and Forestry, Solan, 173230, India	Journal of Agroalimentary Processes and Technologies (2013), 19(4), 419-428, 10 CODEN: JAPTCV; ISSN: 2068-9551 URL: http://www.journal-of-?agroalimentary.ro/admin/articole/19172L67_Vol_19(4)_2013_419-428.pdf	20130101	other	We studied the bioconversion of untreated/ pretreated lignocellulosic biomass by fungus Myceliophthora thermophila SH1 under solid state fermn. Pretreated lignocellulosic biomass has emerged as a suitable substrate for cellulase and xylanase prodn. by M. thermophila SH1 reaching upto 35.32 U/g cellulase and 203.20U/g of xylanase in NaOH+H2O2 pretreated Populus deltoides wood. Comparative study of bioethanol prodn. from P. deltoides wood was carried out using different methods of non-detoxification / detoxification , enzymic hydrolysis and fermn. In method I and II supernatant of alk. hydrogen peroxide pretreated P. deltoides wood was pooled together with enzyme saccharified residue fraction, while in method III and IV supernatant after pretreatment was discarded and only enzymic hydrolyzate contg. simple sugars was used for the fermn. Different ethanologenic yeasts and bacteria were employed under monoculture and co-culture combinations for fermn. The highest ethanol yield of 13.40 g/l was obtained in method IV by monoculture of S. cerevisiae II.	CAPLUS/2015:333791
Production of .beta.-galactosidases from Sporotrichum thermophile	Petrova, S. D. Bakalova, N. G. Ilieva, S. Z. Atev, A. P. Bhat, M. K. Kolev, D. N.	Department of Biochemistry, Faculty of Biology, University of Sofia 'St. Kl. Ohridski', Sofia, Bulg.	Biotechnology + Biotechnological Equipment (2002), 16(2), 127-131 CODEN: BTTEEJ; ISSN: 0205-2067	20020101	other	(b) (4)	CAPLUS/2003:128622
In situ generation of mediator - A new concept for laccase-catalyzed delignification of kraft pulp	Amann, M. Candussio, A. Muller, R. Frey, V. Paice, M. G. Bourbonnais, R. Labonte, S. Sacciadis, G. Renaud, S.	Consortium fuer elektrochemische Industrie GmbH, Munich, D-81379, Germany	TAPPI Pulping/Process and Product Quality Conference, Boston, MA, United States, Nov. 5-8, 2000 (2000), 930-937 Publisher: TAPPI Press, Atlanta, Ga. CODEN: 69BXJI	20000101	other	A very promising laccase mediator system (LMS) had been developed with N-OH-acetanilide (NHA) as the mediator component. However, the high cost due to high mediator dosage and the potential for generating toxic by- products are concerns when implementing such a process in a mill's bleaching sequence. Changing the system from a batch process to a continuous dosing technol. can solve both of these problems. To adapt the proposed new procedure to existing mill equipment, a diacetylated mediator precursor was developed (DiAc; N-acetoxy-N-phenylacetamide), which generates NHA during the laccase stage (L). Mild alk. conditions are optimal for the L-stage with DiAc to assure a quant. NHA release. The process was analyzed in the lab. first, and then transferred to a pilot plant scale. Due to the pH requirements laccase from Myceliophthora thermophila was used. Running a five stage ECF sequence LEpD0EoD1 produced a pulp comparable to a mill ref. pulp (D0EopD1EpD2) with significant saving of bleaching chems. (8,5 kg/ton). Further work is required to develop an enzyme that is optimal for the DiAc mediator reaction conditions.	CAPLUS/2002:62744
Quantifying the heterogeneity of macromolecular machines by mass photometry	Sonn-Segev, Adar Belacic, Katarina Bodrug, Tatyana Young, Gavin VanderLinden, Ryan T. Schulman, Brenda A. Schimpf, Johannes Friedrich, Thorsten Dip, Phat Vinh Schwartz, Thomas U. Bauer, Benedikt Peters, Jan-Michael Struwe, Weston B. Benesch, Justin L. P. Brown, Nicholas G. Haselbach, David Kukura, Philipp	Physical and Theoretical Chemistry Laboratory, Department of Chemistry, University of Oxford, Oxford, OX1 3QZ, UK	Nature Communications (2020), 11(1), 1772 CODEN: NCAOBW; ISSN: 2041-1723	20200101	other	Abstr.: Sample purity is central to in vitro studies of protein function and regulation, and to the efficiency and success of structural studies using techniques such as x-ray crystallog. and cryo-electron microscopy (cryo-EM). Here, we show that mass photometry (MP) can accurately characterize the heterogeneity of a sample using minimal material with high resoln. within a matter of minutes. To benchmark our approach, we use neg. stain electron microscopy (nsEM), a popular method for EM sample screening. We include typical workflows developed for structure detn. that involve multi-step purifn. of a multi-subunit ubiquitin ligase and chem. crosslinking steps. When assessing the integrity and stability of large mol. complexes such as the proteasome, we detect and quantify assemblies invisible to nsEM. Our results illustrate the unique advantages of MP over current methods for rapid sample characterization, prioritization and workflow optimization.	CAPLUS/2020:717233

Myceliophthora thermophila Xyr1 is predominantly involved in xylan degradation and xylose catabolism	dos Santos Gomes, Ana Carolina Falkoski, Daniel Battaglia, Evy Peng, Mao Nicolau de Almeida, Maira Coconi Linares, Nancy Meijnen, Jean-Paul Visser, Jaap de Vries, Ronald P.	Fungal Physiology, Westerdijk Fungal Biodiversity Institute + Fungal Molecular Physiology, Utrecht University, Utrecht, 3584 CT, Neth.	Biotechnology for Biofuels (2019), 12(1), 1-18 CODEN: BBIIFL; ISSN: 1754-6834	20190101	other	Background: Myceliophthora thermophila is a thermophilic ascomycete fungus that is used as a producer of enzyme cocktails used in plant biomass saccharification. Further development of this species as an industrial enzyme factory requires a detailed understanding of its regulatory systems driving the prodn. of plant biomass-degrading enzymes. In this study, we analyzed the function of MtXlr1, an ortholog of the (hemi-)cellulolytic regulator XlnR first identified in another industrially relevant fungus, Aspergillus niger. Results: The Mtxlr1 gene was deleted and the resulting strain was compared to the wild type using growth profiling and transcriptomics. The deletion strain was unable to grow on xylan and D-xylose, but showed only a small growth redn. on L-arabinose, and grew similar to the wild type on Avicel and cellulose. These results were supported by the transcriptome analyses which revealed redn. of genes encoding xylan-degrading enzymes, enzymes of the pentose catabolic pathway and putative pentose transporters. In contrast, no or minimal effects were obsd. for the expression of cellulolytic genes. Conclusions: Myceliophthora thermophila MtXlr1 controls the expression of xylanolytic genes and genes involved in pentose transport and catabolism, but has no significant effects on the prodn. of cellulases. It therefore resembles more the role of its ortholog in Neurospora crassa, rather than the broader role described for this regulator in A. niger and Trichoderma reesei. By revealing the range of genes controlled by MtXlr1, our results provide the basic knowledge for targeted strain improvement by overproducing or constitutively activating this regulator, to further improve the biotechnol. value of M. thermophila.	CAPLUS/2019:1820815
Structural Architecture of the Nucleosome Remodeler ISWI Determined from Cross-Linking, Mass Spectrometry, SAXS, and Modeling	Harrer, Nadine Schindler, Christina E. M. Bruetzel, Linda K. Forne, Ignasi Ludwigsen, Johanna Imhof, Axel Zacharias, Martin Lipfert, Jan Mueller-Planitz, Felix	Molecular Biology, Biomedical Center, Faculty of Medicine, Martinsried, 82152, Germany	Structure (Oxford, United Kingdom) (2018), 26(2), 282-294.e6 CODEN: STRUE6; ISSN: 0969-2126	20180101	other	Chromatin remodeling factors assume crit. roles by regulating access to nucleosomal DNA. To det. the architecture of the Drosophila ISWI remodeling enzyme, we developed an integrative structural approach that combines protein crosslinking, mass spectrometry, small-angle X-ray scattering, and computational modeling. The resulting structural model shows the ATPase module in a resting state with both ATPase lobes twisted against each other, providing support for a conformation that was recently trapped by crystallog. The autoinhibiting NegC region does not protrude from the ATPase module as suggested previously. The regulatory NTR domain is located near both ATPase lobes. The full-length enzyme is flexible and can adopt a compact structure in soln. with the C-terminal HSS domain packing against the ATPase module. Our data imply a series of conformational changes upon activation of the enzyme and illustrate how the NTR, NegC, and HSS domains contribute to regulation of the ATPase module.	CAPLUS/2018:239953
Genome-wide comparative analysis of putative Pth11-related G protein-coupled receptors in fungi belonging to Pezizomycotina	Xu, Xihui Li, Guopeng Li, Lu Su, Zhenzhu Chen, Chen	College of Life Sciences, Nanjing Agricultural University, Nanjing, 210095, Peop. Rep. China	BMC Microbiology (2017), 17, 166/1-166/11 CODEN: BMMIBC; ISSN: 1471-2180	20170101	other	G-protein coupled receptors (GPCRs) are the largest family of transmembrane receptors in fungi, where they play important roles in signal transduction. Among them, the Pth11-related GPCRs form a large and divergent protein family, and are only found in fungi in Pezizomycotina. However, the evolutionary process and potential functions of Pth11-related GPCRs remain largely unknown. Twenty genomes of fungi in Pezizomycotina covering different nutritional strategies were mined for putative Pth11-related GPCRs. Phytopathogens encode much more putative Pth11-related GPCRs than symbionts, saprophytes, or entomopathogens . Based on the phylogenetic tree, these GPCRs can be divided into nine clades, with each clade contg. fungi in different taxonomic orders. Instead of fungi from the same order, those fungi with similar nutritional strategies were inclined to share orthologs of putative Pth11-related GPCRs. Most of the CFEM domain-contg. Pth11-related GPCRs, which were only included in two clades, were detected in phytopathogens . Furthermore, many putative Pth11-related GPCR genes of phytopathogens were upregulated during invasive plant infection , but downregulated under biotic stress. The expressions of putative Pth11-related GPCR genes of saprophytes and entomopathogens could be affected by nutrient conditions, esp. the carbon source. The gene expressions revealed that Pth11-related GPCRs could respond to biotic/abiotic stress and invasive plant infection with different expression patterns. Our results indicated that the Pth11-related GPCRs existed before the diversification of Pezizomycotina and have been gained and/or lost several times during the evolutionary process. Tandem duplications and trophic variations have been important factors in this evolution.	CAPLUS/2018:2120396
Recombinant exochitinase of the thermophilic mould Mycelioptora thermophila BJA: Characteristics and utility in generating N-acetyl glucosamine and in biocontrol of phytopathogenic fungi	Dua, Ashima Joshi, Swati Satyanarayana, T.	Dept. of Microbiology, University of Delhi South Campus, New Delhi, 110 021, India	Biotechnology Progress (2017), 33(1), 70-80 CODEN: BIPRET; ISSN: 1520-6033	20170101	other	Chitinase from the thermophilic mold Myceliopthora thermophila BJA (MtChit) is an acid tolerant, thermostable and org. solvent stable biocatalyst which does not require any metal ions for its activity. To produce high enzyme titers, reduce fermn. time and overcome the need for induction, this enzyme has been heterologously expressed under GAP promoter in the GRAS yeast, Pichia pastoris. The prodn. medium supplemented with the permeabilizing agent Tween-20 supported two-fold higher rMtChit prodn. (5.5 .times. 103 U L-1). The consensus sequences S(132)xG(133)G(134) and D(168)xxD(171)xD(173)xE(175) in the enzyme have been found to represent the substrate binding and catalytic sites, resp. The rMtChit, purified to homogeneity by a two-step purifn. strategy, is a monomeric glycoprotein of .apprx.48 kDa, which is optimally active at 55.degree.C and pH 5.0. The enzyme is thermostable with t1/2 values of 113 and 48 min at 65 and 75.degree.C, resp. Kinetic parameters Km, Vmax, kcat, and kcat/Km of the enzyme are 4.655 mg mL-1, 34.246 nmol mg-1 s-1, 3.425 .times. 106 min-1, and 1.36 .times. 10-6 mg mL-1 min-1, resp. rMtChit is an unique exochitinase, since its action on chitin liberates N-acetylglucosamine NAG. The enzyme inhibits the growth of phytopathogenic fungi like Fusarium oxysporum and Curvularia lunata, therefore, this finds application as biofungicide at high temps. during summer in tropics. .COPYRGT. 2016 American Institute of Chem. Engineers Biotechnol. Prog., 2016.	CAPLUS/2016:1753772

Identification and characterization of the glucose dual-affinity transport system in Neurospora crassa: pleiotropic roles in nutrient transport, signaling, and carbon catabolite repression	Wang, Bang Li, Jingen Gao, Jingfang Cai, Pengli Han, Xiaoyun Tian, Chaoguang	Key Laboratory of Systems Microbial Biotechnology, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin, 300308, Peop. Rep. China	Biotechnology for Biofuels (2017), 10, 17/1-17/22 CODEN: BBIIFL; ISSN: 1754-6834	20170101	other	Background: The glucose dual-affinity transport system (low- and high-affinity) is a conserved strategy used by microorganisms to cope with natural fluctuations in nutrient availability in the environment. The glucose-sensing and uptake processes are believed to be tightly assocd. with cellulase expression regulation in cellulolytic fungi. However, both the identities and functions of the major mol. components of this evolutionarily conserved system in filamentous fungi remain elusive. Here, we systematically identified and characterized the components of the glucose dual-affinity transport system in the model fungus Neurospora crassa. Results: Using RNA sequencing coupled with functional transport analyses, we assigned GLT-1 (Km = 18.42 .+-. 3.38 mM) and HGT-1/-2 (Km = 16.13 .+-. 0.95 and 98.97 .+-. 22.02 .mu.M) to the low- and high-affinity glucose transport systems, resp. The high-affinity transporters hgt-1/-2 complemented a moderate growth defect under high glucose when glt-1 was deleted. Simultaneous deletion of hgt-1/-2 led to extensive derepression of genes for plant cell wall deconstruction in cells grown on cellulose. The suppression by HGT-1/-2 was connected to both carbon catabolite repression (CCR) and the cyclic adenosine monophosphate-protein kinase A pathway. Alteration of a residue conserved across taxa in hexose transporters resulted in a loss of glucose-transporting function, whereas CCR signal transduction was retained, indicating dual functions for HGT-1/-2 as 'transceptors.' Conclusions: In this study, GLT-1 and HGT-1/-2 were identified as the key components of the glucose dual-affinity transport system, which plays diverse roles in glucose transport and carbon metab. Given the wide conservation of the glucose dual-affinity transport system across fungal species, the identification of its components and their pleiotropic roles in this study shed important new light on the mol. basis of nutrient transport, signaling, and plant cell wall degrdn. in fungi.	CAPLUS/2018:381003
Targeted synthesis of novel .beta.-lactam antibiotics by laccase-catalyzed reaction of aromatic substrates selected by pre-testing for their antimicrobial and cytotoxic activity	Mikolasch, Annett Hildebrandt, Oliver Schlueter, Rabea Hammer, Elke Witt, Sabine Lindequist, Ulrike	Institute of Microbiology, Ernst-Moritz-Arndt-University Greifswald, Greifswald, 17487, Germany	Applied Microbiology and Biotechnology (2016), 100(11), 4885-4899 CODEN: AMBiDG; ISSN: 0175-7598	20160101	other	The rapidly increasing problem of antimicrobial-drug resistance requires the development of new antimicrobial agents. The laccase-catalyzed amination of dihydroxy aroms. is a new and promising method to enlarge the range of currently available antibiotics. Thirty-eight potential 1,2- and 1,4-hydroquinoid laccase substrates were screened for their antibacterial and cytotoxic activity to select the best substrates for laccase-catalyzed coupling reaction resulting in potent antibacterial derivs. As a result, methyl-1,4-hydroquinone and 2,3-dimethyl-1,4-hydroquinone were used as parent compds. and 14 novel cephalosporins, penicillins, and carbacephems were synthesized by amination with amino-.beta.-lactam structures. All purified products were stable in aq. buffer and resistant to the action of .beta.-lactamases, and in agar diffusion and broth micro-diln. assays, they inhibited the growth of several Gram-pos. bacterial strains including multidrug-resistant Staphylococcus aureus and Enterococci. Their in vivo activity and cytotoxicity in a Staphylococcus-infected , immune-suppressed mouse model are discussed.	CAPLUS/2016:87112
Fungal communities in pressmud composting harbour beneficial and detrimental fungi for human welfare	Brito de Oliveira, Tassio Lopes, Viviane Cristina Padilha Barbosa, Fernando Nogueira Ferro, Milene Meirelles, Lucas Andrade Sette, Lara Duraes Gomes, Eleni Rodrigues, Andre	Department of Biochemistry and Microbiology, UNESP - Univ Estadual Paulista, Rio Claro, SP, Brazil	Microbiology (London, United Kingdom) (2016), 162(7), 1147-1156 CODEN: MROBEO; ISSN: 1350-0872	20160101	other	Pressmud is a substrate derived from sugarcane juice filtrate, and .apprx.26-40 kg of this residue are produced per ton of sugarcane. It is mainly used as fertilizer in crops, and its application in the field is often made without any prior treatment, but, in this research, it was studied for the risk this practice poses for human health. This research was stimulated by previous results indicating the presence of opportunistic pathogens in residues used in various composting systems and the extensive use of fresh pressmud in agriculture. It was assessed the fungal diversity present in both fresh and composting pressmud using 454 pyrosequencing. In addn., heat-tolerant fungi were isolated and surveyed for their enzymic repertoire of biomass-degrading enzymes (cellulase, xylanase, laccase, and polygalacturonase). A wide range of opportunistic pathogens was found among the most abundant taxa in the fresh pressmud, such as Lomentospora prolificans (43.13 %), Trichosporon sp. (10.07%), Candida tropicalis (7.91%), and Hormographiella aspergillata (8.19%). This indicates that fresh pressmud might be a putative source of human pathogenic fungi, presenting a potential threat to human health if applied as fertilizer without any treatment. With regard to the heat-tolerant fungi found in this substrate, all the 110 isolates screened were able to produce .gtoreq.1 of the tested enzymes. The pressmud composting process not only effectively reduces the load of pathogenic fungi, but also creates an interesting environment for fungi able to produce thermostable hydrolytic and oxidative enzymes with biotechnol. applications.	CAPLUS/2016:1596450
Microbiological consequences of indoor composting	Naegele, A. Reboux, G. Vacheyrou, M. Valot, B. Millon, L. Roussel, S.	UMR/CNRS 6249 Chrono-Environnement, University of Bourgogne - Franche-Comte, Besancon, Fr.	Indoor Air (2016), 26(4), 605-613 CODEN: INAIE5; ISSN: 1600-0668	20160101	other	Recycling of org. waste appeals to more and more people. The aim of this study was to evaluate the microbiol. contamination around org. waste bins at three distances over a 12-mo period. Contamination near the customary trash of control households was evaluated at the beginning to ensure that there is no recruitment bias. Air samples using the MAS 100 impactor were carried out in 38 dwellings that do household waste composting and in 10 dwellings of controls. Collection of particles by CIP 10 rotating cup sampler and dust samples collected by electrostatic dust collector cloths were acquired in dwellings that do household waste composting. Samples were analyzed by culture and by real-time quant. PCR. Information about dwelling characteristics and inhabitant practices was obtained by a standardized questionnaire. The genera most often isolated were Penicillium,Aspergillus,Cladosporium and Streptomyces. Near the org. waste bins, bioaerosol samples showed an increase of Acarus siro (P = 0.001). Sedimented dust analyses highlighted an increase of A. siro,Wallemia sebi,Aspergillus versicolor, and Cladosporium sphaerospermum concns. after a 12-mo survey compared to the beginning. Composting favors microorganism development over time, but does not seem to have an effect on the bioaerosol levels and the surface microbiota beyond 0.5 m from the waste bin.	CAPLUS/2016:1167128

Rubrolide analogues and their derived lactams as potential anticancer agents	Pereira, U. A. Moreira, T. A. Barbosa, L. C. A. Maltha, C. R. A. Bomfim, I. S. Maranhao, S. S. Moraes, M. O. Pessoa, C. Barros-Nepomuceno, F. W. A.	Department of Chemistry, Federal University of Vicosa, Vicosa, Brazil	MedChemComm (2016), 7(2), 345-352 CODEN: MCCEAY; ISSN: 2040-2503	20160101	other	Seven .beta.-aryl-substituted .gamma.-alkylidene-.gamma.-lactone analogs of rubrolides were synthesized from mucochloric acid and converted into their corresponding .gamma.-hydroxy-.gamma.-lactams (76-85%) by a reaction with isobutylamine and propylamine. Further dehydration of the .gamma.-hydroxy-.gamma.-lactams led to the corresponding (Z)- and (E)-.gamma.-alkylidene-.gamma.-lactams (23-45%). All compds. were fully characterized by spectroscopic methods. These 14 compds., together with 32 other rubrolide analogs, were assayed against four human tumor cell lines (HL-60, leukemia; HCT-116, colon; SF-295, central nervous system; and OVCAR-8, ovarian). Of the 46 compds. assayed, 7 caused a large redn. in cell viability (% RCV > 80%) in the tested cell lines and the most active compds. had halogen substituents on the arom. ring. Compds. 10a and 14i were the most active (RC50 = 3.00 and 3.58 .mu.M, resp.) against HL-60 and were not cytotoxic to L929 normal cells at the concns. tested (RC50 > 50 .mu.M). To further understand the mechanism underlying the cytotoxicity of 10a and 14i, studies involving DNA fragmentation, cell cycle anal., phosphatidyl serine externalization and mitochondrial depolarization were performed in the HL-60 cells, using doxorubicin as a pos. control. The results indicated that the cytotoxicity of 10a and 14i involved the induction of cell death by apoptosis. The cell cycle anal. showed that 14i caused the accumulation of cells in the G0/G1 phase at 2.5 and 5 .mu.M.	CAPLUS/2015:2007888
Genetic screening of biogenic amines production capacity from some lactic acid bacteria strains	Elsanhoty, Rafaat M. Ramadan, Mohamed Fawzy	Department of Industrial Biotechnology, Institute of Genetic Engineering and Biotechnology, Branch of Food and Dairy Biotechnology, Sadat City University, Egypt	Food Control (2016), 68, 220-228 CODEN: FOOCEV; ISSN: 0956-7135	20160101	other	There is an increasing interest for using lactic acid bacteria (LAB) as a starter and adjunct cultures for producing novel foods with particular functional traits. The ability of the starter to produce biogenic amines (BA) should be taken into account wherein protective starters should be selected to avoid hygienic and toxicol . risks. This work aimed to study the possibilities of forming BA (histamine, putrescine, agmatine and tyramine) from thirty two LAB strains belonging to species of the genera Lactobacillus and Streptococcus that used in food products as well as strains isolated from healthy breast-fed infants. The anal. protocol involved using polymerase chain reaction (PCR) and thin layer chromatog. (TLC) techniques to det. the ability of LAB strains to form BA. The presence of key genes involved in the biosynthetic pathways of the BA was also assessed by PCR. Six LAB strains gave pos. results for putrescine prodn. wherein the max. level was 14.6 mg/kg. Six strains gave pos. results for histamine prodn. (max. level was 31.7 mg/kg) and were pos. for the presence of histidine decarboxylase (HDC) gene. Seven strains exhibited pos. results for tyramines prodn. (max. level was 2.85 mg/kg) and were pos. for the presence of tyrosine decarboxylase (TDC) gene. Eight strains gave pos. results for agmatine prodn. (max. level was 174.5 mg/kg) and were pos. for the presence of dihydrolase (deiminase) gene that responsible for agmatine formation. It could be concluded that the microorganisms used in food and dairy prodn. should be screened carefully by PCR for their ability to produce BA.	CAPLUS/2016:582543
Effect of Chinese medicinal herbal residues on microbial community succession and anti-pathogenic properties during co-composting with food waste	Zhou, Ying Selvam, Ammaiyappan Wong, Jonathan W. C.	Sino-Forest Applied Research Centre for Pearl River Delta Environment and Department of Biology, Hong Kong Baptist University, Hong Kong	Bioresource Technology (2016), 217, 190-199 CODEN: BIRTEB; ISSN: 0960-8524	20160101	other	This study investigated the antimicrobial properties of Chinese medicinal herbal residues (CMHRs) during its co-composting with food waste (FW) in two different ratios along with a control. Inhibition on total microbial population were assessed while the numerically dominant microbes were isolated and their antagonistic effects were assessed. Results indicate that the active ingredients persist in the composting mass did not affect the microbes unspecifically as revealed from almost similar bacterial and fungal populations. Rather specific inhibitory activities against Alternaria solani and Fusarium oxysporum were obsd. Apart from the CMHR-born active compds., CMHR-induced changes in the antagonistic and mycoparasitic abilities of the bacteria and fungi also contribute to the specific inhibition against the tested pathogens . Therefore use of CMHRs during the composting of CMHRs enhances its antipathogenic property resulting in an anti-pathogenic compost.	CAPLUS/2016:435153
Laccase-cellobiose dehydrogenase-catalyzed detoxification of phenolic-rich olive processing residues	Nugroho Prasetyo, E. Rodriguez, R. D. Lukesch, B. Weiss, S. Murkovic, M. Katsoyannos, E. Sygmund, C. Ludwig, R. Nyanhongo, G. S. Guebitz, G. M.	Institute of Environmental Biotechnology, Graz University of Technology, Graz, 8010, Austria	International Journal of Environmental Science and Technology (2015), 12(4), 1343-1352 CODEN: IJESHU; ISSN: 1735-1472	20150101	other	The combination of a laccase-hydroxybenzotriazole (HBT) mediator system with/without cellobiose dehydrogenase (CDH) or an addnl. Fenton reaction step for the elimination and/or detoxification of phenolic compds. in dry olive mill residues (DOR) and liq. olive mill wastewaters (OMW) was evaluated. The laccase-HBT-CDH and laccase-HBT-CDH-Fenton system were the most effective, removing at least 69 and 72 % of phenolic compds. from a total of 698 and 683 mg in OMW and DOR, resp., in 12 h. The efficient removal of phenolic compds. was also accompanied by >80 % redn. in BOD and COD in both DOR and OMW. Microbial community anal. using single-strand conformation polymorphism (SSCP) gels showed that biogas reactors supplemented with untreated and laccase-HBT-CDH-Fenton-treated DOR and OMW strongly inhibited growth of microorganisms. In contrast, the laccase-HBT- and laccase-HBT-CDH-pretreated OMW and DOR were detoxified as evidenced by SSCP anal., which also indicated a distinct sensitivity of the individual members of the anaerobic population toward the toxicants . Further, although the laccase-HBT-CDH-Fenton system was effective in bleaching and removing phenolic compds. in both OMW and DOR, it was not able to support methane prodn. However, laccase-HBT and laccase-HBT-CDH indeed supported biogas prodn. This study therefore shows that the laccase-HBT-CDH system has a potential for the detoxification of olive mill residues, which can be potentially used as substrates for downstream processes.	CAPLUS/2015:677593

CrGNAT gene regulates excess copper accumulation and tolerance in Chlamydomonas reinhardtii	Wang, Ye Cheng, Zhen Zhen Chen, Xi Zheng, Qi Yang, Zhi Min	Department of Biochemistry and Molecular Biology, College of Life Science, Nanjing Agricultural University, Nanjing, Peop. Rep. China	Plant Science (Shannon, Ireland) (2015), 240, 120-129 CODEN: PLSCE4; ISSN: 0168-9452	20150101	other	Excess copper (Cu) in environment affects the growth and metab. of plants and green algae. However, the mol. mechanism for regulating plant tolerance to excess Cu is not fully understood. Here, we report a gene CrGNAT enconding an acetyltransferase in Chlamydomonas reinhardtii and identified its role in regulating tolerance to Cu toxicity . Expression of CrGNAT was significantly induced by 75-400 .mu.M Cu. The top induction occurred at 100 .mu.M. Transgenic algae overexpressing CrGNAT (35S::CrGNAT) in C. reinhardtii showed high tolerance to excess Cu, with improved cell population, chlorophyll accumulation and photosynthesis efficiency, but with low degree of oxidn. with regard to reduced hydrogen peroxide, lipid peroxides and non-protein thiol compds. In contrast, CrGNAT knock-down lines with antisense led to sensitivity to Cu stress. 35S::CrGNAT algae accumulated more Cu and other metals (Zn, Fe, Cu, Mn and Mg) than wild-type, whereas the CrGNAT down-regulated algae (35S::AntiCrGNAT) had moderate levels of Cu and Mn, but no effects on Zn, Fe and Mg accumulation as compared to wild-type. The elevated metal absorption in CrGNAT overexpression algae implies that the metals can be removed from water media. Quant. RT-PCR anal. revealed that expression of two genes encoding N-lysine histone methyltransferases was repressed in 35S::CrGNAT algae, suggesting that CrGNAT-regulated algal tolerance to Cu toxicity is likely assocd. with histone methylation and chromatin remodeling. The present work provided an example a basis to develop techniques for environmental restoration of metal-contaminated aquatic ecosystems.	CAPLUS/2015:1502200
Alternative splicing acting as a bridge in evolution	Zhou, Kemin Salamov, Asaf Kuo, Alan Aerts, Andrea L. Kong, Xiangyang Grigoriev, Igor V.	US Department of Energy Joint Genome Institute, Walnut Creek, CA, 94598, USA	Stem Cell Investigation (2015), 2, 19/1-19/32 CODEN: SCITE9; ISSN: 2306-9759	20150101	other	Background: Alternative splicing (AS) regulates diverse cellular and developmental functions through alternative protein structures of different isoforms. Alternative exons dominate AS in vertebrates; however, very little is known about the extent and function of AS in lower eukaryotes. To understand the role of introns in gene evolution, we examd. AS from a green algal and five fungal genomes using a novel EST-based gene-modeling algorithm (COMBEST). Methods: AS from each genome was classified with COMBEST that maps EST sequences to genomes to build gene models. Various aspects of AS were analyzed through statistical methods. The interplay of intron 3n length, phase, coding property, and intron retention (RI) were examd. with Chi-square testing. Results: With 3 to 834 times EST coverage, we identified up to 73% of AS in intron-contg. genes and found preponderance of RI among 11 types of AS. The no. of exons, expression level, and max. intron length correlated with no. of AS per gene (NAG), and intron-rich genes suppressed AS. Genes with AS were more ancient, and AS was conserved among fungal genomes. Among stopless introns, non-retained introns (NRI) avoided, but major RI preferred 3n length. In contrast, stop-contg. introns showed uniform distribution among 3n, 3n + 1, and 3n + 2 lengths. We found a clue to the intron phase enigma: it was the coding function of introns involved in AS that dictates the intron phase bias. Conclusions: Majority of AS is non-functional, and the extent of AS is suppressed for intron-rich genes. RI through 3n length, stop codon, and phase bias bridges the transition from functionless to functional alternative isoforms.	CAPLUS/2016:1390369
Development of genetic tools for Myceliophthora thermophila	Xu, Jing Li, Jingen Lin, Liangcai Liu, Qian Sun, Wenliang Huang, Bangquan Tian, Chaoguang	Key Laboratory of Systems Microbial Biotechnology, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin, 300308, Peop. Rep. China	BMC Biotechnology (2015), 15, 1-10 CODEN: BBMIE6; ISSN: 1472-6750	20150101	other	Background: The thermophilic filamentous fungus Myceliophthora thermophila has many suitable characteristics for industrial biotechnol. and could be a promising new chassis system for synthetic biol., particularly the ATCC 42464 strain, whose genome was sequenced in 2011. However, metabolic engineering of this strain using genetic approaches has not been reported owing to a lack of genetic tools for this organism. Results: In the present study, we developed a high efficiency Agrobacterium tumefaciens mediated transformation system for M. thermophila, including an approach for targeted gene deletion using green fluorescence protein (GFP) as a marker for selection. Up to 145 transformants per 105 conidia were obtained in one transformation plate. Moreover, a ku70 deletion mutant was constructed in the ATCC 42464 background using the tools developed in present study and subsequently characterized. The ku70 deletion construct was designed using resistance to phosphinothricin as the selection marker. Addnl., a GFP-encoding cassette was incorporated that allowed for the selection of site-specific (no fluorescence) or ectopic (fluorescence) integration of the ku70 construct. Transformants with ectopically integrated ku70 deletion constructs were therefore identified using the fluorescent signal of GFP. PCR and Southern blotting analyses of non-fluorescent putative ku70 deletion transformants revealed all 11 tested transformants to be correct deletions. The deletion frequency in a pool of 116 transformants analyzed was 58 %. Moreover, the homologous rate improved about 3 folds under ku70 mutant using the pyrG as a test gene to disrupt in M. thermophila. Conclusions: We successfully developed an efficient transformation and target gene disruption approach for M. thermophila ATCC 42464 mediated by A. tumefaciens. The tools and the ku70 deletion strain developed here should advance the development of M. thermophila as an industrial host through metabolic engineering and accelerate the elucidation of the mechanism of rapid cellulose degrdn. in this thermophilic fungus.	CAPLUS/2015:1232928
Determinants of Regioselective Hydroxylation in the Fungal Polysaccharide Monooxygenases	Vu, Van V. Beeson, William T. Phillips, Christopher M. Cate, Jamie H. D. Marletta, Michael A.	Department of Chemistry, The Scripps Research Institute, La Jolla, CA, 92037, USA	Journal of the American Chemical Society (2014), 136(2), 562-565 CODEN: JACSAT; ISSN: 0002-7863	20140101	other	The ubiquitous fungal polysaccharide monooxygenases (PMOs) (also known as GH61 proteins, LPMOs, and AA9 proteins) are structurally related but have significant variation in sequence. A heterologous expression method in Neurospora crassa was developed as a step toward connecting regioselectivity of the chem. to PMO phylogeny. Activity assays, as well as sequence and phylogenetic analyses, showed that the majority of fungal PMOs fall into three major groups with distinctive active site surface features. PMO1s and PMO2s hydroxylate glycosidic positions C1 and C4, resp. PMO3s hydroxylate both C1 and C4. A subgroup of PMO3s (PMO3*) hydroxylate C1. Mutagenesis studies showed that an extra subdomain of about 12 amino acids contribute to C4 oxidn. in the PMO3 family.	CAPLUS/2013:1948280

Comparative genomic and transcriptomic analysis of Wangiella dermatitidis, a major cause of phaeohyphomycosis and a model black yeast human pathogen	Chen, Zehua Martinez, Diego A. Gujja, Sharvari Sykes, Sean M. Zeng, Qiandong Szaniszlo, Paul J. Wang, Zheng Cuomo, Christina A.	Broad Institute of MIT and Harvard, Cambridge, MA, 02142, USA	G3: Genes, Genomes, Genetics (2014), 4(4), 561-578, 18 CODEN: GGGGBN; ISSN: 2160-1836	20140101	other	Black or dark brown (phaeoid) fungi cause cutaneous, s.c., and systemic infections in humans. Black fungi thrive in stressful conditions such as intense light, high radiation, and very low pH. Wangiella (Exophiala) dermatitidis is arguably the most studied phaeoid fungal pathogen of humans. Here, we report our comparative anal. of the genome of W. dermatitidis and the transcriptional response to low pH stress. This revealed that W. dermatitidis has lost the ability to synthesize alpha-glucan, a cell wall compd. many pathogenic fungi use to evade the host immune system. In contrast, W. dermatitidis contains a similar profile of chitin synthase genes as related fungi and strongly induces genes involved in cell wall synthesis in response to pH stress. The large portfolio of transporters may provide W. dermatitidis with an enhanced ability to remove harmful products as well as to survive on diverse nutrient sources. The genome encodes three independent pathways for producing melanin, an ability linked to pathogenesis ; these are active during pH stress, potentially to produce a barrier to accumulated oxidative damage that might occur under stress conditions. In addn., a full set of fungal light-sensing genes is present, including as part of a carotenoid biosynthesis gene cluster. Finally, we identify a two-gene cluster involved in nucleotide sugar metab. conserved with a subset of fungi and characterize a horizontal transfer event of this cluster between fungi and algal viruses. This work reveals how W. dermatitidis has adapted to stress and survives in diverse environments, including during human infections .	CAPLUS/2014:1603341
Secretome weaponries of Cochliobolus lunatus interacting with potato leaf at different temperature regimes reveal a CL[xxxx]LHM - motif	Louis, Bengyella Waikhom, Sayanika Devi Roy, Pranab Bhardwaj, Pardeep Kumar Singh, Mohendro Wakambam Goyari, Sailendra Sharma, Chandradev K. Talukdar, Narayan Chandra	Institute of Bioresources and Sustainable Development (IBSD), Imphal, India	BMC Genomics (2014), 15, 213/1-213/18, 18 pp. CODEN: BGMEET; ISSN: 1471-2164	20140101	other	Background: Plant and animal pathogenic fungus Cochliobolus lunatus cause great economic damages worldwide every year. C. lunatus displays an increased temp. dependent-virulence to a wide range of hosts. Nonetheless, this phenomenon is poorly understood due to lack of insights on the coordinated secretome weaponries produced by C. lunatus under heat-stress conditions on putative hosts. To understand the mechanism better, we dissected the secretome of C. lunatus interacting with potato (Solanum tuberosum L.) leaf at different temp. regimes. Results: C. lunatus produced melanized colonizing hyphae in and on potato leaf, finely modulated the ambient pH as a function of temp. and secreted diverse set of proteins. Using two dimensional gel electrophoresis (2-D) and mass spectrometry (MS) technol., we obsd. discrete secretomes at 20.degree., 28.degree. and 38.degree.. A total of 21 differentially expressed peptide spots and 10 unique peptide spots (that did not align on the gels) matched with 28 unique protein models predicted from C. lunatus m118 v.2 genome peptides. Furthermore, C. lunatus secreted peptides via classical and non-classical pathways related to virulence, proteolysis, nucleic acid metab., carbohydrate metab., heat stress, signal trafficking and some with unidentified catalytic domains. Conclusions: We have identified a set of 5 sol. candidate effectors of unknown function from C. lunatus secretome weaponries against potato crop at different temp. regimes. Our findings demonstrate that C. lunatus has a repertoire of signature secretome which mediates thermo-pathogenicity and share a leucine rich 'CL[xxxx]LHM' - motif. Considering the rapidly evolving temp. dependent-virulence and host diversity of C. lunatus, this data will be useful for designing new protection strategies.	CAPLUS/2014:1841622
New colorimetric screening assays for the directed evolution of fungal laccases to improve the conversion of plant biomass	Pardo, Isabel Chanaga, Xiomara Vicente, Ana Isabel Alcalde, Miguel Camarero, Susana	Centro de Investigaciones Biologicas, CSIC, Madrid, 28040, Spain	BMC Biotechnology (2013), 13, 90 CODEN: BBMIE6; ISSN: 1472-6750	20130101	other	Background: Fungal laccases are multicopper oxidases with huge applicability in different sectors. Here, we describe the development of a set of high-throughput colorimetric assays for screening laccase libraries in directed evolution studies. Results: Firstly, we designed three colorimetric assays based on the oxidn. of sinapic acid, acetosyringone and syringaldehyde with .lambda.max of 512, 520 and 370 nm, resp. These syringyl-type phenolic compds. are released during the degrdn. of lignocellulose and can act as laccase redox mediators. The oxidn. of the three compds. by low- and high-redox potential laccases evolved in Saccharomyces cerevisiae produced quantifiable and linear responses, with detection limits around 1 mU/mL and CV values below 16%. The phenolic substrates were also suitable for pre-screening mutant libraries on solid phase format. Intense colored-halos were developed around the yeast colonies secreting laccase. Furthermore, the oxidn. of violuric acid to its iminoxyl radical (.lambda.max of 515 nm and CV below 15%) was devised as reporter assay for laccase redox potential during the screening of mutant libraries from high-redox potential laccases. Finally, we developed three dye-decolorizing assays based on the enzymic oxidn. of Methyl orange (470 nm), Evans Blue (605 nm) and Remazol Brilliant Blue (640 nm) giving up to 40% decolorization yields and CV values below 18%. The assays were reliable for direct measurement of laccase activity or to indirectly explore the oxidn. of mediators that do not render colored products (but promote dye decolorization). Every single assay reported in this work was tested by exploring mutant libraries created by error prone PCR of fungal laccases secreted by yeast. Conclusions: The high-throughput screening methods reported in this work could be useful for engineering laccases for different purposes. The assays based on the oxidn. of syringyl-compds. might be valuable tools for tailoring laccases precisely enhanced to aid biomass conversion processes. The violuric assay might be useful to preserve the redox potential of laccase while evolving towards new functions. The dye-decolorizing assays are useful for engineering ad hoc laccases for detoxification of textile wastewaters, or as indirect assays to explore laccase activity on other natural mediators.	CAPLUS/2013:1824184

Evaluation of the potential of fungal and plant laccases for active-packaging applications	Chatterjee, Robin Johansson, Kristin Jaernstrom, Lars Joensson, Leif J.	Department of Chemistry, Umeaa University, Umeaa, SE-901 87, Swed.	Journal of Agricultural and Food Chemistry (2011), 59(10), 5390-5395 CODEN: JAFCAU; ISSN: 0021-8561	20110101	other	Laccases from Trametes versicolor (TvL), Myceliophthora thermophila (MtL), and Rhus vernicifera (RvL) were investigated with regard to their potential utilization as oxygen scavengers in active packages contg. food susceptible to oxidn. reactions. The substrate selectivity of the laccases was investigated with a set of 17 reducing substrates, mainly phenolic compds. The temp. dependence of reactions performed at low temps. (4-31 .degree.C) was studied. Furthermore, the laccases were subjected to immobilization in a latex/clay matrix and drying procedures performed at temps. up to 105 .degree.C. The results show that it is possible to immobilize the laccases with retained activity after dispersion coating, drying at 75-105 .degree.C, and subsequent storage of the enzyme-contg. films at 4 .degree.C. TvL and, to some extent, MtL were promiscuous with regard to their reducing substrate, in the sense that the difference in activity with the 17 substrates tested was relatively small. RvL, on the other hand, showed high selectivity, primarily toward substrates resembling its natural substrate urushiol. When tested at 7 .degree.C, all three laccases retained >20% of the activity they had at 25 .degree.C, which suggests that it would be possible to utilize the laccases also in refrigerated food packages. Coating and drying resulted in a remaining enzymic activity ranging from 18 to 53%, depending on the drying conditions used. The results indicate that laccases are useful for active-packaging applications and that the selectivity for reducing substrates is an important characteristic of laccases from different sources.	CAPLUS/2011:524447
Insights into the Phylogeny or Arylamine N-Acetyltransferases in Fungi	Martins, Marta Dairou, Julien Rodrigues-Lima, Fernando Dupret, Jean-Marie Silar, Philippe	Unite de Biologie Fonctionnelle et Adaptative (BFA), CNRS EAC 4413, Laboratoire des Reponses Moleculaires et Cellulaires aux Xenobiotiques, Univ Paris Diderot-Paris 7, Paris, 75013, Fr.	Journal of Molecular Evolution (2010), 71(2), 141-152 CODEN: JMEVAU; ISSN: 0022-2844	20100101	other	Previous studies have shown that Eumycetes fungi can acylate arylamine thanks to arylamine N-acetyltransferases, xenobiotic-metabolizing enzymes also found in animals and bacteria. In this article, we present the results of mining 96 available fungal genome sequences for arylamine N-acetyltransferase genes and propose their phylogeny. The filamentous Pezizomycotina are shown to possess many putative N-acetyltransferases, while these are often lacking in other fungal groups. The evolution of the N-acetyltransferases is best explained by the presence of at least one gene in the opisthokont ancestor of the fungi and animal kingdoms, followed by recurrent gene losses and gene duplications. A possible horizontal gene transfer event may have occurred from bacteria to the basidiomycetous yeast Malassezia globosa.	CAPLUS/2010:1044521
The Lignozym process - a biotechnological process for conservation of chlorine dioxide in the pulp industry	Amann, Manfred Pfaller, Rupert	Consortium fur Elektrochemische Industrie, Munchen, Germany	GSF-Bericht (2001), 01/01, 73-81 CODEN: GSFBEM; ISSN: 0721-1694	20010101	other	The development of biotechnol. alternatives to the classical chem. processes in the pulp prodn. process are highly challenging from the scientific as well as from the economic view. Enzymic systems using low mol. compds. as redox carriers (mediator) between lignin and the active site of the enzyme (e.g. laccase) are promising candidates for a biotechnol. pulp delignification system. A very powerful laccase mediator system (LMS) had been developed with N-OH-acetanilide (NHA) as the mediator component. However, the high cost due to high mediator dosage and the potential for generating toxic byproducts are concern when implementing such a process in a mill's bleaching sequence. Changing the system from a batch process to a continuous dosing technol. can solve both of these problems. To adapt the proposed new procedure to existing mill equipment, a diacetylated mediator precursor was developed (DiAc; N-acetoxy-N-phenylacetamide), which generates NHA during the laccase stage (L). Mild alk. conditions are optimal for the L-stage with DiAc to assure a quant. NHA release. The process was analyzed in the lab first, and then transferred to a pilot plant scale. Due to the pH requirements laccase from Myceliophthora thermophila wa used. Running a five stage ECF sequence LEpD0EoD1 produced a pulp comparable to a mill ref. pulp (D0EopD1EpD2) with significant saving of bleaching chems. (8,5 kg/ton). Further work is required to develop an enzyme that is optimal for the conservation of chlorine dioxide in the pulp industry.	CAPLUS/2001:524558
Biophysical characterization of fungal phytases (myo-inositol hexakisphosphate phosphohydrolases): molecular size, glycosylation pattern, and engineering of proteolytic resistance.	Wyss, M. Pasamontes, L. Friedlein, A. Remy, R. Tessier, M. Kronenberger, A. Middendorf, A. Lehmann, M. Schnoebelen, L. Roethlisberger, U. Kusznir, E. Wahl, G. Mueller, F. Lahm, H. W. Vogel, K. Loon, A. P. G. M. van van Loon, A. P. G. M.	VFB Department, F. Hoffmann-La Roche Ltd., CH-4070 Basel, Switzerland.	Applied and Environmental Microbiology (1999), Volume 65, Number 2, pp. 359-366, 32 refs. ISSN: 0099-2240	19990101	other	Phytases (myo-inositol hexakisphosphate phosphohydrolases) are found naturally in plants and microorganisms, particularly fungi. Interest in these enzymes has been stimulated by the fact that phytase supplements increase the availability of phosphorus in pig and poultry feed and thereby reduce environmental pollution due to excess phosphate excretion in areas where there is intensive livestock production. The wild-type phytases from 6 different fungi, Aspergillus niger, A. terreus, A. fumigatus, Emericella nidulans, Myceliophthora thermophila and Talaromyces thermophilus, were overexpressed in filamentous fungi or yeasts and purified, and their biophysical properties were compared with those of a phytase from Escherichia coli. All of the phytases examined are monomeric proteins. While E. coli phytase is a nonglycosylated enzyme, the glycosylation patterns of the fungal phytases proved to be highly variable, differing for individual phytases, for a given phytase produced in different expression systems, and for individual batches of a given phytase produced in a particular expression system. Whereas the extents of glycosylation were moderate when the fungal phytases were expressed in filamentous fungi, they were excessive when the phytases were expressed in yeasts. However, the different extents of glycosylation had no effect on the specific activity, the thermostability, or the refolding properties of individual phytases. When expressed in A. niger, several fungal phytases were susceptible to limited proteolysis by proteases present in the culture supernatant. N-terminal sequencing of the fragments revealed that cleavage invariably occurred at exposed loops on the surface of the molecule. Site-directed mutagenesis of A. fumigatus and E. nidulans phytases at the cleavage sites yielded mutants that were considerably more resistant to proteolytic attack. Therefore, engineering of exposed surface loops may be a strategy for improving phytase stability during feed processing and in the digestive tract.	CABA/1999:135711

Site-directed mutations in fungal laccase: effect on redox potential, activity and pH profile.	Xu Feng Berka, R. M. Wahleithner, J. A. Nelson, B. A. Shuster, J. R. Brown, S. H. Palmer, A. E. Solomon, E. I. Xu, F.	Novo Nordisk Biotech, 1445 Drew Avenue, Davis, CA 95616, USA.	Biochemical Journal (London) (1998), Volume 334, Number 1, pp. 63-70, 42 refs. ISSN: 0264-6021	19980101	other	A Myceliophthora thermophila laccase and a Rhizoctonia solani laccase were mutated on a pentapeptide segment believed to be near the type-1 Cu site. The mutation L513F in Myceliophthora laccase and the mutation L470F in Rhizoctonia laccase took place at a position corresponding to the type-1 Cu axial methionine (M517) ligand in Zucchini ascorbate oxidase. The triple mutations V509L, S510E, G511A in Myceliophthora laccase and L466V, E467S, A468G in Rhizoctonia laccase involved a sequence segment whose homologue in ascorbate oxidase is flanked by the M517 and a type-1 Cu-ligating histidine (H512). The single mutation did not yield significant changes in the enzymic properties (including any significant increase in the redox potential of the type-1 Cu). In contrast, the triple mutation resulted in several significant changes. In comparison with the wild type, the Rhizoctonia and Myceliophthora laccase triple mutants had a phenol-oxidase activity whose pH optimum shifted 1 unit lower and higher, respectively. Although the redox potentials were not significantly altered, the Km, kcat and fluoride inhibition of the laccases were greatly changed by the mutations . The observed effects were interpreted as possible mutation -induced structural perturbations on the molecular recognition between the reducing substrate and laccase and on the electron transfer from the substrate to the type-1 Cu centre.	CABA/1998:151803
CLR-4, a novel conserved transcription factor for cellulase gene expression in ascomycete fungi.	Liu Qian Li JinGen Gao RanRan Li JinYang Ma GuoLi Tian ChaoGuang Liu, Q. Li, J. G. Gao, R. R. Li, J. Y. Ma, G. L. Tian, C. G.	Key Laboratory of Systems Microbial Biotechnology, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin 300308, China. EMAIL: tian_cg@tib.cas.cn	Molecular Microbiology (2019), Volume 111, Number 2, pp. 373-394 ISSN: 0950-382X Published by: Wiley, Oxford	20190101	other	Fungal degradation of lignocellulosic biomass requires various (hemi-)cellulases and is an important part of the natural carbon cycle. Although induction of cellulases has been described for some saprobic filamentous fungi, the regulation of cellulase transcription is complex and many aspects are still poorly understood. Here, we identified and characterized the novel cellulase regulation factor NcCLR-4 in Neurospora crassa and its ortholog MtCLR-4 in Myceliophthora thermophila . Deletion of CLR-4 resulted in similarly defective cellulolytic enzyme production and activities. Transcriptome analyses of .DELTA.Ncclr-4/.DELTA.Mtclr-4 revealed the down-regulation of genes encoding (hemi-)cellulases and pivotal regulators (clr-1, clr-2 and xyr-1) and key genes in the cAMP signaling pathway such as adenylate cyclase Nccr-1. Intracellular cAMP levels were markedly lower in .DELTA.Ncclr-4/.DELTA.Mtclr-4 than in wild-type during cellulose utilization. In electrophoretic mobility shift (EMSA) and DNase I footprinting assays, NcCLR-4/MtCLR-4 can directly bound to the promoters of Nccr-1/Mtcr-1 (encoding adenylyl cyclase). EMSAs also demonstrated that NcCLR-4/MtCLR-4 could directly bound to clr-1 (encoding a key cellulase regulator), Mtclr-2 and Mtxyr-1 (encoding biomass deconstruction regulators). These findings about the novel cellulase expression regulators NcCLR-4 and MtCLR-4 enrich our understanding of how cellulose degradation is regulated and provide new targets for engineering fungi to deconstruct plant biomass in biorefineries.	CABA/2020:48543
Characteristics and applications of a thermostable and acidic exochitinase of the thermophilic mould Myceliophthora thermophila .	Bharti Rohatgi Satyanarayana, T. Rohatgi, B.	Department of Microbiology, University of Delhi South campus, Benito Juarez Road, New Delhi-110021, India. EMAIL: tsnarayana@gmail.com	Kavaka (2019), Volume 52, pp. 52-61, many ref. ref. ISSN: 0379-5179 Published by: Mycological Society of India, Chennai	20190101	other	The thermophilic mould Myceliophthora thermophila (Apinis) van Oorschot produces an extracellular thermo-acid-stable chitinase, which has been purified to apparent homogeneity by affinity adsorption followed by hydrophobic interaction chromatography. The pure enzyme is a monomer with a molecular mass of 43.0 kD on SDS-PAGE and pI of 4.0. Based on MALDI-ToF-MS/MS and LC-MS analysis of peptides, it was identified as glycosyl hydrolase family 18 protein. The chitinase is optimally active at pH 4.0 and 55.degree.C with T1/2 values of 9 and 3 h at 55 and at 70.degree.C, respectively. The Km and Vmax values (for colloidal chitin hydrolysis) are 0.396 mg mlsup-1 and 25.25 nkats mgsup-1 Ssup-1. It is strongly inhibited by Hgsup2+, Alsup3+ and Fesup3+, but stimulated by Mnsup2+, Casup2+ and Basup2+. The chitinase is tolerant to organic solvents and detergents. It liberates N-acetylglucosamine and chitobiose from colloidal chitin degradation and has a potential to degrade powdered chitin and chitosan. The high temperature optimum and thermostability makes it a suitable candidate for the production of pharmacologically important N-acetylglucosamine and chitobiose from chitin. The cell walls of spores and hyphae are disrupted in Fusarium oxysporum, Curvularia sp. and Stachybotrys sp. due to the degradation of chitin in the cell walls. The enzyme arrests hatching of eggs in the nematode, Meloidogyne incognita and induces mortality in the insect larvae of Aedes aegypti as well as mealy bug (Maconellicoccus hirsutus).	CABA/2020:124310
Production of cellobiose dehydrogenase from a newly isolated white rot fungus Termitomyces sp. OE147.	Gupteshwar Gupta Rishabh Gangwar Ashwani Gautam Lalit Kumar Anuj Dhariwal Vikram Sahai Saroj Mishra Gupta, G. Gangwar, R. Gautam, A. Kumar, L. Dhariwal, A. Sahai, V. Mishra, S.	Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology Delhi, New Delhi, 110 016, India. EMAIL: saroj@dbeb.iitd.ac.in	Applied Biochemistry and Biotechnology (2014), Volume 173, Number 8, pp. 2099-2115, 41 refs. ISSN: 0273-2289 Published by: Springer US, Philadelphia	20140101	other	Class I cellobiose dehydrogenases (CDHs) are extracellular hemoflavo enzymes produced at low levels by the Basidiomycetes (white rot fungi). In presence of suitable electron acceptors, e.g., cytochrome c, 2,6-dichlorophenol-indophenol, or metal ions, it oxidizes cellobiose to cellobionolactone. A stringent requirement for disaccharides makes CDH also useful for conversion of lactose to lactobionic acid, an important ingredient in pharma and detergent industry. In this work, class I CDH was produced using a newly identified white rot fungus Termitomyces sp. OE147. Four media were evaluated for CDH production, and maximum enzyme activity of 0.92 international unit (IU)/ml was obtained on Ludwig medium under submerged conditions. Statistical optimization of N source, which had significant effect on CDH production, using Box-Behnken design followed by optimization of inoculum size and age resulted in an increase in activity to 2.9 IU/ml and a productivity of 25 IU/l/h. The nearly purified CDH exhibited high activity of 26.4 IU/mg protein on lactose indicating this enzyme to be useful for lactobionic acid synthesis. Some of the internal peptide sequences bore 100% homology to the CDH produced in Myceliophthora thermophila . The fungal isolate was amenable to scale up, and an overall productivity of 18 IU/l/h was obtained at 14-l level.	CABA/2014:275576

Isolation and identification of actinomycetes from termite's gut against human pathogen .	Khucharoenphaisan, K. Sripairoj, N. Sinma, K.	Faculty of Science and Technology, Phranakhon Rajabhat University, Bangkok 10220, Thailand.	Asian Journal of Animal and Veterinary Advances (2012), Volume 7, Number 1, pp. 68-73, 25 refs. ISSN: 1683-9919 DOI: 10.3923/ajava.2012.68.73 Published by: Academic Journals Inc., New York	20120101	other	Actinomycetes are a group of prokaryotic organisms belonging to Gram-positive bacteria and play an important ecological role in recycling substances in the nature. The objective of this study was to isolate and identify actinomycetes from termite's gut against human pathogen . Total isolates were examined for antimicrobial activity and selected isolate was identified using morphological characters and molecular technique. The results showed that eighty-three strains of actinomycetes were isolated from guts of Termes sp. Among these, 66, 67, 7, 9 and 3 isolated strains were active against the tested pathogenic microorganism of Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Candida utilis, respectively. Furthermore, some isolate of actinomycetes was able to inhibit both Gram-positive and Gram-negative pathogen . The isolated actinomycetes strain FSPNRU 102 having broad spectrum of inhibition was selected. The morphological character of this strain showed aerial mycelium with longitudinal spirales-type spore chain and light black soluble pigment. The aerial spore color varied from white to gray. Moreover, this strain contained LL-diaminopimelic acid of the peptidoglycan in the whole-cell hydrolysate of chemotaxonomical characteristic. These results assigned strain FSPNRU 102 to genus Streptomyces. Based on its 16S rDNA sequence and phylogenetic tree analysis, this new isolate belong to the Streptomyces niveoruber.	CABA/2012:180180
Comparative spore germination of filamentous fungi on solid state fermentation under different culture conditions.	Hassouni, H. Ismaili-Alaoui, M. Lamrani, K. Gaime-Perraud, I. Augur, C. Roussos, S.	Institut Agronomique et Veterinaire (IAV)-Hassan II, Laboratoire des Bioconversions, BP. 6202-Instituts, 10101 Rabat, Morocco. EMAIL: s.roussos@univ.u-3mrs.fr s.roussos@univ-cezanne.fr	Micologia Aplicada International (2007), Volume 19, Number 1, pp. 7-14, 18 refs. ISSN: 1534-2581 Published by: Micologia Aplicada International, Berkeley	20070101	other	Environmental factors influencing spore germination of three filamentous fungi (Aspergillus niger, Myceliophthora thermophila , Rhizopus microsporus [Rhizopus microsporus var. microsporus]) were studied. Four substrates, all containing sugarcane bagasse as support, were tested for spore germination. Different temperature, moisture, aeration and humidity conditions were evaluated for each species. Spore germination was assessed by observing emergence of the germ tube. Germination rates in A. niger, M. thermophila, and R. microsporus cultivated in solid state fermentation were affected by the substrate, incubation temperature, and initial moisture. A mixture of sugarcane bagasse and wheat bran (80:20 w/w) resulted in high germination rates for all species tested, and was selected for additional experiments. The optimal temperature for spore germination and initial growth was 30.degree.C for A. niger and 45.degree.C for M. thermophila and R. microsporus. Higher germination rates were recorded in all species at 85% (aw 0.99) initial moisture after 6 h of incubation at optimal temperature. Forced humid aeration increased germination rates and decreased the time required for complete spore germination in all species studied.	CABA/2007:185684
Xylanases as a tool for the production of novel phytopharmaceuticals.	Katapodis, P. Christakopoulos, P.	Biotechnology Laboratory, Chemical Engineering Department, National Technical University of Athens, 5 Iroon Polytechniou Str, Zografou Campus, 15700, Athens, Greece. EMAIL: hristako@orfeas.chemeng.ntua.gr	NutraCos (2005), Volume 4, Number 2, pp. 17-21, 10 refs. ISSN: 1720-4011 Published by: B5 srl, Milano	20050101	other	Results are presented of a study on the production and biological activities of acidic and feruloyl oligosaccharides from xylan by family 10 and 11 endoxylanases [endo-1,4-.beta.-xylanase] from Thermoascus aurantiacus and Sporotrichum thermophile [Myceliophthora thermophila]. Both family 10 and 11 endoxylanases were able to release branched xylooligosaccharides from xylan. Feruloyl oligosaccharides acted as antioxidants and showed protective effects against copper-catalysed peroxidation of low-density lipoproteins. Acidic xylooligosaccharides, products of 4-O-methyl-D-glucurono-D-xylan hydrolysis, functioned as plant growth regulators and antibacterial agents. Aldotetrauronic acid induced callus and somatic embryogenesis in culture explants of common mallow (Malva sylvestris) and cotton (Gossypium hirsutum). Aldopentauronic acid caused an increase in both the number of regenerants and their fresh weight to cucumber (cultivars Stella and Gilda) liquid culture in plant airlift bioreactors. Both acidic xylooligosaccharides were active against Bacillus cereus, Micrococcus flavus and Helicobacter pylori.	CABA/2005:153021
Two new records of thermophilic Myceliophthora in China.	Wang DongMei Li DuoChuan Zhao ChunQing Wang, D. M. Li, D. C. Zhao, C. Q.	College of Plant Protection, Shandong Agricultural University, Taian, Shandong 271018, China. EMAIL: lidc20@sdau.edu.cn	Journal of Fungal Research (2004), Volume 2, Number 2, pp. 47-49, 6 refs. ISSN: 1672-3538 Published by: Jilin Agricultural University, Changchun	20040101	other	[redacted]	CABA/2005:39802
Purification and characterization of a Fusarium oxysporum feruloyl esterase (FoFAE-I) catalysing transesterification of phenolic acid esters.	Topakas, E. Stamatis, H. Mastihubova, M. Biely, P. Kekos, D. Macris, B. J. Christakopoulos, P.	Biotechnology Laboratory, Chemical Engineering Department, National Technical University of Athens, 5 Iroon Polytechniou Str, Zografou Campus, Athens 15700, Greece. EMAIL: pc@biocentrum.dtu.dk	Enzyme and Microbial Technology (2003), Volume 33, Number 5, pp. 729-737, 61 refs. ISSN: 0141-0229 DOI: 10.1016/S0141-0229(03)00213-8 Published by: Elsevier Science Inc., New York	20030101	other	An extracellular feruloyl esterase (FoFAE-I) from the culture filtrates of Fusarium oxysporum F3 was purified to homogeneity by ion-exchange, hydrophobic interaction and gel filtration chromatographies. The protein corresponded to molecular mass and pI values of 31 kDa and 9.5, respectively. The enzyme was optimally active at pH 7.0 and 55 .degree.C. The purified esterase was fully stable at pH 7.0-9.0 and temperature up to 30 .degree.C. Determination of kcat/Km revealed that the enzyme hydrolysed methyl p-coumarate (MpCA) 4.5, 9, and 239 times more efficiently than methyl caffeate (MCA), methyl ferulate (MFA) and methyl sinapinate (MSA), respectively. The enzyme was active on substrates containing ferulic acid ester linked to the C-5 and C-2 linkages of arabinofuranose but showed preference for the ester at position 2. 4-Nitrophenyl-2-O-trans-feruloyl-.alpha.-L-arabinofuranoside (NPh-5-Fe-Araf) was hydrolysed 100 times more efficiently than 4-nitrophenyl-5-O-trans-feruloyl-.alpha.-L-arabinofuranoside (NPh-2-Fe-Araf). Ferulic acid (FA) was efficiently released from destarched wheat bran (DSWB) when the esterase was incubated together with xylanase from Sporotrichum thermophile (a maximum of 92% total ferulic acid released after 4 h incubation). FoFAE-I by itself could release FA but at a level almost five-fold lower than that obtained in the presence of xylanase. The potential of FAE-I for the synthesis of various phenolic acid esters was tested using as a reaction system a surfactantless microemulsions formed in ternary mixture consisting of n-hexane, 1-butanol and water.	CABA/2003:182363

Studies on thermophilic fungi of Agaricus bisporus compost.	Vijay, B. Sharma, S. R. Lakhanpal, T. N.	National Research Centre for Mushroom, Chambaghat, Solan 173 213, India.	Journal of Mycology and Plant Pathology (2002), Volume 32, Number 2, pp. 240-243, 15 refs. ISSN: 0971-9393 Published by: Indian Society of Mycology and Plant Pathology, Udaipur	20020101	other	An in vitro study was conducted to determine whether thermophilic fungi as well as their culture filtrates could hamper or increase the growth of Agaricus bisporus and its fungal competitors/pathogenic fungi. The thermophilic fungi used in the study include Chaetomium thermophilium, Humicola grisea, H. insolens, Scytalidium thermophilium, Sporotrichum thermophilum [Myceliophthora thermophila] and Thermomyces lanuginosus. The culture filtrates used were C. indicum, Cladobotryum dendroides [Hypomyces rosellus], Sepedonium maheshwarianum, Trichoderma viride and Verticillium fungicola. Most of the thermophilic fungi reduced the growth of A. bisporus and its competitors, whereas H. insolens and Scytalidium thermophilium increased the growth of A. bisporus by 4.3 and 22.6%, respectively. A marked reduction in the growth of T. viride was observed with all thermophilic fungi, wherein the maximum was by C. thermophilium and the least was by S. thermophilium. Almost all the culture filtrates reduced the growth of A. bisporus and its competitors. However, a marginal increase in the growth of A. bisporus, Chaetomium indicum and V. fungicola was observed in case of H. insolens, C. thermophilium and H. grisea, respectively. Cladobotryum dendroides was the most sensitive fungus towards different culture filtrates and H. grisea reduced its growth by 86.1%. The least inhibition in this case was recorded with Chaetomium thermophilium. A maximum reduction in the growth due to culture filtrates was recorded in V. fungicola compared with the other test fungi.	CABA/2003:145678
Effects of an organophosphorus insecticide on the growth and cellulolytic activity of fungi.	Omar, S. A. Moharram, A. M. Abd-Alla, M. H.	Department of Botany, Faculty of Science, University of Assiut, Assiut, Egypt.	International Biodeterioration + Biodegradation (1993), Volume 31, Number 4, pp. 305-310, 13 refs. ISSN: 0964-8305 DOI: 10.1016/0964-8305(93)90025-W	19930101	other	Respiration, mycelial protein, extracellular protein and mycelial dry weight were significantly reduced in Aspergillus fumigatus, A. terreus and Myceliophthora thermophila that had been treated with Selecron [profenofos] at 10 and 50 p.p.m. The activity of the Cx and C1 cellulases of these fungi were significantly reduced, with the exception of the C1 cellulase of A. fumigatus.	CABA/1995:158521
Effect of elevated amendments of urea on soil ureolytic activity and mycofloral count.	Salama, A. M. Abdel-Rahman, T. M. A. Abo-Ellil, A. H. Aly, M. I. A. El-Selhdar, A. A. H.	Botany Department, Faculty of Science, Cairo University, Cairo, Egypt.	Egyptian Journal of Microbiology (1992), Volume 27, Number 2, pp. 197-214, 38 refs. ISSN: 0022-2704	19920101	other	Increasing concn of urea decreased total fungal counts in a clay soil (pH 6.5). No fungi were isolated in soil amended with 12.5% urea. Urea concn of >4.5% inhibited ammonification, accelerated the oxidation of ammonia to nitrates, inhibited oxidation of nitrites to nitrates and accumulation of nitrites resulting in a reduced fungal count. Fungal counts were increased by application of lower concn of urea by promotion of ammonification, reinitiation of nitrate accumulation and accumulation of nitrites. Soil urease activity was max. at urea concn of 2.5, 3.5 and 4.5% but was inhibited by urea levels above and below these concn. A total of 36 species of fungi was isolated including Aspergillus fumigatus, A. terreus, Mucor pusillus [Rhizomucor pusillus], Humicola grisea and Sporotrichum thermophilum [Myceliophthora thermophila]. No fungal species was isolated at a density of >50% of the total count at all urea concn. An increase in urea concn led to the rapid elimination of fungal species from soil. A. fumigatus, A. flavus, A. niger and Penicillium notatum were the fungi most tolerant of increasing urea concn.	CABA/1995:43213
Fungal flora associated with combine harvester wheat and sorghum dusts from Egypt.	Abdel-Hafez, S. I. I. Moubasher, A. H. Shoreit, A. A. M. Ismail, M. A.		Journal of Basic Microbiology (1990), Volume 30, Number 7, pp. 467-479, 47 refs. ISSN: 0233-111X DOI: 10.1002/jobm.3620300702	19900101	other	A total of 107 species and 8 species varieties belonging to 44 genera were collected from combine harvester wheat and sorghum dusts (35 genera and 91 species + 4 varieties) and from the atmosphere of their hay sites (26 genera and 69 species + 4 varieties) on glucose- and cellulose-Czapek's Dox agar at 28.degree.C and 45.degree.C. The mycoflora of wheat and sorghum dusts were basically similar on the 2 types of media and the most common fungi were: Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, A. ochraceous, A. sydowii, A. terreus, Cochliobolus spicifer, Emericella nidulans, Fusarium moniliforme [Gibberella fujikuroi], Penicillium chrysogenum, P. duclauxii, P. funiculosum and P. oxalicum. Thermophilic species were frequently encountered from the 2 substrates: Chaetomium thermophilum, Humicola grisea var. thermoidea, H. insolens, Malbranchea pulchella var. sulphurea, Rhizomucor pusillus, Sporotrichum thermophilum [Myceliophthora thermophila], Talaromyces thermophilus, Thermoascus thermophilus and Thermomyces lanuginosus. The airborne fungi in the 2 atmospheres were basically similar and the most prevalent species were members of Alternaria (3 spp.), Aspergillus (19 spp. + 2 var.), Cladosporium (2 spp.), Cochliobolus (3 spp.), Emericella (2 spp. + 2 var.), Fusarium (5 spp.), Mucor (1 sp.), Penicillium (14 spp.) and Stachybotrys (1 sp.).	CABA/1993:103964
Studies on the mycoflora of Aswan High Dam Lake, Egypt: monthly variations.	El-Hissy, F. T. Moharram, A. M. El-Zayat, S. A.	Botany Department, Faculty of Science, Assiut University, Assiut, Egypt.	Journal of Basic Microbiology (1990), Volume 30, Number 2, pp. 81-94, 23 refs. ISSN: 0233-111X DOI: 10.1002/jobm.3620300207	19900101	other	Fifty-one species and one variety appertaining to 21 genera of mesophilic fungi were recovered from the monthly samples of marginal water (44 spp., 1 var. and 18 genera) and submerged mud (78 spp., 1 var. and 30 genera) of Aswan High Dam Lake during July 1985-Dec. 1986. The most common species were Aspergillus fumigatus, A. flavus, A. terreus, A. niger and Penicillium funiculosum. The highest fungal populations were detected in Oct., in Dec. 1985 or in Feb. 1986. Of the 12 thermophilic and thermotolerant fungal species, A. fumigatus and A. nidulans were the most common. Paecilomyces variotii, Rhizomucor pusillus, Thermomyces lanuginosus, Thermoascus thermophilus and Sporotrichum thermophilum [Myceliophthora thermophila] were fairly common in one or more localities. The physico-chemical characteristics of water and mud samples were also studied.	CABA/1990:143695
Production of protein by thermophilic fungi from sugar-beet pulp in solid-state fermentation.	Grajek, W.	Acad. Agric., 60-624 Poznan, Poland.	Biotechnology and Bioengineering (1988), Volume 32, Number 1, pp. 255-260, 5 refs. ISSN: 0006-3592 DOI: 10.1002/bit.260320218	19880101	other	(b) (4)	CABA/1988:93800

Utilization and treatment of palm oil mill effluent by selected fungi.	Vikineswary Sabaratnam Sabaratnam, V.		Wallaceana (1988), Volume 52-53, 26 p. ISSN: 0126-8236	19880101	other	(b) (4) ilic (28 spp.) and thermophilic (8 spp.) fungi were isolated from the effluent (POME). Aspergillus niger hoderma harzianum were the most frequently occurring mesophilic while Chaetomium thermophila var. dissitum, Myceliophthora thermophila , Rhizomucor pusillus, Thermoascus aurantiacus and Thermomyces lanuginosus the most frequently isolated thermophilic spp. Mycelial extracts of M. thermophila and T. harzianum were non-toxic to mice while that of C. thermophila var. dissitum caused 50% mortality of the mice within 48 h and that of R. pusillus caused weight loss. In residues after harvesting the fungal biomass (M. thermophila and T. harzianum) did not inhibit germination of Brassica and maize seeds. The latter 2 fungi lowered BOD (by 72%) and COD (74%) and produced appreciable amounts of amylase, cellulase and lipase. The results are discussed.	CABA/1989:35840
Thermophilic and thermotolerant fungi associated with seeds of five members of Umbelliferae from Egypt.	Abdel-Hafez, A. I. I. Moharram, A. M. M. Abdel-Mallek, A. Y.	Dep. Bot., Fac. Sci., Sohag, Univ. Assiut, Egypt.	Cryptogamie, Mycologie (1987), Volume 8, Number 4, pp. 315-320, 22 refs. ISSN: 0181-1584	19870101	other	(b) (4)	ABA/1988:93553
Degradation of tetracyclines and sulfonamides by stevensite- and biochar-immobilized laccase systems and impact on residual antibiotic activity.	Garcia-Delgado, Carlos (correspondence) Eymar, Enrique Camacho-Arevalo, Raquel Garcia-Delgado, Carlos (correspondence) Petruccioli, Maurizio Crognale, Silvia D'Annibale, Alessandro	Department of Agricultural Chemistry and Food Sciences, Autonomous University of Madrid, Madrid, Spain. carlos.garciadelgado@uam.es	Journal of Chemical Technology and Biotechnology, (December 2018) Vol. 93, No. 12, pp. 3394-3409. Refs: 55 ISSN: 0268-2575; E-ISSN: 1097-4660 CODEN: JCTBDC	20180101	other	thermophila (MtL) and Pleurotus eryngii (PeL) through the sequential application of aminopropyltriethoxysilane and glutaraldehyde. The immobilized preparations were tested to remove three tetracyclines and six sulfonamides at 0.1 mmol L-1 of each antibiotic. Degradation experiments were conducted both in the absence and in the presence (0.2 mmol L-1) of ABTS, 1-hydroxybenzotriazol (HBT), syringaldehyde or violuric acid. The residual antibiotic activity was tested towards five bacterial species and a bacterial consortium from wastewater. RESULTS: Higher values of activity yields (74% and 70.3%) and catalytic capabilities (1426 and 1405 IU g-1) were obtained with PeL on stevensite and biochar than with MtL. Stevensite enabled higher reusability and storage stability than biochar. Best removals of tetracyclines and sulfonamides were obtained with immobilized-laccase systems coupled to ABTS or syringaldehyde. Immobilized-laccase/ABTS systems removed 100% of tetracyclines while only chlortetracycline was completely removed in the presence of syringaldehyde. With ABTS, the most effectively removed sulfonamides were sulfathiazole and sulfadiazine (up to 100% and 54%), while syringaldehyde best supported the removal of sulfanilamide, sulfamethazine and sulfamethoxazole (up to 42%, 45% and 46%, respectively). In some cases, an effective antibiotics removal led to either low or no residual antibiotic activity. CONCLUSION: MtL and PeL were immobilized successfully on biochar and stevensite. The addition of either ABTS or syringaldehyde enhanced significant removals, up to 100%, of tetracyclines and sulfonamides by the immobilized laccase systems. Noteworthy, biochar-immobilized laccases/ABTS led to complete suppression of the antibiotic activity of tetracyclines. .COPYRGT. 2018 Society of Chemical Industry.	MBASE/20180666626
The Kinase USK1 Regulates Cellulase Gene Expression and Secondary Metabolite Biosynthesis in Trichoderma reesei.	Beier, Sabrina Hinterdobler, Wolfgang Monroy, Alberto Alonso Bazafkan, Hoda Schmoll, Monika (correspondence)	Center for Health and Bioresources, AIT Austrian Institute of Technology GmbH, Tulln, Austria. monika.schmoll@ait.ac.at	Frontiers in Microbiology, (20 May 2020) Vol. 11. arn. 974. Refs: 75 E-ISSN: 1664-302X	20200101	other	The complex environment of fungi requires a delicate balance between the efforts to acquire nutrition, to reproduce, and to fend off competitors. In Trichoderma reesei, an interrelationship between regulation of enzyme gene expression and secondary metabolism was shown. In this study, we investigated the physiological relevance of the unique YPK1-type kinase USK1 of T. reesei. Usk1 is located in the vicinity of the SOR cluster and is involved in regulation of several genes from this secondary metabolite cluster as well as dihydrotrichotetronine and other secondary metabolites. Moreover, USK1 is required for biosynthesis of normal levels of secondary metabolites in liquid culture. USK1 positively influences cellulase gene regulation, secreted cellulase activity, and biomass formation upon growth in constant darkness on cellulose. Positive effects of USK1 on transcript abundance of the regulator of secondary metabolism, vel1, and the carbon catabolite repressor gene cre1 are in agreement with these functions. In summary, we found that with USK1, T. reesei comprises a unique kinase that adds an additional layer of regulation to the connection of secondary metabolism and enzyme production in fungi.	EMBASE/20200450004
Additional sequence and structural characterization of an endo-processive GH74 xyloglucanase from Myceliophthora thermophila and the revision of the EC 3.2.1.155 entry.	Gusakov, Alexander V.	Department of Chemistry, M. V. Lomonosov Moscow State University, Vorobyovy Gory 1/11, Moscow, 119991, Russian Federation. avgusakov@enzyme. chem.msu.ru	Biochimica et Biophysica Acta - General Subjects, (March 2020) Vol. 1864, No. 3. arn. 129511. Refs: 15 ISSN: 0304-4165; E-ISSN: 1872-8006 CODEN: BBGSB3		other		EMBASE/20200019219

Cryo-EM structures of remodeler-nucleosome intermediates suggest allosteric control through the nucleosome.	Armache, Jean Paul Gamarra, Nathan Johnson, Stephanie L. Leonard, John D. Narlikar, Geeta J. (correspondence) Cheng, Yifan Gamarra, Nathan Leonard, John D. Wu, Shenping Cheng, Yifan Leonard, John D. Wu, Shenping	Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, United States. Geeta.Narlikar@ucsf.edu ycheng@ucsf.edu	eLife, (June 2019) Vol. 8. arn. e46057. Refs: 65 E-ISSN: 2050-084X		other	The SNF2h remodeler slides nucleosomes most efficiently as a dimer, yet how the two protomers avoid a tug-of-war is unclear. Furthermore, SNF2h couples histone octamer deformation to nucleosome sliding, but the underlying structural basis remains unknown. Here we present cryo-EM structures of SNF2h-nucleosome complexes with ADP-BeFx that capture two potential reaction intermediates. In one structure, histone residues near the dyad and in the H2A-H2B acidic patch, distal to the active SNF2h protomer, appear disordered. The disordered acidic patch is expected to inhibit the second SNF2h protomer, while disorder near the dyad is expected to promote DNA translocation. The other structure doesn't show octamer deformation, but surprisingly shows a 2 bp translocation. FRET studies indicate that ADP-BeFx predisposes SNF2h-nucleosome complexes for an elemental translocation step. We propose a model for allosteric control through the nucleosome, where one SNF2h protomer promotes asymmetric octamer deformation to inhibit the second protomer, while stimulating directional DNA translocation.	EMBASE/20190641255
Characterization and dye decolorization potential of two laccases from the marine-derived fungus Pestalotiopsis sp..	Wikee, Saowanee Lumyong, Saisamorn Sciara, Giuliano Faulds, Craig B. Record, Eric (correspondence) Wikee, Saowanee Lumyong, Saisamorn Hatton, Juliette Turbe-Doan, Annick Mathieu, Yann Daou, Marianne Lomascolo, Anne Kumar, Abhishek	Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, 50200, Thailand. eric.record@inra.fr giuliano.sciara@inra.fr scboi009@gmail.com craig.faulds@univ-amu.fr wikeemammaam@gmail.com	International Journal of Molecular Sciences, (2 Apr 2019) Vol. 20, No. 8. arn. 1864. Refs: 66 ISSN: 1661-6596; E-ISSN: 1422-0067	20190101	other	Two laccase-encoding genes from the marine-derived fungus Pestalotiopsis sp. have been cloned in Aspergillus niger for heterologous production, and the recombinant enzymes have been characterized to study their physicochemical properties, their ability to decolorize textile dyes for potential biotechnological applications, and their activity in the presence of sea salt. The optimal pH and temperature of PsLac1 and PsLac2 differed in relation to the substrates tested, and both enzymes were shown to be extremely stable at temperatures up to 50 .degree.C, retaining 100% activity after 3 h at 50 .degree.C. Both enzymes were stable between pH 4-6. Different substrate specificities were exhibited, and the lowest Km and highest catalytic efficiency values were obtained against syringaldazine and 2,6-dimethoxyphenol (DMP) for PsLac1 and PsLac2, respectively. The industrially important dyes-Acid Yellow, Bromo Cresol Purple, Nitrosulfonazo III, and Reactive Black 5-were more efficiently decolorized by PsLac1 in the presence of the redox mediator 1-hydroxybenzotriazole (HBT). Activities were compared in saline conditions, and PsLac2 seemed more adapted to the presence of sea salt than PsLac1. The overall surface charges of the predicted PsLac three-dimensional models showed large negatively charged surfaces for PsLac2, as found in proteins for marine organisms, and more balanced solvent exposed charges for PsLac1, as seen in proteins from terrestrial organisms.	EMBASE/20190320451
Microbiology of Pediatric Orbital Cellulitis and Trends in Methicillin-Resistant Staphylococcus aureus Cases.	Hsu, Jerry Treister, Alison D. Rowley, Anne H. Rahmani, Bahram, MD, MPH (correspondence) Hsu, Jerry Treister, Alison D. Ralay Ranaivo, Hantamalala Rowley, Anne H. Rahmani, Bahram, MD, MPH (correspondence)	Northwestern University, Chicago, IL, United States. brahmani@luriechildre ns.org	Clinical Pediatrics, (1 Sep 2019) Vol. 58, No. 10, pp. 1056-1062. Refs: 25 ISSN: 0009-9228; E-ISSN: 1938-2707 CODEN: CPEDAM	20190101	other	We reviewed medical records of children with orbital cellulitis with positive cultures at a tertiary institution from 2005 to 2018 to identify microbiology trends and features associated with methicillin-resistant Staphylococcus aureus (MRSA) cases. Cultures obtained from the orbits (n = 33), sinuses (n = 31), and dural cavities (n = 4) had yields of 66.7%, 61.3%, and 75%, respectively, compared with 17.6% of blood cultures (n = 69). Fifty-five patients had positive culture results. Staphylococcus aureus was the most common pathogen isolated (n = 19), followed by Streptococcus species, most commonly Streptococcus anginosus (n = 8). The most frequently prescribed antibiotic combination regimen was ampicillin-sulbactam followed by amoxicillin-clavulanate. There were 8 cases of MRSA. MRSA was associated with an age of presentation <1 year old (P =.034). Other clinical features were similar between MRSA and non-MRSA cases. In infants and neonates, or those with epidemiologic risk factors, MRSA should also be considered.	EMBASE/20190564376
Linking enzymatic oxidative degradation of lignin to organics detoxification .	Wang, Xiaolu Yao, Bin (correspondence) Su, Xiaoyun	Key Laboratory for Feed Biotechnology of the Ministry of Agriculture, Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing, 100081, China. suxiaoyun@caas.cn binyao@caas.cn xiaolu4444@126.com	International Journal of Molecular Sciences, (November 2018) Vol. 19, No. 11. arn. 3373. Refs: 138 ISSN: 1661-6596; E-ISSN: 1422-0067		other	The major enzymes involved in lignin degradation are laccase, class II peroxidases (lignin peroxidase, manganese peroxidase, and versatile peroxidase) and dye peroxidase, which use an oxidative or peroxidative mechanism to deconstruct the complex and recalcitrant lignin. Laccase and manganese peroxidase directly oxidize phenolic lignin components, while lignin peroxidase and versatile peroxidase can act on the more recalcitrant non-phenolic lignin compounds. Mediators or co-oxidants not only increase the catalytic ability of these enzymes, but also largely expand their substrate scope to those with higher redox potential or more complicated structures. Neither laccase nor the peroxidases are stringently selective of substrates. The promiscuous nature in substrate preference can be employed in detoxification of a range of organics.	EMBASE/20190016616
CRISPR-based genomic tools for the manipulation of genetically intractable microorganisms.	Shapiro, Rebecca S. Collins, James J. (correspondence) Shapiro, Rebecca S. Collins, James J. (correspondence) Shapiro, Rebecca S. Chavez, Alejandro Collins, James J. (correspondence)	Department of Biological Engineering, Institute for Medical Engineering and Science, Synthetic Biology Center, MIT, Cambridge, MA, United States. jimjc@mit.edu	Nature Reviews Microbiology, (1 Jun 2018) Vol. 16, No. 6, pp. 333-339. Refs: 94 ISSN: 1740-1526; E-ISSN: 1740-1534 CODEN: NRMACK	20180101	other	Genetic manipulation of microorganisms has been crucial in understanding their biology, yet for many microbial species, robust tools for comprehensive genetic analysis were lacking until the advent of CRISPR-Cas-based gene editing techniques. In this Progress article, we discuss advances in CRISPR-based techniques for the genetic analysis of genetically intractable microorganisms, with an emphasis on mycobacteria, fungi and parasites. We discuss how CRISPR-based analyses in these organisms have enabled the discovery of novel gene functions, the investigation of genetic interaction networks and the identification of virulence factors.	EMBASE/20180225028

Lahun pyramid, more surprising treasures are revealed: Fungal diversity analysis using amplicon-basedmetagenomic approach.	Soliman, S. (correspondence) Magdy, M.	Ain Shams University, Cairo, Egypt.	Medical Mycology, (June 2018) Vol. 56, Supp. Supplement 2, pp. S21. Abstract Number: S8.2d. Meeting Info: 20th Congress of the International Society for Human and Animal Mycology, ISHAM 2018. Amsterdam, Netherlands. 30 Jun 2018-04 Jul 2018 ISSN: 1460-2709		other	(b) (4)	EMBASE/0053126870
Charged residues on the side of the nucleosome contribute to normal Spt16-gene interactions in budding yeast.	Nyamugenda, Eugene Cox, A. Brandon Pierce, Jacob B. Banning, Ryan C. Huynh, Michelle L. May, Catey Marshall, Sarah Turkal, Claire E. Duina, Andrea A. (correspondence)	Biology Department, Hendrix College, 1600 Washington Avenue, Conway, AR, 72032, United States. duina@hendrix.edu	Epigenetics, (2 Jan 2018) Vol. 13, No. 1, pp. 1-7. Refs: 36 ISSN: 1559-2294; E-ISSN: 1559-2308	20180101	other	Previous work in Saccharomyces cerevisiae identified three residues located in close proximity to each other on the side of the nucleosome whose integrity is required for proper association of the Spt16 component of the FACT complex across transcribed genes. In an effort to gain further insights into the parameters that control Spt16 interactions with genes in vivo, we tested the effects of additional histone mutants on Spt16 occupancy across two constitutively transcribed genes. These studies revealed that mutations in several charged residues in the vicinity of the three residues originally identified as important for Spt16-gene interactions also significantly perturb normal association of Spt16 across genes. Based on these and our previous findings, we propose that the charge landscape across the region encompassed by these residues, which we refer to as the Influences Spt16-Gene Interactions or ISGI region, is an important contributor to proper Spt16-gene interactions in vivo.	EMBASE/20180095141

Neanderthal behaviour, diet, and disease inferred from ancient DNA in dental calculus.	Weyrich, Laura S. (correspondence) Soubrier, Julien Arriola, Luis Llamas, Bastien Breen, James Farrer, Andrew G. Haak, Wolfgang Cooper, Alan Duchene, Sebastian Holmes, Edward C. Morris, Alan G. Alt, Kurt W. Alt, Kurt W. Dresely, Veit Alt, Kurt W. Dresely, Veit Alt, Kurt W. Caramelli, David Farrell, Milly Francken, Michael Harvati, Katerina Gully, Neville Kaidonis, John Townsend, Grant Hardy, Karen Hardy, Karen Held, Petra	Australian Centre for Ancient DNA, School of Biological Sciences and the Environment Institute, University of Adelaide, Adelaide, SA, Australia. alan.cooper@adelaide.edu.au laura.weyrich@adelaide.edu.au	Nature, (20 Apr 2017) Vol. 544, No. 7650, pp. 357-361. Refs: 46 ISSN: 0028-0836; E-ISSN: 1476-4687 CODEN: NATUAS	20170101	other	Recent genomic data have revealed multiple interactions between Neanderthals and modern humans, but there is currently little genetic evidence regarding Neanderthal behaviour, diet, or disease . Here we describe the shotgun-sequencing of ancient DNA from five specimens of Neanderthal calcified dental plaque (calculus) and the characterization of regional differences in Neanderthal ecology. At Spy cave, Belgium, Neanderthal diet was heavily meat based and included woolly rhinoceros and wild sheep (mouflon), characteristic of a steppe environment. In contrast, no meat was detected in the diet of Neanderthals from El Sidron cave, Spain, and dietary components of mushrooms, pine nuts, and moss reflected forest gathering. Differences in diet were also linked to an overall shift in the oral bacterial community (microbiota) and suggested that meat consumption contributed to substantial variation within Neanderthal microbiota. Evidence for self-medication was detected in an El Sidron Neanderthal with a dental abscess and a chronic gastrointestinal pathogen (Enterocytozoon bieneusi). Metagenomic data from this individual also contained a nearly complete genome of the archaeal commensal Methanobrevibacter oralis (10.2.times. depth of coverage) - the oldest draft microbial genome generated to date, at around 48,000 years old. DNA preserved within dental calculus represents a notable source of information about the behaviour and health of ancient hominin specimens, as well as a unique system that is useful for the study of long-term microbial evolution.	EMBASE/20170272729
Synergistically enhanced stability of laccase immobilized on synthesized silver nanoparticles with water-soluble polymers.	Cunha, M.N.M. Felgueiras, H.P. (correspondence) Zille, A. Gouveia, I.	2C2T, Centre for Science and Textile Technology, Department of Textile Engineering, University of Minho, Campus of Azurem, Guimaraes, 4800-058, Portugal. helena.felgueiras@2c2t.uminho.pt	Colloids and Surfaces B: Biointerfaces, (1 Jun 2017) Vol. 154, pp. 210-220. Refs: 73 ISSN: 0927-7765; E-ISSN: 1873-4367 CODEN: CSBBEQ	20170101	other	Silver nanoparticles (AgNPs) were synthesized by citrate reduction method in the presence of polymers, poly(ethylene glycol) (PEG), poly(vinyl alcohol) (PVA) and chitosan, used as stabilizing agents, and an oxidoreductase enzyme, laccase (Lac), with the goal of expanding the NPs antimicrobial action. AgNPs were characterized by UV-vis spectrometry, dynamic light scattering and transmission electron microscopy. As protecting agents, PEG and PVA promoted the formation of spherical uniformly-shaped, small-sized, monodispersed AgNPs (.apprxeq.20 nm). High Mw polymers were established as most effective in producing small-sized NPs. Chitosan's viscosity led to the formation of aggregates. Despite the decrease in Lac activity registered for the hybrid formulation, AgNPs-polymer-Lac, a significant augment in stability over time (up to 13 days, at 50 .degree.C) was observed. This novel formulation displays improved synergistic performance over AgNPs-Lac or polymer-Lac conjugates, since in the former the Lac activity becomes residual at the end of 3 days. By enabling many ionic interactions, chitosan restricted the mass transfer between Lac and substrate and, thus, inhibited the enzymatic activity. These hybrid nanocomposites made up of inorganic NPs, organic polymers and immobilized antimicrobial oxidoreductive enzymes represent a new class of materials with improved synergistic performance. Moreover, the Lac and the AgNPs different antimicrobial action, both in time and mechanism, may also constitute a new alternative to reduce the probability of developing resistance-associated mutations .	EMBASE/20170221110

Aspergillus galactomannan detection: Is Trichoderma a cause of positive results?.	Markantonatou, A.M. (correspondence) Samaras, K. Zachrou, E. Vyzantiadis, T.A.	Aristotle University of Thessaloniki, Thessaloniki, Greece.	Mycoses, (September 2017) Vol. 60, Supp. Supplement 2, pp. 114. Abstract Number: P118. Meeting Info: 8th Congress on Trends in Medical Mycology, TIMM 2017. Belgrade, Serbia. 06 Oct 2017-09 Oct 2017 ISSN: 1439-0507		other	(b) (4)	MBASE/0052759854
Microbial flavoprotein monooxygenases as mimics of mammalian flavin-containing monooxygenases for the enantioselective preparation of drug metabolites.	Gul, Turan Permentier, Hjalmar P. Bischoff, Rainer Krzek, Marzena Fraaije, Marco W., Dr. (correspondence) Permentier, Hjalmar P.	Analytical Biochemistry, Groningen Research Institute of Pharmacy, University of Groningen, Groningen, Netherlands.	Drug Metabolism and Disposition, (August 2016) Vol. 44, No. 8, pp. 1270-1276. Refs: 38 ISSN: 0090-9556; E-ISSN: 1521-009X CODEN: DMDSAI	20160101	other	Mammalian flavin-containing monooxygenases, which are difficult to obtain and study, play a major role in detoxifying various xenobiotics. To provide alternative biocatalytic tools to generate flavin-containing monooxygenases (FMO)-derived drug metabolites, a collection of microbial flavoprotein monooxygenases, sequence-related to human FMOs, was tested for their ability to oxidize a set of xenobiotic compounds. For all tested xenobiotics [nicotine, lidocaine, 3-(methylthio)aniline, albendazole, and fenbendazole], one or more monooxygenases were identified capable of converting the target compound. Chiral liquid chromatography with tandem mass spectrometry analyses of the conversions of 3-(methylthio) aniline, albendazole, and fenbendazole revealed that the respective sulfoxides are formed in good to excellent enantiomeric excess (e.e.) by several of the tested monooxygenases. Intriguingly, depending on the chosen microbial monooxygenase, either the (R)- or (S)-sulfoxide was formed. For example, when using a monooxygenase from Rhodococcus jostii the (S)-sulfoxide of albendazole (ricobendazole) was obtained with a 95% e.e. whereas a fungal monooxygenase yielded the respective (R)-sulfoxide in 57% e.e. For nicotine and lidocaine, monooxygenases could be identified that convert the amines into their respective N-oxides. This study shows that recombinantly expressed microbial monooxygenases represent a valuable toolbox of mammalian FMO mimics that can be exploited for the production of FMO-associated xenobiotic metabolites.	EMBASE/20160524231
Structure and regulation of the chromatin remodeller ISWI.	Yan, Lijuan Wang, Li Tian, Yuanyuan Xia, Xian Chen, Zhucheng (correspondence) Yan, Lijuan Wang, Li Tian, Yuanyuan Xia, Xian Chen, Zhucheng (correspondence)	MOE Key Laboratory of Protein Science, Tsinghua University, Beijing, 100084, China. Zhucheng_chen@tsinghua.edu.cn	Nature, (15 Dec 2016) Vol. 540, No. 7633, pp. 466-469. Refs: 31 ISSN: 0028-0836; E-ISSN: 1476-4687 CODEN: NATUAS	20160101	other	ISWI is a member of the SWI2/SNF2 family of chromatin remodellers, which also includes Snf2, Chd1, and Ino80. ISWI is the catalytic subunit of several chromatin remodelling complexes, which mobilize nucleosomes along genomic DNA, promoting replication progression, transcription repression, heterochromatin formation, and many other nuclear processes. The ATPase motor of ISWI is an autonomous remodelling machine, whereas its carboxy (C)-terminal HAND-SAND-SLIDE (HSS) domain functions in binding extranucleosomal linker DNA. The activity of the catalytic core of ISWI is inhibited by the regulatory AutoN and NegC domains, which are in turn antagonized by the H4 tail and extranucleosomal DNA, respectively, to ensure the appropriate chromatin landscape in cells. How AutoN and NegC inhibit ISWI and regulate its nucleosome-centring activity remains elusive. Here we report the crystal structures of ISWI from the thermophilic yeast Myceliophthora thermophila and its complex with a histone H4 peptide. Our data show the amino (N)-terminal AutoN domain contains two inhibitory elements, which collectively bind the second RecA-like domain (core2), holding the enzyme in an inactive conformation. The H4 peptide binds to the core2 domain coincident with one of the AutoN-binding sites, explaining the ISWI activation by H4. The H4-binding surface is conserved in Snf2 and functions beyond AutoN regulation. The C-terminal NegC domain is involved in binding to the core2 domain and functions as an allosteric element for ISWI to respond to the extranucleosomal DNA length.	EMBASE/20170229993

Microbial inhibitors of cysteine proteases.	Kedzior, Mateusz (correspondence) Seredynski, Rafal Gutowicz, Jan	Department of Physical Chemistry of Microorganisms, Institute of Genetics and Microbiology, University of Wroclaw, Przybyszewskiego 63/77, Wroclaw, 51-148, Poland. mateusz.kedzior@uwr.edu.pl	Medical Microbiology and Immunology, (1 Aug 2016) Vol. 205, No. 4, pp. 275-296. Refs: 187 ISSN: 0300-8584; E-ISSN: 1432-1831 CODEN: MMIYAO	20160101	other	Cysteine proteases are one of the major classes of proteolytic enzymes involved in a number of physiological and pathological processes in plants, animals and microorganisms. When their synthesis, activity and localization in mammalian cells are altered, they may contribute to the development of many diseases , including rheumatoid arthritis, osteoporosis and cancer . Therefore, cysteine proteases have become promising drug targets for the medical treatment of these disorders. Inhibitors of cysteine proteases are also produced by almost every group of living organisms, being responsible for the control of intracellular proteolytic activity. Microorganisms synthesize cysteine protease inhibitors not only to regulate the activity of endogenous, often virulent enzymes, but also to hinder the host's proteolytic defense system and evade its immune responses against infections . Present work describes known to date microbial inhibitors of cysteine proteases in terms of their structure, enzyme binding mechanism, specificity and pathophysiological roles. The overview of both proteinaceous and small-molecule inhibitors produced by all groups of microorganisms (bacteria, archaea, fungi, protists) and viruses is provided. Subsequently, possible applications of microbial inhibitors in science, medicine and biotechnology are also highlighted.	EMBASE/20160285899
Limitations of the ambush hypothesis' at the single-gene scale: what codon biases are to blame?.	Bertrand, Robert L. (correspondence) Abdel-Hameed, Mona Sorensen, John L.	Department of Chemistry, University of Manitoba, Winnipeg, R3T 2N2, Canada . bertrarl@cc.umanitoba.ca	Molecular Genetics and Genomics, (2015) Vol. 290, No. 2, pp. 493-504. Refs: 95 ISSN: 1617-4615; E-ISSN: 1617-4623 CODEN: MGGOAA	20150101	other	Ribosomal frameshifting, a translational error, catastrophically alters the amino acid composition of the nascent protein by shifting the reading frame from the intended contiguous trinucleotide reading. Frameshift events waste energy and resources, and peptide products have unpredictable cytotoxic effects. The Ambush Hypothesis' (Seligmann and Pollock 2004, DNA Cell Biol 23:701-5) suggests there is a selective pressure favouring the evolution of out-of-frame (hidden') stop codons. Although this hypothesis has gained empirical support through whole-genome studies, it is presently unknown whether it can be applied at a single-gene scale. Herein, we report such an investigation using the gene, polyketide synthase (PKS), among species of fungi. Contrary to expectation, genes presented with significantly lower number of hidden stop codons than expected in a selection-neutral model ($p < 0.0005$), suggesting both non-adherence to the ambush hypothesis as well as suppression of hidden stop codon evolution. It is known that there are multiple adaptive considerations determining codon selection during evolution, and that the information-holding potential of the genetic code is finite. We hypothesize that the reason for low hidden stops in PKS genes is due to competing codon biases' that are prioritized over the selective pressure favouring the emergence of hidden stops. Future studies of the ambush hypothesis in the context of other drivers of codon bias may allow this hypothesis to be molded into a comprehensive genetic theory that can be integrated within the broader genetic theory of codon bias and applied to the genetic code at any scale of analysis.	EMBASE/2014831875
Sensitivity of members of thermophilic compost mycobiota to toxic compounds.	Sebok, Flora (correspondence) Keresz, Gyorgy Dobolyi, Csaba Szoboszlay, Sandor Kriszt, Balazs	Department of Environmental Protection and Environmental Safety, Szent Istvan University, Godollo, Hungary.	Acta Microbiologica et Immunologica Hungarica, (June 2015) Vol. 62, Supp. SUPPL. 1, pp. 94-95. Editor: Marialigeti, K. Editor: Dobay, O. Meeting Info: Annual Meeting of the Hungarian Society for Microbiology and EU FP7 PROMISE Regional Meeting 2014. Keszthely, Hungary. 15 Oct 2014-17 Oct 2014 ISSN: 1217-8950		other	(b) (4)	EMBASE/0052077238
Laccase-based biosensors for detection of phenolic compounds.	Rodriguez-Delgado, Melissa M. Aleman-Nava, Gibran S. Parra, Roberto (correspondence) Rodriguez-Delgado, Jose Manuel Dieck-Assad, Graciano Martinez-Chapa, Sergio Omar Barcelo, Dami`a	Tecnologico de Monterrey, Campus Monterrey, Ave. Eugenio Garza Sada 2501, CP 64849, Monterrey, NL, Mexico. r.parra@itesm.mx	TrAC - Trends in Analytical Chemistry, (December 01, 2015) Vol. 74, pp. 21-45. Refs: 147 ISSN: 0165-9936; E-ISSN: 1879-3142 CODEN: TTAEDJ		other	Monitoring of phenolic compounds in the food industry and for environmental and medical applications has become more relevant in recent years. Conventional methods for detection and quantification of these compounds, such as spectrophotometry and chromatography, are time consuming and expensive. However, laccase biosensors represent a fast method for on-line and in situ monitoring of these compounds. We discuss the main transduction principles. We divide the electrochemical principle into amperometric, voltammetric, potentiometric and conductometric sensors. We divide optical transducers into fluorescence and absorption. The amperometric transducer method is the most widely studied and used for laccase biosensors. Optical biosensors present higher sensitivity than the other biosensors. Laccase production is dominated by a few fungus genera: Trametes, Aspergillus, and Ganoderma. We present an overview of laccase biosensors used for the determination of phenolic compounds in industrial applications.	EMBASE/2015472924

Application of response surface methodology to study the removal of estrogens in a laccase-mediated continuous membrane reactor.	Lloret, Lucia (correspondence) Eibes, Gemma Feijoo, Gumersindo Moreira, M. Teresa Lema, Juan M.	Department of Chemical Engineering, School of Engineering, University of Santiago de Compostela, E-15782 Santiago de Compostela, Spain. lucia.lloret@usc.es	Biocatalysis and Biotransformation, (August 2013) Vol. 31, No. 4, pp. 197-207. Refs: 34 ISSN: 1024-2422; E-ISSN: 1029-2446 CODEN: BOBOEQ	20130101	other	A three-level Box-Behnken factorial design combined with response surface methodology (RSM) was applied as a tool to study the laccase-catalyzed removal of three estrogenic compounds: estrone (E1), estradiol (E2), and ethinylestradiol (EE2), in a continuous enzymatic membrane reactor (EMR). Three main factors affecting the treatment efficiency were considered: enzyme activity, hydraulic residence time (HRT) and oxygenation rate. As expected, laccase activity and HRT showed large effects and, interestingly, the relevance of oxygen in improving the oxidation kinetics through raising the dissolved oxygen above saturation levels was demonstrated. When considering elimination rates as the response, optimal conditions were: 1,000 U/L of laccase, 1 h HRT and 60 mgO2/(L.cntdot.h) of oxygenation rate, predicting 2.82-3.24 mg eliminated/(L.cntdot.h), (71-81% of oxidation). These optimum conditions were successfully validated, and 75% of estrogenicity reduction was achieved. On the other hand, only 100 U/L were found as optimal to maximize the efficacy of the enzyme: E1 was oxidized by 0.06 mg/(L.cntdot.h.cntdot.U), although the removal of estrogenicity decreased to 60%. The methodology was also applied to maximize the reduction of estrogenic activity: the highest values assayed [1,000 U/L, HRT 4 h and 60 mgO2/(L.cntdot.h)] provided 99% detoxification . .COPYRGT. 2013 Informa UK, Ltd.	EMBASE/2013524970
Two novel GH11 endo-xylanases from Myceliophthora thermophila C1 act differently toward soluble and insoluble xylans.	van Gool, M.P. Schols, H.A. (correspondence) Gruppen, H. van Muiswinkel, G.C.J. Hinz, S.W.A. Sinitsyn, A.P.	Laboratory of Food Chemistry, Wageningen University, P.O. Box 8129, 6700 EV Wageningen, Netherlands. henk.schols@wur.nl	Enzyme and Microbial Technology, (10 Jun 2013) Vol. 53, No. 1, pp. 25-32. Refs: 33 ISSN: 0141-0229; E-ISSN: 1879-0909 CODEN: EMTED2	20130101	other	Two novel GH11 endo-xylanases from Myceliophthora thermophila C1 (C1), Xyl7 and Xyl8, were purified and the influence of solubility and molecular structure of various xylans on their efficiency was investigated. Both endo-xylanases were hindered by a high degree of substitution of a xylan. The two GH11 xylanases released different products from the xylans, in which Xyl7 displayed a degradation product composition closer to GH10 xylanases. A correlation of the degradation product composition with a specific residue at position 163 in the amino acid sequence of Xyl8 is suggested: tyrosine in Xyl8; valine in Xyl7. This is confirmed with examples of various endo-xylanases reported in literature.The C1 GH11 xylanases were more efficient on self-associated xylan compared to C1 GH10 endo-xylanases and they released more small xylooligomers from these xylans. This is contrary to the general assumption that GH10 xylanases degrade xylans to a higher degree than GH11 xylanases. .COPYRGT. 2013 Elsevier Inc.	EMBASE/2013324309
Laccase-catalyzed degradation of anti-inflammatories and estrogens.	Lloret, L. Eibes, G. Lu-Chau, T.A. Moreira, M.T. (correspondence) Feijoo, G. Lema, J.M.	Dept. of Chemical Engineering, School of Engineering, University of Santiago de Compostela, Santiago de Compostela E-15782, Spain. maite.morei ra@usc.es	Biochemical Engineering Journal, (Septemper 2010) Vol. 51, No. 3, pp. 124-131. Refs: 32 ISSN: 1369-703X CODEN: BEJOFV	20100101	other	Pharmaceuticals are regarded as emerging environmental pollutants since many of them are ubiquitous, recalcitrant and biologically active. In this paper, the degradation of several pharmaceuticals such as anti-inflammatory drugs (diclofenac and naproxen) and estrogen hormones (estrone, 17.beta.-estradiol, 17.alpha.-ethinylestradiol) was assessed by means of the commercial laccase (Lac) from Myceliophthora thermophila . The influence of different mediators (synthetic and natural) and their concentration on the Lac-based oxidation system were evaluated. Estrogens were completely degraded after only 15. min while the other types of pharmaceuticals presented higher persistence since 1. h of incubation was required for total removal of diclofenac and 8. h to attain up to 60% of naproxen degradation. Among the different natural mediators, syringaldehyde greatly enhanced the action of the Lac, similarly to the synthetic mediator 1-hydroxibenzotriazole (HBT) in the case of estrogens and diclofenac. The other natural mediators presented significantly high efficiency, obtaining removal percentages ranging from 80% to 100% after 24. h of enzymatic reaction. .COPYRGT. 2010 Elsevier B.V.	EMBASE/2010430631
Immobilization of Myceliophthora thermophila laccase on poly(glycidyl methacrylate) microspheres enhances the degradation of azinphos-methyl	Vera, Myleidi Rivas, Bernabe L. (Reprint) Nyanhongo, Gibson S. Pellis, Alessandro Guebitz, Georg M.	Univ Concepcion, Fac Chem, Dept Polymer, Casilla 160-C, Edmundo Larenas 129, Concepcion, Chile (Reprint) E-mail: brivas@udec.cl Univ Nat Resources + Life Sci Vienna, Dept Agrobiotechnol IFA Tulln, Inst Environm Biotechnol, Konrad Lorenz Str 20, A-3430 Tulln, Austria Univ York, Dept Chem, Green Chem Ctr Excellence, York YO10 5DD, N Yorkshire, England Austrian Ctr Ind Biotechnol, Div Polymers + Enzymes, A-3430 Tulln, Austria	JOURNAL OF APPLIED POLYMER SCIENCE, (20 APR 2019) Vol. 136, No. 16. ISSN: 0021-8995.	20190101	other	Laccase enzymes are multicopper oxidases capable of oxidizing different compounds. However, to be able to use this biocatalyst for industrial applications, their immobilization is needed. The present work investigates the immobilization of Myceliophthora thermophila laccase (MtL) on monodisperse microspheres of poly(glycidyl methacrylate) (PGMA) to be used in azinphos-methyl degradation. The immobilization was optimized to achieve the highest activity of the immobilized enzyme. As result, the protein load obtained was 2.5 mg protein g(-1) carrier (35 U g(-1) of carrier). The immobilized enzymes showed a broadened pH and temperature range of optimum activity and significantly improved the storage and operational stability. Finally, the complete degradation of azinphos-methyl using immobilized laccase was achieved after 1 h of reaction. The collected data indicate that the immobilization of MtL on PGMA microspheres is an excellent alternative to improve biochemical properties in the enzyme and to allow their efficient use in pesticides degradation. (c) 2018 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 2019, 136, 47417.	SCISEARCH/2019:254151
Secretory expression of recombinant small laccase from Streptomyces coelicolor A3(2) in Pichia pastoris	Yadav, Deepti Ranjan, Bibhuti Mchunu, Nokuthula Kudanga, Tukayi (Reprint) Le Roes-Hill, Marilize	Durban Univ Technol, Fac Appl Sci, Dept Biotechnol + Food Technol, POB 1334, ZA-4000 Durban, South Africa (Reprint) E-mail: tukayik@dut.ac.za Cape Peninsula Univ Technol, Inst Biomed + Microbial Biotechnol, Biocatalysis + Tech Biol Res Grp, Bellville Campus, Symphony Way, POB 1906, ZA-7535 Bellville, South Africa	INTERNATIONAL JOURNAL OF BIOLOGICAL MACROMOLECULES, (MAR 2018) Vol. 108, pp. 642-649. ISSN: 0141-8130.	20180101	other	This work reports for the first time the secretory expression of the small laccase (SLAC) from Streptomyces coelicolor A3(2) in Pichia pastoris. Using an AOX1 promoter and alpha factor as a secretion signal, the recombinant P. pastoris harbouring the laccase gene (rSLAC) produced high titres of extracellular laccase (500 +/- 10 U/l), which were further increased seven fold by pre-incubation at 80 degrees C for 30 min. The enzyme (similar to 38 kDa) had an optimum activity at 80 degrees C, but optimum pH varied with substrate used. K-m values for ABTS, SGZ and 2,6-DMP were 142.85 mu M, 10 mu M and 54.55 mu M and the corresponding k(cat) values were 60.6 s(-1), 25.36 s(-1) and 27.84 s(-1), respectively. The t(1/2) values of the rSLAC at 60 degrees C, 70 degrees C, 80 degrees C were 60 h, 32 h and 10 h, respectively. The enzyme deactivation energy (E-d) was 117.275 kJ/mol while Delta G, Delta H and Delta S for thermal inactivation of the rSLAC were all positive. The rSLAC decolourised more than 90% of Brilliant Blue G and Trypan Blue dye in 6 h without the addition of a mediator. High titres of SLAC expressed in P. pastoris enhance its potential for various industrial applications. (C) 2017 Elsevier B.V. All rights reserved.	SCISEARCH/2018:395929

Characterization of a New Glyoxal Oxidase from the Thermophilic Fungus Myceliophthora thermophila M77: Hydrogen Peroxide Production Retained in 5-Hydroxymethylfurfural Oxidation	Seiki Kadowaki, Marco Antonio de Godoy, Mariana Ortiz Kumagai, Patricia Suemy Mort, Andrew Prade, Rolf Alexander (Reprint) Polikarpov, Igor (Reprint) da Costa-Filho, Antonio Jose	Univ Sao Paulo, Sao Carlos Inst Phys, Av Trabalhador Sao Carlense 400, BR-13566590 Sao Carlos, SP, Brazil E-mail: marcokadowaki@gmail.com marianaort@gmail.com patysuemy@gmail.com Oklahoma State Univ, Dept Biochem + Mol Biol, Stillwater, OK 74078 USA (Reprint) E-mail: marcokadowaki@gmail.com andrew.mort@okstate.edu rolf.prade@okstate.edu ipolikarpov@ifsc.usp.br Oklahoma State Univ, Dept Microbiol + Mol Genet, Stillwater, OK 74078 USA E-mail: marcokadowaki@gmail.com Univ Sao Paulo, Ribeirao Preto Sch Philosophy Sci + Literature, Dept Phys, BR-14040901 Ribeirao Preto, Brazil E-mail: ajcosta@usp.br	CATALYSTS, (OCT 2018) Vol. 8, No. 10. ISSN: 2073-4344.	20180101	other	Myceliophthora thermophyla is a thermophilic industrially relevant fungus that secretes an assortment of hydrolytic and oxidative enzymes for lignocellulose degradation. Among them is glyoxal oxidase (MtGLOx), an extracellular oxidoreductase that oxidizes several aldehydes and a-hydroxy carbonyl substrates coupled to the reduction of O-2 to H2O2. This copper metalloprotein belongs to a class of enzymes called radical copper oxidases (CRO) and to the 'auxiliary activities' subfamily AA5_1 that is based on the Carbohydrate-Active enZYmes (CAZy) database. Only a few members of this family have been characterized to date. Here, we report the recombinant production, characterization, and structure-function analysis of MtGLOx. Electron Paramagnetic Resonance (EPR) spectroscopy confirmed MtGLOx to be a radical-coupled copper complex and small angle X-ray scattering (SAXS) revealed an extended spatial arrangement of the catalytic and four N-terminal WSC domains. Furthermore, we demonstrate that methylglyoxal and 5-hydroxymethylfurfural (HMF), a fermentation inhibitor, are substrates for the enzyme.	SCISEARCH/2018:2001267
Efficacy of ionic liquids on the growth and simultaneous xylanase production by Sporotrichum thermophile : membrane integrity, composition and morphological investigation	Sadaf, Ayesha Khare, S. K. (Reprint)	Indian Inst Technol, Dept Chem, Enzyme + Microbial Biochem Lab, New Delhi 110016, India (Reprint) E-mail: skhare@rocketmail.com	RSC ADVANCES, (2017) Vol. 7, No. 34, pp. 21114-21123. ISSN: 2046-2069.	20170101	other	Ionic liquids (ILs) are emerging as promising solvent systems for carrying out various biochemical reactions. The application of whole microbial cells for carrying out catalysis in the presence of ILs is gaining interest due to the advantages of being cost effective and providing a natural environment for enzymes. Present study elucidates the tolerance of a thermophilic fungus, S. thermophile, towards various hydrophilic imidazolium based ILs. [EMIM][OAc], [BMIM][MeSO4] and [BMIM][Otf] were found to be compatible and did not affect the growth and xylanase production whereas [HMIM][BF4] and [BMIM][BF4] were not compatible and inhibited the growth drastically. The impact of two types of the ILs systems, compatible [EMIM][OAc] and non-compatible [BMIM][BF4], on S. thermophile membrane permeability, surface morphology, membrane characteristics and fatty acid composition were studied by SEM, TEM, AFM and FAME analysis. [EMIM][OAc] was tolerated by S. thermophile upto 4.0% (v/v) concentrations and the cellular response in this particular IL was comparable with that of a control. In order to explore the feasibility of using whole cells of S. thermophile in place of its crude xylanase, a study was conducted for in situ pre-treatment and saccharification of [EMIM][OAc] treated wheat straw and a high amount of reducing sugars were released as compared to controls. IL stable cells can thus be utilised for simultaneous pre-treatment and saccharification.	SCISEARCH/2017:923621
Ultrasound-assisted swelling of bacterial cellulose	Song, Ji Eun (Reprint) Kim, Hye Rim Su, Jing Cavaco-Paulo, Artur Loureiro, Ana Martins, Madalena Silva, Carla	Univ Minho, Ctr Biol Engn, Campus Gualtar, P-4710057 Braga, Portugal (Reprint) Sookmyung Womens Univ, Dept Clothing + Text, Seoul 04310, South Korea E-mail: khyerim@sookmyung.ac.kr Jiangnan Univ, Coll Text, Wuxi, Jiangsu, Peoples R China E-mail: carla.silva@ceb.uminho.pt Sookmyung Womens Univ, Dept Clothing + Text, Seoul 04310, South Korea (Reprint)	ENGINEERING IN LIFE SCIENCES, (2017 OCT 2017) Vol. 17, No. 10, pp. 1108-1117. ISSN: 1618-0240.	20170101	other	Bacterial cellulose (BC) was obtained by static cultivation using commercial BC gel from scoby. BC membranes (oven dried and freeze-dried) were swelled with 8% NaOH, in the absence and in the presence of ultrasound (US), for 30, 60, and 90 min. The influence of swelling conditions on both physico-chemical properties and molecules entrapment was evaluated. Considering the highest levels of entrapment, an optimum swelling procedure was established: 8% NaOH for 30min at room temperature in the presence of US. Native and PEGylated laccase from Myceliophthora thermophila was immobilized on BC membranes and a different catalytic behaviour was observed after immobilization. Native laccase presented activity values similar to published reports (5-7U/gBC) after immobilization whereas PEGylated enzymes showed much lower activity (1-2U/gBC). BC swelled membranes are presented herein as a potential support for the preparation of immobilized enzymes for industrial applications, like phenolics polymerization.	SCISEARCH/2017:1826246
Expression of a novel recombinant cyanate hydratase (rTI-Cyn) in Pichia pastoris, characteristics and applicability in the detoxification of cyanate	Ranjan, Bibhuti (Reprint) Pillai, Santhosh Permaul, Kugenthiren Singh, Suren	E-mail: singhs@dut.ac.za Durban Univ Technol, Fac Appl Sci, Dept Biotechnol + Food Technol, ZA-4000 Durban, South Africa (Reprint)	BIORESOURCE TECHNOLOGY, (2017 AUG 2017) Vol. 238, pp. 582-588. ISSN: 0960-8524.	20170101	other	A recombinant Pichia pastoris harbouring the cyanate hydratase gene (rTI-Cyn) from the thermophilic fungus Thermomyces lanuginosus SSBP yielded a high titre of extracellular cyanate hydratase (100 +/- 13 U mL(-1)) which was similar to 10-fold higher than the native fungal strain. The purified rTI-Cyn had a molecular mass of similar to 20 kDa on SDS-PAGE, with K-m, V-max, k(cat) and k(cat)/K-m values of 0.34 mM, 2857.14 mu moles mg(-1) min(-1), 2.14 x 10(4) s(-1) and 6.3 x 10(7) M-1 s(-1), respectively. Its properties of thermostability, pH stability, and heavy metals insensitivity, make it a suitable candidate for bioremediation in extreme environments. The rTI-Cyn was able to degrade toxic cyanate completely with the liberation of ammonia, which was confirmed by FTIR analysis. This is the first report of any known cyanate hydratase that has been expressed in P. pastoris, characterized and effectively evaluated for cyanate detoxification . (C) 2017 Elsevier Ltd. All rights reserved.	SCISEARCH/2017:1127134

Heterologous production of extreme alkaline thermostable NAD(+)-dependent formate dehydrogenase with wide-range pH activity from Myceliophthora thermophila	Altas, Nilay Aslan, Askin Sevinc Karatas, Ersin Chronopoulou, Evangelia Labrou, Nikolaos E. Binay, Baris (Reprint)	Yildiz Tech Univ, Fac Sci + Letters, Dept Chem, TR-34210 Istanbul, Turkey Gebze Tech Univ, Dept Chem, TR-41400 Gebze, Kocaeli, Turkey Gebze Tech Univ, Dept Mol Biol + Genet, TR-41400 Gebze, Kocaeli, Turkey Agr Univ Athens, Lab Enzyme Technol, Dept Biotechnol, Sch Food Biotechnol + Dev, 75 Iera Odos St, GR-11855 Athens, Greece Gebze Tech Univ, Dept Bioengn, TR-41400 Gebze, Kocaeli, Turkey (Reprint) E-mail: binay@gtu.edu.tr	PROCESS BIOCHEMISTRY, (OCT 2017) Vol. 61, pp. 110-118. ISSN: 1359-5113.	20170101	other	NAD(+)-dependent formate dehydrogenase(s) (EC 1.2.1.2, FDH) catalyzes the interconversion of formate anion to carbon dioxide coupled with the conversion of NAD(+) or NADH. FDHs attract significant attention in biotechnology due to their potential applications in NAD(H)-dependent industrial biocatalysis as well as in the production of renewable fuels and chemicals from carbon dioxide. In the present work, a new FDH from thermophilic fungus Myceliophthora thermophik (MtFDH) was characterized. The gene of the enzyme was synthesised, cloned, expressed in E. colt, as 6His-tagged protein, and purified to homogeneity by metal chelate affinity chromatography. Kinetic analysis suggested that MtFDH exhibits higher catalytic efficiency on NaHCO3 compared to formate. Notable, recombinant MtFDH displays a pH optimum for the conversion of formate anion to carbon dioxide at extreme alkaline pH (pH 10.5). Thermal stability analysis showed that the enzyme displays good thermostability with T-m 48 degrees C. Homology modelling and phylogenetic analysis suggested that the enzyme belongs to the D-specific 2-hydroxy acid dehydrogenases family. The active-site residues are well conserved compared to other homologous FDHs. The results of the present work provide new knowledge on the structure, function and diversity of FDHs and indicate that MtFDH possess a huge potential for CO2 reduction or NADH generation and under extreme alkaline conditions.	SCISEARCH/2017:1916648
Biochemical Characterization of a Family 15 Carbohydrate Esterase from a Bacterial Marine Arctic Metagenome	De Santi, Concetta Willassen, Nils Peder Williamson, Adele (Reprint)	UiT Arctic Univ Norway, Norwegian Struct Biol Ctr, Dept Chem, Tromso, Norway (Reprint) E-mail: adele.k.williamson@uit.no	PLOS ONE, (19 JUL 2016) Vol. 11, No. 7. ISSN: 1932-6203.	20160101	other	Background The glucuronoyl esterase enzymes of wood-degrading fungi (Carbohydrate Esterase family 15; CE15) form part of the hemicellulolytic and cellulolytic enzyme systems that break down plant biomass, and have possible applications in biotechnology. Homologous enzymes are predicted in the genomes of several bacteria, however these have been much less studied than their fungal counterparts. Here we describe the recombinant production and biochemical characterization of a bacterial CE15 enzyme denoted MZ0003, which was identified by in silico screening of a prokaryotic metagenome library derived from marine Arctic sediment. MZ0003 has high similarity to several uncharacterized gene products of polysaccharide-degrading bacterial species, and phylogenetic analysis indicates a deep evolutionary split between these CE15s and fungal homologs. Results MZ0003 appears to differ from previously-studied CE15s in some aspects. Some glucuronoyl esterase activity could be measured by qualitative thin-layer chromatography which confirms its assignment as a CE15, however MZ0003 can also hydrolyze a range of other esters, including p-nitrophenyl acetate, which is not acted upon by some fungal homologs. The structure of MZ0003 also appears to differ as it is predicted to have several large loop regions that are absent in previously studied CE15s, and a combination of homology-based modelling and site-directed mutagenesis indicate its catalytic residues deviate from the conserved Ser-His-Glu triad of many fungal CE15s. Taken together, these results indicate that potentially unexplored diversity exists among bacterial CE15s, and this may be accessed by investigation of the microbial metagenome. The combination of low activity on typical glucuronoyl esterase substrates, and the lack of glucuronic acid esters in the marine environment suggest that the physiological substrate of MZ0003 and its homologs is likely to be different from that of related fungal enzymes.	SCISEARCH/2016:1406254
Characteristics and Applicability of Phytase of the Yeast Pichia anomala in Synthesizing Haloperoxidase	Satyanarayana, Tulasi (Reprint) Joshi, Swati	Univ Delhi, Dept Microbiol, South Campus, New Delhi 110021, India (Reprint) E-mail: tsnarayana@gmail.com Univ Delhi, Dept Microbiol, New Delhi 110021, India E-mail: swati.joshi.aj@gmail.com tsnarayana@gmail.com	APPLIED BIOCHEMISTRY AND BIOTECHNOLOGY, (JUL 2015) Vol. 176, No. 5, pp. 1351-1369. ISSN: 0273-2289.	20150101	other	The phytase of the yeast Pichia anomala is a histidine acid phosphatase based on signature sequences and catalytic amino acids identified by site-directed mutagenesis . Among modulators, N-bromosuccinimide and butanedione inhibit phytase, while Ca2+ and Ni2+ stimulate slightly. Vanadate exhibits competitive inhibition of phytase, making it bifunctional to act as haloperoxidase. Molecular docking supports vanadate to share its binding site with phytate. The T (1/2), activation energy (E (a)), temperature quotient (Q (10)), activation energy of thermal inactivation (E-d), and enthalpy (Delta H (d) (0)) of the enzyme are 4.0 min (80 A degrees C), 27.72 kJ mol(-1), 2.1, 410.62 kJ mol(-1), and 407.8 kJ mol(-1) (65-80 A degrees C), respectively. The free energy of the process (Delta G (d) (o)) increases from 49.56 to 71.58 kJ mol(-1) with rise in temperature, while entropy of inactivation (Delta S (d) (0)) remains constant at 1.36 kJ mol(-1) K-1. The supplementation of whole wheat dough with rPPHY resulted in 72.5 % reduction in phytic acid content of bread. These characteristics confirm that the phytase has adequate thermostability for its applicability as a food and feed additive.	SCISEARCH/2015:1218167
Widening the pH Activity Profile of a Fungal Laccase by Directed Evolution	Torres-Salas, Pamela (Reprint) Mate, Diana M. Ghazi, Iraj Plou, Francisco J. Ballesteros, Antonio O. Alcalde, Miguel	CSIC, Inst Catalysis, Dept Biocatalysis, Marie Curie St 2, Madrid 28049, Spain (Reprint) CSIC, Inst Catalysis, Dept Biocatalysis, Madrid 28049, Spain E-mail: malcalde@icp.csic.es	CHEMBIOCHEM, (27 2013 MAY 2013) Vol. 14, No. 8, pp. 934-937. ISSN: 1439-4227.	20130101	other		SCISEARCH/2013:786744

Combinatorial approach of statistical optimization and mutagenesis for improved production of acidic phytase by Aspergillus niger NCIM 563 under submerged fermentation condition	Bhavsar, K. Gujar, P. Shah, P. Khire, J. M. (Reprint) Kumar, V. Ravi	Natl Chem Lab, NCIM Resource Ctr, Pune 411008, Maharashtra, India (Reprint) E-mail: jmkhire@yahoo.com Natl Chem Lab, Chem Engn + Proc Dev Div, Pune 411008, Maharashtra, India	APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, (JAN 2013) Vol. 97, No. 2, pp. 673-679. ISSN: 0175-7598.	20130101	other	Combination of statistical optimization and mutagenesis to isolate hypersecretory strains is studied to maximize phytase production from Aspergillus niger NCIM 563 under submerged fermentation. The overall results obtained show a remarkable 5.98-fold improvement in phytase production rates when compared to that using basal medium. Optimization of culture conditions from parent strain is studied first by the Plackett-Burman technique to evaluate the effects of 11 variables for phytase production. The results showed that glucose, MgSO4, KCl, incubation period, and MnSO4 are the most significant variables affecting enzyme production. Further optimization in these variables, using a central composite design technique, resulted in 3.74-fold increase in the yield of phytase production to 254,500 U/l when compared with the activity observed with basal media (68,000 U/l) in shake flask. Our experiments show that the phytase from A. niger NCIM 563 exhibits desirable activity in simulated gastric fluid conditions with low pH and also improved thermostability when compared to commercial phytase. The improved yield demonstrates the potential applicability of phytase enzyme as a source of phytase supplement for phosphorus nutrition and environmental protection in animal feed industry. Physical and chemical mutagenesis experiments were carried out in parallel to isolate hypersecretory mutants that could possibly further enhance the enzyme production. Using optimized media conditions of the parent strain, our results show that mutant strain A. niger NCIM 1359 increased the phytase activity by another 1.6-fold to 407,200 U/l.	SCISEARCH/2013:193567
Corynascus verrucosus, second record for science and new for India	Sharma, Rohit (Reprint) Shouche, Yogesh S. Kulkarni, Girish	Natl Ctr Cell Sci, Pune, Maharashtra, India (Reprint) E-mail: rohit@nccs.res.in Natl Ctr Cell Sci, Mol Biol Unit, Pune, Maharashtra, India	NOVA HEDWIGIA, (2013) Vol. 97, No. 3-4, pp. 485-494. ISSN: 0029-5035.	20130101	other	Colynascus verrucosus, a member of Chaetomiaceae, is described and illustrated as second record for science and new to India. It was isolated as endophyte from flower bud of Jatropha podagrica. It is characterized by non-ostiolate ascomata, peridium with brown appendages and ellipsoidal ascospores with acute ends and two sub-terminal to oblique germ pores. Its anamorph was also produced on all media tested. The ascomata and ascospores are somewhat smaller than those described for the holotype of this species. The morphological identification is supported by ITS DNA sequence data.	SCISEARCH/2013:1680481
Role of the C-terminus of Pleurotus eryngii Ery4 laccase in determining enzyme structure, catalytic properties and stability	Grieco, Francesco (Reprint) Bleve, Gianluca Spagnolo, Stefano Mita, Giovanni Lezzi, Chiara Rampino, Patrizia Tasco, Gianluca Casadio, Rita Tufariello, Maria	CNR, ISPA, Via Monteroni, I-73100 Lecce, Italy (Reprint) E-mail: francesco.grieco@ispa.cnr.it CNR, ISPA, I-73100 Lecce, Italy E-mail: francesco.grieco@ispa.cnr.it Univ Salento, Dipartimento Sci + Tecnol Biol + Ambientali, I-73100 Lecce, Italy Univ Bologna, Dipartimento Biol Evoluzionist Sperimentale, I-40126 Bologna, Italy CNR, IMM, I-73100 Lecce, Italy	PROTEIN ENGINEERING DESIGN + SELECTION, (JAN 2013) Vol. 26, No. 1, pp. 1-13. ISSN: 1741-0126.	20130101	other	The ERY4 laccase gene of Pleurotus eryngii is not biologically active when expressed in yeast. To explain this finding, we analysed the role of the C-terminus of Ery4 protein by producing a number of its different mutant variants. Two different categories of ERY4 mutant genes were produced and expressed in yeast: (i) mutants carrying C-terminal deletions and (ii) mutants carrying different site-specific mutations at their C-terminus. Investigation of the catalytic properties of the recombinant enzymes indicated that each novel variant acquired different affinities and catalytic activity for various substrates. Our results highlight that C-terminal processing is fundamental for Ery4 laccase enzymatic activities allowing substrate accessibility to the enzyme catalytic core. Apparently, the last 18 amino acids in the C-terminal end of the Ery4 laccase play a critical role in enzyme activity, stability and kinetic and, in particular biochemical and structural data indicate that the K532 residue is fundamental for enzyme activation. These studies shed light on the structure/function relationships of fungal laccases and will enhance the development of biotechnological strategies for the industrial exploitation of these enzymes.	SCISEARCH/2013:74473
Polymerization study of the aromatic amines generated by the biodegradation of azo dyes using the laccase enzyme	Franciscon, Elisangela (Reprint) Piubeli, Francine de Menezes, Cristiano Ragagnin Silva, Isis Serrano Durrant, Lucia Regina Cavaco-Paulo, Artur Fantinatti-Garboggini, Fabiana Grossman, Matthew James	Univ Estadual Campinas, Dept Food Sci, BR-13083970 Campinas, SP, Brazil (Reprint) E-mail: elisfran@fea.unicamp.br Univ Minho, Dept Text Engn, P-4800058 Guimaraes, Portugal Univ Estadual Campinas, Chem Biol + Agr Pluridisciplinary Res Ctr CPQBA, BR-13083970 Campinas, SP, Brazil BioSage, Lawrenceville, NJ 08648 USA	ENZYME AND MICROBIAL TECHNOLOGY, (5 APR 2010) Vol. 46, No. 5, pp. 360-365. ISSN: 0141-0229.	20100101	other	Four different azo dyes were decolorized (color reduction >90%) by bacteria isolated from a textile wastewater effluent. Dye decolorizing was carried out under microaerobic conditions until completion, after which the aromatic amine concentration was determined. A laccase from Myceliophthora thermophila was used to catalyze coupling reactions of the aromatic amines produced from decolorizing the dyes. The reaction was carried out with stirring (100 rpm) in a weak acidic buffer solution (pH 5.0) at 45 degrees C for 3 days. The presence of aromatic amines in the samples after bacterial decolorizing confirmed the azo bond was reduced in the process. In addition, the UV-vis spectrum was shifted significantly after the sequential bacterial-laccase treatment also indicating a chemical transformation of the dyes. After laccase treatment the solutions formed colored soluble and precipitated products. The particles sizes making up the precipitates formed after laccase treatment varied between 105 and 483 nm as determined by Photon Correlation Spectroscopy (PCS). The laccase treatment also reduced the COD of the dye solutions by similar to 20%. We show that successive bacterial-laccase treatment is effective in decolorized azo dyes by reduction of the azo bonds, and promoting coupling reactions between the aromatic amines formed. Promoting coupling reactions between the aromatic amines using enzymes may prove useful for the physical removal and reuse of these amines. (C) 2010 Published by Elsevier Inc.	SCISEARCH/2010:317171
Activation of laccase by penicillin and derivatives	Xu, Feng (Reprint) Ransbarger, Darron	Novozymes Inc, 1445 Drew Ave, Davis, CA 95616 USA (Reprint) Novozymes Inc, Davis, CA 95616 USA E-mail: fxu@novozymes.com	PROCESS BIOCHEMISTRY, (SEP 2006) Vol. 41, No. 9, pp. 2082-2086. ISSN: 1359-5113.	20060101	other	Capable of oxidizing a wide range of reducing substances, laccase has a great potential for various biocatalytic applications. Extensive research effort is being made for improving laccase's activity, specificity, and stability. We observed that pre-incubating with penicillin led to a significant activation of a Rhizoctonia solani laccase, although penicillin was not a redox substrate of the enzyme. The activation impacted mainly the k(cat) of the laccase, and the effect was optimal at pH 5. Ampicillin and penicilloic acid were also active on the laccase (although their effect were weaker than that of penicillin), but 6-aminopenicillanic acid, phenylacetic acid, and cephalexin were not active. The apparent effect from penicillin was attributed to a non-redox interaction with the polypeptide backbone of the laccase. The effect was significantly weaker on Myceliophthora thermophila and Trametes villosa laccases. (c) 2006 Elsevier Ltd. All rights reserved.	SCISEARCH/2006:827264

Thermal denaturation of a blue-copper laccase: Formation of a compact denatured state with residual structure linked to pH changes in the region of histidine protonation	Toledo-Nunez, Citlali Hernandez-Arana, Andres Lopez-Cruz, Javier I.	Departamento de Quimica, Area de Biofisicoquimica, Universidad Autonoma Metropolitana-Iztapalapa, D.F. 09340, Mexico (MX)) Departamento de Biotecnologia, Universidad Autonoma Metropolitana-Iztapalapa, D.F. 09340, Mexico (MX))	Biophysical Chemistry (Jun 2012) Volume 167, pp. 36-42, 43 refs. CODEN: BICIAZ ISSN: 0301-4622 E-ISSN: 1873-4200 DOI: 10.1016/j.bpc.2012.04.004 Published by: Elsevier B.V.,	20120101	other	The partial (absolute) heat capacity of a laccase enzyme from Myceliophthora thermophila (MtL) was determined from calorimetric scans in the 4.5-10.0 pH range. Above pH 7.5, the heat capacity of the thermally denatured state (C p D) of this blue-copper glycoprotein is consistent with that for an unfolded, fully solvated polypeptide chain, if its carbohydrate content is taken into account. Below pH 7.5, C p D decreases and eventually levels off within the 5.5-4.5 pH region, where a compact, partially solvated denatured state is formed. In the compact state, denatured MtL is an oligomer, and exhibits considerable native-like secondary structure and a perturbed environment of its copper atoms. Analysis of the pH dependence of C p D and the content of secondary structure gives results implying that His residues play an important role in the stability of the compact denatured state. .COPYRGT. 2012 Elsevier B.V. All rights reserved.	ESBIOBASE/2012196481
Growth of Trichoderma harzianum and Myceliophthora thermophila in palm oil sludge	Vikineswary, S. Ravoof, A.A. Kuthubutheen, A.J.	Institute of Advanced Studies, University of Malaya, 50603 Kuala Lumpur (MY)) Department of Botany, Faculty of Science, University of Malaya, (MY))	World Journal of Microbiology and Biotechnology (1997) Volume 13, Number 2, pp. 189-194, 21 refs. CODEN: WJMBEY ISSN: 0959-3993 DOI: 10.1023/A:1018541831195 Published by: Kluwer Academic Publishers,	19970101	other	Uncentrifuged palm oil mill sludge (POS) diluted to about 50% (v/v in tap water) supports good mycelial growth of Myceliophthora thermophila and Trichoderma harzianum. Both of the selected fungi are non-toxic to mice. After 24 h M. thermophila grown in batch culture in POS yielded 28.6 g/l of mycelial biomass with biological oxygen demand (BOD) and chemical oxidation demand (COD) reductions of 72% and 74% respectively. T. harzianum yielded 24.4 g/l of mycelial biomass with BOD and COD reductions of 67% and 68% respectively. The crude protein of the mycelial biomass of M. thermophila and T. harzianum was twice that of untreated POS. T. harzianum showed amylolytic activity while M. thermophila was cellulolytic and lipolytic.	ESBIOBASE/1997093993
Upgrading of efficient and scalable CRISPR-Cas-mediated technology for genetic engineering in thermophilic fungus Myceliophthora thermophila	Liu, Qian Zhang, Yongli Li, Fangya Li, Jingen Sun, Wenliang Tian, Chaoguang		Biotechnology for Biofuels, Vol. 12, pp. 1-19, 20190101 E-ISSN: 1754-6834 DOI: 10.1186/s13068-019-1637-y Published by: BioMed Central, London		other	Background Thermophilic filamentous fungus Myceliophthora thermophila has great capacity for biomass degradation and is an attractive system for direct production of enzymes and chemicals from plant biomass. Its industrial importance inspired us to develop genome editing tools to speed up the genetic engineering of this fungus. First-generation CRISPR-Cas9 technology was developed in 2017 and, since then, some progress has been made in thermophilic fungi genetic engineering, but a number of limitations remain. They include the need for complex independent expression cassettes for targeting multiplex genomic loci and the limited number of available selectable marker genes. Results In this study, we developed an Acidaminococcus sp. Cas12a-based CRISPR system for efficient multiplex genome editing, using a single-array approach in M. thermophila. These CRISPR-Cas12a cassettes worked well for simultaneous multiple gene deletions/insertions. We also developed a new simple approach for marker recycling that relied on the novel cleavage activity of the CRISPR-Cas12a system to make DNA breaks in selected markers. We demonstrated its performance by targeting nine genes involved in the cellulase production pathway in M. thermophila via three transformation rounds, using two selectable markers neo and bar. We obtained the nonuple mutant M9 in which protein productivity and lignocellulase activity were 9.0- and 18.5-fold higher than in the wild type. We conducted a parallel investigation using our transient CRISPR-Cas9 system and found the two technologies were complementary. Together we called them CRISPR-Cas-assisted marker recycling technology (Camr technology). Conclusions Our study described new approaches (Camr technology) that allow easy and efficient marker recycling and iterative stacking of traits in the same thermophilic fungus strain either, using the newly established CRISPR-Cas12a system or the established CRISPR-Cas9 system. This Camr technology will be a versatile and efficient tool for engineering, theoretically, an unlimited number of genes in fungi. We expect this advance to accelerate biotechnology-oriented engineering processes in fungi.	PQSCITECH/2020:22576
Optimization of Transesterification Reactions with CLEA-Immobilized Feruloyl Esterases from Thermothelomyces thermophila and Talaromyces wortmannii	Zerva, Anastasia Antonopoulou, Io Enman, Josefine Iancu, Laura Juetten, Peter Rova, Ulrika Christakopoulos, Paul		Molecules, Vol. 23, No. 9, 20180101 E-ISSN: 1420-3049 DOI: 10.3390/molecules23092403 Published by: MDPI AG, Basel		other	Feruloyl esterases (FAEs, E.C. 3.1.1.73) are biotechnologically important enzymes with several applications in ferulic acid production from biomass, but also in synthesis of hydroxycinnamic acid derivatives. The use of such biocatalysts in commercial processes can become feasible by their immobilization, providing the advantages of isolation and recycling. In this work, eight feruloyl esterases, immobilized in cross-linked enzyme aggregates (CLEAs) were tested in regard to their transesterification performance, towards the production of prenyl ferulate (PFA) and arabinose ferulate (AFA). After solvent screening, comparison with the activity of respective soluble enzymes, and operational stability tests, FAE125 was selected as the most promising biocatalyst. A central composite design revealed the optimum conditions for each transesterification product, in terms of water content, time, and substrate ratio for both products, and temperature and enzyme load additionally for prenyl ferulate. The optimum product yields obtained were 83.7% for PFA and 58.1% for AFA. FAE125 CLEAs are stable in the optimum conditions of transesterification reactions, maintaining 70% residual activity after five consecutive reactions. Overall, FAE125 CLEAs seem to be able to perform as a robust biocatalyst, offering satisfactory yields and stability, and thus showing significant potential for industrial applications.	PQSCITECH/2019:464973

Combined genome and transcriptome sequencing to investigate the plant cell wall degrading enzyme system in the thermophilic fungus Malbranchea cinnamomea	Huttner, Silvia Nguyen, Thanh Thuy Granchi, Zoraide Chin-A-Woeng, Thomas Ahren, Dag Larsbrink, Johan Vu Nguyen Thanh Olsson, Lisbeth		Biotechnology for Biofuels, Vol. 10, 20170101 E-ISSN: 1754-6834 DOI: 10.1186/s13068-017-0956-0 Published by: BioMed Central, London		other	(b) (4)	PQSCITECH/2018:199811
Myceliophthora thermophila lytic polysaccharide monooxygenase mutant and application in cellulose degradation	Liu, Fufeng Guo, Xiao An, Yajing Chai, Chengcheng Lu, Fuping	ASSIGNEE: Tianjin University of Science + Technology	(2019) Faming Zhuanli Shenqing, 12pp. CODEN: CNXXEV.	20190101	other	(b) (4)	TOXCENTER/2019:634959
One kind of cadmium pollution of biological repair agent and preparation method thereof [Machine Translation].	Xing, Jun Gao, Xueyan Zhang, Jian	ASSIGNEE: Tianjin Tianfeng Zetian Biotechnology Co., Ltd.	(2019) Faming Zhuanli Shenqing, 5pp. CODEN: CNXXEV.	20190101	other	(b) (4)	TOXCENTER/2019:212835
Preparation method of biological pigsty deodorant	Su, Jinping	ASSIGNEE: Changsha Manwang Bioengineering Co., Ltd.	(2019) Faming Zhuanli Shenqing, 4pp. CODEN: CNXXEV.	20190101	other	(b) (4)	TOXCENTER/2019:15997

Method for preparing organic fertilizer special for tobacco	Yuan, Cheng Li, Baocai Ren, Wanyun Chen, Chaochao Zeng, Tianzhu Pu, Xinjiang	ASSIGNEE: Yunnan Shangcheng Biotechnology Co., Ltd.	(2017) Faming Zhuanli Shenqing, 7pp. CODEN: CNXXEV.	20170101	other	(b) (4)	TOXCENTER/2017:415885
Preparation method of tea tree-specific organic fertilizer	Yuan, Cheng Ren, Wanyun Li, Baocai Chen, Chaochao Zeng, Tianzhu Pu, Xinjiang	ASSIGNEE: Yunnan Shangcheng Biotechnology Co., Ltd.	(2017) Faming Zhuanli Shenqing, 7pp. CODEN: CNXXEV.	20170101	other	(b) (4)	TOXCENTER/2017:405673
Organic fertilizer special for tea leaf and its preparation method	Du, Wei Zhang, Jiecun Han, Yongcai Zhao, Yanzhou Li, Ruoqing Tu, Jijun Xiu, Kui Li, Zhi Pei, Zhijun	ASSIGNEE: Xuancheng Nanyang Biotechnology Co., Ltd.	(2016) Faming Zhuanli Shenqing, 5pp. CODEN: CNXXEV.	20160101	other	(b) (4)	TOXCENTER/2016:359633

Green bio-organic fertilizer produced by multifunctional decomposing inoculant and natural organic materials [Machine Translation].	He, Zongjun Liang, Haitian Zhao, Linna Tian, Yang	ASSIGNEE: Tianjin Institute of Agricultural Resources and Environmental Sciences	(2014) Faming Zhuanli Shenqing, 13pp. CODEN: CNXXEV.	20140101	other	(b) (4)	TOXCENTER/2014:307170
Increasing the effectiveness of filamentous fungal saccharification of lignocellulosic biomass by selective removal of inhibition-sensitive enzymes	Emalfarb, Mark Sinitsyn, Arkady Jundzil, Richard Wery, Jan Visser, Jacob Joosten, Rob Koetsier, Martijn	ASSIGNEE: Dyadic International (Usa) Ltd.	(2013) PCT Int. Appl., 93pp. CODEN: PIXXD2.	20130101	other	(b) (4)	TOXCENTER/2013:462992
Endoglucanase 1B variants with increased catalytic activity towards cellulosic biomass and their uses	Ang, Ee Lui Eberhard, Ellen Zhang, Xiyun Zhang, Wei Tian, Jing Smith, Derek Mitchell, Vesna	ASSIGNEE: Codexis, Inc.	(2013) PCT Int. Appl., 89pp. CODEN: PIXXD2.	20130101	other	(b) (4)	TOXCENTER/2013:311825
Generation of protein variants by functional domain-specific mutagenesis and DNA shuffling	Clark, Louis Choudhary, Trish	ASSIGNEE: Codexis, Inc.	(2013) PCT Int. Appl., 73pp. CODEN: PIXXD2.	20130101	other	(b) (4)	TOXCENTER/2013:39172
Substitution variants of a GH61 glycoside hydrolase of Mycelophthora with increased catalytic activity for use in the saccharification of cellulosic biomass	Yang, Jie Zhang, Xiyun Yoon, Jungjoo Rao, Kripa Grate, John H. Elgart, David Baidyaroy, Dipnath	ASSIGNEE: Codexis, Inc.	(2013) U.S. Pat. Appl. Publ., 251pp., Cont.-in-part of U.S. Ser. No. 215,193. CODEN: USXXCO.	20130101	other	(b) (4)	TOXCENTER/2013:116355
Substitution variants of cellobiohydrolase with modified catalytic and carbohydrate-binding domains for processing of cellulosic substrates	Montalibet, Jacqueline Gudynaite-Savitch, Loreta Hill, Christopher Hindle, Christopher D. Lavigne, James A. Masri, Nabil Tahna, Fuad Tomashek, John J.	ASSIGNEE: logen Energy Corporation	(2013) U.S. Pat. Appl. Publ., 48pp. CODEN: USXXCO.	20130101	other		TOXCENTER/2013:116379
Method for preparing commercial organic matter decomposing agent via microbial fermentation culture	Zhang, Xinxiong Mao, Guangping	ASSIGNEE: Dongguan Baode Biological Engineering Co., Ltd.	(2013) Faming Zhuanli Shenqing, 28pp. CODEN: CNXXEV.	20130101	other		TOXCENTER/2013:402304

Fermentation broth for urban and rural garbage and its preparation method	Leng, Dengshu		(2011) Faming Zhuanli Shenqing, 7pp. CODEN: CNXXEV.	20110101	other	(b) (4)	TOXCENTER/2011:457986
Method for treating sludge containing limit-exceeded heavy metals by fermenting	Lou, Binfei Sun, Xianfeng	ASSIGNEE: Xi'an Polytechnic University	(2010) Faming Zhuanli Shenqing, 7pp. CODEN: CNXXEV.	20100101	other		TOXCENTER/2010:198996
Laccase for detoxification of feed products	Nielsen, Anders Vikso Sorensen, Birthe Hauerbach	ASSIGNEE: Novozymes A/S	(2009) U.S. Pat. Appl. Publ., 9pp. CODEN: USXXCO.	20090101	other		TOXCENTER/2009:276480
Three-dimensional structure of galactanases for design of enzyme variants with improved properties	De Maria, Leonardo Svendsen, Allan Borchert, Torben Vedel Christensen, Lars Lehmann Hylling Larsen, Sine Ryttersgaard, Carsten	ASSIGNEE: Novozymes A/S	(2004) PCT Int. Appl., 262 pp. CODEN: PIXXD2.	20040101	other		TOXCENTER/2004:165362
Three-dimensional structure of Coprinus cinereus laccase and the design of mutants with improved properties for industrial uses	Svendsen, Allan Xu, Feng	ASSIGNEE: Novo Nordisk A/S	(1998) PCT Int. Appl., 148 pp. CODEN: PIXXD2.	19980101	other		TOXCENTER/1998:186097
Myceliophthora and Scytalidium laccase mutants with improved properties for industrial use	Svendsen, Allan	ASSIGNEE: Novo Nordisk A/S	(1998) PCT Int. Appl., 34 pp. CODEN: PIXXD2.	19980101	other		TOXCENTER/1998:186096
Design of laccase variants having improved stability based on the three-dimensional structure of Coprinus cinereus laccase	Pedersen, Anders Hjelholt Svendsen, Allan Schneider, Palle Rasmussen, Grethe Cherry, Joel R.	ASSIGNEE: Novo Nordisk A/S	(1998) PCT Int. Appl., 168 pp. CODEN: PIXXD2.	19980101	other		TOXCENTER/1998:159126
Myceliophthora and Scytalidium laccase variants having improved stability	Pedersen, Anders Hjelholt Svendsen, Allan Schneider, Palle Rasmussen, Grethe Cherry, Joel R.	ASSIGNEE: Novo Nordisk A/S	(1998) PCT Int. Appl., 31 pp. CODEN: PIXXD2.	19980101	other		TOXCENTER/1998:159125

Microbe and microbial product for fast degradation of agricultural waste solid	He, Cexi Xiao, Wenlin He, Lin		(1998) Faming Zhuanli Shenqing, 13 pp. CODEN: CNXXEV.	19980101	other	(b) (4)	TOXCENTER/2000:113840
Detoxification of tellurium in Aspergillus fumigatus and Sporotrichum thermophile	Hussein, H. El-Sheikh	Botany + Microbiology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt.	Al-Azhar Bulletin of Science, (1996) Vol. 7, No. 1, Pt. 2, pp. 557-568. CODEN: ABSCE7. ISSN: 1110-2535.	19960101	other	(b) (4)	TOXCENTER/1997:218195
THE EFFECTS OF FUNGICIDES ON THE GROWTH RATES OF THERMOPHILOUS FUNGI	KUTHUBUTHEEN A J [Reprint author] PUGH G J F	DEP BOT, UNIV MALAYA, KUALA LUMPUR, MALAYS	Mycopathologia, (1977) Vol. 62, No. 2, pp. 67-76. CODEN: MYCPAH. ISSN: 0301-486X.	19770101	other	The effects of the fungicides captan, dicloran, thiram, Verdasan and mercuric chloride (HgCl2) on the radial and mycelial growth rates of thermophilous fungi isolated from living green leaves were studied. On control plates Thermoascus aurantiacus and Torula thermophila had the highest radial growth rates; Malbranchea pulchella var. sulfurea and Talaromyces duponti had the lowest growth rates. Good sporulation was seen on all control plates. With increasing concentrations of fungicides the growth rates were correspondingly retarded and sporulation was reduced. At higher fungicide concentrations the hyphal tips and young hyphae were thicker and warty or granulated. Aspergillus and Mucor pusillus were the most tolerant species; they were capable of growth in more concentrations of fungicides compared with the other species. T. duponti and Thermomyces lanuginosus were the most sensitive species. Most marked reductions in growth rates were produced by thiram and Verdasan. All 10 thermophilous species studied were capable of growth with more concentrations of HgCl2 than with the organo mercurial Verdasan. Six species studied showed higher radial growth rates compared with the 2 fastest growing mesophilic species studied, Trichoderma viride and Zygorhynchus moelleri. While M. pusillus was capable of growth in dry wt at 0.05 ppm of all 5 fungicides, Sporotrichum thermophile did not show mycelial growth with Verdasan at 0.05 ppm. T. lanuginosus showed reduced growth rates with thiram and no mycelial growth with HgCl2 and Verdasan.	TOXCENTER/1978:56404
THERMOPHILOUS FUNGI PART 1 CONTRIBUTION TO THEIR ISOLATION THEIR METABOLISM AND THEIR ABILITY TO DEGRADE ORGANIC MATTER	JODICE R FERRARA R CERUTI SCURTI J FIUSELLO N OBERT F CORTELLEZZI G C		Allionia (Turin), Vol. 20, pp. 53-73. 1974-1975. CODEN: ALLIAM. ISSN: 0065-6429.		other		TOXCENTER/1976:57856
Improving cellulases production by Myceliophthora thermophila through disruption of protease genes.	Li Xiaolin He Qun Liu Qian Sun Wenliang Tian Chaoguang	State Key Laboratory of Agrobiotechnology and MOA Key Laboratory of Soil Microbiology, College of Biological Sciences, China Agricultural University, Beijing, 100193, China. qunhe@cau.edu.cn Key Laboratory of Systems Microbial Biotechnology, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin, 300308, China. tian_cg@tib.cas.cn	Biotechnology letters, (2020 Feb) Vol. 42, No. 2, pp. 219-229. Electronic Publication Date: 4 Dec 2019 Journal code: 8008051. E-ISSN: 1573-6776. L-ISSN: 0141-5492.	20200101	duplicates	OBJECTIVE: To identify main protease genes for the proteolytic degradation of cellulases in M. thermophila and generate a lower-proteases fungal host that can be used for further metabolic engineering to increase cellulase production and heterologous protein expression. RESULTS: Systematic transcriptomic analysis were conducted on the expression of proteases genes in M. thermophila genome and five highly expressed genes encoding extracellular proteases were selected for mutation analyses. A series of single- and multi-gene mutants of these five selected genes was constructed using the CRISPR-Cas9 technique. Compared with WT, the .DELTA.Mtalp1 and the quintuple mutant showed significantly lower protease activity (decreased 52.7% and 58.4%, respectively) and at least double enhanced cellulase production. CONCLUSIONS: The results indicated that Mtalp1 is a critical protease gene in cellulase degradation in M. thermophila and disruption of protease genes showed significantly decreased protease activity and obviously enhanced cellulase production in the fermentation broth of .DELTA.Mtalp1 and the quintuple mutant.	MEDLINE/2021866634

Thermothelomyces thermophila human infections .	Nourrisson C Garcia-Hermoso D Morio F Kauffmann-Lacroix C Berrette N Bonhomme J Poirier P Lortholary O	Laboratoire de Parasitologie-Mycologie, CHU Clermont-Ferrand, Clermont-Ferrand, France, Clermont Universite, Universite Blaise Pascal, Laboratoire Microorganismes, Genome et Environnement, Clermont-Ferrand, France. Institut Pasteur, Unite de Mycologie Moleculaire, Centre National de Reference Mycoses Invasives et Antifongiques, Paris, France, CNRS URA3012, Paris, France. Laboratoire de Parasitologie-Mycologie, CHU de Nantes, Nantes, France, Departement de Parasitologie et Mycologie Medicale, EA1155-IICiMed, Faculte de Pharmacie, Universite de Nantes, Nantes Atlantique Universites, Nantes, France. Laboratoire de Parasitologie-Mycologie, CHU de Poitiers, 2 rue de la Miletrie 86021 Poitiers Cedex, Poitiers, France. Laboratoire de Microbiologie, Centre Hospitalier du Mans, Le Mans, France. Laboratoire de Microbiologie, Centre Hospitalier Universitaire de Caen, Caen, France. Laboratoire de Parasitologie-Mycologie, CHU Clermont-Ferrand, F-63003 Clermont-Ferrand, France, Clermont Universite, Universite Blaise Pascal, Laboratoire Microorganismes, Genome et Environnement, Clermont-Ferrand, France. Institut Pasteur, Unite de Mycologie Moleculaire,	Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases, (20170500) Vol. 23, No. 5, pp. 338-341. Electronic Publication Date: 2 Nov 2016 Journal code: 9516420. E-ISSN: 1469-0691. L-ISSN: 1198-743X.	20170101	duplicates		MEDLINE/2017633370
WHO Technical Report Series No. 922: Evaluation of certain food additives and contaminants.	Joint FAO/WHO Expert Committee on Food Additives		WHO Technical Report Series, (2004) No. 922, pp. 1-176. print. CODEN: WHOTAC. ISSN: 0512-3054.	20040101	duplicates	This report represents the conclusions of a Joint FAO/WHO Expert Committee convened to evaluate the safety of various food additives, with a view to recommending acceptable daily intakes (ADIs) and to prepare specifications for the identity and purity of food additives. The first part of the report contains a general discussion of the principles governing the toxicological evaluation of food additives (including flavouring agents) and contaminants, assessments of intake, and the establishment and revision of specifications for food additives. A summary follows of the Committee's evaluations of toxicological and intake data on various specific food additives (alpha-amylase from Bacillus lichenformis containing a genetically engineered alpha-amylase gene from B. licheniformis, annatto extracts, curcumin, diacetyl and fatty acid esters of glycerol, D-tagatose, laccase from Myceliophthora thermophila expressed in Aspergillus oryzae, mixed xylanase, beta-glucanase enzyme preparation produced by a strain of Humicola insolens, neotame, polyvinyl alcohol, quillaia extracts and xylanase from Thermomyces lanuginosus expressed in Fusarium venenatum), flavouring agents, a nutritional source of iron (ferrous glycinate, processed with citric acid), a disinfectant for drinking-water (sodium dichloroisocyanurate) and contaminants (cadmium and methylmercury). Annexed to the report are tables summarizing the Committee's recommendations for ADIs of the food additives, recommendations on the flavouring agents considered, and tolerable intakes of the contaminants considered, changes in the status of specifications and further information requested or desired.	BIOSIS/2004:392225
Peptides with antioxidant properties identified from casein, whey, and egg albumin hydrolysates generated by two novel fungal proteases.	Hamin Neto, Youssef A. A. Rosa, Jose Cesar Cabral, Hamilton [Reprint Author] hamilton@fcfrp.usb.br	Univ Sao Paulo, Sch Pharmaceut Sci Ribeirao Preto, Cafe Ave S-N, Sao Paulo, Brazil	Preparative Biochemistry + Biotechnology, (AUG 9 2019) Vol. 49, No. 7. http://www.tandfonline.com/loi/lpbb20. ISSN: 1082-6068. E-ISSN: 1532-2297.	20190101	duplicates	There are many diseases linked to oxidative stress, including cancer . Importantly, endogenous antioxidants are insufficient to protect against this process. Peptides derived from food proteins produced by hydrolysis have been investigated as exogenous antioxidants. The present study aimed to identify novel peptides with antioxidant potential produced from egg and milk proteins hydrolysis with two new fungal proteases isolated from Eupenicillium javanicum and Myceliophthora thermophila . The degree of hydrolysis at several time points was calculated and correlated to DPPH scavenging and metal chelating assays, all hydrolysates presented antioxidant activity. Casein hydrolyzed by the M. thermophila protease showed the best antioxidant activity. The identified sequences showed that the proportions of amino acids that influence antioxidant activity support the antioxidant assay. Our data reveal the conditions necessary for the successful generation of antioxidant peptides using two novel fungal proteases. This opens a potential new avenue for the design and manufacture of antioxidant molecules.	BIOSIS/2019:738883

Decolorization and detoxification of textile wastewaters by recombinant Myceliophthora thermophila and Trametes trogii laccases.	Herkommerova, Klara Dostal, Jiri Pichova, Iva [Reprint Author]	Czech Acad Sci, Inst Organ Chem and Biochem, Lab Viral and Microbial Prot, Prague, Czech Republic iva.pichova@uochb.cas.cz	3 Biotech, (NOV 30 2018) Vol. 8, No. 12, pp. Article No.: 505. http://www.springerlink.com/content/2190-572X/. ISSN: 2190-572X. E-ISSN: 2190-5738.	20180101	duplicates	Laccases are multi-copper oxidoreductases with broad biotechnological applications. Here, we report detailed biochemical characterization of purified recombinant laccases originating from Myceliophthora thermophila (MtL) and Trametes trogii (TtL). We identified optimal conditions for decolorization of commercial dyes and textile wastewater samples. We also tested the toxicity of decolorized wastewater samples using human peripheral blood mononuclear cells. MtL and TtL were expressed in Saccharomyces cerevisiae, and secreted enzymes were purified by consecutive hydrophobic and gel chromatography. The molecular masses of TtL (similar to 65kDa) and MtL (>100kDa) suggested glycosylation of the recombinant enzymes. Deglycosylation of MtL and TtL led to 25% and 10% decreases in activity, respectively. In a thermal stability assay, TtL retained 61% and MtL 86% of the initial activity at 40 degrees C. While TtL retained roughly 50% activity at 60 degrees C, MtL lost stability at temperatures higher than 40 degrees C. MtL and TtL preferred syringaldazine as a substrate, and the catalytic efficiencies for ABTS oxidation were 7.5 times lower than for syringaldazine oxidation. In the presence of the mediator HBT, purified TtL almost completely decolorized dyes within 30min and substantially decolorized wastewater samples from a textile factory (up to 74%) within 20h. However, products of TtL-catalyzed decolorization were more toxic than MtL-decolorized products, which were almost completely detoxified .	BIOSIS/2018:1049495
Isolation and characterization of a new cytotoxic polyketide-amino acid hybrid from Thermothelomyces thermophilus ATCC 42464	Gao, Yang-Le Zhang, Ming-Liang Wang, Xue Zhang, Huai-Dong Huang, Jian-Zhong Li, Li	Engineering Research Center of Industrial Microbiology, College of Life Sciences, Fujian Normal University, Fuzhou, Peop. Rep. China	Natural Product Research (2019) Ahead of Print CODEN: NPRAAT; ISSN: 1478-6419	20190101	duplicates	Fungi are a rich source of novel anticancer compds. Bioassay-guided isolation has led to the isolation of 4 polyketide-amino acid hybrid compds. with trans-fused decalin system from the fungus Thermothelomyces thermophilus ATCC 42464 (=Myceliophthora thermophila ATCC 42464): myceliothermophins A, B, E, and F (I). The structure of the new compd. I was clearly detd. by a combination of NMR anal. and high-resoln. electrospray ionization mass spectroscopy (HRESIMS). I exhibited promising cytotoxicity against some cell lines derived from colorectal carcinoma , hepatic carcinoma , and gastric carcinoma , indicating that compds. with trans-fused decalin system would be promising in the course of developing novel anticancer drugs.	CAPLUS/2019:1399349
Versatile fungal polyphenol oxidase with chlorophenol bioremediation potential: characterization and protein engineering	Nikolaivits, Efstratios Dimarogona, Maria Karagiannaki, Ioanna Chalima, Angelina Fishman, Ayelet Topakas, Evangelos	Biotechnology Laboratory, School of Chemical Engineering, National Technical University of Athens, Athens, Greece	Applied and Environmental Microbiology (2018), 84(23), e01628-18/1-e01628-18/16 CODEN: AEMIDF; ISSN: 1098-5336 URL: http://aem.asm.org/	20180101	duplicates	Polyphenol oxidases (PPOs) have been mostly assocd. with the undesirable postharvest browning in fruits and vegetables and have implications in human melanogenesis. Nonetheless, they are considered useful biocatalysts in the food, pharmaceutical, and cosmetic industries. The aim of the present work was to characterize a novel PPO and explore its potential as a bioremediation agent. A gene encoding an extracellular tyrosinase-like enzyme was amplified from the genome of Thermothelomyces thermophila and expressed in Pichia pastoris. The recombinant enzyme (TtPPO) was purified and biochem. characterized. Its prodn. reached 40 mg/L, and it appeared to be a glycosylated and N-terminally processed protein. TtPPO showed broad substrate specificity, as it could oxidize 28/30 compds. tested, including polyphenols, substituted phenols, catechols, and methoxyphenols. Its optimum temp. was 65.degree.C, with a half-life of 18.3 h at 50.degree.C, while its optimum pH was 7.5. The homol. model of TtPPO was constructed, and site-directed mutagenesis was performed in order to increase its activity on mono- and dichlorophenols (di-CPs). The G292N/Y296V variant of TtPPO 5.3-fold increased activity on 3,5-dichlorophenol (3,5-diCP) compared to the wild type.	CAPLUS/2019:376418
Decolorization and detoxification of textile wastewaters by recombinant Myceliophthora thermophila and Trametes trogii laccases.	Herkommerova, K. Dostal, J. Pichova, I.	Laboratory of Viral and Microbial Proteins, Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic. EMAIL: iva.pichova@uochb.cas.cz	3 Biotech (2018), Volume 8, Number 12, 505 p., 67 refs. ISSN: 2190-572X DOI: 10.1007/s13205-018-1525-3 Published by: Springer Berlin, Heidelberg	20180101	duplicates	Laccases are multi-copper oxidoreductases with broad biotechnological applications. Here, we report detailed biochemical characterization of purified recombinant laccases originating from Myceliophthora thermophila (MtL) and Trametes trogii (TtL). We identified optimal conditions for decolorization of commercial dyes and textile wastewater samples. We also tested the toxicity of decolorized wastewater samples using human peripheral blood mononuclear cells. MtL and TtL were expressed in Saccharomyces cerevisiae, and secreted enzymes were purified by consecutive hydrophobic and gel chromatography. The molecular masses of TtL (65 kDa) and MtL (>100 kDa) suggested glycosylation of the recombinant enzymes. Deglycosylation of MtL and TtL led to 25% and 10% decreases in activity, respectively. In a thermal stability assay, TtL retained 61% and MtL 86% of the initial activity at 40.degree.C. While TtL retained roughly 50% activity at 60.degree.C, MtL lost stability at temperatures higher than 40.degree.C. MtL and TtL preferred syringaldazine as a substrate, and the catalytic efficiencies for ABTS oxidation were 7.5 times lower than for syringaldazine oxidation. In the presence of the mediator HBT, purified TtL almost completely decolorized dyes within 30 min and substantially decolorized wastewater samples from a textile factory (up to 74%) within 20 h. However, products of TtL-catalyzed decolorization were more toxic than MtL-decolorized products, which were almost completely detoxified .	CABA/2019:365537

Development of a genome-editing CRISPR/Cas9 system in thermophilic fungal Myceliophthora species and its application to hyper-cellulase production strain engineering.	Liu Qian Gao RanRan Li JinGen Lin LiangCai Zhao JunQi Sun WenLiang Tian ChaoGuang Liu, Q. Gao, R. R. Li, J. G. Lin, L. C. Zhao, J. Q. Sun, W. L. Tian, C. G.	Key Laboratory of Systems Microbial Biotechnology, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin 300308, China. EMAIL: tian_cg@tib.cas.cn	Biotechnology for Biofuels (2017), Volume 10, Number 1, (3 January 2017) p., 60 refs. ISSN: 1754-6834 Published by: BioMed Central Ltd, London	20170101	duplicates	Background: Over the past 3 years, the CRISPR/Cas9 system has revolutionized the field of genome engineering. However, its application has not yet been validated in thermophilic fungi. Myceliophthora thermophila , an important thermophilic biomass-degrading fungus, has attracted industrial interest for the production of efficient thermostable enzymes. Genetic manipulation of Myceliophthora is crucial for metabolic engineering and to unravel the mechanism of lignocellulose deconstruction. The lack of a powerful, versatile genome-editing tool has impeded the broader exploitation of M. thermophila in biotechnology. Results: In this study, a CRISPR/Cas9 system for efficient multiplexed genome engineering was successfully developed in the thermophilic species M. thermophila and M. heterothallica. This CRISPR/Cas9 system could efficiently mutate the imported amdS gene in the genome via NHEJ-mediated events. As a proof of principle, the genes of the cellulase production pathway, including cre-1, res-1, gh1-1, and alp-1, were chosen as editing targets. Simultaneous multigene disruptions of up to four of these different loci were accomplished with neomycin selection marker integration via a single transformation using the CRISPR/Cas9 system. Using this genome-engineering tool, multiple strains exhibiting pronounced hyper-cellulase production were generated, in which the extracellular secreted protein and lignocellulase activities were significantly increased (up to 5- and 13-fold, respectively) compared with the parental strain. Conclusions: A genome-wide engineering system for thermophilic fungi was established based on CRISPR/Cas9. Successful expansion of this system without modification to M. heterothallica indicates it has wide adaptability and flexibility for use in other Myceliophthora species. This system could greatly accelerate strain engineering of thermophilic fungi for production of industrial enzymes, such as cellulases as shown in this study and possibly bio-based fuels and chemicals in the future.	CABA/2017:54439
Bioremediation of polycyclic aromatic hydrocarbons by fungal laccases engineered by directed evolution.	Zumarraga, M. Plou, F. J. Garcia-Arellano, H. Ballesteros, A. Alcalde, M. Editor(s): Alcalde, M. Ferrer, M. Plou, F. J.	Instituto de Catalisis y Petroleoqui mica (CSIC), Cantoblanco, Madrid, Spain.	Biocatalysis and Biotransformation (2007), Volume 25, Number 2/4, 219 p., 40 refs. ISSN: 1024-2422 DOI: 10.1080/10242420701444272 Published by: Informa Healthcare, Abingdon Conference: International Symposium on Environmental Biocatalysis, Cordoba, Spain, 23-26 April 2006.	20070101	duplicates	Fungal laccases are useful for several remarkable transformations, such as bioremediation of polycyclic aromatic hydrocarbons, synthesis of phenolic-based resins, oxidation of lignin derivatives and others. Most of these substrates are barely water-soluble, and although polar organic co-solvents may be added to enhance their solubility, transformation rates dramatically decrease due to the negative effect of organic solvents on the protein structure. Laccase from Myceliophthora thermophila variant T2 (MtLT2) was submitted to laboratory evolution in Saccharomyces cerevisiae with the aim of improving activity and stability in organic co-solvents. Some 4500 clones created by random mutagenesis were screened in two rounds of directed evolution. Libraries were explored under increasing concentrations of acetonitrile and ethanol, and several mutants with improved features were purified and further characterised. Turnover rates of MtLT2 in 30% (v/v) acetonitrile and 50% (v/v) ethanol were increased up to 6.5- and 7.5-fold, respectively. The best variants showed similar rates in 20% (v/v) acetonitrile or 30% (v/v) ethanol as the parent type in aqueous media. Mutant laccases were also tested for the oxidation of anthracene in the presence of 20% (v/v) acetonitrile.	CABA/2008:212294
Severe osteomyelitis caused by Myceliophthora thermophila after a pitchfork injury.	Destino, L. Sutton, D. A. Helon, A. L. Havens, P. L. Thometz, J. G. Willoughby, R. E., Jr. Chusid, M. J.	Department of Pediatrics, Medical College of Wisconsin, Children's Hospital of Wisconsin, Wisconsin, USA. EMAIL: ldestino@mcw.edu suttond@UTHSCSA.edu anna.helon@gmail.com phavens@mcw.edu jthometz@mcw.edu rewillou@mcw.edu mchusid@mcw.edu	Annals of Clinical Microbiology and Antimicrobials (2006), Volume 5, Number 21, (08 September 2006) p., 18 refs. ISSN: 1476-0711 Published by: BioMed Central Ltd, London	20060101	duplicates	Background: Traumatic injuries occurring in agricultural settings are often associated with infections caused by unusual organisms. Such agents may be difficult to isolate, identify, and treat effectively. Case report: A 4-year-old boy developed an extensive infection of his knee and distal femur following a barnyard pitchfork injury. Ultimately the primary infecting agent was determined to be Myceliophthora thermophila , a thermophilic melanized hyphomycete, rarely associated with human infection , found in animal excreta. Because of resistance to standard antifungal agents including amphotericin B and caspofungin, therapy was instituted with a prolonged course of terbinafine and voriconazole. Voriconazole blood levels demonstrated that the patient required a drug dosage (13.4 mg/kg) several fold greater than that recommended for adults in order to attain therapeutic blood levels. Conclusion: Unusual pathogens should be sought following traumatic farm injuries. Pharmacokinetic studies may be of critical importance when utilizing antifungal therapy with agents for which little information exists regarding drug metabolism in children.	CABA/2007:183845
Evaluation of certain food additives and contaminants.		Viale delle Terme di Caracalla, 00100 Rome, Italy. Joint Food and Agriculture Organization World Health Organization	WHO Technical Report Series (2004), Number 922, x + 176 p., 24 refs. ISSN: 0512-3054 Published by: World Health Organization, Geneva	20040101	duplicates	This report discusses the principles governing the toxicological evaluation of food additives and contaminants, assessments of intake, and the establishment and revision of specifications for food additives. A summary follows of the Committee's evaluations of toxicological and intake data on various specific food additives (.alpha.-amylase from Bacillus licheniformis containing a genetically engineered .alpha.-amylase gene from B. licheniformis, annatto extracts, curcumin, diacetyl and fatty acid esters of glycerol, D-tagatose, laccase from Myceliophthora thermophila expressed in Aspergillus oryzae, mixed xylanase, .beta.-glucanase enzyme preparation produced by a strain of Humicola insolens, neotame, polyvinyl alcohol, quillaia extracts, and xylanase from Thermomyces lanuginosus expressed in Fusarium venenatum), flavouring agents, a nutritional source of iron (ferrous glycinate, processed with citric acid), a disinfectant for drinking water (sodium dichloroisocyanurate), and contaminants (cadmium and methylmercury). The Committee's recommendations for ADIs (acceptable daily intakes) of the food additives, recommendations on the flavouring agents considered, tolerable intakes of the contaminants considered, and changes in the status of specifications and further information requested or desired are summarized in tables.	CABA/2004:163708

Practical guidance for the implementation of the CRISPR genome editing tool in filamentous fungi	Min Jin Kwon Schuetze, Tabea Spohner, Sebastian Haefner, Stefan Meyer, Vera		Fungal Biology and Biotechnology, Vol. 6, 20190101 E-ISSN: 2054-3085 DOI: 10.1186/s40694-019-0079-4 Published by: BioMed Central, London		duplicates	Background Within the last years, numerous reports described successful application of the CRISPR nucleases Cas9 and Cpf1 for genome editing in filamentous fungi. However, still a lot of efforts are invested to develop and improve protocols for the fungus and genes of interest with respect to applicability, scalability and targeting efficiencies. These efforts are often hampered by the fact that-although many different protocols are available-none have systematically analysed and compared different CRISPR nucleases and different application procedures thereof for the efficiency of single- and multiplex-targeting approaches in the same fungus. Results We present here data for successful genome editing in the cell factory Thermothelomyces thermophilus , formerly known as Myceliophthora thermophila , using the three different nucleases SpCas9, FnCpf1, AsCpf1 guided to four different gene targets of our interest. These included a polyketide synthase (pks4.2), an alkaline protease (alp1), a SNARE protein (snc1) and a potential transcription factor (ptf1). For all four genes, guide RNAs were developed which enabled successful single-targeting and multiplex-targeting. CRISPR nucleases were either delivered to T. thermophilus on plasmids or preassembled with in vitro transcribed gRNA to form ribonucleoproteins (RNPs). We also evaluated the efficiency of single oligonucleotides for site-directed mutagenesis . Finally, we were able to scale down the transformation protocol to microtiter plate format which generated high numbers of positive transformants and will thus pave the way for future high-throughput investigations. Conclusion We provide here the first comprehensive analysis and evaluation of different CRISPR approaches for a filamentous fungus. All approaches followed enabled successful genome editing in T. thermophilus; however, with different success rates. In addition, we show that the success rate depends on the respective nuclease and on the targeted gene locus. We finally present a practical guidance for experimental considerations aiming to guide the reader for successful implementation of CRISPR technology for other fungi.	PQSCITECH/2019:405006
Development of a genome-editing CRISPR/Cas9 system in thermophilic fungal Myceliophthora species and its application to hyper-cellulase production strain engineering	Liu, Qian Gao, Ranran Li, Jingen Lin, Liangcai Zhao, Junqi Sun, Wenliang Tian, Chaoguang Liu, Qian Gao, Ranran Li, Jingen Lin, Liangcai Zhao, Junqi Sun, Wenliang Tian, Chaoguang		Biotechnology for Biofuels, Vol. 10, 20170101 E-ISSN: 1754-6834 DOI: 10.1186/s13068-016-0693-9 Published by: BioMed Central, London		duplicates	Background Over the past 3 years, the CRISPR/Cas9 system has revolutionized the field of genome engineering. However, its application has not yet been validated in thermophilic fungi. Myceliophthora thermophila , an important thermophilic biomass-degrading fungus, has attracted industrial interest for the production of efficient thermostable enzymes. Genetic manipulation of Myceliophthora is crucial for metabolic engineering and to unravel the mechanism of lignocellulose deconstruction. The lack of a powerful, versatile genome-editing tool has impeded the broader exploitation of M. thermophila in biotechnology. Results In this study, a CRISPR/Cas9 system for efficient multiplexed genome engineering was successfully developed in the thermophilic species M. thermophila and M. heterothallica. This CRISPR/Cas9 system could efficiently mutate the imported amdS gene in the genome via NHEJ-mediated events. As a proof of principle, the genes of the cellulase production pathway, including cre-1, res-1, gh1-1, and alp-1, were chosen as editing targets. Simultaneous multigene disruptions of up to four of these different loci were accomplished with neomycin selection marker integration via a single transformation using the CRISPR/Cas9 system. Using this genome-engineering tool, multiple strains exhibiting pronounced hyper-cellulase production were generated, in which the extracellular secreted protein and lignocellulase activities were significantly increased (up to 5- and 13-fold, respectively) compared with the parental strain. Conclusions A genome-wide engineering system for thermophilic fungi was established based on CRISPR/Cas9. Successful expansion of this system without modification to M. heterothallica indicates it has wide adaptability and flexibility for use in other Myceliophthora species. This system could greatly accelerate strain engineering of thermophilic fungi for production of industrial enzymes, such as cellulases as shown in this study and possibly bio-based fuels and chemicals in the future.	PQSCITECH/2018:205614
Severe osteomyelitis caused by Myceliophthora thermophila after a pitchfork injury	Destino, Lauren Sutton, Deanna A Helon, Anna L Havens, Peter L Thometz, John G Willoughby Jr, Rodney E Chusid, Michael J	Department of Pediatrics, Medical College of Wisconsin and Children's Hospital of Wisconsin, USA	Annals of Clinical Microbiology and Antimicrobials [Ann. Clin. Microbiol. Antimicro.]. Vol. 5, [np]. 2006. ISSN: 1476-0711 DOI: 10.1186/1476-0711-5-21 Published by: BioMed Central Ltd., Middlesex House 34-42 Cleveland Street London W1T 4LB UK, [mailto:info@biomedcentral.com], [URL:http://www.biomedcentral.com]	20060101	duplicates	Background Traumatic injuries occurring in agricultural settings are often associated with infections caused by unusual organisms. Such agents may be difficult to isolate, identify, and treat effectively. Case report A 4-year-old boy developed an extensive infection of his knee and distal femur following a barnyard pitchfork injury. Ultimately the primary infecting agent was determined to be Myceliophthora thermophila , a thermophilic melanized hyphomycete, rarely associated with human infection , found in animal excreta. Because of resistance to standard antifungal agents including amphotericin B and caspofungin, therapy was instituted with a prolonged course of terbinafine and voriconazole. Voriconazole blood levels demonstrated that the patient required a drug dosage (13.4 mg/kg) several fold greater than that recommended for adults in order to attain therapeutic blood levels. Conclusion Unusual pathogens should be sought following traumatic farm injuries. Pharmacokinetic studies may be of critical importance when utilizing antifungal therapy with agents for which little information exists regarding drug metabolism in children.	PQSCITECH/2006:525520
Fatal disseminated infection in an acute myeloblastic leukemic caused by Myceliophthora thermophila , (Apinis) van Oorschot, a rare agent of mycosis : Clinical and mycologic characteristics	McGough, DA Fraser, H Bourbeau, P Naumovitz, D Shah, N Fothergill, AW	Geisinger Med. Cent., Danville, PA	20001231 Conference: XI Congress of the International Society for Human and Animal Mycology, Montreal, Que. (Canada), 24-28 Jun 1991. (World Meeting Number 912 0046)		duplicates		PQSCITECH/2000:680088

Novel cephalosporins synthesized by amination of 2,5-dihydroxybenzoic acid derivatives using fungal laccases II	Mikolasch, A Niedermeyer, TH Lalk, M Witt, S Lindequist, U	Ernst Moritz Arndt Univ, Inst Microbiol, FL Jahn Str 15, D-17489 Griefswald, Germany annett.mikolasch@gmx.de	Chemical and Pharmaceutical Bulletin (Japan), (2007) Vol. 55, pp. 412-416. 26 Refs. CODEN: CPBTAL. ISSN: 0009-2363.	20070101	duplicates	Sixteen novel cephalosporins were synthesized by amination of 2,5-dihydroxybenzoic acid derivatives with the aminocephalosporins cefadroxil, cefalexin, cefaclor, and the structurally related carbacephem loracarbef using laccases from Trametes sp. or Myceliophthora thermophila . All products inhibited the growth of several Gram positive bacterial strains in the agar diffusion assay, among them methicillin-resistant Staphylococcus aureus and vancomycin-resistant enterococci. The products protected mice against an infection with Staphylococcus aureus lethal to the control animals. Cytotoxicity and acute toxicity of the new compounds were negligible. The results show the usefulness of laccase for the synthesis of potential new antibiotics. The biological activity of the new compounds stimulates intensified pharmacological tests.	TOXCENTER/2007:136339
The Lignozym process - a biotechnological process for conservation of chlorine dioxide in the pulp industry	Amann, Manfred Pfaller, Rupert	Consortium fur Elektrochemische Industrie, Munchen, Germany.	GSF-Bericht, (2001) No. 01/01, pp. 73-81. CODEN: GSFBEM. ISSN: 0721-1694.	20010101	duplicates	(b) (4)	TOXCENTER/2001:137490
Effect of elevated amendments of urea on soil ureolytic activity and mycoflora count	Salama, A. M. Abdel-Rahman, Tahany M. A. Abo-Ellil, Amany H. Aly, M. I. A. El Selhdar, A. H.	Botany Dep., Fac. Sci., Cairo Univ., Cairo, Egypt	Egyptian Journal of Microbiology, (1992 (1993)) Vol. 27, No. 2, pp. 197-214. CODEN: EJMBA2. ISSN: 0301-8172.	19930101	duplicates	(b) (4)	TOXCENTER/1994:106012

Annex 1 - Part B: 2020 Search List - Toxicological Relevant

Title	Author	Company/Institute	PATWWW/AutoDoc	Publ. Date	Category	Abstracts	IDFromSource
Invasive Myceliophthora thermophila infection mimicking invasive aspergillosis in a neutropenic patient : a new cause of cross-reactivity with the Aspergillus galactomannan serum antigen assay.	Morio Florent Fraissinet Francois Gastinne Thomas Le Pape Patrice Delaunay Jacques Sigler Lynne Gibas Connie Fe C Miegeville Michel	Laboratoire de Parasitologie-Mycologie, CHU de Nantes, Nantes, France. florent.morio@chu-nantes.fr	Medical mycology, (2011 Nov) Vol. 49, No. 8, pp. 883-6. Electronic Publication Date: 30 May 2011 Journal code: 9815835. E-ISSN: 1460-2709. L-ISSN: 1369-3786.	20110101	Tox	Myceliophthora thermophila is a thermophilic mould widely found in the environment but rarely responsible for human infections . We describe a case of invasive Myceliophthora thermophila infection mimicking invasive aspergillosis in a neutropenic patient with haematological malignancy. Cross-reactivity with Aspergillus galactomannan assay (GM) was demonstrated by repeated positive results and confirmed by cross-reaction between the fungal isolate and the GM assay. The patient was successfully treated with voriconazole. Potential GM cross-reactivity must be considered in future studies including patients categorized as having probable invasive aspergillosis using the GM as the only mycological criterion.	MEDLINE/2011912310
A fatal case with disseminated Myceliophthora thermophila infection in a lymphoma patient .	Le Naoures Cecile Bonhomme Julie Terzi Nicolas Duhamel Chantal Galateau-Salle Francoise	Department of Pathology, Centre Hospitalier Universitaire de Caen, Caen F-14033, France.	Diagnostic microbiology and infectious disease, (2011 Jun) Vol. 70, No. 2, pp. 267-9. Electronic Publication Date: 22 Mar 2011 Journal code: 8305899. E-ISSN: 1879-0070. L-ISSN: 0732-8893.	20110101	Tox	We report a lethal Myceliophthora thermophila infection in an immunocompromised patient . Based upon the clinical and mycological presentation, an initial diagnosis of invasive aspergillosis was made, possibly delaying optimal management in the patient . Melanized fungi are emerging pathogens that require early identification to improve their unfavorable prognosis. Copyright .COPYRGT. 2011 Elsevier Inc. All rights reserved.	MEDLINE/2011535063
Left ventricular assist device outflow cannula obstruction by the rare environmental fungus Myceliophthora thermophila .	Weitzel Nathaen Puskas Ferenc Cleveland Joseph Levi Marilyn E Seres Tamas	Department of Anesthesiology, University of Colorado Denver and Health Sciences Center, Aurora, Colorado 80045, USA. nathaen.weitzel@uchsc.ed	Anesthesia and analgesia, (2009 Jan) Vol. 108, No. 1, pp. 73-5. Ref: 13 Journal code: 1310650. E-ISSN: 1526-7598. L-ISSN: 0003-2999.	20090101	Tox	Left ventricular assist devices are used to provide mechanical circulatory support during end-stage heart failure either as a destination therapy or as a bridge to heart transplantation. Perioperative transesophageal echocardiography is becoming an invaluable tool to investigate device function during implantation and in case of mechanical malfunction. Most malfunctions are due to inflow graft occlusion, or device malfunction, while outflow graft dysfunction is rare. Here, we present a case of severe outflow conduit obstruction by a rare environmental fungus, Myceliophthora thermophila . After replacement of the infected device and intensive antifungal treatment, heart transplantation was performed 2 yr later.	MEDLINE/2009040788
Cytotoxic polyketides containing tetramic acid moieties isolated from the fungus Myceliophthora Thermophila : elucidation of the relationship between cytotoxicity and stereoconfiguration.	Yang Yu-Liang Lu Chun-Ping Chen Mao-Yen Chen Kuei-Yu Wu Yang-Chang Wu Shih-Hsiung	Institute of Biological Chemistry, Academia Sinica, Taipei 115, Taiwan.	Chemistry (Weinheim an der Bergstrasse, Germany), (2007) Vol. 13, No. 24, pp. 6985-91. Journal code: 9513783. ISSN: 0947-6539. L-ISSN: 0947-6539.	20070101	Tox	Five new polyketides that contain tetramic acids, myceliothermophins A-E, were isolated from the thermophilic fungus Myceliophthora thermophila . Two sets of 5-alkyl-5-hydroxyl (or 5-methoxyl)-1H-pyrrol-2(5H)-one diastereomers, myceliothermophins A/B and C/D, were separated as pure compounds by using silica-gel column chromatography and recycling reverse-phase high-performance liquid chromatography (RP-HPLC). The relative configurations of the chiral centers in 5-alkyl-5-hydroxyl (or 5-methoxyl)-1H-pyrrol-2(5H)-one moieties were deduced from NOESY correlations. In the cytotoxic assay, the 5-(2-methylpropyldiene)-1H-pyrrol-2(5H)-one analogue (myceliothermophin E) exhibited inhibition against four cancer cell lines. In addition, the significant inhibitory effect of myceliothermophins A and C and the inactivity of myceliothermophins B and D revealed the importance of the relative configurations of 5-alkyl-5-hydroxyl (or 5-methoxyl)-1H-pyrrol-2(5H)-one moieties on their cytotoxicity potency against cancer cells.	MEDLINE/2007574317
Severe osteomyelitis caused by Myceliophthora thermophila after a pitchfork injury.	Destino Lauren Sutton Deanna A Helon Anna L Havens Peter L Thometz John G Willoughby Rodney E Jr Chusid Michael J	Department of Pediatrics, Medical College of Wisconsin and Children's Hospital of Wisconsin, USA. ldestino@mcw.edu	Annals of clinical microbiology and antimicrobials, (2006 Sep 08) Vol. 5, pp. 21. Electronic Publication Date: 8 Sep 2006 Journal code: 101152152. E-ISSN: 1476-0711. L-ISSN: 1476-0711. Report No.: PMC-PMC1592504.	20060101	Tox	BACKGROUND: Traumatic injuries occurring in agricultural settings are often associated with infections caused by unusual organisms. Such agents may be difficult to isolate, identify, and treat effectively. CASE REPORT: A 4-year-old boy developed an extensive infection of his knee and distal femur following a barnyard pitchfork injury. Ultimately the primary infecting agent was determined to be Myceliophthora thermophila , a thermophilic melanized hyphomycete, rarely associated with human infection , found in animal excreta. Because of resistance to standard antifungal agents including amphotericin B and caspofungin, therapy was instituted with a prolonged course of terbinafine and voriconazole. Voriconazole blood levels demonstrated that the patient required a drug dosage (13.4 mg/kg) several fold greater than that recommended for adults in order to attain therapeutic blood levels. CONCLUSION: Unusual pathogens should be sought following traumatic farm injuries. Pharmacokinetic studies may be of critical importance when utilizing antifungal therapy with agents for which little information exists regarding drug metabolism in children.	MEDLINE/2006594947
Evaluation of certain food additives and contaminants.	Anonymous		World Health Organization technical report series, (2004) Vol. 922, pp. 1-176. Journal code: 7903212. ISSN: 0512-3054. L-ISSN: 0512-3054.	20040101	Tox	This report represents the conclusions of a Joint FAO/WHO Expert Committee convened to evaluate the safety of various food additives, with a view to recommending acceptable daily intakes (ADIs) and to prepare specifications for the identity and purity of food additives. The first part of the report contains a general discussion of the principles governing the toxicological evaluation of food additives (including flavouring agents) and contaminants, assessments of intake, and the establishment and revision of specifications for food additives. A summary follows of the Committee's evaluations of toxicological and intake data on various specific food additives (alpha-amylase from Bacillus lichenformis containing a genetically engineered alpha-amylase gene from B. licheniformis, annatto extracts, curcumin, diacetyl and fatty acid esters of glycerol, D-tagatose, laccase from Myceliophthora thermophila expressed in Aspergillus oryzae, mixed xylanase, beta-glucanase enzyme preparation produced by a strain of Humicola insolens, neotame, polyvinyl alcohol, quillaia extracts and xylanase from Thermomyces lanuginosus expressed in Fusarium venenatum), flavouring agents, a nutritional source of iron (ferrous glycinate, processed with citric acid), a disinfectant for drinking-water (sodium dichloroisocyanurate) and contaminants (cadmium and methylmercury). Annexed to the report are tables summarizing the Committee's recommendations for ADIs of the food additives, recommendations on the flavouring agents considered, and tolerable intakes of the contaminants considered, changes in the status of specifications and further information requested or desired.	MEDLINE/2004446940

Isolation and characterization of a new cytotoxic polyketide-amino acid hybrid from Thermothelomyces thermophilus ATCC 42464.	Gao Yang-Le Zhang Ming-Liang Wang Xue Zhang Huai-Dong Huang Jian-Zhong Li Li	a) Engineering Research Center of Industrial Microbiology, College of Life Sciences , Fujian Normal University , Fuzhou , China. b) State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica , Chinese Academy of Medical Sciences and Peking Union Medical College , Beijing , China.	Natural product research, (2019 Jul 16) pp. 1-7. Electronic Publication Date: 16 Jul 2019 Journal code: 101167924. E-ISSN: 1478-6427. L ISSN: 1478-6419.	20190101	Tox	Fungi are a rich source of novel anticancer compounds. Bioassay-guided isolation has led to the isolation of four polyketide-amino acid hybrid compounds with trans-fused decalin system from the fungus Thermothelomyces thermophilus ATCC 42464 (=Myceliophthora thermophila ATCC 42464): myceliothermophins A, B, E and F (1-4). The structure of the new compound (myceliothermophin F, compound 4) was clearly determined by a combination of nuclear magnetic resonance (NMR) analysis and high-resolution electrospray ionisation mass spectroscopy (HRESIMS). The new compound exhibited promising cytotoxicity against some cell lines derived from colorectal carcinoma , hepatic carcinoma and gastric carcinoma , indicating that compounds with trans-fused decalin system would be promising in the course of developing novel anticancer drugs.	MEDLINE/2021377838
Posttraumatic gas-containing brain abscess caused by Clostridium perfringens with unique simultaneous fungal suppuration by Myceliophthora thermophila : case report.	Tekkok I H Higgins M J Ventureyra E C	Division of Neurosurgery, Children's Hospital of Eastern Ontario, University of Ottawa Faculty of Medicine, Canada.	Neurosurgery, (1996 Dec) Vol. 39, No. 6, pp. 1247-51. Journal code: 7802914. ISSN: 0148-396X. L-ISSN: 0148-396X.	19960101	Tox	OBJECTIVE AND IMPORTANCE: Gas-containing brain abscesses are rare, and the vast majority are caused by Clostridium perfringens. Significant simultaneous fungal infection in a bacterial abscess is even rarer. We present such a case and review the literature. CLINICAL PRESENTATION: A 21-month-old male patient sustained a penetrating head injury in a barnyard, developed a gas-containing left parietal brain abscess, and presented with high fever, galeal swelling, and seizure. INTERVENTION: The patient initially underwent debridement of his wound and then repeated aspirations. The initial cultures revealed pure growth of Clostridium perfringens. Despite appropriate antibiotic therapy, serial neuroimaging did not demonstrate a decrease in the size of the cavity. An excision had to be undertaken 6 weeks after the injury. The culture from the excised specimen revealed an unexpected growth of a saprophytic and opportunistic fungus, Myceliophthora thermophila . Antifungal treatment consisting of the administration of liposomal amphotericin B and itraconazole was then performed. The child was well and neurologically intact 6 months after the excision. CONCLUSION: Our review revealed 38 cases of clostridial brain abscess in the literature. Despite the reputation of the organism, the outcome with clostridial brain abscesses was relatively benign. The main characteristics of clostridial brain abscesses are highlighted, with reference to their optimal treatment. Our review also revealed that fungal infection after a penetrating head injury is extremely rare and often fatal. Our case seems to be the first in the medical literature with growth of M. thermophila as a causative agent for intracranial suppuration.	MEDLINE/1997093221
Epidemiology of CNS mould infections : Common pathogens and rare exotics.	Sutton, D. A. [Reprint Author]	Univ Texas Hlth Sci Ctr San Antonio, San Antonio, TX 78229 USA	Mycoses, (JUN 2012) Vol. 55, No. Suppl. 4, Sp. Iss. SI, pp. 31. http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1439-0507. Meeting Info.: 18th Congress of the International-Society-for-Human-and-Animal-Mycology. Berlin, GERMANY. June 11 -15, 2012. Int Soc Human + Anim Mycol. CODEN: MYCSEU. ISSN: 0933-7407. E-ISSN: 1439-0507.	20120101	Tox		BIOSIS/2012:513017
Fungal biota associated with bats in Western France.	Larcher, G. [Reprint Author] Bouchara, J. P. Pailley, P. Montfort, D. Beguin, H. De Bievre, C. Chabasse, D.	Laboratoire de Parasitologie-Mycologie, Groupe d'Etude des Interactions Hote-Parasite, UPRES-EA 3142, Centre Hospitalier Universitaire, 4, rue Larrey, 49033, Angers, France gerald.larcher@univ-angers.fr	Journal de Mycologie Medicale, (Mars 2003) Vol. 13, No. 1, pp. 29-34. print. ISSN: 1156-5233.	20030101	Tox	Although the association between tropical bats and pathogenic fungi has been reported long ago, no study has been performed in Europe to our knowledge. The fungal biota associated with European Chiroptera in the region of Pays de la Loire (Western France) was therefore analyzed to determine if these mammals harbor fungi pathogenic for humans or household animals. One hundred and seven samples of guano or hair/skin coming from six bat species were collected from caverns of Western France during the national annual counting of Chiroptera performed in the course of the hibernation period. They consisted of 82 samples of guano and 25 samples of hair/skin swabs. Mycological analysis of these samples revealed the great diversity of the fungal biota, more pronounced for Rhinolophidae Chiroptera than for Vespertilionidae. Among the pathogenic fungi, yeasts of Candida genus (C. glabrata and C. guilliermondii) and eight species of keratinophilic fungi (Trichophyton terrestre, Scopulariopsis brevicaulis, Aphanoascus fulvescens, Myceliophthora thermophila , Chrysosporium lobatum, Chrysosporium merdarium, Chrysosporium pseudomerdarium and Chrysosporium pruinosum) were identified. In addition, an Onygenaceae highly resembling Histoplasma capsulatum was isolated. Our results demonstrate that several human or animal pathogenic fungi may be associated with bats. Moreover, their habit to gather, sometimes in vast colonies in certain caverns, creates an environmental medium suitable for the proliferation of these fungi by enrichment of the soil with hair, skin, feces and bats corpses. However, due to the particular habitat of these animals, one may consider from this study that bats do not constitute a real danger for human or animal health.	BIOSIS/2003:337569

Biochemical characterization of a eukaryotic decalin-forming Diels-Alderase	Li, Li Yu, Peiyuan Tang, Man-Cheng Zou, Yi Gao, Shu-Shan Hung, Yiu-Sun Zhao, Muxun Watanabe, Kenji Houk, K. N. Tang, Yi	Engineering Research Center of Industrial Microbiology (Ministry of Education) and College of Life Sciences, Fujian Normal University, Fuzhou, 350117, Peop. Rep. China	Journal of the American Chemical Society (2016), 138(49), 15837-15840 CODEN: JACSAT; ISSN: 0002-7863	20160101	Tox	The trans-decalin structure formed by intramol. Diels-Alder cycloaddn. is widely present among bioactive natural products isolated from fungi. Here, the authors elucidated the Myceliophthora thermophila concise 3-enzyme biosynthetic pathway of the cytotoxic compd., myceliothermophin , and biochem. characterized the Diels-Alderase that catalyzes the formation of trans-decalin from an acyclic substrate. Computational studies of the reaction mechanism rationalized both the substrate and stereoselectivity of the enzyme.	CAPLUS/2016:1940500
Osteoarticular Infections Caused by Non-Aspergillus Filamentous Fungi in Adult and Pediatric Patients : A Systematic Review	Taj-Aldeen, Saad J. Rammaert, Blandine Gamaletsou, Maria Sipsas, Nikolaos V. Zeller, Valerie Roilides, Emmanuel Kontoyiannis, Dimitrios P. Miller, Andy O. Petraitis, Vidmantas Walsh, Thomas J. Lortholary, Olivier	Mycology Unit, Microbiology Division, Department of Laboratory Medicine and Pathology, (DPK), Hamad Medical Corporation, Qatar, TX, USA	Medicine (Philadelphia, PA, United States) (2015), 94(50), e2078 CODEN: MEDIAV; ISSN: 0025-7974	20150101	Tox	Osteoarticular mycoses due to non-Aspergillus molds are uncommon and challenging infections . A systematic literature review of non-Aspergillus osteoarticular mycoses was performed using PUBMED and EMBASE databases from 1970 to 2013.Among 145 patients were 111 adults (median age 48.5 [16-92 y]) and 34 pediatric patients (median age 7.5 [3-15 y]); 114 (79.7%) were male and 88 (61.9%) were immunocompromised. Osteomyelitis was due to direct inoculation in 54.5%. Trauma and puncture wounds were more frequent in children (73.5% vs 43.5%; P=0.001). Prior surgery was more frequent in adults (27.7% vs 5.9%; P=0.025). Vertebral (23.2%) and craniofacial osteomyelitis (13.1%) with neurol. deficits predominated in adults. Lower limb osteomyelitis (47.7%) and knee arthritis (67.8%) were predominantly seen in children. Hyalohyphomycosis represented 64.8% of documented infections with Scedosporium apiospermum (33.1%) and Lomentospora prolificans (15.8%) as the most common causes. Combined antifungal therapy and surgery was used in 69% of cases with overall response in 85.8%. Median duration of therapy was 115 days (range 5-730). When voriconazole was used as single agent for treatment of hyalohyphomycosis and phaeohyphomycosis, an overall response rate was achieved in 94.1% of cases. Non-Aspergillus osteoarticular mycoses occur most frequently in children after injury and in adults after surgery. Accurate early diagnosis and long-course therapy (median 6 mo) with a combined medical-surgical approach may result in favorable outcome.	CAPLUS/2015:2094386
Fatal aortic Myceliophthora thermophila infection in a patient affected by cystic medial necrosis.	Farina, C. Gamba, A. Tambini, R. Beguin, H. Trouillet, J. L.	Microbiology Institute, Azienda Ospedaliera 'Ospedali Riuniti di Bergamo', 24128 Bergamo, Italy.	Medical Mycology (1998), Volume 36, Number 2, pp. 113-118, 31 refs.	19980101	Tox	(b) (4) .	CABA/1998:121655
Fatal disseminated infection caused by Myceliophthora thermophila , a new agent of mycosis : case history and laboratory characteristics.	Bourbeau, P. McGough, D. A. Fraser, H. Shah, N. Rinaldi, M. G.	Department of Laboratory Medicine, Geisinger Medical Center, Danville, Pennsylvania 17822, USA.	Journal of Clinical Microbiology (1992), Volume 30, Number 11, pp. 3019-3023, 14 refs. ISSN: 0095-1137	19920101	Tox	(b) (4)	CABA/1992:130923
Thermothelomyces thermophila human infections .	Nourrisson, C. Garcia-Hermoso, D. Morio, F. Kauffmann-Lacroix, C. Berrette, N. Bonhomme, J. Poirier, P. Lortholary, O.	Laboratoire de Parasitologie-Mycologie, CHU Clermont-Ferrand, Clermont-Ferrand, France. France, French Mycosis Study Group EMAIL: olivier.lortholary@aphp.fr olivier.lortholary@pasteur.fr	Clinical Microbiology and Infection (2017), Volume 23, Number 5, pp. 338-341 ISSN: 1198-743X Published by: Elsevier, Oxford	20170101	Tox		CABA/2017:250153

Safety evaluation of certain food additives and contaminants.	Anonymous	Viale delle Terme di Caracalla, 00100 Rome, Italy. Joint Food and Agriculture Organization World Health Organization	WHO Food Additives Series (2004), Number 52, vi + 667 p. ISSN: 0300-0923 Published by: World Health Organization, Geneva	20040101	Tox	(b) (4)	CABA/2005:78293
Miscellaneous opportunistic fungi: Microascaceae and other Ascomycetes, Hyphomycetes, Coelomycetes, and Basidiomycetes. Mycology Series Volume 16	Sigler, L. Editor(s): Howard, D. H.	Microfungus Collection and Herbarium, Devonian Botanic Garden and Medical Microbiology and Immunology, University of Alberta, Edmonton, Alberta, Canada.	Pathogenic fungi in humans and animals (2002), Number Ed. 2, pp. 637-676, 119 refs. ISBN: 0-8247-0638-8 Published by: Marcel Dekker, Inc., New York	20020101	Tox		CABA/2003:14805
Experimentally induced mycetoma: species of Sporotrichum and Sporothrix.	Stretton, R. J.	Dep. Chem., Loughborough Univ. Technol., Loughborough, UK.	Mycopathologia (1975), Volume 55, Number 2, pp. 83-90, 13 fig., 39 refs. ISSN: 0301-486X DOI: 10.1007/BF00444276	19750101	Tox	(b) (4)	CABA/1976:61719
Disseminated phaeohyphomycosis: Review of an emerging mycosis	Revankar S G (Reprint) Patterson J E Sutton D A Pullen R Rinaldi M G	Vet Adm Med Ctr, Div Infect Dis 111D, 4500 S Lancaster Rd, Dallas, TX 75216 USA (Reprint) E-mail: sanjay.revankar@med.va.gov Vet Adm Med Ctr, Div Infect Dis 111D, Dallas, TX 75216 USA Univ Texas, SW Med Ctr, Dept Med, Dallas, TX USA Univ Texas, Hlth Sci Ctr, Dept Med, San Antonio, TX 78284 USA Univ Texas, Hlth Sci Ctr, Dept Pathol, San Antonio, TX 78284 USA Audie L Murphy Mem Vet Adm Med Ctr, San Antonio, TX 78284 USA	CLINICAL INFECTIOUS DISEASES, (15 FEB 2002) Vol. 34, No. 4, pp. 467-476. ISSN: 1058-4838.	20020101	Tox	Disseminated phaeohyphomycosis is an uncommon infection caused by dematiaceous fungi, although the number of case reports about this infection has been increasing in recent years. A total of 72 cases are reviewed. Scedosporium prolificans is by far the most common cause. The presence of melanin in their cell walls may be a virulence factor for these fungi. The primary risk factor is decreased host immunity, although cases in apparently immunocompetent patients have been reported. Eosinophilia was seen in 11% of cases. Endocarditis is mostly reported on bioprosthetic valves, particularly those of porcine origin. The outcome of antifungal therapy remains poor, with an overall mortality rate of 79%. Special precautions taken for immunocompromised patients may help prevent exposure to fungi during the patients ' period of greatest risk. The development of newer antifungal agents and combination therapy may hold promise in improving the management of these devastating infections in the future.	SCISEARCH/2002:88161
TOXIGENIC THERMOPHILIC AND THERMO TOLERANT FUNGI	DAVIS N D WAGENER R E MORGAN-JONES G DIENER U L		Applied Microbiology, (1975) Vol. 29, No. 4, pp. 455-457. CODEN: APMBAY. ISSN: 0003-6919.	19750101	Tox		TOXCENTER/1975:53477

Annex 1 - Part C: 2020 Search List - Ecotoxicological Relevant

Title	Author	Company/Institute	PATWWW/AutoDoc	Publ. Date	Category	Abstracts	IDFromSource
Thermophilous fungi of birds' nests.	APINIS, A. E. PUGH, G. J. F.	Dep. Bot. Univ. Nottingham, Engl., UK	MYCOPATHOL MYCOL APPL, (1967) Vol. 33, No. 1, pp. 1-9.	19670101	Ecotox	Twenty-seven spp. of thermophilous fungi were recorded from the nests of 12 passerine bird spp. There was considerable variation in the relative frequency of these fungi in the nests of different bird spp. The most abundant fungi included Allescheria terrestris, Aspergillus fumigatus, Chaetomium thermophile, Coprinus delicatulus, Humicola insolens, Sporotrichum thermophile , Thermoidium sulphureum, Thermomyces lanugihosus, and Torula thermophila. New records for Great Britain luce: Dactylomyces thermophilus, H. insolens, Penicillium duponti, Stilbella thermophila, and T. thermophila. Many of the fungi grow as saprophytes, but others, including Absidia ramosa, Aspergillus fumigatus, Emericella nidulans, Dendrostllbella boydii (conidial Allescheria boydii), Mucor pusillus, Rhizopus arrhizus, and Thermo-myces lanuginosus are also known as potential pathogens of man and animals.	BIOSIS/1968:41601

Annex 2: 89154892 *Thermothelomyces* 2023 Update Search Results

Annex 2 - Part A: 2023 Update Search List

IDFromSource	Title	Company/Institute	Author	Source	Publ. Date	Not relevant	Abstracts
BIOSIS/2023:712066	Production of flu vaccine in Myceliophthora thermophila .	DYADIC INTERNATIONAL INC	Emalfarb, Mark [Inventor] Anonymous Verwoerd, Teunis Cornelis [Inventor] Alfenito, Mark R. [Inventor] Baer, Mark [Inventor] Legastelois, Isabelle [Inventor] Kazek, Marie-Pierre [Inventor] Bernard, Marie-Clotilde [Inventor] Dubayle, Jean [Inventor] Kensinger, Richard [Inventor]	US11738080	20230829	other	(b) (4)
PQSCITECH/2023:326756	Development of the thermophilic fungus Myceliophthora thermophila into glucoamylase hyperproduction system via the metabolic engineering using improved AsCas12a variants		Zhu, Zhijian Zhang, Manyu Liu, Dandan Liu, Defei Sun, Tao Yang, Yujing Dong, Jiacheng Zhai, Huanhuan Sun, Wenliang Liu, Qian Tian, Chaoguang	Microbial Cell Factories, Vol. 22, pp. 1-17, 20230101 E-ISSN: 1475-2859 DOI: https://doi.org/10.1186/s12934-023-02149-4 Published by: BioMed Central, London	20230101	duplicates	BackgroundGlucoamylase is an important enzyme for starch saccharification in the food and biofuel industries and mainly produced from mesophilic fungi such as Aspergillus and Rhizopus species. Enzymes produced from thermophilic fungi can save the fermentation energy and reduce costs as compared to the fermentation system using mesophiles. Thermophilic fungus Myceliophthora thermophila is industrially deployed fungus to produce enzymes and biobased chemicals from biomass during optimal growth at 45 °C. This study aimed to construct the M. thermophila platform for glucoamylase hyper-production by broadening genomic targeting range of the AsCas12a variants, identifying key candidate genes and strain engineering.ResultsIn this study, to increase the genome targeting range, we upgraded the CRISPR-Cas12a-mediated technique by engineering two AsCas12a variants carrying the mutations S542R/K607R and S542R/K548V/N552R. Using the engineered AsCas12a variants, we deleted identified key factors involved in the glucoamylase expression and secretion in M. thermophila, including Mtstk-12, Mtap3m, Mtdsc-1 and Mtsah-2. Deletion of four targets led to more than 1.87- and 1.85-fold higher levels of secretion and glucoamylases activity compared to wild-type strain MtWT. Transcript level of the major amylolytic genes showed significantly increased in deletion mutants. The glucoamylase hyper-production strain MtGM12 was generated from our previously strain MtYM6 via genetically engineering these targets Mtstk-12, Mtap3m, Mtdsc-1 and Mtsah-2 and overexpressing Mtamy1 and Mtpga3. Total secreted protein and activities of amylolytic enzymes in the MtGM12 were about 35.6-fold and 51.9-55.5-fold higher than in MtWT. Transcriptional profiling analyses revealed that the amylolytic gene expression levels were significantly up-regulated in the MtGM12 than in MtWT. More interestingly, the MtGM12 showed predominantly short and highly bulging hyphae with proliferation of rough ER and abundant mitochondria, secretion vesicles and vacuoles when culturing on starch.ConclusionsOur results showed that these AsCas12a variants worked well for gene deletions in M. thermophila. We successfully constructed the glucoamylase hyper-production strain of M. thermophila by the rational redesigning and engineering the transcriptional regulatory and secretion pathway. This targeted engineering strategy will be very helpful to improve industrial fungal strains and promote the morphology engineering for enhanced enzyme production.
BIOSIS/2023:707240	Development of the thermophilic fungus Myceliophthora thermophila into glucoamylase hyperproduction system via the metabolic engineering using improved AsCas12a variants.	Univ Sci and Technol China, Div Life Sci and Med, Hefei 230027, Peoples R China liu_q1@tib.cas.cn; tian_cg@tib.cas.cn	Zhu, Zhijian [Reprint Author] Zhang, Manyu Liu, Dandan Liu, Defei Sun, Tao Yang, Yujing Dong, Jiacheng Zhai, Huanhuan Sun, Wenliang Liu, Qian [Reprint Author] Tian, Chaoguang [Reprint Author]	Microbial Cell Factories, (AUG 11 2023) Vol. 22, No. 1, pp. Article No.: 150. http://www.microbialcellfactories.com/. E-ISSN: 1475-2859.	20230101	duplicates	BackgroundGlucoamylase is an important enzyme for starch saccharification in the food and biofuel industries and mainly produced from mesophilic fungi such as Aspergillus and Rhizopus species. Enzymes produced from thermophilic fungi can save the fermentation energy and reduce costs as compared to the fermentation system using mesophiles. Thermophilic fungus Myceliophthora thermophila is industrially deployed fungus to produce enzymes and biobased chemicals from biomass during optimal growth at 45 + DEG;C. This study aimed to construct the M. thermophila platform for glucoamylase hyper-production by broadening genomic targeting range of the AsCas12a variants, identifying key candidate genes and strain engineering.ResultsIn this study, to increase the genome targeting range, we upgraded the CRISPR-Cas12a-mediated technique by engineering two AsCas12a variants carrying the mutations S542R/K607R and S542R/K548V/N552R. Using the engineered AsCas12a variants, we deleted identified key factors involved in the glucoamylase expression and secretion in M. thermophila, including Mtstk-12, Mtap3m, Mtdsc-1 and Mtsah-2. Deletion of four targets led to more than 1.87- and 1.85-fold higher levels of secretion and glucoamylases activity compared to wild-type strain MtWT. Transcript level of the major amylolytic genes showed significantly increased in deletion mutants. The glucoamylase hyper-production strain MtGM12 was generated from our previously strain MtYM6 via genetically engineering these targets Mtstk-12, Mtap3m, Mtdsc-1 and Mtsah-2 and overexpressing Mtamy1 and Mtpga3. Total secreted protein and activities of amylolytic enzymes in the MtGM12 were about 35.6-fold and 51.9-55.5-fold higher than in MtWT. Transcriptional profiling analyses revealed that the amylolytic gene expression levels were significantly up-regulated in the MtGM12 than in MtWT. More interestingly, the MtGM12 showed predominantly short and highly bulging hyphae with proliferation of rough ER and abundant mitochondria, secretion vesicles and vacuoles when culturing on starch.ConclusionsOur results showed that these AsCas12a variants worked well for gene deletions in M. thermophila. We successfully constructed the glucoamylase hyper-production strain of M. thermophila by the rational redesigning and engineering the transcriptional regulatory and secretion pathway. This targeted engineering strategy will be very helpful to improve industrial fungal strains and promote the morphology engineering for enhanced enzyme production.

MEDLINE/2027616280	Development of the thermophilic fungus Myceliophthora thermophila into glucoamylase hyperproduction system via the metabolic engineering using improved AsCas12a variants.	Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, 230027, China. Key Laboratory of Engineering Biology for Low-Carbon Manufacturing, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin, 300308, China. liu_q1@tib.cas.cn; tian_cg@tib.cas.cn National Technology Innovation Center of Synthetic Biology, Tianjin, 300308, China. liu_q1@tib.cas.cn; tian_cg@tib.cas.cn College of Biotechnology, Tianjin University of Science + Technology, Tianjin, 300457, China. State Key Laboratory of Agrobiotechnology and MOA Key Laboratory of Soil Microbiology, College of Biological Sciences, China Agricultural University, Beijing, 100193, China.	Zhu Zhijian Zhang Manyu Liu Dandan Liu Defei Sun Tao Yang Yujing Dong Jiacheng Sun Wenliang Liu Qian Tian Chaoguang Zhai Huanhuan	Microbial cell factories, (2023 Aug 11) Vol. 22, No. 1, pp. 150. Electronic Publication Date: 11 Aug 2023 Journal code: 101139812. E-ISSN: 1475-2859. L-ISSN: 1475-2859. Report No.: PMC-PMC10416393.	20230101	other	BACKGROUND: Glucoamylase is an important enzyme for starch saccharification in the food and biofuel industries and mainly produced from mesophilic fungi such as Aspergillus and Rhizopus species. Enzymes produced from thermophilic fungi can save the fermentation energy and reduce costs as compared to the fermentation system using mesophiles. Thermophilic fungus Myceliophthora thermophila is industrially deployed fungus to produce enzymes and biobased chemicals from biomass during optimal growth at 45 °C. This study aimed to construct the M. thermophila platform for glucoamylase hyper-production by broadening genomic targeting range of the AsCas12a variants, identifying key candidate genes and strain engineering. RESULTS: In this study, to increase the genome targeting range, we upgraded the CRISPR-Cas12a-mediated technique by engineering two AsCas12a variants carrying the mutations S542R/K607R and S542R/K548V/N552R. Using the engineered AsCas12a variants, we deleted identified key factors involved in the glucoamylase expression and secretion in M. thermophila, including Mtstk-12, Mtap3m, Mtdsc-1 and Mtsah-2. Deletion of four targets led to more than 1.87- and 1.85-fold higher levels of secretion and glucoamylases activity compared to wild-type strain MtWT. Transcript level of the major amylolytic genes showed significantly increased in deletion mutants. The glucoamylase hyper-production strain MtGM12 was generated from our previously strain MtYM6 via genetically engineering these targets Mtstk-12, Mtap3m, Mtdsc-1 and Mtsah-2 and overexpressing Mtamy1 and Mtpga3. Total secreted protein and activities of amylolytic enzymes in the MtGM12 were about 35.6-fold and 51.9-55.5-fold higher than in MtWT. Transcriptional profiling analyses revealed that the amylolytic gene expression levels were significantly up-regulated in the MtGM12 than in MtWT. More interestingly, the MtGM12 showed predominantly short and highly bulging hyphae with proliferation of rough ER and abundant mitochondria, secretion vesicles and vacuoles when culturing on starch. CONCLUSIONS: Our results showed that these AsCas12a variants worked well for gene deletions in M. thermophila. We successfully constructed the glucoamylase hyper-production strain of M. thermophila by the rational redesigning and engineering the transcriptional regulatory and secretion pathway. This targeted engineering strategy will be very helpful to improve industrial fungal strains and promote the morphology engineering for enhanced enzyme production. © 2023. BioMed Central Ltd., part of Springer Nature.
CAPLUS/2023:690181	The putative methyltransferase LaeA regulates mycelium growth and cellulase production in Myceliophthora thermophila	Key Laboratory of Engineering Biology for Low-carbon Manufacturing, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin, 300308, Peop. Rep. China	Zhao, Zhen Gu, Shuying Liu, Defei Liu, Dandan Chen, Bingchen Li, Jingen Tian, Chaoguang	Biotechnology for Biofuels and Bioproducts (2023), 16(1), 58 CODEN: BBBIDI; ISSN: 2731-3654	20230101	other	Filamentous fungi with the ability to use complex carbon sources has been developed as platforms for biochems. prodn. Myceliophthora thermophila has been developed as the cell factory to produce lignocellulolytic enzymes and plant biomass-based biofuels and biochems. in biorefinery. However, low fungal growth rate and cellulose utilization efficiency are significant barriers to the satisfactory yield and productivity of target products, which needs our further exploration and improvement. In this study, we comprehensively explored the roles of the putative methyltransferase LaeA in regulating mycelium growth, sugar consumption, and cellulases expression. Deletion of laeA in thermophile fungus Myceliophthora thermophila enhanced mycelium growth and glucose consumption significantly. Further exploration of LaeA regulatory network indicated that multiple growth regulatory factors (GRF) Cre-1, Grf-1, Grf-2, and Grf-3, which act as neg. repressors of carbon metab., were regulated by LaeA in this fungus. We also detd. that phosphoenolpyruvate carboxykinase (PCK) is the core node of the metabolic network related to fungal vegetative growth, of which enhancement partially contributed to the elevated sugar consumption and fungal growth of mutant ΔlaeA. Noteworthily, LaeA participated in regulating the expression of cellulase genes and their transcription regulator. ΔlaeA exhibited 30.6% and 5.5% increases in the peak values of extracellular protein and endo-glucanase activity, resp., as compared to the WT strain. Furthermore, the global histone methylation assays indicated that LaeA is assocd. with modulating H3K9 methylation levels. The normal function of LaeA on regulating fungal physiol. is dependent on methyltransferase activity. The research presented in this study clarified the function and elucidated the regulatory network of LaeA in the regulation of fungal growth and cellulase prodn., which will significantly deepen our understanding about the regulation mechanism of LaeA in filamentous fungi and provides the new strategy for improvement the fermn. properties of industrial fungal strain by metabolic engineering.
BIOSIS/2023:183980	Breeding of a thermostable xylanase-producing strain of Myceliophthora thermophila by atmospheric room temperature plasma (ARTP) mutagenesis .	Chinese Acad Forestry CAF, Inst Chem Ind Forest Prod, Natl Engn Res Ctr Low Carbon Proc and Utilizat Fores, Jiangsu Coinnovat Ctr Efficient Proc and Utilizat Fo, Nanjing, Jiangsu, Peoples R China Sunyunjuan@icifp.cn; Jiangjianchun@icifp.cn	Zhang, Ning [Reprint Author] Jiang, Yue Sun, Yun-Juan [Reprint Author] Jiang, Jian-Chun [Reprint Author] Tong, Ya-Juan	Frontiers in Bioengineering and Biotechnology, (JAN 5 2023) Vol. 10, pp. Article No.: 1095323. http://journal.frontiersin.org/journal/bioengineerin g-and-biotechnology#. ISSN: 2296-4185. E-ISSN: 2296-4185.	20230101	duplicates	Introduction: Hemicellulose is an important component in lignocellulose materials, which is second only to cellulose, accounting for 15%-35% of the dry weight of plants. In the current situation of energy shortage, making full use of lignocellulose materials to produce fuel ethanol has become an important way to solve the energy problem. Xylanase plays a crucial role in the utilization of hemicellulose. It is a necessary means to reduce the cost of hemicellulose utilization by improving the activity of xylanase. Moreover, most naturally xylanases are mesophilic enzymes, which limits their industrial application.Methods:In this study, Myceliophthora thermophila was used to produce xylanases and a thermostable mutant M 2103 was obtained by atmospheric room temperature plasma (ARTP) mutagenesis . The research work started with exploring the effects of ARTP mutagenesis on the antioxidase system [superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), polyphenol oxidase (PPO), and antioxidant capacity (AOC)] of M. thermophile, and found that superoxide dismutase activity increased by 221.13%, and polyphenol oxidase activity increased by 486.04% as compared with the original strain when the implantation time was 300 s. So as to determine the conditions for subsequent mutagenesis .Results and Discussion:For the mutant M 2103, the reaction temperature for xylanase production remained stable in the range of 70?-85?. Its optimum temperature was 75?, which was 15? higher than that of the original strain. And its xylanase activity increased by 21.71% as compared with the original strain. M 2103 displayed a significantly higher relative xylanase activity than the original strain in the acidic (pH 4.0-7.0) range, and the xylanase activity was relatively stable in the pH range of 6.0-8.5. These results provide an alternative biocatalyst for the production of xylooligosaccharide, and a potential usage of ARTP in the mutagenesis of thermostable mutant.
SCISEARCH/2023:453654	Isolation of the Anti-Inflammatory Agent Mycelliostatin from a Methionine-Enriched Culture of Myceliophthora thermophila ATCC 42464	Shenzhen Univ, Coll Life Sci + Oceanog, Shenzhen Key Lab Marine Bioresource + Ecoenvironm, Shenzhen 518060, Peoples R China (Reprint) Aichi Med Univ, Sch Med, Dept Mol Target Med, Nagoya, Aichi 4801195, Japan E-mail: umezawa@aichi-med-u.ac.jp Aichi Med Univ, Sch Med, Dept Pharmacol, Nagoya, Aichi 4801195, Japan E-mail: lwang@szu.edu.cn Aichi Med Univ, Sch Med, Dept Mol Target Med, Nagoya, Aichi 4801195, Japan (Reprint)	Wu, Yanhua (Reprint) Lin, Yinzhi Kojima, Shiori Okada, Shoshiro Umezawa, Kazuo Shen, Junfeng Wang, Liyan	JOURNAL OF NATURAL PRODUCTS, (3 2023 FEB 2023) . ISSN: 0163-3864.	20230101	duplicates	Safe and effective nonsteroidal anti-inflammatory drugs are needed. Meanwhile, addition of amino acids to cultures of microorganisms is likely to increase the possibility of novel secondary metabolite isolation. In the course of screening for antiinflammatory agents using cellular lipopolysaccharide (LPS)induced nitric oxide (NO) production, two new related compounds with the myceliothermophin structure from a methionine-enriched culture of Myceliophthora thermophila ATCC 42464 were isolated. The new compounds have an additional methylthio group on the myceliothermophin structure and were named myceliostatins A and B. Both compounds inhibited LPS-induced NO production at nontoxic concentrations in macrophage-like mouse monocytic leukemia RAW264.7 cells. Myceliostatin B inhibited the expression of LPS-induced iNOS, IL-6, and IL-1 beta and the upstream NF -KB activity in situ and in vitro. Finally, it was found to inhibit NF -KB binding to DNA in the reconstruction system with purified p65. Myceliostatin B also inhibited LPS-induced reactive oxygen species (ROS) production. Thus, myceliostatin B, a novel compound derived from M. thermophila, was found to be a new anti-inflammatory and antioxidant compound directly inhibiting NF -KB.

BIOSIS/2023:170510	Dissecting key residues of a C4-dicarboxylic acid transporter to accelerate malate export in Myceliophthora.	Chinese Acad Sci, Tianjin Inst Ind Biotechnol, Key Lab Syst Microbial Biotechnol, Tianjin 300308, Peoples R China li_jg@tib.cas.cn; tian_cg@tib.cas.cn	Wu, Taju [Reprint Author] Wang, Yutao Li, Jingen [Reprint Author] Tian, Chaoguang [Reprint Author]	Applied Microbiology and Biotechnology, (FEB 2023) Vol. 107, No. 2-3. http://www.springerlink.com/content/100457/. CODEN: AMBIDG. ISSN: 0175-7598. E-ISSN: 1432-0614.	20230101	duplicates	Efficient transporters are necessary for high concentration and purity of desired products during industrial production. In this study, we explored the mechanism of substrate transport and preference of the C4-dicarboxylic acid transporter AoMAE in the fungus Myceliophthora thermophila , and investigated the roles of 18 critical amino acid residues within this process. Among them, the residue Arg78, forming a hydrogen bond network with Arg23, Phe25, Thr74, Leu81, His82, and Glu94 to stabilize the protein conformation, is irreplaceable for the export function of AoMAE. Furthermore, varying the residue at position 100 resulted in changes to the size and shape of the substrate binding pocket, leading to alterations in transport efficiencies of both malic acid and succinic acid. We found that the mutation T100S increased malate production by 68%. Using these insights, we successfully generated an AoMAE variant with mutation T100S and deubiquitination, exhibiting an 81% increase in the selective export activity of malic acid. Simply introducing this version of AoMAE into M. thermophila wild-type strain increased production of malic acid from 1.22 to 54.88 g/L. These findings increase our understanding of the structure-function relationships of organic acid transporters and may accelerate the process of engineering dicarboxylic acid transporters with high efficiency.
MEDLINE/2026591516	Dissecting key residues of a C4-dicarboxylic acid transporter to accelerate malate export in Myceliophthora.	Key Laboratory of Systems Microbial Biotechnology, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin, 300308, China. li_jg@tib.cas.cn; tian_cg@tib.cas.cn National Technology Innovation Center of Synthetic Biology, Tianjin, 300308, China. li_jg@tib.cas.cn; tian_cg@tib.cas.cn College of Biotechnology, Tianjin University of Science +Technology, Tianjin, 300457, China.	Wu Taju Wang Yutao Li Jingen Tian Chaoguang	Applied microbiology and biotechnology, (2023 Feb) Vol. 107, No. 2-3, pp. 609-622. Electronic Publication Date: 21 Dec 2022 Journal code: 8406612. E-ISSN: 1432-0614. L-ISSN: 0175-7598.	20230101	other	Efficient transporters are necessary for high concentration and purity of desired products during industrial production. In this study, we explored the mechanism of substrate transport and preference of the C4-dicarboxylic acid transporter AoMAE in the fungus Myceliophthora thermophila , and investigated the roles of 18 critical amino acid residues within this process. Among them, the residue Arg78, forming a hydrogen bond network with Arg23, Phe25, Thr74, Leu81, His82, and Glu94 to stabilize the protein conformation, is irreplaceable for the export function of AoMAE. Furthermore, varying the residue at position 100 resulted in changes to the size and shape of the substrate binding pocket, leading to alterations in transport efficiencies of both malic acid and succinic acid. We found that the mutation T100S increased malate production by 68%. Using these insights, we successfully generated an AoMAE variant with mutation T100S and deubiquitination, exhibiting an 81% increase in the selective export activity of malic acid. Simply introducing this version of AoMAE into M. thermophila wild-type strain increased production of malic acid from 1.22 to 54.88 g/L. These findings increase our understanding of the structure-function relationships of organic acid transporters and may accelerate the process of engineering dicarboxylic acid transporters with high efficiency. KEY POINTS: • This is the first systematical analysis of key residues of a malate transporter in fungi. • Protein engineering of AoMAE led to 81% increase of malate export activity. • Arg78 was essential for the normal function of AoMAE in M. thermophila. • Substitution of Thr100 affected export efficiency and substrate selectivity of AoMAE. © 2022. The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature.
CAPLUS/2023:1826375	Bioacetoin Production by Bacillus subtilis subsp. subtilis Using Enzymatic Hydrolysate of Lignocellulosic Biomass	Laboratory of Bioprocess Technology, Department of Microbiology, Maharshi Dayanand University, India	Saini, Meenaxi Anu Rapoport, Alexander Tiwari, Santosh Kumar Singh, Davender Malik, Vinay Kumar, Sandeep Singh, Bijender	Fermentation (2023), 9(8), 698 CODEN: FERMC4; ISSN: 2311-5637 URL: https://www.mdpi.com/journal/fermentation	20230101	other	Acetoin is an important bio-product useful in the chem., food and pharmaceutical industries. Microbial fermn. is the major process for the prodn. of bioacetoin, as the petroleum resources used in chem. methods are depleting day by day. Bioacetoin prodn. using wild microorganisms is an easy, eco-friendly and economical method for the prodn. of bioacetoin. In the present study, culture conditions and nutritional requirements were optimized for bioacetoin prodn. by a wild and non-pathogenic strain of B. subtilis subsp. subtilis JJBS250. The bacterial culture produced max. bioacetoin (259 mg L-1) using peptone (3%) and sucrose (2%) at 30 °C, 150 rpm and pH 7.0 after 24 h. Further supplementation of combinatorial nitrogen sources, i.e., peptone (1%) and urea (0.5%), resulted in enhanced titer of bioacetoin (1017 mg L-1) by the bacterial culture. An approx. 46.22-fold improvement in bioacetoin prodn. was achieved after the optimization process. The anal. of samples using thin layer chromatog. confirmed the presence of bioacetoin in the culture filtrate. The enzymic hydrolyzate was obtained by saccharification of pretreated rice straw and sugarcane bagasse using cellulase from Myceliophthora thermophila . Fermn. of the enzymic hydrolyzate (3%) of pretreated rice straw and sugarcane bagasse by the bacterial culture resulted in 210 and 473.17 mgL-1 bioacetoin, resp. Enzymic hydrolyzates supplemented with peptone as a nitrogen source showed a two to four-fold improvement in the prodn. of bioacetoin. Results have demonstrated the utility of wild type B. subtilis subsp. subtilis JJBS250 as a potential source for economical bioacetoin prodn. by making use of renewable and cost-effective lignocellulosic substrate. Therefore, this study will help in the sustainable management of agricultural waste for the industrial prodn. of bioacetoin, and in combating environmental pollution.
PQSCITECH/2023:336918	Bioacetoin Production by Bacillus subtilis subsp. subtilis Using Enzymatic Hydrolysate of Lignocellulosic Biomass		Saini, Meenaxi Anu Rapoport, Alexander Rapoport, Alexander Tiwari, Santosh Kumar Singh, Davender Malik, Vinay Kumar, Sandeep Singh, Bijender Singh, Bijender	Fermentation, Vol. 9, No. 8, 20230101 E-ISSN: 2311-5637 DOI: https://doi.org/10.3390/fermentation9080698 Published by: MDPI AG, Basel	20230101	duplicates	Acetoin is an important bio-product useful in the chemical, food and pharmaceutical industries. Microbial fermentation is the major process for the production of bioacetoin, as the petroleum resources used in chemical methods are depleting day by day. Bioacetoin production using wild microorganisms is an easy, eco-friendly and economical method for the production of bioacetoin. In the present study, culture conditions and nutritional requirements were optimized for bioacetoin production by a wild and non-pathogenic strain of B. subtilis subsp. subtilis JJBS250. The bacterial culture produced maximum bioacetoin (259 mg L-1) using peptone (3%) and sucrose (2%) at 30 °C, 150 rpm and pH 7.0 after 24 h. Further supplementation of combinatorial nitrogen sources, i.e., peptone (1%) and urea (0.5%), resulted in enhanced titre of bioacetoin (1017 mg L-1) by the bacterial culture. An approximately 46.22-fold improvement in bioacetoin production was achieved after the optimization process. The analysis of samples using thin layer chromatography confirmed the presence of bioacetoin in the culture filtrate. The enzymatic hydrolysate was obtained by saccharification of pretreated rice straw and sugarcane bagasse using cellulase from Myceliophthora thermophila . Fermentation of the enzymatic hydrolysate (3%) of pretreated rice straw and sugarcane bagasse by the bacterial culture resulted in 210 and 473.17 mgL-1 bioacetoin, respectively. Enzymatic hydrolysates supplemented with peptone as a nitrogen source showed a two to four-fold improvement in the production of bioacetoin. Results have demonstrated the utility of wild type B. subtilis subsp. subtilis JJBS250 as a potential source for economical bioacetoin production by making use of renewable and cost-effective lignocellulosic substrate. Therefore, this study will help in the sustainable management of agricultural waste for the industrial production of bioacetoin, and in combating environmental pollution.
EMBASE/20230373120	Insight into phytase-producing microorganisms for phytate solubilization and soil sustainability.	Department Food Science and Technology, Graphic Era (Deemed to be University), Dehradun, India. binnaik@gmail.com Himalayan School of Biosciences, Swami Rama Himalayan University, Dehradun, India. Department of Biotechnology, Graphic Era (Deemed to be University), Dehradun, India. Faculty of Agricultural Sciences, GLA University, Mathura, India. Department of Microbiology, Graphic Era (Deemed to be University), Dehradun, India. Department of Microbiology, Faculty of Agriculture and Forestry, University of Helsinki, Helsinki, Finland. per.saris@helsinki.fi	Rizwanuddin, Sheikh Naik, Bindu Kumar, Vijay Verma, Ankit Kumar, Vivek Singh, Pallavi Mishra, Sadhna Chauhan, Mansi Saris, Per Erik Joakim (correspondence)	Frontiers in Microbiology, (2023) Vol. 14. arn. 1127249. Refs: 109 E-ISSN: 1664-302X	20230101	other	The increasing demand for food has increased dependence on chemical fertilizers that promote rapid growth and yield as well as produce toxicity and negatively affect nutritional value. Therefore, researchers are focusing on alternatives that are safe for consumption, non-toxic , cost-effective production process, and high yielding, and that require readily available substrates for mass production. The potential industrial applications of microbial enzymes have grown significantly and are still rising in the 21st century to fulfill the needs of a population that is expanding quickly and to deal with the depletion of natural resources. Due to the high demand for such enzymes, phytases have undergone extensive research to lower the amount of phytate in human food and animal feed. They constitute efficient enzymatic groups that can solubilize phytate and thus provide plants with an enriched environment. Phytases can be extracted from a variety of sources such as plants, animals, and microorganisms. Compared to plant and animal-based phytases, microbial phytases have been identified as competent, stable, and promising bioinoculants. Many reports suggest that microbial phytase can undergo mass production procedures with the use of readily available substrates. Phytases neither involve the use of any toxic chemicals during the extraction nor release any such chemicals; thus, they qualify as bioinoculants and support soil sustainability. In addition, phytase genes are now inserted into new plants/crops to enhance transgenic plants reducing the need for supplemental inorganic phosphates and phosphate accumulation in the environment. The current review covers the significance of phytase in the agriculture system, emphasizing its source, action mechanism, and vast applications.

EMBASE/20230599117	A fungal lytic polysaccharide monooxygenase is required for cell wall integrity, thermotolerance, and virulence of the fungal human pathogen Cryptococcus neoformans.	Department of Medicine, Duke University School of Medicine, Durham, NC, United States. andrew.alspaugh@duke.edu Department of Molecular Genetics and Microbiology, Duke University School of Medicine, Durham, NC, United States. andrew.alspaugh@duke.edu Department of Geoscience and Natural Resource Management, University of Copenhagen, Frederiksberg, Denmark. Department of Physics, Astronomy Department, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States. INRAE, Aix-Marseille Univ., Biodiversite et Biotechnologie Fongiques (BBF), Marseille, France.	Probst, Corinna Nichols, Connie B. Alspaugh, J. Andrew (correspondence) Hallas-Moller, Magnus Ipsen, Johan O. Andersen, Karsten Martens, Helle J. Johansen, Katja S. Brooks, Jacob T. Haon, Mireille Berrin, Jean-Guy	PLoS Pathogens, (April 2023) Vol. 19, No. 4. arn. e1010946. Refs: 96 ISSN: 1553-7366; E-ISSN: 1553-7374	20230101	other	Fungi often adapt to environmental stress by altering their size, shape, or rate of cell division. These morphological changes require reorganization of the cell wall, a structural feature external to the cell membrane composed of highly interconnected polysaccharides and glycoproteins. Lytic polysaccharide monooxygenases (LPMOs) are copper-dependent enzymes that are typically secreted into the extracellular space to catalyze initial oxidative steps in the degradation of complex biopolymers such as chitin and cellulose. However, their roles in modifying endogenous microbial carbohydrates are poorly characterized. The CEL1 gene in the human fungal pathogen Cryptococcus neoformans (Cn) is predicted by sequence homology to encode an LPMO of the AA9 enzyme family. The CEL1 gene is induced by host physiological pH and temperature, and it is primarily localized to the fungal cell wall. Targeted mutation of the CEL1 gene revealed that it is required for the expression of stress response phenotypes, including thermotolerance, cell wall integrity, and efficient cell cycle progression. Accordingly, a cel1Δ deletion mutant was avirulent in two models of C. neoformans infection . Therefore, in contrast to LPMO activity in other microorganisms that primarily targets exogenous polysaccharides, these data suggest that CnCel1 promotes intrinsic fungal cell wall remodeling events required for efficient adaptation to the host environment.
SCISEARCH/2023:162211	Enhanced production and immobilization of phytase from Aspergillus oryzae: a safe and ideal food supplement for improving nutrition	Cent Univ Haryana, Dept Biotechnol, Jant Pali 123031, Mahendergarh, India (Reprint) Maharshi Dayanand Univ, Dept Microbiol, Lab Bioproc Technol, Rohtak 124001, India E-mail: ohlanbs@gmail.com Maharshi Dayanand Univ, Dept Microbiol, Lab Enzymol + Recombinant DNA Technol, Rohtak 124001, India Shobhit Inst Engn + Technol Deemed Univ, Dept Biotechnol, Meerut 250110, India Chinmaya Degree Coll, Dept Bot, Haridwar 249403, India RPS Degree Coll, Dept Phys, Mahendergarh 123029, India Univ Delhi, Shivaji Coll, Dept Bot, New Delhi 110027, India E-mail: ohlanbs@gmail.com Maharshi Dayanand Univ, Dept Microbiol, Lab Bioproc Technol, Rohtak 124001, India (Reprint)	Pragya, Krishna Kant (Reprint) Singh, Bijender Sharma, Krishna Kant Kumar, Sandeep Manisha, Vijay Singh, Davender Kumar, Vijay	LETTERS IN APPLIED MICROBIOLOGY, (16 2023 FEB 2023) Vol. 76, No. 2. ISSN: 0266-8254.	20230101	other	Microbial phytases are potentially excellent candidates for eliminating anti-nutrient i.e. phytic acid, due to hydrolysis of phospho-monoester linkages present in the phytic acid. An average 2.29-fold increase in phytase production was obtained after statistical optimization in solid-state fermentation. Aspergillus oryzae SBS50 phytase was immobilized on a Ca-alginate matrix with an effectiveness of 53%. Immobilized-phytase retained > 50% activity after recycling for five cycles and also displayed more stability in the presence of organic solvents, metal ions, and detergents as compared to free enzyme. Values of K-m and V-max of immobilized phytase were recorded as 0.66 mM and 666.6 nmol/sec, respectively. Immobilized phytase efficiently hydrolyzed the phytate contents in wheat and pearl millet flours, exhibiting > 70% catalytic activity even after three cycles. Phytase supplementation resulted in the improved nutritional quality of these flours. Furthermore, the safety assessment of the treated and untreated samples reveals the absence of any aflatoxin in the phytase produced by the mould. The results revealed the improved stability of phytase after immobilization and as a safe and significant additive for application in the food industry.
CAPLUS/2023:470533	In Silico Analysis of the Structural Dynamics and Substrate Recognition Determinants of the Human Mitochondrial Carnitine/Acylcarnitine SLC25A20 Transporter	Department of Sciences, University of Roma Tre, Italy	Pasquadibisceglie, Andrea Quadrotta, Virginia Polticelli, Fabio	International Journal of Molecular Sciences (2023), 24(4), 3946 CODEN: IJMCFK; ISSN: 1422-0067 URL: https://www.mdpi.com/journal/ijms	20230101	other	The Carnitine-Acylcarnitine Carrier is a member of the mitochondrial Solute Carrier Family 25 (SLC25), known as SLC25A20, involved in the electroneutral exchange of acylcarnitine and carnitine across the inner mitochondrial membrane. It acts as a master regulator of fatty acids beta-oxidn. and is known to be involved in neonatal pathologies and cancer . The transport mechanism, also known as 'alternating access', involves a conformational transition in which the binding site is accessible from one side of the membrane or the other. In this study, through a combination of state-of-the-art modeling techniques, mol. dynamics, and mol. docking, the structural dynamics of SLC25A20 and the early substrates recognition step have been analyzed. The results obtained demonstrated a significant asymmetry in the conformational changes leading to the transition from the c- to the m-state, confirming previous observations on other homologous transporters. Moreover, anal. of the MD simulations' trajectories of the apo-protein in the two conformational states allowed for a better understanding of the role of SLC25A20 Asp231His and Ala281Val pathogenic mutations , which are at the basis of Carnitine-Acylcarnitine Translocase Deficiency. Finally, mol. docking coupled to mol. dynamics simulations lend support to the multi-step substrates recognition and translocation mechanism already hypothesized for the ADP/ATP carrier.
EMBASE/20230157984	Hierarchically-structured laccase@Ni3(PO4)2 hybrid nanoflowers for antibiotic degradation: Application in real wastewater effluent and toxicity evaluation.	Department of Drug and Food Control, Faculty of Pharmacy, Tehran University of Medical Sciences, P.O. Box 14155-6451, Tehran, 1417614411, Iran, Islamic Republic of. samadin@tums.ac.ir Department of Pharmaceutical Biotechnology, Faculty of Pharmacy + Biotechnology Research Center, Tehran University of Medical Sciences, P.O. Box 14155-6451, Tehran, 1417614411, Iran, Islamic Republic of. faramarz@tu ms.ac.ir Pharmaceutical Quality Assurance Research Center, The Institute of Pharmaceutical Sciences (TIPS), Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of. samadin@tums.ac.ir	Jafari-Nodoushan, Hossein Fazeli, Mohammad Reza Samadi, Nasrin (correspondence) Faramarzi, Mohammad Ali	International Journal of Biological Macromolecules, (15 Apr 2023) Vol. 234. arn. 123574. Refs: 108 ISSN: 0141 8130; E-ISSN: 1879-0003 CODEN: IJBMDR	20230101	other	Laccase@Ni3(PO4)2 hybrid nanoflowers (HNFs) were prepared by the anisotropic growth of biomineralized nickel phosphate. The immobilization yield was 77.5 ± 3.6 %, and the immobilized enzyme retained 50 % of its initial activity after 18 reusability cycles. The immobilized and free enzymes lost 80 % of their activity after 18 and 6 h incubation in municipal wastewater effluent (MWWE), respectively. The increase in α-helix content (8 %) following immobilization led to a more rigid enzyme structure, potentially contributing to its improved stability. The removal of ciprofloxacin from MWWE by laccase@Ni3(PO4)2·HNFs/p-coumaric acid oxidation system was optimized using a Box-Behnken design. Under the optimized conditions [initial laccase activity (0.05 U mL-1), the concentration of p-coumaric acid (2.9 mM), and treatment time (4.9 h)], the biocatalyst removed 90 % of ciprofloxacin (10 mg L-1) from MWWE. The toxicity of ciprofloxacin against some G+ and G- bacteria was reduced by 35-70 %, depending on their strain. The EC50 of ciprofloxacin for the alga Raphidocelis subcapitata reduced from 3.08 to 1.07 mg L-1 (p-value toxicity of identified biodegradation products was lower than ciprofloxacin at three trophic levels, as predicted by ECOSAR software.
CAPLUS/2023:693658	Effect of Laccase Detoxification on Bioethanol Production from Liquid Fraction of Steam-Pretreated Olive Tree Pruning	Forest Sciences Institute (ICIFOR-INIA), CSIC, Spain	Ibarra, David Eugenio, Maria E. Alvira, Pablo Ballesteros, Ignacio Ballesteros, Mercedes Negro, Maria J.	Fermentation (2023), 9(3), 214 CODEN: FERMC4; ISSN: 2311-5637 URL: https://www.mdpi.com/journal/fermentation	20230101	duplicates	During lignocellulosic bioethanol prodn., the whole slurry obtained by steam explosion is filtered, generating a water-insol. fraction rich in cellulose which is used for saccharification and ethanol fermn., as well as a liq. fraction contg. solubilised glucose and xylose but also some inhibitory byproducts (furan derivs., weak acids and phenols), which limits its use for this purpose. Since utilization of this liq. fraction to ethanol is essential for an economically feasible cellulosic ethanol process, this work studied a laccase from Myceliophthora thermophila to detoxify the liq. fraction obtained from steam-pretreated olive tree pruning (OTP) and to overcome the effects of these inhibitors. Then, the fermn. of laccase-treated liq. fraction was evaluated on ethanol prodn. by different Saccharomyces cerevisiae strains, including the Ethanol Red, with the capacity to ferment glucose but not xylose, and the xylose-fermenting recombinant strain F12. Laccase treatment reduced total phenols content by 87% from OTP liq. fraction, not affecting furan derivs. and weak acids concn. Consequently, the fermentative behavior of both Ethanol Red and F12 strains was improved, and ethanol prodn. and yields were increased. Moreover, F12 strain was capable of utilizing some xylose, which increased ethanol prodn. (10.1 g/L) compared to Ethanol Red strain (8.6 g/L).

BIOSIS/2023:362839	Effect of Laccase Detoxification on Bioethanol Production from Liquid Fraction of Steam-Pretreated Olive Tree Pruning.	CSIC, Forest Sci Inst, ICIFOR, INIA, Ctra Coruna Km 7-5, Madrid 28040, Spain ibarra.david@inia.csic.es	Ibarra, David [Reprint Author] Eugenio, Maria E. Alvira, Pablo Ballesteros, Ignacio Ballesteros, Mercedes Negro, Maria J.	Fermentation-Basel, (MAR 2023) Vol. 9, No. 3, pp. Article No.: 214. http://www.mdpi.com/journal/fermentation. E-ISSN: 2311-5637.	20230101	other	During lignocellulosic bioethanol production, the whole slurry obtained by steam explosion is filtered, generating a water-insoluble fraction rich in cellulose which is used for saccharification and ethanol fermentation, as well as a liquid fraction containing solubilised glucose and xylose but also some inhibitory by-products (furan derivatives, weak acids and phenols), which limits its use for this purpose. Since utilization of this liquid fraction to ethanol is essential for an economically feasible cellulosic ethanol process, this work studied a laccase from Myceliophthora thermophila to detoxify the liquid fraction obtained from steam-pretreated olive tree pruning (OTP) and to overcome the effects of these inhibitors. Then, the fermentation of laccase-treated liquid fraction was evaluated on ethanol production by different Saccharomyces cerevisiae strains, including the Ethanol Red, with the capacity to ferment glucose but not xylose, and the xylose-fermenting recombinant strain F12. Laccase treatment reduced total phenols content by 87% from OTP liquid fraction, not affecting furan derivatives and weak acids concentration. Consequently, the fermentative behavior of both Ethanol Red and F12 strains was improved, and ethanol production and yields were increased. Moreover, F12 strain was capable of utilizing some xylose, which increased ethanol production (10.1 g/L) compared to Ethanol Red strain (8.6 g/L).
EMBASE/20221033285	TARGETING SORBED PAHS IN HISTORICALLY CONTAMINATED SOIL - CAN LACCASE MEDIATOR SYSTEMS OR FENTON'S REAGENT REMOVE INACCESSIBLE PAHS?.	Institute of Environmental Biotechnology, BOKU - University of Natural Resources and Life Sciences, Vienna, Tulln, A-3430, Austria. andreas.loibner@boku.ac.at Department of Environmental and Resource Engineering, Technical University of Denmark, Kongens Lyngby, Denmark. BOKU - University of Natural Resources and Life Sciences, Vienna, Department of Agrobiotechnology (IFA-Tulln), Institute of Environmental Biotechnology, Konrad-Lorenz-Strasse 20, Tulln, 3430, Austria. andreas.loi bner@boku.ac.at	Humel, Stefan Fuhrer, Bernadette Svetitsch, Michaela Loibner, Andreas P. (correspondence) Mayer, Philipp	Journal of Hazardous Materials, (5 Feb 2023) Vol. 443, Part Part B, arn. 130286. Refs: 56 ISSN: 0304-3894; E-ISSN: 1873-3336 CODEN: JHMAD9	20230101	duplicates	This laboratory study investigates the potential of two innovative laccase-mediator systems for removing PAHs from historically contaminated field soil and focuses on the treatment effect on the accessible and desorption resistant PAH fraction. Laccase degraded accessible PAHs when applied in combination with the mediator TEMPO (up to 24 % within 48 h). The mediator HBT did not induce degradation but mobilized desorption resistant PAHs from high affinity sorption sites via a competitive sorption mechanism. Enzymatic degradation of inaccessible PAHs was not observed with neither of the two enzyme-mediator systems. To verify a potential radical susceptibility of contaminants inaccessible to microorganisms, PAH contaminated biochar was treated with hydroxyl radicals generated by Fenton's reaction. These radical species reduced the desorption resistant fraction of phenanthrene (13 ± 10 %), fluoranthene (33 ± 8 %) and benzo(a)pyrene (69 ± 5 %). In conclusion, laccase-mediator systems can interact with accessible and inaccessible PAHs, whereas direct degradation of desorption resistant contaminants required highly active hydroxyl radicals. Further studies should develop enzyme-mediator systems establishing a sufficient oxidation potential to attack the desorption resistant contaminant fraction.
BIOSIS/2023:175796	Targeting sorbed PAHs in historically contaminated soil - Can laccase mediator systems or Fenton's reagent remove inaccessible PAHs?.	BOKU Univ Nat Resources and Life Sci, Inst Environm Biotechnol, Dept Agrobiotechnol IFA Tulln, Konrad Lorenz Str 20, A-3430 Vienna, Tulln, Austria andreas.loibner@boku.ac.at	Humel, Stefan Fuehrer, Bernadette Svetitsch, Michaela Mayer, Philipp Loibner, Andreas P. [Reprint Author]	Journal of Hazardous Materials, (FEB 5 2023) Vol. 443, No. Part B, pp. Article No.: 130286. http://www.journals.elsevier.com/journal-of-hazardous-materials/#description. CODEN: JHMAD9. ISSN: 0304-3894. E-ISSN: 1873-3336.	20230101	other	This laboratory study investigates the potential of two innovative laccase-mediator systems for removing PAHs from historically contaminated field soil and focuses on the treatment effect on the accessible and desorption resistant PAH fraction. Laccase degraded accessible PAHs when applied in combination with the mediator TEMPO (up to 24 % within 48 h). The mediator HBT did not induce degradation but mobilized desorption resistant PAHs from high affinity sorption sites via a competitive sorption mechanism. Enzymatic degradation of inaccessible PAHs was not observed with neither of the two enzyme-mediator systems. To verify a potential radical susceptibility of contaminants inaccessible to microorganisms, PAH contaminated biochar was treated with hydroxyl radicals generated by Fenton's reaction. These radical species reduced the desorption resistant fraction of phenanthrene (13 +/- 10 %), fluoranthene (33 +/- 8 %) and benzo(a)pyrene (69 +/- 5 %). In conclusion, laccase-mediator systems can interact with accessible and inaccessible PAHs, whereas direct degradation of desorption resistant contaminants required highly active hydroxyl radicals. Further studies should develop enzyme-mediator systems establishing a sufficient oxidation potential to attack the desorption resistant contaminant fraction.
BIOSIS/2023:682420	Recent advances in the use of laccase enzyme in deep eutectic solvents.	Mizoram Univ, Sch Phys Sci, Dept Ind Chem, Aizawl 796004, Mizoram, India pabbathi@mzu.edu.in	Hatimuria, Madushmita Das, Joydeep Gavvala, Krishna Bag, Soumabha Pabbathi, Ashok [Reprint Author]	Sustainable Chemistry and Pharmacy, (JUN 2023) Vol. 33, pp. Article No.: 101148. http://www.sciencedirect.com/science/journal/23525541. E-ISSN: 2352-5541.	20230101	other	Laccase enzymes have enormous applications in various fields including textile, bioremediation, water purification and biofuel industries. However, the utility of laccases is limited due to poor stability and activity under different industrial operating conditions. There has been extensive research on improving the activity of laccases by enzyme engineering and solvent engineering approaches. In solvent engineering approaches, laccase has been studied in non-aqueous solvents such as ionic liquids (ILs) and deep eutectic solvents (DESs). DESs are greener and more sustainable in nature compared to ILs. DESs have many advantages, for example, inexpensive, less toxic , and easy preparation methods. However, to the best of our knowledge, there are no comprehensive reviews focusing specifically on studies related to laccase in DESs. In this review, we discussed the laccase activity and stability in various DESs considering the existing literature. In addition, we also discussed about the correlation between laccase interaction with different types of DESs components and descriptors which can regulate the activity of laccase. Finally, we highlighted the need for future research along the lines of the laccase-DESs greener systems which can provide significant insights into the development of laccase-DESs systems leading to the green and sustainable methods for wide range of applications.
MEDLINE/2027227188	Construction and characterization of a Myceliophthora thermophila lytic polysaccharide monooxygenase mutant S174C/A93C with improved thermostability.	College of Chemical and Biological Engineering, Shandong University of Science and Technology, Qingdao 266590, PR China, Key Laboratory of Industrial Fermentation Microbiology of the Ministry of Education, College of Biotechnology, Tianjin University of Science and Technology, Tianjin 300450, PR China. Key Laboratory of Industrial Fermentation Microbiology of the Ministry of Education, College of Biotechnology, Tianjin University of Science and Technology, Tianjin 300450, PR China. Key Laboratory of Industrial Fermentation Microbiology of the Ministry of Education, College of Biotechnology, Tianjin University of Science and Technology, Tianjin 300450, PR China. fufengliu@tust.edu.cn College of Chemical and Biological Engineering, Shandong University of Science and Technology, Qingdao 266590, PR China. wb@sdust.edu.cn	Guo Xiao Jiang Luying An Yajing Lu Fuping Liu Fufeng Wang Bo	Enzyme and microbial technology, (2023 Aug) Vol. 168, pp. 110255. Electronic Publication Date: 10 May 2023 Journal code: 8003761. E-ISSN: 1879-0909. L-ISSN: 0141-0229.	20230101	other	Lytic polysaccharide monooxygenases (LPMOs) can oxidatively cleave the glycosidic bonds of crystalline polysaccharides, providing more accessible sites for polysaccharide hydrolases and promoting efficient conversion of biomass. In order to promote industrial applications of LPMOs, the stability of an LPMO of Myceliophthora thermophila C1 (MtC1LPMO) was improved by adding disulfide bonds in this study. Firstly, the structural changes of wild-type (WT) MtC1LPMO at different temperatures were explored using molecular dynamics simulations, and eight mutants were selected by combining the predicted results from Disulfide by Design (DBD), Multi agent stability prediction upon point mutations (Maestro) and Bridge disulfide (BridgeD) websites. Then, the enzymatic properties of the different mutants were determined after their expression and purification, and the mutant S174C/A93C with the highest thermal stability was obtained. The specific activities of unheated S174C/A93C and WT were 160.6 ± 1.7 U/g and 174.8 ± 7.5 U/g, respectively, while those of S174C/A93C and WT treated at 70 °C for 4 h were 77.7 ± 3.4 U/g and 46.1 ± 0.4 U/g, respectively. The transition midpoint temperature of S174C/A93C was 2.7 °C higher than that of WT. The conversion efficiency of S174C/A93C for both microcrystalline cellulose and corn straw was about 1.5 times higher than that of WT. Finally, molecular dynamics simulations revealed that the introduction of disulfide bonds increased the β-sheet content of the H1-E34 region, thus improving the rigidity of the protein. Therefore, the overall structural stability of S174C/A93C was improved, which in turn improved its thermal stability. Copyright © 2023 Elsevier Inc. All rights reserved.

PQSCITECH/2023:162082	The arabinose transporter MtLat-1 is involved in hemicellulase repression as a pentose transceptor in Myceliophthora thermophila		Gu, Shuying Zhao, Zhen Xue, Fanglei Liu, Defei Liu, Qian Li, Jingen Tian, Chaoguang	Biotechnology for Biofuels, Vol. 16, pp. 1-16, 20230101 E-ISSN: 1754-6834 DOI: https://doi.org/10.1186/s13068-023-02305-3 Published by: BioMed Central, London	20230101	duplicates	BackgroundFilamentous fungi possess an array of secreted enzymes to depolymerize the structural polysaccharide components of plant biomass. Sugar transporters play an essential role in nutrient uptake and sensing of extracellular signal molecules to inhibit or trigger the induction of lignocellulolytic enzymes. However, the identities and functions of transceptors associated with the induction of hemicellulase genes remain elusive.ResultsIn this study, we reveal that the l-arabinose transporter MtLat-1 is associated with repression of hemicellulase gene expression in the filamentous fungus Myceliophthora thermophila . The absence of Mtlat-1 caused a decrease in l-arabinose uptake and consumption rates. However, mycelium growth, protein production, and hemicellulolytic activities were markedly increased in a ΔMtlat-1 mutant compared with the wild-type (WT) when grown on arabinan. Comparative transcriptomic analysis showed a different expression profile in the ΔMtlat-1 strain from that in the WT in response to arabinan, and demonstrated that MtLat-1 was involved in the repression of the main hemicellulase-encoding genes. A point mutation that abolished the l-arabinose transport activity of MtLat-1 did not impact the repression of hemicellulase gene expression when the mutant protein was expressed in the ΔMtlat-1 strain. Thus, the involvement of MtLat-1 in the expression of hemicellulase genes is independent of its transport activity. The data suggested that MtLat-1 is a transceptor that senses and transduces the molecular signal, resulting in downstream repression of hemicellulolytic gene expression. MtAra-1 protein directly regulated the expression of Mtlat-1 by binding to its promoter region. Transcriptomic profiling indicated that the transcription factor MtAra-1 also plays an important role in expression of arabinanolytic enzyme genes and l-arabinose catabolism.ConclusionsM. thermophila MtLat-1 functions as a transceptor that is involved in l-arabinose transport and signal transduction associated with suppression of the expression of hemicellulolytic enzyme-encoding genes. The data presented in this study add to the models of the regulation of hemicellulases in filamentous fungi.
MEDLINE/2027015264	The arabinose transporter MtLat-1 is involved in hemicellulase repression as a pentose transceptor in Myceliophthora thermophila .	Key Laboratory of Engineering Biology for Low-carbon Manufacturing, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin, 300308, China. li_jg@tib.cas.cn; tian_cg@tib.cas.cn National Technology Innovation Center of Synthetic Biology, Tianjin, 300308, China. li_jg@tib.cas.cn; tian_cg@tib.cas.cn University of Chinese Academy of Sciences, Beijing, 100049, China. College of Biotechnology, Tianjin University of Science and Technology, Tianjin, 300457, China.	Gu Shuying Zhao Zhen Xue Fanglei Liu Defei Liu Qian Li Jingen Tian Chaoguang	Biotechnology for biofuels and bioproducts, (2023 Mar 25) Vol. 16, No. 1, pp. 51. Electronic Publication Date: 25 Mar 2023 Journal code: 9918300888906676. E-ISSN: 2731-3654. L-ISSN: 2731-3654. Report No.: PMC-PMC10040116.	20230101	other	BACKGROUND: Filamentous fungi possess an array of secreted enzymes to depolymerize the structural polysaccharide components of plant biomass. Sugar transporters play an essential role in nutrient uptake and sensing of extracellular signal molecules to inhibit or trigger the induction of lignocellulolytic enzymes. However, the identities and functions of transceptors associated with the induction of hemicellulase genes remain elusive. RESULTS: In this study, we reveal that the L-arabinose transporter MtLat-1 is associated with repression of hemicellulase gene expression in the filamentous fungus Myceliophthora thermophila . The absence of Mtlat-1 caused a decrease in L-arabinose uptake and consumption rates. However, mycelium growth, protein production, and hemicellulolytic activities were markedly increased in a ΔMtlat-1 mutant compared with the wild-type (WT) when grown on arabinan. Comparative transcriptomic analysis showed a different expression profile in the ΔMtlat-1 strain from that in the WT in response to arabinan, and demonstrated that MtLat-1 was involved in the repression of the main hemicellulase-encoding genes. A point mutation that abolished the L-arabinose transport activity of MtLat-1 did not impact the repression of hemicellulase gene expression when the mutant protein was expressed in the ΔMtlat-1 strain. Thus, the involvement of MtLat-1 in the expression of hemicellulase genes is independent of its transport activity. The data suggested that MtLat-1 is a transceptor that senses and transduces the molecular signal, resulting in downstream repression of hemicellulolytic gene expression. MtAra-1 protein directly regulated the expression of Mtlat-1 by binding to its promoter region. Transcriptomic profiling indicated that the transcription factor MtAra-1 also plays an important role in expression of arabinanolytic enzyme genes and L-arabinose catabolism. CONCLUSIONS: M. thermophila MtLat-1 functions as a transceptor that is involved in L-arabinose transport and signal transduction associated with suppression of the expression of hemicellulolytic enzyme-encoding genes. The data presented in this study add to the models of the regulation of hemicellulases in filamentous fungi. © 2023. The Author(s).
MEDLINE/2027407888	Preclinical immunogenicity and protective efficacy of a SARS-CoV-2 RBD-based vaccine produced with the thermophilic filamentous fungal expression system Thermothelomyces heterothallica C1.	Research Center for Emerging Infections and Zoonoses, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany. Institute for Biochemistry, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany. Department of Pathology, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany. Viroclinics Xplore, Schaijk, Netherlands. Dyadic International, Inc., Jupiter, FL, United States. VTT Technical Research Centre of Finland Ltd., Espoo, Finland. Virology Section, Infectious Diseases and Immunology Division, Department of Biomolecular Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands. Department of Viroscience, Erasmus Medical Center, Rotterdam, Netherlands.	Gonzalez-Hernandez Mariana Kaiser Franziska Karola Steffen Imke Osterhaus Albert D M E Ciurkiewicz Malgorzata Baumgartner Wolfgang van Amerongen Geert Tchelet Ronen Emalfarb Mark Saloheimo Markku Wiebe Marilyn G Vitikainen Marika Albulescu Irina C Bosch Berend-Jan Haagmans Bart L	Frontiers in immunology, (2023) Vol. 14, pp. 1204834. Electronic Publication Date: 9 Jun 2023 Journal code: 101560960. E-ISSN: 1664-3224. L-ISSN: 1664-3224. Report No.: PMC-PMC10289020.	20230101	other	Introduction: The emergency use of vaccines has been the most efficient way to control the coronavirus disease 19 (COVID-19) pandemic. However, the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants of concern has reduced the efficacy of currently used vaccines. The receptor-binding domain (RBD) of the SARS-CoV-2 spike (S) protein is the main target for virus neutralizing (VN) antibodies. Methods: A SARS-CoV-2 RBD vaccine candidate was produced in the Thermothelomyces heterothallica (formerly, Myceliophthora thermophila) C1 protein expression system and coupled to a nanoparticle. Immunogenicity and efficacy of this vaccine candidate was tested using the Syrian golden hamster (Mesocricetus auratus) infection model. Results: One dose of 10-µg RBD vaccine based on SARS-CoV-2 Wuhan strain, coupled to a nanoparticle in combination with aluminum hydroxide as adjuvant, efficiently induced VN antibodies and reduced viral load and lung damage upon SARS-CoV-2 challenge infection . The VN antibodies neutralized SARS-CoV-2 variants of concern: D614G, Alpha, Beta, Gamma, and Delta. Discussion: Our results support the use of the Thermothelomyces heterothallica C1 protein expression system to produce recombinant vaccines against SARS-CoV-2 and other virus infections to help overcome limitations associated with the use of mammalian expression system. Copyright © 2023 Gonzalez-Hernandez, Kaiser, Steffen, Ciurkiewicz, van Amerongen, Tchelet, Emalfarb, Saloheimo, Wiebe, Vitikainen, Albulescu, Bosch, Baumgartner, Haagmans and Osterhaus.
SCISEARCH/2023:509533	Enzyme-Catalyzed Polymerization of Kraft Lignin from Eucalyptus globulus: Comparison of Bacterial and Fungal Laccases Efficacy	E-mail: mariaeugenia@inia.csic.es Univ Alcala, Dept Biomed + Biotechnol, Alcala De Henares 28805, Spain Univ Basque Country UPV EHU, Gen Serv Res SGIKER, Edificio Joxe Mari Korta, Avda Tolosa 72, San Sebastian 20018, Spain CSIC, Forest Sci Inst ICIFOR INIA, Ctra Coruna Km 7-5, Madrid 28040, Spain (Reprint)	Garcia-Fuentevilla, Luisa (Reprint) Martin-Sampedro, Raquel Ibarra, David Eugenio, Maria E. Dominguez, Gabriela Hernandez, Manuel Arias, Maria E. Santos, Jose I.	POLYMERS, (2023 FEB 2023) Vol. 15, No. 3.	20230101	other	Kraft lignin, a side-stream from the pulp and paper industry, can be modified by laccases for the synthesis of high added-value products. This work aims to study different laccase sources, including a bacterial laccase from Streptomyces ipomoeae (SiLA) and a fungal laccase from Myceliophthora thermophila (MtL), for kraft lignin polymerization. To study the influence of some variables in these processes, a central composite design (CCD) with two continuous variables (enzyme concentration and reaction time) and three levels for each variable was used. The prediction of the behavior of the output variables (phenolic content and molecular weight of lignins) were modelled by means of response surface methodology (RSM). Moreover, characterization of lignins was performed by Fourier-transform infrared (FTIR) spectroscopy and different nuclear magnetic resonance (NMR) spectroscopy techniques. In addition, antioxidant activity was also analyzed. Results showed that lignin polymerization (referring to polymerization as lower phenolic content and higher molecular weight) occurred by the action of both laccases. The enzyme concentration was the most influential variable in the lignin polymerization reaction within the range studied for SiLA laccase, while the most influential variable for MtL laccase was the reaction time. FTIR and NMR characterization analysis corroborated lignin polymerization results obtained from the RSM.

EMBASE/20230816177	Multimodal metagenomic analysis reveals microbial single nucleotide variants as superior biomarkers for early detection of colorectal cancer .	Department of Gastroenterology, the Shanghai Tenth People's Hospital, School of Medicine, School of Life Sciences and Technology, Tongji University, Shanghai, China. rxzhu@tongji.edu.cn Guangdong Institute of Gastroenterology, Guangdong Provincial Key Laboratory of Colorectal and Pelvic Floor Diseases, Biomedical Innovation Center, Sun Yat-Sen University, Guangzhou, China. zhulx6@mail.sysu.edu.cn Department of General Surgery, The Sixth Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China. zhulx6@mail.sysu.edu.cn National Clinical Research Center for Child Health, the Children's Hospital, Zhejiang University School of Medicine, Hangzhou, China. jiaona@zju.edu.cn Center for IBD Research, Department of Gastroenterology, The Shanghai Tenth People's Hospital, School of Medicine, Tongji University, Shanghai, 200072, China. liuzhanju88@126.com Research Institute, GloriousMed Clinical Laboratory Co, Ltd, Shanghai, China. rxzhu@tongji.edu.cn	Gao, Wenxing Gao, Xiang Gao, Sheng Sun, Ruicong Feng, Zhongsheng Zhu, Ruixin Zhu, Lixin Wu, Dingfeng Jiao, Na Liu, Zhanju (correspondence)	Gut Microbes, (2023) Vol. 15, No. 2. arn. 2245562. Refs: 57 ISSN: 1949-0976; E-ISSN: 1949-0984	20230101	other	Microbial signatures show remarkable potentials in predicting colorectal cancer (CRC). This study aimed to evaluate the diagnostic powers of multimodal microbial signatures, multi-kingdom species, genes, and single-nucleotide variants (SNVs) for detecting precancerous adenomas. We performed cross-cohort analyses on whole metagenome sequencing data of 750 samples via xMarkerFinder to identify adenoma-associated microbial multimodal signatures. Our data revealed that fungal species outperformed species from other kingdoms with an area under the ROC curve (AUC) of 0.71 in distinguishing adenomas from controls. The microbial SNVs, including dark SNVs with synonymous mutations , displayed the strongest diagnostic capability with an AUC value of 0.89, sensitivity of 0.79, specificity of 0.85, and Matthews correlation coefficient (MCC) of 0.74. SNV biomarkers also exhibited outstanding performances in three independent validation cohorts (AUCs = 0.83, 0.82, 0.76; sensitivity = 1.0, 0.72, 0.93; specificity = 0.67, 0.81, 0.67, MCCs = 0.69, 0.83, 0.72) with high disease specificity for adenoma. In further support of the above results, functional analyses revealed more frequent inter-kingdom associations between bacteria and fungi, and abnormalities in quorum sensing, purine and butanoate metabolism in adenoma, which were validated in a newly recruited cohort via qRT-PCR. Therefore, these data extend our understanding of adenoma-associated multimodal alterations in the gut microbiome and provide a rationale of microbial SNVs for the early detection of CRC.
CAPLUS/2023:417549	Safety and efficacy of a feed additive consisting of endo-1, 4-β-D-mannanase produced by Thermothelomyces thermophilus DSM 33149 (Natupulse TS / TS L) for chickens and turkeys for fattening, minor poultry species for fattening and ornamental birds (BASF SE)		EFSA Panel on Additives and Products or Substances used in Animal Feed Bampidis, Vasileios Azimonti, Giovanna Bastos, Maria de Lourdes Christensen, Henrik Durjava, Mojca Dusemund, Birgit Kouba, Maryline Lopez-Alonso, Marta Lopez Puente, Secundino Marcon, Francesca Mayo, Baltasar Pechova, Alena Petkova, Mariana Ramos, Fernando Sanz, Yolanda Villa, Roberto Edoardo Woutersen, Ruud Brantom, Paul Herman, Lieve Louro, Henriqueta Anguita, Montserrat Brozzi, Rosella Galobart, Jaume Innocenti, Matteo	EFSA Journal (2023), 21(2), e07873 CODEN: EJFOA6; ISSN: 1831-4732	20230101	duplicates	Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of endo-1,4-β-D-mannanase produced by Thermothelomyces thermophilus DSM 33149 (Natupulse TS/TS L) as a zootech. feed additive for chickens and turkeys for fattening, minor poultry species for fattening and ornamental birds. The additive under assessment, Natupulse TS/TS L, does not pose any safety concern regarding the prodn. strain. The FEEDAP Panel concluded that the additive is tolerated by chickens for fattening, and this conclusion can be extrapolated to all poultry for fattening. Due to the lack of reliable data regarding the potential of the additive to induce chromosomal damage, the FEEDAP Panel cannot conclude on the safety of the additive for the target species and on the safety for consumer. The use of the additive in animal nutrition is considered safe for the environment. The additive is considered not to be irritant to the skin and eyes but is considered a respiratory sensitizer, although exposure by inhalation is unlikely. The Panel could not conclude on the potential of the additive to be a skin sensitizer. Due to the lack of reliable data, the FEEDAP Panel considered that a potential of the additive to induce chromosomal damage in exposed unprotected users cannot be excluded. Consequently, exposure of users should be minimised. The Panel concluded that the additive Natupulse TS/TS L has the potential to be efficacious in chickens for fattening at the proposed conditions of use and this conclusion can be extrapolated to turkeys for fattening, minor poultry species for fattening and ornamental birds.
MEDLINE/2026290115	Structural and thermostability insights into cellobiohydrolase of a thermophilic mould Myceliophthora thermophila : in-silico studies.	Department of Biological Sciences and Engineering, Netaji Subhas University of Technology, Dwarka, New Delhi, India. Department of Applied Sciences and Humanities (Faculty of Technology), University of Delhi, Delhi, India. Department of Applied Sciences, Indian Institute of Information Technology Allahabad, Allahabad, Uttar Pradesh, India.	Dadwal Anica Sharma Shilpa Satyanarayana Tulasi Singh Vishal Sahoo Amaresh Kumar	Journal of biomolecular structure + dynamics, (2023 Oct-Nov) Vol. 41, No. 17, pp. 8373-8382. Electronic Publication Date: 13 Oct 2022 Journal code: 8404176. E-ISSN: 1538-0254. L-ISSN: 0739-1102.	20230101	other	Cellobiohydrolase (CBH) is one of the cellulases with a wide range of industrial applications; it plays a pivotal role in cellulose hydrolysis and thus in biofuel production. The structural and thermostability analysis of a CBHII of the thermophilic mold Myceliophthora thermophila (MtCel6A) had been carried out using various in-silico approaches. The validation of 3 D model by the Ramachandran plot indicated 88.5% amino acid residues in the favoured regions. Docking analysis suggested MtCel6A to display a high affinity towards cellotetraose as compared to other substrates. The enzyme exhibited a high tolerance to the end product, cellobiose. The thermostability evaluation by molecular dynamic simulations and principal component analysis confirmed its tolerance to elevated temperatures. The identified thermolabile regions could be targeted for site-directed mutagenesis in order to ameliorate thermostability further. Our experimental data published earlier confirmed the present findings of in-silico studies. The structural and functional characteristics of MtCel6A highlighted its critical features that make it a useful biocatalyst in several industrial processes.Communicated by Ramaswamy H. Sarma.

CAPLUS/2022:2780712	Electrochemical characterization of a family AA10 LPMO and the impact of residues shaping the copper site on reactivity	LAQV, REQUIMTE, NOVA School of Sciences and Technology, Universidade NOVA de Lisboa, Caparica, 2829-516, Port.	Cordas, Cristina M. Valerio, Gabriel N. Stepnov, Anton Kommedal, Eirik Kjendseth, Asmund R. Forsberg, Zarah Eijsink, Vincent G. H. Moura, Jose J. G.	Journal of Inorganic Biochemistry (2023), 238, 112056 CODEN: JIBIDJ; ISSN: 0162-0134	20230101	other	Research on enzymes for lignocellulose biomass degrdn. has progressively increased in recent years due to the interest in taking advantage of this natural resource. Among these enzymes are the lytic polysaccharide monooxygenases (LPMOs) that oxidatively depolymerize cryst. cellulose using a reactive oxygen species generated in a reduced mono-copper active site. The copper site comprises of a highly conserved histidine-brace, providing three equatorial nitrogen ligands, whereas less conserved residues close to the copper contribute to shaping and confining the site. The catalytic copper site is exposed to the solvent and to the cryst. substrates, and as so, the influence of the copper environment on LPMO properties, including the redox potential, is of great interest. In the current work, a direct electrochem. study of an LPMO (ScLPMO10C) was conducted allowing to retrieve kinetic and thermodn. data assocd. with the redox transition in the catalytic center. Moreover, two residues that do not bind to the copper but shape the copper sites were mutated , and the properties of the mutants were compared with those of the wild-type enzyme. The direct electrochem. studies, using cyclic voltammetry, yielded redox potentials in the +200 mV range, well in line with LPMO redox potentials detd. by other methods. Interestingly, while the mutations hardly affected the formal redox potential of the enzyme, they drastically affected the reactivity of the copper site and enzyme functionality.
SCISEARCH/2023:552396	Safety and efficacy of a feed additive consisting of endo-1,4-b-D-mannanase produced by Thermothelomyces thermophilus DSM 33149 (Natupulse (R) TS/TS L) for chickens and turkeys for fattening, minor poultry species for fattening and ornamental birds (BASF SE)		Bampidis, Vasileios (Reprint) Azimonti, Giovanna Bastos, Maria de Lourdes Christensen, Henrik Durjava, Mojca Dusemund, Birgit Kouba, Maryline Lopez-Alonso, Marta Lopez Puente, Secundino Marcon, Francesca Mayo, Baltasar Pechova, Alena Petkova, Mariana Ramos, Fernando Sanz, Yolanda Villa, Roberto Edoardo Woutersen, Ruud Brantom, Paul Herman, Lieve Louro, Henriqueta Anguita, Montserrat Brozzi, Rosella Galobart, Jaume Innocenti, Matteo Lorenzo Ortuno, Jordi Pettenati, Elisa	EFSA JOURNAL, (2023 FEB 2023) Vol. 21, No. 2.	20230101	duplicates	Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of endo-1,4-beta-d-mannanase produced by Thermothelomyces thermophilus DSM 33149 (Natupulse (R) TS/TS L) as a zootechnical feed additive for chickens and turkeys for fattening, minor poultry species for fattening and ornamental birds. The additive under assessment, Natupulse (R) TS/TS L, does not pose any safety concern regarding the production strain. The FEEDAP Panel concluded that the additive is tolerated by chickens for fattening, and this conclusion can be extrapolated to all poultry for fattening. Due to the lack of reliable data regarding the potential of the additive to induce chromosomal damage, the FEEDAP Panel cannot conclude on the safety of the additive for the target species and on the safety for consumer. The use of the additive in animal nutrition is considered safe for the environment. The additive is considered not to be irritant to the skin and eyes but is considered a respiratory sensitiser, although exposure by inhalation is unlikely. The Panel could not conclude on the potential of the additive to be a skin sensitiser. Due to the lack of reliable data, the FEEDAP Panel considered that a potential of the additive to induce chromosomal damage in exposed unprotected users cannot be excluded. Consequently, exposure of users should be minimised. The Panel concluded that the additive Natupulse (R) TS/TS L has the potential to be efficacious in chickens for fattening at the proposed conditions of use and this conclusion can be extrapolated to turkeys for fattening, minor poultry species for fattening and ornamental birds.

CABA/2023:165913	Safety and efficacy of a feed additive consisting of endo-1,4-β-D-mannanase produced by Thermothelomyces thermophilus DSM 33149 (Natupulse® TS/TS L) for chickens and turkeys for fattening, minor poultry species for fattening and ornamental birds (BASF SE).		Bampidis, V. Azimonti, G. Lourdes Bastos, M. de Christensen, H. Durjava, M. Dusemund, B. Kouba, M. Lopez-Alonso, M. Puente, S. L. Marcon, F. Mayo, B. Pechova, A. Petkova, M. Ramos, F. Sanz, Y. Villa, R. E. Woutersen, R. Brantom, P. Herman, L. Louro, H. Anguita, M. Brozzi, R. Galobart, J. Innocenti, M. L. Ortuno, J. Pettenati, E. Pizzo, F. Revez, J.	EFSA Journal (2023), Volume 21, Number 2, 7 refs. ISSN: 1831-4732 DOI: https://doi.org/10.2903/j.efsa.2023.7873 Published by: Wiley, Oxford	20230101	duplicates	Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of endo-1,4-β-d-mannanase produced by Thermothelomyces thermophilus DSM 33149 (Natupulse® TS/TS L) as a zootechnical feed additive for chickens and turkeys for fattening, minor poultry species for fattening and ornamental birds. The additive under assessment, Natupulse® TS/TS L, does not pose any safety concern regarding the production strain. The FEEDAP Panel concluded that the additive is tolerated by chickens for fattening, and this conclusion can be extrapolated to all poultry for fattening. Due to the lack of reliable data regarding the potential of the additive to induce chromosomal damage, the FEEDAP Panel cannot conclude on the safety of the additive for the target species and on the safety for consumer. The use of the additive in animal nutrition is considered safe for the environment. The additive is considered not to be irritant to the skin and eyes but is considered a respiratory sensitiser, although exposure by inhalation is unlikely. The Panel could not conclude on the potential of the additive to be a skin sensitiser. Due to the lack of reliable data, the FEEDAP Panel considered that a potential of the additive to induce chromosomal damage in exposed unprotected users cannot be excluded. Consequently, exposure of users should be minimised. The Panel concluded that the additive Natupulse® TS/TS L has the potential to be efficacious in chickens for fattening at the proposed conditions of use and this conclusion can be extrapolated to turkeys for fattening, minor poultry species for fattening and ornamental birds.
CAPLUS/2021:1691828	A greener, mild, and efficient bioprocess for the pretreatment and saccharification of rice straw	Laboratory of Bioprocess Technology, Department of Microbiology, Maharshi Dayanand University, Haryana, 124001, India	Anu Kumar, Vinod Singh, Davender Singh, Bijender	Biomass Conversion and Biorefinery (2023), 13(5), 4121-4133 CODEN: BCBIBN; ISSN: 2190-6823	20230101	other	Open rice straw burning has created hazardous effects on the environment and human health. Biol. pretreatment of rice straw has been proven as an environmental-benign and economical feasible process for the prodn. of biofuels and value-added products under milder and greener conditions by avoiding harmful chems. and toxic reagents. A consolidated bioprocess for the prodn. of cellulolytic enzymes and biol. pretreatment was developed to achieve a cost-effective saccharification of rice straw. Biodegrdn. of biomass was slightly enhanced with increase in cultivation time of microbial pretreatment. Furthermore, the modification in biomass due to biol. pretreatment weakened the interaction between hemicellulose and lignin that resulted in significant redn. in lignin using Bacillus subtilis subsp. subtilis JJBS300, Myceliophthora thermophila BJTLRMDU3, and Aspergillus oryzae SBS50 for biol. pretreatment of rice straw. Bacterial culture produced max. FPase, CMCase, and β-glucosidase of 28, 17, and 20.36 U/g DMR after 2 days during pretreatment process, whereas M. thermophila produced max. FPase (85.10 U/g DMR), CMCase (96.89 U/g DMR), and β-glucosidase (91.92 U/g DMR) after 9 days. The mold A.oryzae SBS50 also produced max. FPase (57.41 U/g DMR), CMCase (55.36 U/g DMR), and β-glucosidase (48.04 U/g DMR) after 9 days of pretreatment. Maximum amt. of reducing sugars of 52.41, 86.74, and 49.59 mg/g substrate were liberated from 6-, 5-, and 3-day-old biol. pretreated rice straw by B. subtilis, M. thermophila, and A. oryzae, resp., after enzymic hydrolysis for 6 h at 60 oC and pH 5.0. Laccase-pretreated rice straw followed by enzymastic saccharification resulted in liberation of 162.82 mg/g reducing sugars at pH 5.0 and 60 °C after 6h using 20 U/g cellulase. Simultaneous laccase pretreatment and saccharification (SLPS) process further enhanced the liberated reducing sugars, i.e., 179.47 mg/g substrate. FTIR, XRD, and SEM analyses showed marked morphol. changes as a result of delignification after biol. pretreatment of rice straw. Biol. pretreatment being an environmental-benign process causing no harm to the environment in comparison to phys. and chem. pretreatments of lignocellulosic biomass could be a better pretreatment strategy for bioremediation of lignocellulosic substrates.
EMBASE/20220002180	Improvement of catalytic performance of endoglucanase CgEndo from Colletotrichum graminicola by site-directed mutagenesis .	Key Laboratory of Bioorganic Synthesis of Zhejiang Province, College of Biotechnology and Bioengineering, Zhejiang University of Technology, Hangzhou, 310014, China. microliu@zjut.edu.cn The National and Local Joint Engineering Research Center for Biomanufacturing of Chiral Chemicals, Zhejiang University of Technology, Hangzhou, 310014, China. microliu@zjut.edu.cn Zhejiang Huakang Pharmaceutical Co., LTD, 18 Huagong Road, Huabu Town, Kaihua, 324302, China. College of Biotechnology and Bioengineering, Zhejiang University of Technology, No. 18 Chaowang Road, Hangzhou, Zhejiang Province, 310014, China. microliu@zjut.edu.cn	Zhou, Hai-Yan Yi, Xiao-Nan Chen, Qi Zhou, Jian-Bao Li, Shu-Fang Cai, Xue Liu, Zhi-Qiang (correspondence) Zheng, Yu-Guo Chen, De-Shui Cheng, Xin-Ping Li, Mian Wang, Hong-Yan Chen, Kai-Qian	Enzyme and Microbial Technology, (March 2022) Vol. 154. arn. 109963. Refs: 45 ISSN: 0141-0229; E-ISSN: 1879-0909 CODEN: EMTED2	20220101	other	In order to improve the catalytic efficiency of cellulase for more effective utilization of lignocellulose, a novel endoglucanase (CgEndo) from Colletotrichum graminicola was expressed by Pichia pastoris X33 and modified by site-directed mutagenesis . Two mutants, Y63S and N20D/S113T, with 62.31% and 57.14% increased enzyme activities were obtained, respectively. On this basis, their biochemical properties, kinetic parameters, structural information as well as the application in biomass degradation were investigated and compared with the wild-type CgEngo. The results indicated that the mutation Y63S and N20D/S113T resulted in an improvement of proximity between enzyme and substrate through conformational changes of the catalytic region, which might contribute to the higher enzyme activities and catalysis efficiency (Kcat/Km) of Y63S and N20D/S113T. These findings laid important foundation for the further engineering of this endoglucanase and practical application in efficient degradation of cellulosic biomass in nature.

EMBASE/20220012976	Fungal cellulases: protein engineering and post-translational modifications.	College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou, 310058, China. liyanjunspkx@sina.com Key Laboratory of Food and Biological Engineering of Zhejiang Province, Research and Development Department, Hangzhou Wahaha Technology Co. Ltd., Hangzhou Wahaha Group Co. Ltd., Hangzhou, 310018, China. liyanjunspkx@sina .com	Zhang, Ruiqin Li, Yanjun (correspondence) Cao, Chenghao Bi, Jiahua	Applied Microbiology and Biotechnology, (January 2022) Vol. 106, No. 1. Refs: 226 ISSN: 0175-7598; E-ISSN: 1432-0614 CODEN: AMBIDG	20220101	other	Abstract: Enzymatic degradation of lignocelluloses into fermentable sugars to produce biofuels and other biomaterials is critical for environmentally sustainable development and energy resource supply. However, there are problems in enzymatic cellulose hydrolysis, such as the complex cellulase composition, low degradation efficiency, high production cost, and post-translational modifications (PTMs), all of which are closely related to specific characteristics of cellulases that remain unclear. These problems hinder the practical application of cellulases. Due to the rapid development of computer technology in recent years, computer-aided protein engineering is being widely used, which also brings new opportunities for the development of cellulases. Especially in recent years, a large number of studies have reported on the application of computer-aided protein engineering in the development of cellulases; however, these articles have not been systematically reviewed. This article focused on the aspect of protein engineering and PTMs of fungal cellulases. In this manuscript, the latest literatures and the distribution of potential sites of cellulases for engineering have been systematically summarized, which provide reference for further improvement of cellulase properties. Key points: •Rational design based on virtual mutagenesis can improve cellulase properties. •Modifying protein side chains and glycans helps obtain superior cellulases. •N-terminal glutamine-pyroglutamate conversion stabilizes fungal cellulases.
EMBASE/20220051092	Berberine bridge enzyme-like oxidase-catalysed double bond isomerization acts as the pathway switch in cytochalasin synthesis.	College of Pharmaceutical Sciences, Southwest University, Chongqing, 400715, China. zouyi31@swu.edu.cn Key Laboratory of Marine Drugs, Chinese Ministry of Education, School of Medicine and Pharmacy, Ocean University of China, Qingdao, 266003, China.	Zhang, Jin-Mei Liu, Xuan Wei, Qian Zou, Yi (correspondence) Ma, Chuanteng Li, Dehai	Nature Communications, (December 2022) Vol. 13, No. 1. arn. 225. Refs: 42 E-ISSN: 2041-1723	20220101	other	Cytochalasans (CYTs), as well as their polycyclic (pcCYTs) and polymerized (meCYTs) derivatives, constitute one of the largest families of fungal polyketide-nonribosomal peptide (PK-NRP) hybrid natural products. However, the mechanism of chemical conversion from mono-CYTs (moCYTs) to both pcCYTs and meCYTs remains unknown. Here, we show the first successful example of the reconstitution of the CYT core backbone as well as the whole pathway in a heterologous host. Importantly, we also describe the berberine bridge enzyme (BBE)-like oxidase AspoA, which uses Glu538 as a general acid biocatalyst to catalyse an unusual protonation-driven double bond isomerization reaction and acts as a switch to alter the native (for moCYTs) and nonenzymatic (for pcCYTs and meCYTs) pathways to synthesize aspochalasin family compounds. Our results present an unprecedented function of BBE-like enzymes and highly suggest that the isolated pcCYTs and meCYTs are most likely artificially derived products.
EMBASE/20220594807	DEVELOPMENT OF AN EFFICIENT C-TO-T BASE-EDITING SYSTEM AND ITS APPLICATION TO CELLULASE TRANSCRIPTION FACTOR PRECISE ENGINEERING IN THERMOPHILIC FUNGUS MYCELIOPHTHORA THERMOPHILA .	Key Laboratory of Systems Microbial Biotechnology, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin, China. tian_cg@tib.cas.cn liu_q1@tib.cas.cn National Technology Innovation Center of Synthetic Biology, Tianjin, China . tian_cg@tib.cas.cn University of Chinese Academy of Sciences, Beijing, China. tian_cg@tib.cas .cn College of Biotechnology, Tianjin University of Science + Technology, Tianjin, China.	Zhang, Chenyang Li, Nan Rao, Lang Li, Jingen Liu, Qian Tian, Chaoguang (correspondence)	Microbiology Spectrum, (June 2022) Vol. 10, No. 3. Refs: 72 E-ISSN: 2165-0497	20220101	duplicates	Myceliophthora thermophila is a thermophilic fungus with great potential in biorefineries and biotechnology. The base editor is an upgraded version of the clustered regularly interspaced short palindromic repeats (CRISPR)-dependent genome-editing tool that introduces precise point mutations without causing DNA double-strand breaks (DSBs) and has been used in various organisms but rarely in filamentous fungi, especially thermophilic filamentous fungi. Here, for the first time, we constructed three cytosine base editors (CBEs) in M. thermophila, namely, evolved apolipoprotein B mRNA-editing enzyme catalytic subunit 1 (APOBEC1) cytosine base editor 4 max (MtevoBE4max), bacteriophage Mu Gam protein cytosine base editor 4 max (MtGAM-BE4max), and evolved CDA1 deaminase cytosine base editor (Mtevo-CDA1), and efficiently inactivated genes by precisely converting three codons (CAA, CAG, and CGA) into stop codons without DSB formation. The Mtevo-CDA1 editor with up to 92.6% editing efficiency is a more suitable tool for cytosine base editing in thermophilic fungi. To investigate the function of each motif of the cellulase transcription factor M. thermophila CLR-2 (MtCLR-2), we used the Mtevo-CDA1 editor. The fungal-specific motif of MtCLR-2 was found to be strongly involved in cellulase secretion, conidium formation, hyphal branching, and colony formation. Mutation of the fungus-specific motif caused significant defects in these characteristics. Thus, we developed an efficient thermophilic fungus-compatible base-editing system that could also be used for genetic engineering in other relevant filamentous fungi. IMPORTANCE A CRISPR/Cas-based base-editing approach has been developed to introduce point mutations without inducing double-strand breaks (DSBs) and attracted substantial academic and industrial interest. Our study developed the deaminase-cytosine base-editing system to efficiently edit three target genes, amdS, cre-1, and the essential cellulase regulator gene Mtclr-2, in Myceliophthora thermophila . A variety of point mutations in the target loci of the DNA-binding domain and fungus-specific motif of M. thermophila CLR-2 (MtCLR-2) were successfully generated via our base editor Mtevo-CDA1 to elucidate its function. Here, we show that the DNA-binding domain of MtCLR-2 is important for the fungal response to cellulose conditions, while its fungus-specific motif is involved in fungal growth. These findings indicate that our base editor can be an effective tool for elucidating the functions of motifs of target genes in filamentous fungi and for metabolic engineering in the field of synthetic biology.
CAPLUS/2022:1794558	Development of an efficient C-to-T base-editing system and its application to cellulase transcription factor precise engineering in thermophilic fungus Myceliophthora thermophila	Key Laboratory of Systems Microbial Biotechnology, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin, Peop. Rep. China	Zhang, Chenyang Li, Nan Rao, Lang Li, Jingen Liu, Qian Tian, Chaoguang	Microbiology Spectrum (2022), 10(3), 1-21 CODEN: MSIPC7; ISSN: 2165-0497 URL: https://journals.asm.org/journal/spectrum	20220101	duplicates	Myceliophthora thermophila is a thermophilic fungus with great potential in biorefineries and biotechnol. The base editor is an upgraded version of the clustered regularly interspaced short palindromic repeats (CRISPR)-dependent genome-editing tool that introduces precise point mutations without causing DNA double-strand breaks (DSBs) and has been used in various organisms but rarely in filamentous fungi, esp. thermophilic filamentous fungi. Here, for the first time, we constructed three cytosine base editors (CBEs) in M. thermophila, namely, evolved apolipoprotein B mRNA-editing enzyme catalytic subunit 1 (APOBEC1) cytosine base editor 4 max (Mtevo-BE4max), bacteriophage Mu Gam protein cytosine base editor 4 max (MtGAM-BE4max), and evolved CDA1 deaminase cytosine base editor (Mtevo-CDA1), and efficiently inactivated genes by precisely converting three codons (CAA, CAG, and CGA) into stop codons without DSB formation. The Mtevo-CDA1 editor with up to 92.6% editing efficiency is a more suitable tool for cytosine base editing in thermophilic fungi. To investigate the function of each motif of the cellulase transcription factor M. thermophila CLR-2 (MtCLR-2), we used the Mtevo-CDA1 editor. The fungal-specific motif of MtCLR-2 was found to be strongly involved in cellulase secretion, conidium formation, hyphal branching, and colony formation. Mutation of the fungus-specific motif caused significant defects in these characteristics. Thus, we developed an efficient thermophilic fungus-compatible base-editing system that could also be used for genetic engineering in other relevant filamentous fungi.
MEDLINE/2026735628	Breeding of a thermostable xylanase-producing strain of Myceliophthora thermophila by atmospheric room temperature plasma (ARTP) mutagenesis .	Key Lab of Biomass Energy and Material, Key Lab of Chemical Engineering of Forest Products, National Engineering Research Center of Low-Carbon Processing and Utilization of Forest Biomass, Jiangsu Co-Innovation Center of Efficient Processing and Utilization of Forest Resource, National Forestry and Grassland Administration, Institute of Chemical Industry of Forest Products, Chinese Academy of Forestry (CAF), Nanjing, Jiangsu, China. Co-Innovation Center of Efficient Processing and Utilization of Forest Resources, Nanjing Forestry University, Nanjing, China.	Zhang Ning Jiang Yue Sun Yun-Juan Jiang Jian-Chun Tong Ya-Juan	Frontiers in bioengineering and biotechnology, (2022) Vol. 10, pp. 1095323. Electronic Publication Date: 5 Jan 2023 Journal code: 101632513. ISSN: 2296-4185. L-ISSN: 2296-4185. Report No.: PMC-PMC9849395.	20220101	other	Introduction: Hemicellulose is an important component in lignocellulose materials, which is second only to cellulose, accounting for 15%-35% of the dry weight of plants. In the current situation of energy shortage, making full use of lignocellulose materials to produce fuel ethanol has become an important way to solve the energy problem. Xylanase plays a crucial role in the utilization of hemicellulose. It is a necessary means to reduce the cost of hemicellulose utilization by improving the activity of xylanase. Moreover, most naturally xylanases are mesophilic enzymes, which limits their industrial application. Methods:In this study, Myceliophthora thermophila was used to produce xylanases and a thermostable mutant M 2103 was obtained by atmospheric room temperature plasma (ARTP) mutagenesis . The research work started with exploring the effects of ARTP mutagenesis on the antioxidase system [superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), polyphenol oxidase (PPO), and antioxidant capacity (AOC)] of M. thermophile, and found that superoxide dismutase activity increased by 221.13%, and polyphenol oxidase activity increased by 486.04% as compared with the original strain when the implantation time was 300 s. So as to determine the conditions for subsequent mutagenesis . Results and Discussion:For the mutant M 2103, the reaction temperature for xylanase production remained stable in the range of 70°C-85°C. Its optimum temperature was 75°C, which was 15°C higher than that of the original strain. And its xylanase activity increased by 21.71% as compared with the original strain. M 2103 displayed a significantly higher relative xylanase activity than the original strain in the acidic (pH 4.0-7.0) range, and the xylanase activity was relatively stable in the pH range of 6.0-8.5. These results provide an alternative biocatalyst for the production of xylooligosaccharide, and a potential usage of ARTP in the mutagenesis of thermostable mutant. Copyright © 2023 Zhang, Jiang, Sun, Jiang and Tong.

MEDLINE/2025659041	Development of an Efficient C-to-T Base-Editing System and Its Application to Cellulase Transcription Factor Precise Engineering in Thermophilic Fungus Myceliophthora thermophila .	Key Laboratory of Systems Microbial Biotechnology, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin, People's Republic of China. National Technology Innovation Center of Synthetic Biology, Tianjin, People's Republic of China. University of Chinese Academy of Sciences, Beijing, People's Republic of China. College of Biotechnology, Tianjin University of Science + Technology, Tianjin, People's Republic of China.	Zhang Chenyang Li Nan Rao Lang Li Jingen Liu Qian Tian Chaoguang	Microbiology spectrum, (20220629) Vol. 10, No. 3, pp. e0232121. Electronic Publication Date: 24 May 2022 Journal code: 101634614. E-ISSN: 2165-0497. L-ISSN: 2165-0497. Report No.: PMC-PMC9241923.	20220101	other	Myceliophthora thermophila is a thermophilic fungus with great potential in biorefineries and biotechnology. The base editor is an upgraded version of the clustered regularly interspaced short palindromic repeats (CRISPR)-dependent genome-editing tool that introduces precise point mutations without causing DNA double-strand breaks (DSBs) and has been used in various organisms but rarely in filamentous fungi, especially thermophilic filamentous fungi. Here, for the first time, we constructed three cytosine base editors (CBEs) in M. thermophila, namely, evolved apolipoprotein B mRNA-editing enzyme catalytic subunit 1 (APOBEC1) cytosine base editor 4 max (Mtevo-BE4max), bacteriophage Mu Gam protein cytosine base editor 4 max (MtGAM-BE4max), and evolved CDA1 deaminase cytosine base editor (Mtevo-CDA1), and efficiently inactivated genes by precisely converting three codons (CAA, CAG, and CGA) into stop codons without DSB formation. The Mtevo-CDA1 editor with up to 92.6% editing efficiency is a more suitable tool for cytosine base editing in thermophilic fungi. To investigate the function of each motif of the cellulase transcription factor M. thermophila CLR-2 (MtCLR-2), we used the Mtevo-CDA1 editor. The fungal-specific motif of MtCLR-2 was found to be strongly involved in cellulase secretion, conidium formation, hyphal branching, and colony formation. Mutation of the fungus-specific motif caused significant defects in these characteristics. Thus, we developed an efficient thermophilic fungus-compatible base-editing system that could also be used for genetic engineering in other relevant filamentous fungi. IMPORTANCE A CRISPR/Cas-based base-editing approach has been developed to introduce point mutations without inducing double-strand breaks (DSBs) and attracted substantial academic and industrial interest. Our study developed the deaminase-cytosine base-editing system to efficiently edit three target genes, amdS, cre-1, and the essential cellulase regulator gene Mtclr-2, in Myceliophthora thermophila . A variety of point mutations in the target loci of the DNA-binding domain and fungus-specific motif of M. thermophila CLR-2 (MtCLR-2) were successfully generated via our base editor Mtevo-CDA1 to elucidate its function. Here, we show that the DNA-binding domain of MtCLR-2 is important for the fungal response to cellulose conditions, while its fungus-specific motif is involved in fungal growth. These findings indicate that our base editor can be an effective tool for elucidating the functions of motifs of target genes in filamentous fungi and for metabolic engineering in the field of synthetic biology.
MEDLINE/2025975629	QM/MM Study of the Reaction Mechanism of Thermophilic Glucuronoyl Esterase for Biomass Treatment.	Departamento de Quimica e Bioquimica, Faculdade de Ciencias, Universidade do Porto, Rua do Campo Alegre s/n, 4169-007, Porto, Portugal.	Viegas Matilde F Neves Rui P P Ramos Maria J Fernandes Pedro A	Chemphyschem : a European journal of chemical physics and physical chemistry, (20221019) Vol. 23, No. 20, pp. e202200269. Electronic Publication Date: 4 Aug 2022 Journal code: 100954211. E-ISSN: 1439-7641. L-ISSN: 1439-4235.	20220101	other	Hydrolysis of lignocellulosic biomass, composed of a lignin-carbohydrate-complex (LCC) matrix, is critical for producing bioethanol from glucose. However, current methods for LCC processing require costly and polluting processes. The fungal Thermothelomyces thermophila glucuronoyl esterase (TtGE) is a promising thermophilic enzyme that hydrolyses LCC ester bonds. This study describes the TtGE catalytic mechanism using QM/MM methods. Two nearly-degenerate rate-determining transition states were found, with barriers of 16 and 17 kcal · mol(-1) , both with a zwitterionic nature that results from a proton interplay from His346 to either the Ser213-hydroxyl or the lignin leaving group and the rehybridisation of the ester moiety of the substrate to an alkoxide. An oxyanion hole, characteristic of esterases, was provided by the conserved Arg214 through its backbone and sidechain. Our work further suggests that a mutation of Glu267 to a non-negative residue will decrease the energetic barrier in ca. -5 kcal · mol(-1) , improving the catalytic rate of TtGE. © 2022 Wiley-VCH GmbH.
CAPLUS/2022:3198157	Diarylation of thiazolopyrimidines by laccase and their in vitro evaluation as antitumor agents	Department of Organic and Inorganic Chemistry, Faculty of Chemistry, Shahid Beheshti University, Tehran, Iran	Shahedi, Mansour Shahani, Rojina Habibi, Zohreh Yousefi, Maryam Brask, Jesper Minaei-Tehrani, Arash Samadi, Fatemeh Yazdi Mohammadi, Mehdi	Scientific Reports (2022), 12(1), 22326 CODEN: SRCEC3; ISSN: 2045-2322	20220101	duplicates	A mild and efficient method was developed for the synthesis of new derivs. of thiazolo[3,2-a] pyrimidin-3(2H)-ones from available starting materials based on the oxidn. of catechols to ortho-quinone by Myceliophthora thermophila laccase (Novozym 51,003) and 1,4-addn. of active methylene carbon to these in situ generated intermediates in moderate to good yields (35-93%). The structure of the products was confirmed through 1H NMR, 13C NMR, HMBC, HSQC, DEPT-135, and mass spectroscopy techniques. These novel compds. were evaluated as active antitumor agents against human colorectal adenocarcinoma and liver adenocarcinoma cell lines. All compds. displayed potent inhibition activities against the HT-29 cell line with IC50 values of 9.8-35.9 μM, superior to the pos. control doxorubicin, and most showed potent anticancer activities against the HepG2 cell line.
BIOSIS/2023:179410	Diarylation of thiazolopyrimidines by laccase and their in vitro evaluation as antitumor agents.	Shahid Beheshti Univ, Fac Chem, Dept Organ and Inorgan Chem, GC, Tehran, Iran Z_habibi@sbu.ac.ir; M.yousefi@ari.ir	Shahedi, Mansour [Reprint Author] Shahani, Rojina Habibi, Zohreh [Reprint Author] Yousefi, Maryam [Reprint Author] Brask, Jesper Minaei-Tehrani, Arash Samadi, Fatemeh Yazdi Mohammadi, Mehdi	Scientific Reports, (DEC 25 2022) Vol. 12, No. 1, pp. Article No.: 22326. https://www.nature.com/srep. ISSN: 2045-2322. E-ISSN: 2045-2322.	20220101	other	A mild and efficient method was developed for the synthesis of new derivatives of thiazolo[3,2-a] pyrimidin-3(2H)-ones from available starting materials based on the oxidation of catechols to ortho-quinone by Myceliophthora thermophila laccase (Novozym 51,003) and 1,4-addition of active methylene carbon to these in situ generated intermediates in moderate to good yields (35-93%). The structure of the products was confirmed through H-1 NMR, C-13 NMR, HMBC, HSQC, DEPT-135, and mass spectroscopy techniques. These novel compounds were evaluated as active antitumor agents against human colorectal adenocarcinoma and liver adenocarcinoma cell lines. All compounds displayed potent inhibition activities against the HT-29 cell line with IC50 values of 9.8-35.9 mu M, superior to the positive control doxorubicin, and most showed potent anticancer activities against the HepG2 cell line.
EMBASE/20220435259	Toxicity and Local Tolerance of a Novel Spike Protein RBD Vaccine Against SARS-CoV-2, Produced Using the C1 Thermothelomyces Heterothallica Protein Expression Platform.	The Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel. Department of Dermatology, Hadassah Medical Center, Jerusalem, Israel. Envigo CRS Israel Limited, Ness Ziona, Israel. Israel Institute for Biological Research, Ness Ziona, Israel. Technical Research Centre of Finland Ltd, Espoo, Finland. BTG-Biotechnology General (Israel) Ltd, Kiryat Malachi, Israel. Dyadic International, Inc, Jupiter, FL, United States. Tel Aviv University, Tel Aviv, Israel. anyska@nyska.net	Ramot, Yuval Kronfeld, Noam Ezov, Nati Friedman, Sheli Ophir, Yakir Saloheimo, Markku Vitikainen, Marika Ben-Artzi, Hanna Avigdor, Avi Tchelet, Ronen Valbuena Crespo, Noelia Emalfarb, Mark Nyska, Abraham (correspondence)	Toxicologic Pathology, (April 2022) Vol. 50, No. 3, pp. 294-307. Refs: 28 ISSN: 0192-6233; E-ISSN: 1533-1601 CODEN: TOPADD	20220101	other	Coronavirus disease 2019 (COVID-19) has caused the ongoing COVID-19 pandemic and there is a growing demand for safe and effective vaccines. The thermophilic Thermothelomyces heterothallica filamentous fungal host, C1-cell, can be utilized as an expression platform for the rapid production of large quantities of antigens for developing vaccines. The aim of this study was to evaluate the local tolerance and the systemic toxicity of a C1-cell expressed receptor-binding domain (C1-RBD) vaccine, following repeated weekly intramuscular injections (total of 4 administrations), in New Zealand White rabbits. The animals were sacrificed either 3 days or 3 weeks following the last dose. No signs of toxicity were observed, including no injection site reactions. ELISA studies revealed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific immunoglobulin G antibodies in the sera of C1-RBD-treated animals starting from day 13 post injection, that were further elevated. Histopathology evaluation and immunohistochemical staining revealed follicular hyperplasia, consisting of B-cell type, in the spleen and inguinal lymph nodes of the treated animals that were sustained throughout the recovery phase. No local or systemic toxicity was observed. In conclusion, the SARS-CoV-2 C1-RBD vaccine candidate demonstrated an excellent safety profile and a lasting immunogenic response against receptor-binding domain, thus supporting its further development for use in humans.

PQSCITECH/2022:502992	Thermophilic Filamentous Fungus C1-Cell-Cloned SARS-CoV-2-Spike-RBD-Subunit-Vaccine Adjuvanted with Aldydrogel®85 Protects K18-hACE2 Mice against Lethal Virus Challenge		Nechooshtan, Ram Ehrlich, Sharon Vitikainen, Marika Makovitzki, Arik Dor, Eyal Hadar, Marcus Hefetz, Idan Pitel, Shani Wiebe, Marilyn Huuskonen, Anne Cherry, Lilach Lupu, Edith Sapir, Yehuda Holtzman, Tzvi Aftalion, Moshe Gur, David Tamir, Hadas Yahalom-Ronen, Yfat Ramot, Yuval Kronfeld, Noam Zarling, David Vallerga, Anne Tchelet, Ronen Nyska, Abraham Saloheimo, Markku Emalfarb, Mark Ophir, Yakir Ophir, Yakir	Vaccines, Vol. 10, No. 12, 20220101 E-ISSN: 2076-393X DOI: https://doi.org/10.3390/vaccines10122119 Published by: MDPI AG, Basel	20220101	duplicates	SARS-CoV-2 is evolving with increased transmission, host range, pathogenicity , and virulence. The original and mutant viruses escape host innate (Interferon) immunity and adaptive (Antibody) immunity, emphasizing unmet needs for high-yield, commercial-scale manufacturing to produce inexpensive vaccines/boosters for global/equitable distribution. We developed DYAI-100A85, a SARS-CoV-2 spike receptor binding domain (RBD) subunit antigen vaccine expressed in genetically modified thermophilic filamentous fungus, Thermothelomyces heterothallica C1, and secreted at high levels into fermentation medium. The RBD-C-tag antigen strongly binds ACE2 receptors in vitro. Alhydrogel® 85'-adjuvanted RDB-C-tag-based vaccine candidate (DYAI-100A85) demonstrates strong immunogenicity, and antiviral efficacy, including in vivo protection against lethal intranasal SARS-CoV-2 (D614G) challenge in human ACE2-transgenic mice. No loss of body weight or adverse events occurred . DYAI-100A85 also demonstrates excellent safety profile in repeat-dose GLP toxicity study . In summary, subcutaneous prime/boost DYAI-100A85 inoculation induces high titers of RBD-specific neutralizing antibodies and protection of hACE2-transgenic mice against lethal challenge with SARS-CoV-2. Given its demonstrated safety, efficacy , and low production cost, vaccine candidate DYAI-100 received regulatory approval to initiate a Phase 1 clinical trial to demonstrate its safety and efficacy in humans.
EMBASE/20221144132	THERMOPHILIC FILAMENTOUS FUNGUS C1-CELL-CLONED SARS-COV-2-SPIKE-RBD-SUBUNIT-VACCINE ADJUVANTED WITH ALDYDROGEL®85 PROTECTS K18-HACE2 MICE AGAINST LETHAL VIRUS CHALLENGE.	Department of Biotechnology, Israel Institute for Biological Research, Ness-Ziona, 7410001, Israel. yophir@ucdavis.edu VTT Technical Research Centre of Finland Ltd, Espoo, 02150, Finland. Department of Biochemistry and Molecular Genetics, Israel Institute for Biological Research, Ness-Ziona, 7410001, Israel. Department of Infectious Diseases, Israel Institute of Biological Research (IIBR), Ness-Ziona, 7410001, Israel. Faculty of Medicine, Hebrew University of Jerusalem, Jerusalem, 9112102, Israel. Department of Dermatology, Hadassah Medical Center, Jerusalem, 9112102, Israel. Envigo CRS Israel Limited, Ness-Ziona, 7414001, Israel. Dyadic International, Inc, Jupiter, FL, 33477-5094, United States. Dyadic Netherland B.V, Nieuwe Kanaal 7-S, Wageningen, 6709 PA, Netherlands . Department of Pathology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, 6997801, Israel. Department of Chemical Engineering, University of California, Davis, CA, 95616, United States. yophir@ucdavis.edu	Nechooshtan, Ram Ehrlich, Sharon Makovitzki, Arik Dor, Eyal Marcus, Hadar Hefetz, Idan Pitel, Shani Cherry, Lilach Lupu, Edith Sapir, Yehuda Holtzman, Tzvi Ophir, Yakir (correspondence) Vitikainen, Marika Wiebe, Marilyn Huuskonen, Anne Saloheimo, Markku Aftalion, Moshe Gur, David Tamir, Hadas Yahalom-Ronen, Yfat Ramot, Yuval Kronfeld, Noam Zarling, David Vallerga, Anne Emalfarb, Mark Tchelet, Ronen Nyska, Abraham	Vaccines, (December 2022) Vol. 10, No. 12. arn. 2119. Refs: 67 E-ISSN: 2076-393X	20220101	duplicates	SARS-CoV-2 is evolving with increased transmission, host range, pathogenicity , and virulence. The original and mutant viruses escape host innate (Interferon) immunity and adaptive (Antibody) immunity, emphasizing unmet needs for high-yield, commercial-scale manufacturing to produce inexpensive vaccines/boosters for global/equitable distribution. We developed DYAI-100A85, a SARS-CoV-2 spike receptor binding domain (RBD) subunit antigen vaccine expressed in genetically modified thermophilic filamentous fungus, Thermothelomyces heterothallica C1, and secreted at high levels into fermentation medium. The RBD-C-tag antigen strongly binds ACE2 receptors in vitro. Alhydrogel® 85'-adjuvanted RDB-C-tag-based vaccine candidate (DYAI-100A85) demonstrates strong immunogenicity, and antiviral efficacy, including in vivo protection against lethal intranasal SARS-CoV-2 (D614G) challenge in human ACE2-transgenic mice. No loss of body weight or adverse events occurred. DYAI-100A85 also demonstrates excellent safety profile in repeat-dose GLP toxicity study. In summary, subcutaneous prime/boost DYAI-100A85 inoculation induces high titers of RBD-specific neutralizing antibodies and protection of hACE2-transgenic mice against lethal challenge with SARS-CoV-2. Given its demonstrated safety , efficacy, and low production cost, vaccine candidate DYAI-100 received regulatory approval to initiate a Phase 1 clinical trial to demonstrate its safety and efficacy in humans.

SCISEARCH/2023:138504	Thermophilic Filamentous Fungus C1-Cell-Cloned SARS-CoV-2-Spike-RBD-Subunit-Vaccine Adjuvanted with Aldydrogel (R) 85 Protects K18-hACE2 Mice against Lethal Virus Challenge	Univ Calif Davis, Dept Chem Engn, Davis, CA 95616 USA (Reprint) Israel Inst Biol Res, Dept Biotechnol, IL-7410001 Ness Ziona, Israel E-mail: yophir@ucdavis.edu VTT Tech Res Ctr Finland Ltd, Espoo 02150, Finland Israel Inst Biol Res, Dept Biochem + Mol Genet, IL-7410001 Ness Ziona, Israel Israel Inst Biol Res IIBR, Dept Infect Dis, IL-7410001 Ness Ziona, Israel Hebrew Univ Jerusalem, Fac Med, IL-9112102 Jerusalem, Israel Hadassah Med Ctr, Dept Dermatol, IL-9112102 Jerusalem, Israel Envigo CRS Israel Ltd, IL-7414001 Ness Ziona, Israel Dyad Int Inc, Jupiter, FL 33477 USA Dyad Netherland BV, Nieuwe Kanaal 7-S, NL-6709 PA Wageningen, Netherlands Tel Aviv Univ, Sackler Fac Med, Dept Pathol, IL-6997801 Tel Aviv, Israel E-mail: yophir@ucdavis.edu Israel Inst Biol Res, Dept Biotechnol, IL-7410001 Ness Ziona, Israel (Reprint)	Nechooshtan, Ram (Reprint) Ehrlich, Sharon Makovitzki, Arik Dor, Eyal Marcus, Hadar Hefetz, Idan Pitel, Shani Cherry, Lilach Lupu, Edith Sapir, Yehuda Holtzman, Tzvi Ophir, Yakir Vitikainen, Marika Wiebe, Marilyn Huuskonen, Anne Saloheimo, Markku Aftalion, Moshe Gur, David Tamir, Hadas Yahalom-Ronen, Yfat Ramot, Yuval Kronfeld, Noam Zarling, David Vallerga, Anne Emalfarb, Mark Tchelet, Ronen Nyska, Abraham	VACCINES, (2022 DEC 2022) Vol. 10, No. 12.	20220101	duplicates	SARS-CoV-2 is evolving with increased transmission, host range, pathogenicity , and virulence. The original and mutant viruses escape host innate (Interferon) immunity and adaptive (Antibody) immunity, emphasizing unmet needs for high-yield, commercial-scale manufacturing to produce inexpensive vaccines/boosters for global/equitable distribution. We developed DYAI-100A85, a SARS-CoV-2 spike receptor binding domain (RBD) subunit antigen vaccine expressed in genetically modified thermophilic filamentous fungus, Thermothelomyces heterothallica C1, and secreted at high levels into fermentation medium. The RBD-C-tag antigen strongly binds ACE2 receptors in vitro. Alhydrogel (R)'85'-adjuvanted RDB-C-tag-based vaccine candidate (DYAI-100A85) demonstrates strong immunogenicity, and antiviral efficacy, including in vivo protection against lethal intranasal SARS-CoV-2 (D614G) challenge in human ACE2-transgenic mice. No loss of body weight or adverse events occurred. DYAI-100A85 also demonstrates excellent safety profile in repeat-dose GLP toxicity study. In summary, subcutaneous prime/boost DYAI-100A85 inoculation induces high titers of RBD-specific neutralizing antibodies and protection of hACE2-transgenic mice against lethal challenge with SARS-CoV-2. Given its demonstrated safety , efficacy, and low production cost, vaccine candidate DYAI-100 received regulatory approval to initiate a Phase 1 clinical trial to demonstrate its safety and efficacy in humans.
MEDLINE/2026609970	Thermophilic Filamentous Fungus C1-Cell-Cloned SARS-CoV-2-Spike-RBD-Subunit-Vaccine Adjuvanted with Aldydrogel(®)85 Protects K18-hACE2 Mice against Lethal Virus Challenge.	Department of Biotechnology, Israel Institute for Biological Research, Ness-Ziona 7410001, Israel. VTT Technical Research Centre of Finland Ltd., 02150 Espoo, Finland. Department of Biochemistry and Molecular Genetics, Israel Institute for Biological Research, Ness-Ziona 7410001, Israel. Department of Infectious Diseases, Israel Institute of Biological Research (IIBR), Ness-Ziona 7410001, Israel. Faculty of Medicine, Hebrew University of Jerusalem, Jerusalem 9112102, Israel. Department of Dermatology, Hadassah Medical Center, Jerusalem 9112102, Israel. Envigo CRS Israel Limited, Ness-Ziona 7414001, Israel. Dyadic International, Inc., Jupiter, FL 33477-5094, USA. Dyadic Netherland B.V., Nieuwe Kanaal 7-S, 6709 PA Wageningen, The Netherlands. Department of Pathology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 6997801, Israel. Department of Chemical Engineering, University of California, Davis, CA 95616, USA.	Nechooshtan Ram Ehrlich Sharon Makovitzki Arik Dor Eyal Marcus Hadar Hefetz Idan Pitel Shani Cherry Lilach Lupu Edith Sapir Yehuda Holtzman Tzvi Ophir Yakir Vitikainen Marika Wiebe Marilyn Huuskonen Anne Saloheimo Markku Aftalion Moshe Gur David Tamir Hadas Yahalom-Ronen Yfat Ramot Yuval Kronfeld Noam Zarling David Vallerga Anne Emalfarb Mark Tchelet Ronen Nyska Abraham	Vaccines, (2022 Dec 11) Vol. 10, No. 12. Electronic Publication Date: 11 Dec 2022 Journal code: 101629355. ISSN: 2076-393X. L-ISSN: 2076-393X. Report No.: PMC-PMC9783968.	20220101	other	SARS-CoV-2 is evolving with increased transmission, host range, pathogenicity , and virulence. The original and mutant viruses escape host innate (Interferon) immunity and adaptive (Antibody) immunity, emphasizing unmet needs for high-yield, commercial-scale manufacturing to produce inexpensive vaccines/boosters for global/equitable distribution. We developed DYAI-100A85, a SARS-CoV-2 spike receptor binding domain (RBD) subunit antigen vaccine expressed in genetically modified thermophilic filamentous fungus, Thermothelomyces heterothallica C1, and secreted at high levels into fermentation medium. The RBD-C-tag antigen strongly binds ACE2 receptors in vitro. Alhydrogel(®)'85'-adjuvanted RDB-C-tag-based vaccine candidate (DYAI-100A85) demonstrates strong immunogenicity, and antiviral efficacy, including in vivo protection against lethal intranasal SARS-CoV-2 (D614G) challenge in human ACE2-transgenic mice. No loss of body weight or adverse events occurred. DYAI-100A85 also demonstrates excellent safety profile in repeat-dose GLP toxicity study. In summary, subcutaneous prime/boost DYAI-100A85 inoculation induces high titers of RBD-specific neutralizing antibodies and protection of hACE2-transgenic mice against lethal challenge with SARS-CoV-2. Given its demonstrated safety , efficacy, and low production cost, vaccine candidate DYAI-100 received regulatory approval to initiate a Phase 1 clinical trial to demonstrate its safety and efficacy in humans.
BIOSIS/2022:332605	Laccase-Catalyzed Derivatization of Aminoglycoside Antibiotics and Glucosamine.	Univ Greifswald, Inst Microbiol, Felix Hausdorff Str 8, D-17489 Greifswald, Germany annett.mikolasch@uni-greifswald.de; lindequi@uni-greifswald.de; witt@biometec.de; veronikahahn@gmx.at	Mikolasch, Annett [Reprint Author] Lindequist, Ulrike Witt, Sabine Hahn, Veronika [Reprint Author]	Microorganisms, (MAR 2022) Vol. 10, No. 3, pp. Article No.: 626. http://www.mdpi.com/journal/microorganisms. E-ISSN: 2076-2607.	20220101	other	The increasing demand for new and effective antibiotics requires intelligent strategies to obtain a wide range of potential candidates. Laccase-catalyzed reactions have been successfully applied to synthesize new beta-lactam antibiotics and other antibiotics. In this work, laccases from three different origins were used to produce new aminoglycoside antibiotics. Kanamycin, tobramycin and gentamicin were coupled with the laccase substrate 2,5-dihydroxy-N-(2-hydroxyethyl)-benzamide. The products were isolated, structurally characterized and tested in vitro for antibacterial activity against various strains of Staphylococci, including multidrug-resistant strains. The cytotoxicity of these products was tested using FL cells. The coupling products showed comparable and, in some cases, better antibacterial activity than the parent antibiotics in the agar diffusion assay, and they were not cytotoxic . The products protected mice against infection with Staphylococcus aureus, which was lethal to the control animals. The results underline the great potential of laccases in obtaining new biologically active compounds, in this case new antibiotic candidates from the class of aminoglycosides.

EMBASE/20211126087	Applications of CRISPR/Cas gene-editing technology in yeast and fungi.	State Key Laboratory of Oral Diseases, National Clinical Research Center for Oral Diseases, West China School of Stomatology, Sichuan University, Chengdu, 610064, China. renbiao@scu.edu.cn chenglei@scu.edu.cn Department of Operative Dentistry and Endodontics, West China Hospital of Stomatology, Sichuan University, Chengdu, 610041, China. chenglei@scu.edu. cn	Liao, Binyou Chen, Xi Zhou, Xuedong Zhou, Yujie Shi, Yangyang Ye, Xingchen Liao, Min Zhou, Ziyi Cheng, Lei Ren, Biao (correspondence)	Archives of Microbiology, (January 2022) Vol. 204, No. 1. arn. 79. Refs: 140 ISSN: 0302-8933; E-ISSN: 1432-072X CODEN: AMICCW	20220101	other	Genome editing technology has progressed rapidly in recent years. Although traditional gene-editing methods, including homologous recombination, zinc finger endonucleases, and transcription activator-like effector nucleases, have substantial implications for research in genetics and molecular biology, but they have remarkable limitations, including their low efficiency, high error rate, and complex design. A new gene-editing technology, the CRISPR/Cas system, was developed based on studies of archaeal and bacterial immune responses to viruses. Owing to its high target efficiency, simple primer design, and wide applications, the CRISPR/Cas system, whose developers were awarded the Nobel Prize in Chemistry in 2020, has become the dominant genomic editing technology in academia and the pharmaceutical industry. Here, we briefly introduce the CRISPR/Cas system and its main applications for genome engineering, metabolic engineering, and transcriptional regulation in yeast, filamentous fungi, and macrofungi. The polygene and polyploid editing, construction of yeast chromosomes, yeast library creation, regulation of metabolic pathways, and CRISPR activation/CRISPR interference systems are mainly summarized and discussed. The potential applications for the treatment of fungal infections and the further transformation and application of the CRISPR/Cas system in fungi are also proposed and discussed.
BIOSIS/2022:231084	A recombinant SARS-CoV-2 receptor-binding domain expressed in an engineered fungal strain of Thermothelomyces heterothallica induces a functional immune response in mice.	Ctr Genet Engn and Biotechnol CIGB, Ave 31,POB 6162, Havana 610600, Cuba laura.lazo@cigb.edu.cu	Lazo, Laura [Reprint Author] Bequet-Romero, Monica Lemos, Gilda Musacchio, Alexis Cabrales, Ania Bruno, Andy J. Espinosa, Luis Ariel Saloheimo, Markku Vitikainen, Marika Hernandez, Amalia Emalfarb, Mark Tchelet, Ronen Suzarte, Edith Guillen, Gerardo	Vaccine, (FEB 16 2022) Vol. 40, No. 8, pp. 1162-1169. http://www.journals.elsevier.com/vaccine/#description. CODEN: VACCDE. ISSN: 0264-410X. E-ISSN: 1873-2518.	20220101	other	Since the beginning of the COVID-19 pandemic, the development of effective vaccines against this pathogen has been a priority for the scientific community. Several strategies have been developed including vaccines based on recombinant viral protein fragments. The receptor-binding domain (RBD) in the S1 subunit of S protein has been considered one of the main targets of neutralizing antibodies. In this study we assess the potential of a vaccine formulation based on the recombinant RBD domain of SARS-CoV-2 expressed in the thermophilic filamentous fungal strain Thermothelomyces heterothallica and the hepatitis B virus (HBV) core protein. Functional humoral and cellular immune responses were detected in mice. To our knowledge, this is the first report on the immune evaluation of a biomedical product obtained in the fungal strain T. heterothallica. These results together with the intrinsic advantages of this expression platform support its use for the development of biotechnology products for medical purpose. (c) 2022 Elsevier Ltd. All rights reserved.
EMBASE/20221043695	Cms1 coordinates stepwise local 90S pre-ribosome assembly with timely snR83 release.	Heidelberg University Biochemistry Center (BZH), Im Neuenheimer Feld 328, Heidelberg, 69120, Germany. ed.hurt@bzh.uni-heidelberg.de Institutes of Biomedical Sciences, Shanghai Key Laboratory of Medical Epigenetics, International Co-laboratory of Medical Epigenetics and Metabolism, Fudan University, Dong'an Road 131, Shanghai, 200032, China. Department of Biology, University of Fribourg, Chemin du Musee 10, Fribourg, 1700, Switzerland. Gene Center, Ludwig-Maximilians-Universitat Munchen, Feodor-Lynen-Strasse 25, Munich, 81377, Germany. beckmann@genzentrum.lmu.de Laboratoire de Biologie Moleculaire Eucaryote du CNRS, Universite Paul Sabatier, 118 Route de Narbonne, Toulouse Cedex 9, 31062, France.	Lau, Benjamin Beine-Golovchuk, Olga Kornprobst, Markus Hurt, Ed (correspondence) Cheng, Jingdong Kressler, Dieter Beckmann, Roland Jady, Beata Kiss, Tamas	Cell Reports, (22 Nov 2022) Vol. 41, No. 8. arn. 111684. Refs: 79 E-ISSN: 2211-1247	20220101	other	Ribosome synthesis begins in the nucleolus with 90S pre-ribosome construction, but little is known about how the many different snoRNAs that modify the pre-rRNA are timely guided to their target sites. Here, we report a role for Cms1 in such a process. Initially, we discovered CMS1 as a null suppressor of a nop14 mutant impaired in Rrp12-Enp1 factor recruitment to the 90S. Further investigations detected Cms1 at the 18S rRNA 3' major domain of an early 90S that carried H/ACA snR83, which is known to guide pseudouridylation at two target sites within the same subdomain. Cms1 co-precipitates with many 90S factors, but Rrp12-Enp1 encircling the 3' major domain in the mature 90S is decreased. We suggest that Cms1 associates with the 3' major domain during early 90S biogenesis to restrict premature Rrp12-Enp1 binding but allows snR83 to timely perform its modification role before the next 90S assembly steps coupled with Cms1 release take place.
CAPLUS/2022:217473	Evaluation of Antibacterial and Antifungal Properties of Low Molecular Weight Chitosan Extracted from Hermetia illucens Relative to Crab Chitosan	Institute of Bioengineering, Research Centre of Biotechnology, Russian Academy of Sciences, Moscow, 119071, Russia	Khayrova, Adelya Lopatin, Sergey Shagdarova, Balzhima Sinitsyna, Olga Sinitsyn, Arkady Varlamov, Valery	Molecules (2022), 27(2), 577 CODEN: MOLEFW; ISSN: 1420-3049 URL: https://www.mdpi.com/journal/molecules	20220101	duplicates	This study shows the research on the depolymn. of insect and crab chitosans using novel enzymes. Enzyme prepns. contg. recombinant chitinase Chi 418 from Trichoderma harzianum, chitinase Chi 403, and chitosanase Chi 402 from Myceliophthora thermophila , all belonging to the family GH18 of glycosyl hydrolases, were used to depolymerise a biopolymer, resulting in a range of chitosans with av. mol. wts. (Mw) of 6-21 kDa. The depolymerised chitosans obtained from crustaceans and insects were studied, and their antibacterial and antifungal properties were evaluated. The results proved the significance of the chitosan's origin, showing the potential of Hermetia illucens as a new source of low mol. wt. chitosan with an improved biol. activity.
MEDLINE/2025109064	Evaluation of Antibacterial and Antifungal Properties of Low Molecular Weight Chitosan Extracted from Hermetia illucens Relative to Crab Chitosan.	Institute of Bioengineering, Research Centre of Biotechnology, Russian Academy of Sciences, 119071 Moscow, Russia. Entoprotech Ltd., Skolkovo Innovation Centre, 121205 Moscow, Russia. Department of Chemistry, Moscow State University, 119991 Moscow, Russia.	Khayrova Adelya Lopatin Sergey Shagdarova Balzhima Varlamov Valery Sinitsyna Olga Sinitsyn Arkady	Molecules (Basel, Switzerland), (2022 Jan 17) Vol. 27, No. 2. Electronic Publication Date: 17 Jan 2022 Journal code: 100964009. E-ISSN: 1420-3049. L-ISSN: 1420-3049. Report No.: PMC-PMC8777618.	20220101	other	This study shows the research on the depolymerisation of insect and crab chitosans using novel enzymes. Enzyme preparations containing recombinant chitinase Chi 418 from Trichoderma harzianum, chitinase Chi 403, and chitosanase Chi 402 from Myceliophthora thermophila , all belonging to the family GH18 of glycosyl hydrolases, were used to depolymerise a biopolymer, resulting in a range of chitosans with average molecular weights (Mw) of 6-21 kDa. The depolymerised chitosans obtained from crustaceans and insects were studied, and their antibacterial and antifungal properties were evaluated. The results proved the significance of the chitosan's origin, showing the potential of Hermetia illucens as a new source of low molecular weight chitosan with an improved biological activity.

SCISEARCH/2022:219931	Laccase: Various types and applications	Abadan Univ Med Sci, Dept Basic Sci, Abadan, Iran (Reprint) Shahid Beheshti Univ Med Sci, Sch Med, Dept Clin Biochem, Tehran, Iran Isfahan Univ Med Sci, Sch Pharm + Pharmaceut Sci, Dept Clin Biochem, Esfahan, Iran Behbahan Fac Med Sci, Behbahan, Iran Tabriz Univ Med Sci, Sch Adv Med Sci, Dept Med Biotechnol, Tabriz, Iran Abadan Fac Med Sci, Abadan, Iran E-mail: clinicalbiochem.1365@gmail.com Shiraz Univ Med Sci, Sch Adv Med Sci + Technol, Dept Med Biotechnol, Shiraz, Iran E-mail: mortazataheri@yahoo.com Shiraz Univ Med Sci, Sch Adv Med Sci + Technol, Dept Med Biotechnol, Shiraz, Iran (Reprint)	Khatami, Seyyed Hossein (Reprint) Vakili, Omid Movahedpour, Ahmad Ghesmati, Zeinab Ghasemi, Hassan Taheri-Anganeh, Mortaza	BIOTECHNOLOGY AND APPLIED BIOCHEMISTRY, (2022 DEC 2022) Vol. 69, No. 6, pp. 2658-2672. ISSN: 0885-4513.	20220101	other	Laccase belongs to the polyphenol oxidase family and is very important in removing environmental pollutants due to its structural and functional properties. Recently, the ability of laccase to oxidize phenolic and nonphenolic substances has been considered by many researchers. This enzyme's application scope includes a broad range of chemical processes and industrial usages, such as bioremediation, nanobiotechnology, woodworking industries, bleaching of paper pulp, dyeing in the textile industry, biotechnological uses in food industries, biorefining, detoxification from wastewater, production of organic matter from phenolic and amine substrates, and biofuels. Although filamentous fungi produce large amounts of laccase, high-yield industrial-scale production of laccase is still faced with many problems. At present, researchers are trying to increase the efficiency and productivity and reduce the final price of laccase by finding suitable microorganisms and improving the process of production and purification of laccase. This article reviews the introduction of laccase, its properties, production processes, and the effect of various factors on the enzyme's stability and activity, and some of its applications in various industries.
CAPLUS/2022:828848	The Highly Productive Thermothelomyces heterothallica C1 Expression System as a Host for Rapid Development of Influenza Vaccines	Dyadic International Inc., USA	Keresztes, Gabor Baer, Mark Alfenito, Mark R. Verwoerd, Theo C. Kovalchuk, Andriy Wiebe, Marilyn G. Andersen, Tor Kristian Saloheimo, Markku Tchelet, Ronen Kensinger, Richard Groedeland, Gunnveig Emalfarb, Mark	Vaccines (Basel, Switzerland) (2022), 10(2), 148 CODEN: VBSABP; ISSN: 2076-393X URL: https://www.mdpi.com/journal/vaccines	20220101	duplicates	(1) Influenza viruses constantly change and evade prior immune responses, forcing seasonal re-vaccinations with updated vaccines. Current FDA-approved vaccine manufg. technologies are too slow and/or expensive to quickly adapt to mid-season changes in the virus or to the emergence of pandemic strains. Therefore, cost-effective vaccine technologies that can quickly adapt to newly emerged strains are desirable. (2) The filamentous fungal host Thermothelomyces heterothallica C1 (C1, formerly Myceliophthora thermophila) offers a highly efficient and cost-effective alternative to reliably produce immunogens of vaccine quality at large scale. (3) We showed the utility of the C1 system expressing hemagglutinin (HA) and a HA fusion protein from different H1N1 influenza A virus strains. Mice vaccinated with the C1-derived HA proteins elicited anti-HA immune responses similar, or stronger than mice vaccinated with HA products derived from prototypical expression systems. A challenge study demonstrated that vaccinated mice were protected against the aggressive homologous viral challenge. (4) The C1 expression system is proposed as part of a set of protein expression systems for plug-and-play vaccine manufg. platforms. Upon the emergence of pathogens of concern these platforms could serve as a quick soln. for producing enough vaccines for immunizing the world population in a much shorter time and more affordably than is possible with current platforms.
MEDLINE/2025266225	The Highly Productive Thermothelomyces heterothallica C1 Expression System as a Host for Rapid Development of Influenza Vaccines.	Dyadic International Inc., 140 Intracoastal Pointe Drive, Suite 404, Jupiter, FL 33477, USA. EnGen Bio LLC, 61 Avondale Ave., Redwood City, CA 94062, USA. VTT Technical Research Centre of Finland Ltd., P.O. Box 1000, 02044 Espoo, Finland. Institute of Clinical Medicine, University of Oslo, 0027 Oslo, Norway. Sanofi Pasteur, 1541 Ave. Marcel Merieux, 69280 Marcy l'Etoile, France. Department of Immunology and Transfusion Medicine, Oslo University Hospital, 0027 Oslo, Norway.	Keresztes Gabor Verwoerd Theo C Tchelet Ronen Emalfarb Mark Baer Mark Alfenito Mark R Kovalchuk Andriy Wiebe Marilyn G Saloheimo Markku Andersen Tor Kristian Grodeland Gunnveig Kensinger Richard	Vaccines, (2022 Jan 20) Vol. 10, No. 2. Electronic Publication Date: 20 Jan 2022 Journal code: 101629355. ISSN: 2076-393X. L-ISSN: 2076-393X. Report No.: PMC-PMC8877961.	20220101	other	(1) Influenza viruses constantly change and evade prior immune responses, forcing seasonal re-vaccinations with updated vaccines. Current FDA-approved vaccine manufacturing technologies are too slow and/or expensive to quickly adapt to mid-season changes in the virus or to the emergence of pandemic strains. Therefore, cost-effective vaccine technologies that can quickly adapt to newly emerged strains are desirable. (2) The filamentous fungal host Thermothelomyces heterothallica C1 (C1, formerly Myceliophthora thermophila) offers a highly efficient and cost-effective alternative to reliably produce immunogens of vaccine quality at large scale. (3) We showed the utility of the C1 system expressing hemagglutinin (HA) and a HA fusion protein from different H1N1 influenza A virus strains. Mice vaccinated with the C1-derived HA proteins elicited anti-HA immune responses similar, or stronger than mice vaccinated with HA products derived from prototypical expression systems. A challenge study demonstrated that vaccinated mice were protected against the aggressive homologous viral challenge. (4) The C1 expression system is proposed as part of a set of protein expression systems for plug-and-play vaccine manufacturing platforms. Upon the emergence of pathogens of concern these platforms could serve as a quick solution for producing enough vaccines for immunizing the world population in a much shorter time and more affordably than is possible with current platforms.
EMBASE/20221046951	Role of aIF5B in archaeal translation initiation.	Laboratoire de Biologie Structurale de la Cellule, BIOC, Ecole Polytechnique, CNRS, Institut Polytechnique de Paris, Palaiseau, Cedex 91128, France. emmanuelle.schmitt@polytechnique.edu pierre-damien.coureux@polytechnique.edu yves.mechulam@polytechnique.edu Laboratoire de Physique de la Matiere Condensee, PMC, Ecole Polytechnique, CNRS, Institut Polytechnique de Paris, Palaiseau, Cedex 91128, France.	Kazan, Ramy Bourgeois, Gabrielle Lazennec-Schurdevin, Christine Mechulam, Yves Coureux, Pierre-Damien Schmitt, Emmanuelle (correspondence) Larquet, Eric	Nucleic Acids Research, (24 Jun 2022) Vol. 50, No. 11, pp. 6532-6548. Refs: 98 ISSN: 0305-1048; E-ISSN: 1362-4962 CODEN: NARHAD	20220101	other	In eukaryotes and in archaea late steps of translation initiation involve the two initiation factors e/aIF5B and e/aIF1A. In eukaryotes, the role of eIF5B in ribosomal subunit joining is established and structural data showing eIF5B bound to the full ribosome were obtained. To achieve its function, eIF5B collaborates with eIF1A. However, structural data illustrating how these two factors interact on the small ribosomal subunit have long been awaited. The role of the archaeal counterparts, aIF5B and aIF1A, remains to be extensively addressed. Here, we study the late steps of Pyrococcus abyssi translation initiation. Using in vitro reconstituted initiation complexes and light scattering, we show that aIF5B bound to GTP accelerates subunit joining without the need for GTP hydrolysis. We report the crystallographic structures of aIF5B bound to GDP and GTP and analyze domain movements associated to these two nucleotide states. Finally, we present the cryo-EM structure of an initiation complex containing 30S bound to mRNA, Met-tRNAi Met, aIF5B and aIF1A at 2.7 Å resolution. Structural data shows how archaeal 5B and 1A factors cooperate to induce a conformation of the initiator tRNA favorable to subunit joining. Archaeal and eukaryotic features of late steps of translation initiation are discussed.
BIOSIS/2023:91994	PFK2/FBPase-2 is a potential target for metabolic engineering in the filamentous fungus Myceliophthora thermophila .	Tianjin Univ Sci and Technol, Coll Biotechnol, Tianjin, Peoples R China tian_cg@tib.cas.cn	Hu, Die [Reprint Author] Zhang, Yongli Liu, Defei Wang, Depei Tian, Chaoguang [Reprint Author]	Frontiers in Microbiology, (NOV 21 2022) Vol. 13, pp. Article No.: 1056694. http://www.frontiersin.org/microbiology/. E-ISSN: 1664-302X.	20220101	other	The key enzyme 6-phosphofructo-2-kinase (PFK2)/fructose-2,6-bisphosphatase (FBPase-2) is responsible for regulating the rates of glycolysis and gluconeogenesis in eukaryotes. However, its functions and mechanisms in filamentous fungi remain largely enigmatic. In this study, we systematically investigated the function of this enzyme in Myceliophthora thermophila , a thermophilic filamentous fungus with great capacity to produce industrial enzymes and organic acids. Our results showed that the M. thermophila genome encodes three isomers, all with the PFK2/FBPase-2 structure: pfk2-a, pfk2-b, and pfk2-c. Overexpression of each gene revealed that endogenous expression of pfk2-c (PFK2 activity) promoted glucose metabolism, while overexpression of pfk2-a (FBPase-2 activity) inhibited strain growth. Using knockouts, we found that each gene was individually non-essential, but the triple knockout led to significantly slower growth compared with the wild-type strain. Only the pfk2-a single knockout exhibited 22.15% faster sugar metabolism, exerted through activation of 6-phosphofructo-1-kinase (PFK1), thereby significantly promoting glycolysis and the tricarboxylic acid cycle. The FBPase-2 deletion mutant strain also exhibited overflow metabolism, and knocking out pfk2-a was proved to be able to improve the production and synthesis rate of various metabolites, such as glycerol and malate. This is the first study to systematically investigate the function of PFK2/FBPase-2 in a thermophilic fungus, providing an effective target for metabolic engineering in filamentous fungi.

EMBASE/20220833224	PROLINE/GLYCINE RESIDUES OF THE PG-LEVELS GUIDE CONFORMATIONAL CHANGES ALONG THE TRANSPORT CYCLE IN THE MITOCHONDRIAL CARNITINE/ACYLCARNITINE CARRIER (SLC25A20).	CNR Institute of Biomembranes, Bioenergetics and Molecular Biotechnologies (IBIOM), Via Amendola 122/O, Bari, 70126, Italy. n.giangregorio@ibiom.cnr. it Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, Italy, Via E. Orabona, 4, Bari, 70126, Italy. ciro.pierri@uniba.it Department DiBEST (Biologia, Ecologia, Scienze della Terra) Unit of Biochemistry and Molecular Biotechnology, University of Calabria, Via Bucci 4C, Arcavacata di Rende, 87036, Italy.	Giangregorio, Nicola (correspondence) Tonazzi, Annamaria Tragni, Vincenzo Indiveri, Cesare Pierri, Ciro Leonardo Incampo, Giovanna De Grassi, Anna	International Journal of Biological Macromolecules, (30 Nov 2022) Vol. 221, pp. 1453-1465. Refs: 89 ISSN: 0141-8130; E-ISSN: 1879-0003 CODEN: IJBMDR	20220101	other	Mitochondrial carnitine/acylcarnitine carrier (CAC) is a member of the mitochondrial carrier (MC) family and imports acylcarnitine into the mitochondrial matrix in exchange for carnitine, playing a pivotal role in carnitine shuttle, crucial for fatty acid oxidation. The crystallized structure of CAC has not been solved yet, however, the availability of several in vitro/in silico studies, also based on the crystallized structures of the ADP/ATP carrier in the cytosolic-conformation and in the matrix-conformation, has made possible to confirm the hypothesis of the single-binding centered-gated pore mechanism for all the members of the MC family. In addition, our recent bioinformatics analyses allowed quantifying in silico the importance of protein residues of MC substrate binding region, of those involved in the formation of the matrix and cytosolic gates, and of those belonging to the Pro/Gly (PG) levels, proposed to be crucial for the tilting/kinking/bending of the six MC transmembrane helices, funneling the substrate translocation pathway. Here we present a combined in silico/in vitro analysis employed for investigating the role played by a group of 6 proline residues and 6 glycine residues, highly conserved in CAC, belonging to MC PG-levels. Residues of the PG-levels surround the similarly located MC common substrate binding region, and were proposed to lead conformational changes and substrate translocation, following substrate binding. For our analysis, we employed 3D molecular modeling approaches, alanine scanning site-directed mutagenesis and in vitro transport assays. Our analysis reveals that P130 (H3), G268 (H6) and G220 (H5), mutated in alanine, affect severely CAC transport activity (mutant catalytic efficiency lower than 5 % compared to the wild type CAC), most likely due to their major role in triggering CAC conformational changes, following carnitine binding. Notably, P30A (H1) and G121A (H3) CAC mutants, increase the carnitine uptake up to 217 % and 112 %, respectively, compared to the wild type CAC.
BIOSIS/2022:825836	Eight (histone H4 genes) is too much: A CRISPR/Cas9 strategy to replace highly duplicated genes with a single modified version for mutational analysis.	Amer Soc Plant Biologists, Rockville, MD 20855 USA Suzanne.de-bruijn@jic.ac.uk	de Bruijn, Suzanne [Reprint Author]	Plant Cell, (SEP 27 2022) Vol. 34, No. 10. https://academic.oup.com/plcell. CODEN: PLCEEW. ISSN: 1040-4651. E-ISSN: 1532-298X.	20220101	other	
SCISEARCH/2022:2312673	Structural and thermostability insights into cellobiohydrolase of a thermophilic mould Myceliophthora thermophila : in-silico studies	E-mail: tsnarayana@gmail.com Univ Delhi, Dept Appl Sci + Humanities, Fac Technol, Delhi, India Indian Inst Informat Technol Allahabad, Dept Appl Sci, Allahabad, Uttar Pradesh, India Netaji Subhas Univ Technol, Dept Biol Sci + Engn, Azad Hind Fauj Marg, Sect 3, New Delhi 110078, India (Reprint)	Dadwal, Anica (Reprint) Sharma, Shilpa Satyanarayana, Tulasi Singh, Vishal Sahoo, Amaresh Kumar	JOURNAL OF BIOMOLECULAR STRUCTURE + DYNAMICS, (6 2022 OCT 2022) . ISSN: 0739-1102.	20220101	duplicates	Cellobiohydrolase (CBH) is one of the cellulases with a wide range of industrial applications; it plays a pivotal role in cellulose hydrolysis and thus in biofuel production. The structural and thermostability analysis of a CBHII of the thermophilic mold Myceliophthora thermophila (MtCel6A) had been carried out using various in-silico approaches. The validation of 3 D model by the Ramachandran plot indicated 88.5% amino acid residues in the favoured regions. Docking analysis suggested MtCel6A to display a high affinity towards cellotetraose as compared to other substrates. The enzyme exhibited a high tolerance to the end product, cellobiose. The thermostability evaluation by molecular dynamic simulations and principal component analysis confirmed its tolerance to elevated temperatures. The identified thermolabile regions could be targeted for site-directed mutagenesis in order to ameliorate thermostability further. Our experimental data published earlier confirmed the present findings of in-silico studies. The structural and functional characteristics of MtCel6A highlighted its critical features that make it a useful biocatalyst in several industrial processes. Communicated by Ramaswamy H. Sarma
MEDLINE/2023469717	Structural aspects of β-glucosidase of Myceliophthora thermophila (MtBgl3c) by homology modelling and molecular docking.	Department of Biological Sciences and Engineering, Netaji Subhas Institute of Technology (University of Delhi), New Delhi, India. Department of Applied Sciences, Indian Institute of Information Technology Allahabad, Allahabad, Uttar Pradesh, India.	Dadwal Anica Sharma Shilpa Satyanarayana Tulasi Singh Vishal	Journal of biomolecular structure + dynamics, (20220700) Vol. 40, No. 11, pp. 5211-5228. Electronic Publication Date: 8 Jan 2021 Journal code: 8404176. E-ISSN: 1538-0254. L-ISSN: 0739-1102.	20220101	other	Cellulases are the enzymes with diverse range of industrial applications. Cellulases degrade cellulose into monomeric glucose units by hydrolysing β-1,4-glycosidic bonds. There are three components of cellulases: a) endoglucanase, b) exoglucanase and c) β-glucosidase which act synergistically in cellulose bioconversion. The cellulases are the third largest industrial enzymes with a great potential in bioethanol production. In this investigation, a β-glucosidase of a thermophilic fungus Myceliophthora thermophila (MtBgl3c) was analysed for its structural characterization using in silico approaches. The protein structure of MtBgl3c is unknown, therefore an attempt has been made to model 3D structure using Modeller 9.23 software. The MtBgl3c protein model generated was validated from Verify 3D and ERRAT scores of 89.37% and 71.25%, respectively derived from SAVES. Using RAMPAGE the Ramachandran plot was generated, which predicted the accuracy of the 3D model with 91.5% amino acid residues in the favored region. The ion binding and N-glycosylation sites were also predicted. The generated model was docked with cellobiose to predict the most favorable binding sites of MtBgl3c. The key amino acid residues involved in cellobiose bonding are Val88, Asp106, Asp287, Tyr255, Arg170, Glu514. The catalytic conserved amino residues of MtBgl3c were identified. The dock score of cellobiose with MtBgl3c is much lower (-6.46 kcal/mol) than that of glucose (-5.61 kcal/mol), suggesting its high affinity for cellobiose. The docking data of MtBgl3c with glucose illustrate its tolerance to glucose. The present study provides insight into structural characteristics of the MtBgl3c which can be further validated by experimental data. Highlights3D structure of β-glucosidase (MtBgl3c) of Myceliophthora thermophila is being proposed based on computational analysesThe amino acid residues Asp106, Asp287, Tyr255, Arg170 and Glu514 have been identified to play catalytically important role in substrate bindingDocking and interaction of MtBgl3c with cellobiose and glucose has been confirmedDocking analysis of MtBgl3c with glucose suggested its glucose toleranceThe data would be useful in engineering enzymes for attaining higher catalytic efficiencyCommunicated by Ramaswamy H. Sarma.
CAPLUS/2021:235712	Mechanism of membrane-curvature generation by ER-tubule shaping proteins	Howard Hughes Medical Institute and Department of Cell Biology, Harvard Medical School, Boston, MA, 02115, USA	Wang, Ning Clark, Lindsay D. Gao, Yuan Kozlov, Michael M. Shemesh, Tom Rapoport, Tom A.	Nature Communications (2021), 12(1), 568 CODEN: NCAOBW; ISSN: 2041-1723	20210101	other	The endoplasmic reticulum (ER) network consists of tubules with high membrane curvature in cross-section, generated by the reticulons and REEPs. These proteins have two pairs of trans-membrane (TM) segments, followed by an amphipathic helix (APH), but how they induce curvature is poorly understood. Here, we show that REEPs form homodimers by interaction within the membrane. When overexpressed or reconstituted at high concns. with phospholipids, REEPs cause extreme curvature through their TMs, generating lipoprotein particles instead of vesicles. The APH facilitates curvature generation, as its mutation prevents ER network formation of reconstituted proteoliposomes, and synthetic L- or D-amino acid peptides abolish ER network formation in Xenopus egg exts. In Schizosaccharomyces japonicus, the APH is required for reticulon's exclusive ER-tubule localization and restricted mobility. Thus, the TMs and APH cooperate to generate high membrane curvature. We propose that the formation of splayed REEP/reticulon dimers is responsible for ER tubule formation.
BIOSIS/2021:516939	Transcriptional Profiling of Myceliophthora thermophila on Galactose and Metabolic Engineering for Improved Galactose Utilization.	Tianjin Univ Sci and Technol, Coll Biotechnol, Tianjin, Peoples R China li_jg@tib.cas.cn	Wang, Hanyu [Reprint Author] Sun, Tao Zhao, Zhen Gu, Shuying Liu, Qian Wu, Taju Wang, Depei Tian, Chaoguang Li, Jingen [Reprint Author]	Frontiers in Microbiology, (APR 28 2021) Vol. 12, pp. Article No.: 664011. http://www.frontiersin.org/microbiology/. E-ISSN: 1664-302X.	20210101	other	Efficient biological conversion of all sugars from lignocellulosic biomass is necessary for the cost-effective production of biofuels and commodity chemicals. Galactose is one of the most abundant sugar in many hemicelluloses, and it will be important to capture this carbon for an efficient bioconversion process of plant biomass. Thermophilic fungus Myceliophthora thermophila has been used as a cell factory to produce biochemicals directly from renewable polysaccharides. In this study, we draw out the two native galactose utilization pathways, including the Leloir pathway and oxido-reductive pathway, and identify the significance and contribution of them, through transcriptional profiling analysis of M. thermophila and its mutants on galactose. We find that galactokinase was necessary for galactose transporter expression, and disruption of galK resulted in decreased galactose utilization. Through metabolic engineering, both galactokinase deletion and galactose transporter overexpression can activate internal the oxido-reductive pathway and improve the consumption rate of galactose. Finally, the heterologous galactose-degradation pathway, De Ley-Doudoroff (DLD) pathway, was successfully integrated into M. thermophila, and the consumption rate of galactose in the engineered strain was increased by 57%. Our study focuses on metabolic engineering for accelerating galactose utilization in a thermophilic fungus that will be beneficial for the rational design of fungal strains to produce biofuels and biochemicals from a variety of feedstocks with abundant galactose.

EMBASE/20210649152	A review of the enzymatic, physical, and chemical modification techniques of xanthan gum.	State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu, 214122, China. bjiang@jiangnan.edu.cn International Joint Laboratory on Food Safety, Jiangnan University, Wuxi, Jiangsu, 214122, China. bjiang@jiangnan.edu.cn School of Food and Biological Engineering, Jiangsu University, Zhenjiang, China.	Riaz, Tahreem Jiang, Bo (correspondence) Chen, Jingjing Iqbal, Muhammad Waheed	International Journal of Biological Macromolecules, (1 Sep 2021) Vol. 186, pp. 472-489. Refs: 182 ISSN: 0141-8130; E-ISSN: 1879-0003 CODEN: IJBMDR	20210101	other	Xanthan gum (XG), a bacterial polysaccharide has numerous valuable characteristics in the food, biomedical, pharmaceuticals, and agriculture sector. However, XG has also its particular limitations such as its vulnerability to microbial contamination , inadequate mechanical and thermal stability, unusable viscosity, and poor water solubility. Therefore, XG's structure and conformation need to be modified enzymatically, chemically, or physically to improve its optimistic features and decrease the formation of crystals, increase antioxidant ability, and radical scavenging activity. We have found out different means to modify XG and elaborate the importance and significance of the modified structure of XG. In this review, different enzymes are reviewed for XG degradation, which modifies their structure from different points (main chain or side chain). This article also reviews various physical methods (ultrasound, shear, pressure, sonication, annealing, and heat treatments) based on prevailing publications to alter XG conformation and produce low molecular weight (LMW) and less viscous end-product. Moreover, some chemical means are also discussed that result in modified XG through crosslinking, grafting, acetylation, pyruvation, as well as by applying different chemical agents. Overall, the current progress on XG degradation is very auspicious to develop a new molecule with considerable uses, in various industries with future assessments.
CAPLUS/2021:2526470	Laccase-Mediator System Using a Natural Mediator as a Whitening Agent for the Decolorization of Melanin	Department of Biological Engineering, Konkuk University, S. Korea	Park, Saerom Jung, Dahun Do, Hyejin Yun, Jonghyeon Lee, Dongjun Hwang, Soeun Lee, Sang Hyun	Polymers (Basel, Switzerland) (2021), 13(21), 3671 CODEN: POLYCK; ISSN: 2073-4360 URL: https://www.mdpi.com/journal/polymers	20210101	duplicates	In this study, a laccase-mediator system (LMS) using a natural mediator was developed as a whitening agent for melanin decolorization. Seven natural mediators were used to replace synthetic mediators and successfully overcome the low redox potential of laccase and limited access of melanin to the active site of laccase. The melanin decolorization activity of laccases from Trametes versicolor (lacT) and Myceliophthora thermophila (lacM) was significantly enhanced using natural mediators including acetosyringone, syringaldehyde, and acetovanillone, which showed low cytotoxicity . The methoxy and ketone groups of natural mediators play an important role in melanin decolorization. The specificity consts. of lacT and lacM for melanin decolorization were enhanced by 247 and 334, resp., when acetosyringone was used as a mediator. LMS using lacM and acetosyringone could also decolorize the melanin present in the cellulose hydrogel film, which mimics the skin condition. Furthermore, LMS could decolorize not only synthetic eumelanin analogs prepd. by the oxidn. of tyrosine but also natural melanin produced by melanoma cells.
MEDLINE/2024823967	Laccase-Mediator System Using a Natural Mediator as a Whitening Agent for the Decolorization of Melanin.	Department of Biological Engineering, Konkuk University, Seoul 05029, Korea.	Park Saerom Jung Dahun Do Hyejin Yun Jonghyeon Lee Dongjun Hwang Soeun Lee Sang Hyun	Polymers, (2021 Oct 25) Vol. 13, No. 21. Electronic Publication Date: 25 Oct 2021 Journal code: 101545357. E-ISSN: 2073-4360. L-ISSN: 2073-4360. Report No.: PMC-PMC8587086.	20210101	other	In this study, a laccase-mediator system (LMS) using a natural mediator was developed as a whitening agent for melanin decolorization. Seven natural mediators were used to replace synthetic mediators and successfully overcome the low redox potential of laccase and limited access of melanin to the active site of laccase. The melanin decolorization activity of laccases from Trametes versicolor (lacT) and Myceliophthora thermophila (lacM) was significantly enhanced using natural mediators including acetosyringone, syringaldehyde, and acetovanillone, which showed low cytotoxicity . The methoxy and ketone groups of natural mediators play an important role in melanin decolorization. The specificity constants of lacT and lacM for melanin decolorization were enhanced by 247 and 334, respectively, when acetosyringone was used as a mediator. LMS using lacM and acetosyringone could also decolorize the melanin present in the cellulose hydrogel film, which mimics the skin condition. Furthermore, LMS could decolorize not only synthetic eumelanin analogs prepared by the oxidation of tyrosine but also natural melanin produced by melanoma cells.
CAPLUS/2021:1284836	Considerations regarding activity determinants of fungal polyphenol oxidases based on mutational and structural studies	Industrial Biotechnology + Biocatalysis Group, Biotechnology Laboratory, School of Chemical Engineering, National Technical University of Athens, Athens, Greece	Nikolaivits, Efstratios Valmas, Alexandros Dedes, Grigorios Topakas, Evangelos Dimarogona, Maria	Applied and Environmental Microbiology (2021), 87(11), e00396-21 CODEN: AEMIDF; ISSN: 1098-5336 URL: http://aem.asm.org/	20210101	duplicates	Polyphenol oxidases (PPOs) are an industrially relevant family of enzymes, being involved in the postharvest browning of fruits and vegetables, as well as in human melanogenesis. Their involvement lies in their ability to oxidize phenolic or polyphenolic compds., which subsequently form pigments. The PPO family includes tyrosinases and catechol oxidases, which, in spite of their high structural similarity, exhibit different catalytic activities. Long-standing research efforts have not yet managed to decipher the structural determinants responsible for this differentiation, as every new theory is disproved by a more recent study. In the present work, we combined biochem. along with structural data in order to better understand the function of a previously characterized PPO from Thermothelomyces thermophila (TtPPO). The crystal structure of a TtPPO variant, detd. at 1.55 Å resoln., represents the second known structure of an ascomycete PPO. Kinetic data for structure-guided mutants prove the implication of 'gate' residue L306, residue HB1 + 1 (G292), and HB2 + 1 (Y296) in TtPPO function against various substrates. Our findings demonstrate the role of L306 in the accommodation of bulky substrates and show that residue HB1 + 1 is unlikely to det. monophenolase activity, as was suggested from previous studies.
ESBIOBASE/2021291649	Considerations Regarding Activity Determinants of Fungal Polyphenol Oxidases Based on Mutational and Structural Studies	Industrial Biotechnology + Biocatalysis Group, Biotechnology Laboratory, School of Chemical Engineering, National Technical University of Athens, Athens (GR)) Department of Biology, University of Patras, Patras (GR)) Laboratory of Structural Biology and Biotechnology, Department of Chemical Engineering, University of Patras, Patras (GR))	Nikolaivits, Efstratios Dedes, Grigorios Topakas, Evangelos Valmas, Alexandros Dimarogona, Maria	Applied and Environmental Microbiology (1 Jun 2021) Volume 87, Number 11, pp. 1-14, arn: e00396-21, 51 refs. CODEN: AEMIDF ISSN: 0099-2240 E-ISSN: 1098-5336 DOI: 10.1128/AEM.00396-21 Published by: American Society for Microbiology,	20210101	duplicates	Polyphenol oxidases (PPOs) are an industrially relevant family of enzymes, being involved in the postharvest browning of fruits and vegetables, as well as in human melanogenesis. Their involvement lies in their ability to oxidize phenolic or polyphenolic compounds, which subsequently form pigments. The PPO family includes tyrosinases and catechol oxidases, which, in spite of their high structural similarity, exhibit different catalytic activities. Long-standing research efforts have not yet managed to decipher the structural determinants responsible for this differentiation, as every new theory is disproved by a more recent study. In the present work, we combined biochemical along with structural data in order to better understand the function of a previously characterized PPO from Thermothelomyces thermophila (TtPPO). The crystal structure of a TtPPO variant, determined at 1.55 A resolution, represents the second known structure of an ascomycete PPO. Kinetic data for structure-guided mutants prove the implication of 'gate' residue L306, residue H B1 +1 (G292), and H B2 +1 (Y296) in TtPPO function against various substrates. Our findings demonstrate the role of L306 in the accommodation of bulky substrates and show that residue H B1 +1 is unlikely to determine monophenolase activity, as was suggested from previous studies. Importance PPOs are enzymes of biotechnological interest. They have been extensively studied both biochemically and structurally, with a special focus on the plantderived counterparts. Even so, explicit description of the molecular determinants of their substrate specificity is still pending. For ascomycete PPOs, only one crystal structure has been determined so far, thus limiting our knowledge on this tree branch of the family. In the present study, we report the second crystal structure of an ascomycete PPO. Combined with site-directed mutagenesis and biochemical studies, we depict the amino acids in the vicinity of the active site that affect enzyme activity and perform a detailed analysis on a variety of substrates. Our findings improve current understanding of structure-function relations of microbial PPOs, which is a prerequisite for the engineering of biocatalysts of desired properties.

MEDLINE/2024614177	Unique features of the bifunctional GH30 from Thermothelomyces thermophila revealed by structural and mutational studies.	Industrial Biotechnology + Biocatalysis Group, Biotechnology Laboratory, School of Chemical Engineering, National Technical University of Athens, Athens, Greece. Laboratory of Structural Biology and Biotechnology, Department of Chemical Engineering, University of Patras, Patras, Greece. Helmholtz-Zentrum Berlin, Macromolecular Crystallography (HZB-MX), Berlin, Germany. Department of Biology, University of Patras, Patras, Greece. Laboratory of Structural Biology and Biotechnology, Department of Chemical Engineering, University of Patras, Patras, Greece. mdimarog@chemeng.upatras.gr Industrial Biotechnology + Biocatalysis Group, Biotechnology Laboratory, School of Chemical Engineering, National Technical University of Athens, Athens, Greece. vtopakas@chemeng.ntua.gr	Nikolaivits Efstratios Pentari Christina Kosinas Christos Feiler Christian G Weiss Manfred S Spiliopoulou Maria Dimarogona Maria Topakas Evangelos	Carbohydrate polymers, (2021 Dec 01) Vol. 273, pp. 118553. Electronic Publication Date: 12 Aug 2021 Journal code: 8307156. E-ISSN: 1879-1344. L-ISSN: 0144-8617.	20210101	other	Fungal xylanases belonging to family GH30_7, initially categorized as endo-glucuronoxylanases, are now known to differ both in terms of substrate specificity, as well as mode of action. Recently, TtXyn30A, a GH30_7 xylanase from Thermothelomyces thermophila , was shown to possess dual activity, acting on the xylan backbone in both an endo- and an exo- manner. Here, in an effort to identify the structural characteristics that append these functional properties to the enzyme, we present the biochemical characterization of various TtXyn30A mutants as well as its crystal structure, alone, and in complex with the reaction product. An auxiliary catalytic amino acid has been identified, while it is also shown that glucuronic acid recognition is not mediated by a conserved arginine residue, as shown by previously determined GH30 structures. Copyright © 2021 Elsevier Ltd. All rights reserved.
MEDLINE/2023797384	Considerations Regarding Activity Determinants of Fungal Polyphenol Oxidases Based on Mutational and Structural Studies.	Industrial Biotechnology + Biocatalysis Group, Biotechnology Laboratory, School of Chemical Engineering, National Technical University of Athens, Athens, Greece. Department of Biology, University of Patras, Patras, Greece. Laboratory of Structural Biology and Biotechnology, Department of Chemical Engineering, University of Patras, Patras, Greece. mdimarog@chemeng.upatras.gr	Nikolaivits Efstratios Dedes Grigorios Topakas Evangelos Valmas Alexandros Dimarogona Maria	Applied and environmental microbiology, (20210511) Vol. 87, No. 11. Electronic Publication Date: 11 May 2021 Journal code: 7605801. E-ISSN: 1098-5336. L-ISSN: 0099-2240. Report No.: PMC-PMC8208164.	20210101	other	Polyphenol oxidases (PPOs) are an industrially relevant family of enzymes, being involved in the postharvest browning of fruits and vegetables, as well as in human melanogenesis. Their involvement lies in their ability to oxidize phenolic or polyphenolic compounds, which subsequently form pigments. The PPO family includes tyrosinases and catechol oxidases, which, in spite of their high structural similarity, exhibit different catalytic activities. Long-standing research efforts have not yet managed to decipher the structural determinants responsible for this differentiation, as every new theory is disproved by a more recent study. In the present work, we combined biochemical along with structural data in order to better understand the function of a previously characterized PPO from Thermothelomyces thermophila (TtPPO). The crystal structure of a TtPPO variant, determined at 1.55 ÅA resolution, represents the second known structure of an ascomycete PPO. Kinetic data for structure-guided mutants prove the implication of 'gate' residue L306, residue HB1+1 (G292), and HB2+1 (Y296) in TtPPO function against various substrates. Our findings demonstrate the role of L306 in the accommodation of bulky substrates and show that residue HB1+1 is unlikely to determine monophenolase activity, as was suggested from previous studies.IMPORTANCE PPOs are enzymes of biotechnological interest. They have been extensively studied both biochemically and structurally, with a special focus on the plant-derived counterparts. Even so, explicit description of the molecular determinants of their substrate specificity is still pending. For ascomycete PPOs, only one crystal structure has been determined so far, thus limiting our knowledge on this tree branch of the family. In the present study, we report the second crystal structure of an ascomycete PPO. Combined with site-directed mutagenesis and biochemical studies, we depict the amino acids in the vicinity of the active site that affect enzyme activity and perform a detailed analysis on a variety of substrates. Our findings improve current understanding of structure-function relations of microbial PPOs, which is a prerequisite for the engineering of biocatalysts of desired properties. Copyright © 2021 American Society for Microbiology.
EMBASE/20200900480	Molecular engineering to improve lignocellulosic biomass based applications using filamentous fungi.	Fungal Physiology, Westerdijk Fungal Biodiversity Institute + Fungal Molecular Physiology, Utrecht University, Utrecht, Netherlands. r.devries@ wi.knaw.nl Department of Microbiology, University of Helsinki, Helsinki, Finland.	Meng, Jiali de Vries, Ronald P. (correspondence) Makela, Miia R.	Advances in Applied Microbiology, (January 2021) Vol. 114, pp. 73-109. Editor: Gadd, Geoffrey Michael, Dundee, Scotland Editor: Sariaslani, Sima, Wilmington, Delaware Refs: 159 ISSN: 0065-2164 ISBN: 9780128245927 CODEN: ADAMAP	20210101	other	Lignocellulosic biomass is an abundant and renewable resource, and its utilization has become the focus of research and biotechnology applications as a very promising raw material for the production of value-added compounds. Filamentous fungi play an important role in the production of various lignocellulolytic enzymes, while some of them have also been used for the production of important metabolites. However, wild type strains have limited efficiency in enzyme production or metabolic conversion, and therefore many efforts have been made to engineer improved strains. Examples of this are the manipulation of transcriptional regulators and/or promoters of enzyme-encoding genes to increase gene expression, and protein engineering to improve the biochemical characteristics of specific enzymes. This review provides and overview of the applications of filamentous fungi in lignocellulosic biomass based processes and the development and current status of various molecular engineering strategies to improve these processes.
BIOSIS/2021:181480	Thermophilic Chitinases: Structural, Functional and Engineering Attributes for Industrial Applications.	CSIR Natl Inst Interdisciplinary Sci and Technol, Microbial Proc and Technol Div, Trivandrum 695019, Kerala, India ashok.pandey1@iitr.res.in	Mathew, Gincy M. [Reprint Author] Madhavan, Aravind Arun, K. B. Sindhu, Raveendran Binod, Parameswaran Singhania, Reeta Rani Sukumaran, Rajeev K. Pandey, Ashok [Reprint Author]	Applied Biochemistry and Biotechnology, (JAN 2021) Vol. 193, No. 1, pp. 142-164. https://www.springer.com/chemistry/biotechnology/journal/12010. CODEN: ABIBDL. ISSN: 0273-2289. E-ISSN: 1559-0291.	20210101	other	Chitin is the second most widely found natural polymer next to cellulose. Chitinases degrade the insoluble chitin to bioactive chitooligomers and monomers for various industrial applications. Based on their function, these enzymes act as biocontrol agents against pathogenic fungi and invasive pests compared with conventional chemical fungicides and insecticides. They have other functional roles in shellfish waste management, fungal protoplast generation, and Single-Cell Protein production. Among the chitinases, thermophilic and thermostable chitinases are gaining popularity in recent years, as they can withstand high temperatures and maintain the enzyme stability for longer periods. Not all chitinases are thermostable; hence, tailor-made thermophilic chitinases are designed to enhance their thermostability by direct evolution, genetic engineering involving mutagenesis , and proteomics approach. Although research has been done extensively on cloning and expression of thermophilic chitinase genes, there are only few papers discussing on the mechanism of chitin degradation using thermophiles. The current review discusses the sources of thermophilic chitinases, improvement of protein stability by gene manipulation, metagenomics approaches, chitin degradation mechanism in thermophiles, and their prospective applications for industrial, agricultural, and pharmaceutical purposes.
BIOSIS/2021:530986	Production and properties of non-cytotoxic pyomelanin by laccase and comparison to bacterial and synthetic pigments.	Aix Marseille Univ, Mediterranean Inst Oceanol MIO, 163 Ave Luminy, F-13288 Marseille 9, France jean.lorquin@mio.osupytheas.fr	Lorquin, Faustine Ziarelli, Fabio Amouric, Agnes Di Giorgio, Carole Robin, Maxime Piccerelle, Philippe Lorquin, Jean [Reprint Author]	Scientific Reports, (APR 20 2021) Vol. 11, No. 1, pp. Article No.: 8538. http://www.nature.com/srep. ISSN: 2045-2322. E-ISSN: 2045-2322.	20210101	other	Pyomelanin is a polymer of homogentisic acid synthesized by microorganisms. This work aimed to develop a production process and evaluate the quality of the pigment. Three procedures have been elaborated and optimized, (1) an HGA-Mn2+ chemical autoxidation (Pyo(CHEM) yield 0.317 g/g substrate), (2) an induced bacterial culture of Halomonas titanicae through the 4-hydroxyphenylacetic acid-1-hydroxylase route (Pyo(BACT), 0.55 g/L), and (3) a process using a recombinant laccase extract with the highest level produced (Pyo(ENZ), 1.25 g/g substrate) and all the criteria for a large-scale prototype. The chemical structures had been investigated by C-13 solid-state NMR (CP-MAS) and FTIR. C-ar-C-ar bindings predominated in the three polymers, C-ar-O-C-ar (ether) linkages being absent, proposing mainly C-3-C-6 (alpha-bindings) and C-4-C-6 (beta-bindings) configurations. This work highlighted a biological decarboxylation by the laccase or bacterial oxidase(s), leading to the partly formation of gentisyl alcohol and gentisaldehyde that are integral parts of the polymer. By comparison, Pyo(ENZ) exhibited an M-w of 5,400 Da, was hyperthermostable, non-cytotoxic even after irradiation, scavenged ROS induced by keratinocytes, and had a highly DPPH-antioxidant and Fe3+-reducing activity. As a representative pigment of living cells and an available standard, Pyo(ENZ) might also be useful for applications in extreme conditions and skin protection.

CAPLUS/2021:2756429	Characterization of the blood and cerebrospinal fluid microbiome in children with bacterial meningitis and its potential correlation with inflammation	Key Laboratory of Molecular Genetics, Shanghai Institute of Nutrition and Health, University of Chinese Academy of Sciences, Chinese Academy of Sciences (CAS), Shanghai, Peop. Rep. China	Liao, Huiping Zhang, Yuchao Guo, Wei Wang, Xi Wang, Hailong Ye, Haocheng Wu, Kai Zhang, Yu-Hang Guo, Lingyun Zhu, Yufei Guo, Yongli Hu, Landian Liu, Gang Kong, Xiangyin	mSystems (2021), 6(3), e00049 CODEN: MSYSBR; ISSN: 2379-5077 URL: https://journals.asm.org/journal/msystems	20210101	duplicates	Bacterial meningitis shows a higher incidence in children than adults, but signs may be scarce. Although some pathogenic microorganisms of meningitis from cerebrospinal fluid (CSF) have been reported, the signature of the representative microbiota in CSF and blood samples from patients remains incompletely revealed. To extend the understanding of the microbiome in patients , we recruited 32 children with bacterial meningitis, 30 undiagnosed infectious children, and 10 matched healthy individuals, which was followed by untargeted metagenomic next-generation sequencing (mNGS) and bioinformatic anal. Our results showed that children with bacterial meningitis exhibited different microbiome signatures in their CSF and blood compared with undiagnosed and healthy children, and patients could be divided into varied subsets according to these signatures, including Escherichia coli, Klebsiella pneumoniae, Thermothelomyces thermophila , Lactobacillus acidophilus, and Staphylococcus haemolyticus. To further explore their potential role in patients ' conditions, we examd. their correlation with clin. parameters. Importantly, microbiome signatures with compositional changes were correlated with the C-reactive protein (CRP) level in blood and granulocyte percentage in CSF. Moreover, the blood in subsets of patients with a predominance of Klebsiella pneumoniae could replace CSF as the main specimen for clin. monitoring.
EMBASE/20210516691	Characterization of the blood and cerebrospinal fluid microbiome in children with bacterial meningitis and its potential correlation with inflammation.	Key Laboratory of Molecular Genetics, Shanghai Institute of Nutrition and Health, University of Chinese Academy of Sciences, Chinese Academy of Sciences (CAS), Shanghai, China. xykong@sibs.ac.cn ldhu2013@163.com Key Laboratory of Major Diseases in Children, Ministry of Education, Department of Infectious Diseases, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing, China. liugang@bch.com.cn Research Unit of Critical Infection in Children, Chinese Academy of Medical Sciences, Beijing, China. liugang@bch.com.cn	Liao, Huiping Zhang, Yuchao Guo, Wei Wang, Hailong Ye, Haocheng Wu, Kai Zhang, Yu-Hang Zhu, Yufei Hu, Landian Kong, Xiangyin Wang, Xi Guo, Lingyun Guo, Yongli Liu, Gang (correspondence)	mSystems, (June 2021) Vol. 6, No. 3. arn. e00049-21. Refs: 66 E-ISSN: 2379-5077	20210101	duplicates	Bacterial meningitis shows a higher incidence in children than adults, but signs may be scarce. Although some pathogenic microorganisms of meningitis from cerebrospinal fluid (CSF) have been reported, the signature of the representative microbiota in CSF and blood samples from patients remains incompletely revealed. To extend the understanding of the microbiome in patients , we recruited 32 children with bacterial meningitis, 30 undiagnosed infectious children, and 10 matched healthy individuals, which was followed by untargeted metagenomic next-generation sequencing (mNGS) and bioinformatic analysis. Our results showed that children with bacterial meningitis exhibited different microbiome signatures in their CSF and blood compared with undiagnosed and healthy children, and patients could be divided into varied subsets according to these signatures, including Escherichia coli, Klebsiella pneumoniae, Thermothelomyces thermophila , Lactobacillus acidophilus, and Staphylococcus haemolyticus. To further explore their potential role in patients ' conditions, we examined their correlation with clinical parameters. Importantly, microbiome signatures with compositional changes were correlated with the C-reactive protein (CRP) level in blood and granulocyte percentage in CSF. Moreover, the blood in subsets of patients with a predominance of Klebsiella pneumoniae could replace CSF as the main specimen for clinical monitoring.
BIOSIS/2021:1016504	Characterization of the Blood and Cerebrospinal Fluid Microbiome in Children with Bacterial Meningitis and Its Potential Correlation with Inflammation.	Chinese Acad Sci, Univ Chinese Acad Sci, Shanghai Inst Nutr and Hlth, Key Lab Mol Genet, Shanghai, Peoples R China ldhu2013@163.com; liugang@bch.com.cn; xykong@sibs.ac.cn	Liao, Huiping [Reprint Author] Zhang, Yuchao Guo, Wei Wang, Xi Wang, Hailong Ye, Haocheng Wu, Kai Zhang, Yu-Hang Guo, Lingyun Zhu, Yufei Guo, Yongli Hu, Landian [Reprint Author] Liu, Gang [Reprint Author] Kong, Xiangyin [Reprint Author]	mSystems, (MAY 2021) Vol. 6, No. 3, pp. Article No.: e00049-21. msystems.asm.org. ISSN: 2379-5077. E ISSN: 2379-5077.	20210101	duplicates	Bacterial meningitis shows a higher incidence in children than adults, but signs may be scarce. Although some pathogenic microorganisms of meningitis from cerebrospinal fluid (CSF) have been reported, the signature of the representative microbiota in CSF and blood samples from patients remains incompletely revealed. To extend the understanding of the microbiome in patients , we recruited 32 children with bacterial meningitis, 30 undiagnosed infectious children, and 10 matched healthy individuals, which was followed by untargeted metagenomic next-generation sequencing (mNGS) and bioinformatic analysis. Our results showed that children with bacterial meningitis exhibited different microbiome signatures in their CSF and blood compared with undiagnosed and healthy children, and patients could be divided into varied subsets according to these signatures, including Escherichia coli, Klebsiella pneumoniae, Thermothelomyces thermophila , Lactobacillus acidophilus, and Staphylococcus haemolyticus. To further explore their potential role in patients ' conditions, we examined their correlation with clinical parameters. Importantly, microbiome signatures with compositional changes were correlated with the C-reactive protein (CRP) level in blood and granulocyte percentage in CSF. Moreover, the blood in subsets of patients with a predominance of Klebsiella pneu-moniae could replace CSF as the main specimen for clinical monitoring.IMPORTANCE This study revealed the microbial compositions in children with bacterial meningitis who were treated with antibiotics and made a comprehensive comparison between blood and CSF specimens for the risk and prognosis assessment. We found that microbiome signatures could distinguish patient subsets in the chil-dren and were correlated with the CRP level in blood and granulocyte percentage in CSF. The compositional changes in representative microbiota constituents could pro-vide guidance for clinical monitoring and antibiotic intervention.
BIOSIS/2021:980410	Production of antimicrobial powders of guaiacol oligomers by a laccase-catalyzed synthesis reaction.	Jiangnan Univ, Jiangsu Engn Technol Res Ctr Funct Text, 1800 Lihu Ave, Wuxi, Jiangsu, Peoples R China sujing@jiangnan.edu.cn; artur@deb.uminho.pt	Li, Nannan [Reprint Author] Su, Jing [Reprint Author] Wang, Hongbo Cavaco-Paulo, Artur [Reprint Author]	Process Biochemistry, (DEC 2021) Vol. 111, No. Part 1, pp. 213-220. http://www.journals.elsevier.com/process-biochemistry/#description. ISSN: 1359-5113. E-ISSN: 1873-3298.	20210101	other	Guaiacol oligomers were synthesized by enzyme-catalyzed polymerization with laccase as the catalyst. The polymerization procedure in aqueous media was followed by UV-vis spectra. The oligomers were characterized by NMR and MALDI-TOF spectra. The UV-vis spectra observation showed a new peak formed in the range of 380-530 nm indicating that new substances formed. The NMR results indicated that phenolic hydroxyl participated in the polymerization. The MALDI-TOF spectra revealed the weight average polymerization degree of guaiacol oligomers is 10 and the mass of the repeating unit is 122. The thermal stability was studied by TGA/ DSC analysis. Guaiacol oligomers were more thermally stable than guaiacol, which can be ascribed to the long pi-conjugated chains. Antioxidant behavior based on ABTS center dot+ radical showed a concentration-dependent activity and reached a plateau at 800 mu g/mL. The prepared guaiacol oligomers displayed antimicrobial activity against Staphylococcus aureus and Escherichia coli, in which the inhibition percentage is up to 99 %.
CAPLUS/2021:1230837	Accessing chemo- and regioselective benzylic and arom. oxidns. by protein engineering of an unspecific peroxygenase	Bioorganic Chemistry, Leibniz Institute of Plant Biochemistry, Halle, 06120, Germany	Knorrscheidt, Anja Soler, Jordi Huenecke, Nicole Puellmann, Pascal Garcia-Borras, Marc Weissenborn, Martin J.	ACS Catalysis (2021), 11(12), 7327-7338 CODEN: ACCACS; ISSN: 2155-5435	20210101	other	Unspecific peroxygenases (UPOs) enable oxyfunctionalizations of a broad substrate range with unparalleled activities. Tailoring these enzymes for chemo- and regioselective transformations represents a grand challenge due to the difficulties in their heterologous productions. Herein, we performed protein engineering in Saccharomyces cerevisiae using the MthUPO from Myceliophthora thermophila . More than 5300 transformants were screened. This protein engineering led to a significant reshaping of the active site as elucidated by computational modeling. The reshaping was responsible for the increased oxyfunctionalization activity, with improved kcat/Km values of up to 16.5-fold for the model substrate 5-nitro-1,3-benzodioxole. Moreover, variants were identified with high chemo- and regioselectivities in the oxyfunctionalization of arom. and benzylic carbons, resp. The benzylic hydroxylation was demonstrated to perform with enantioselectivities of up to 95% ee. The proposed evolutionary protocol and rationalization of the enhanced activities and selectivities acquired by MthUPO variants represent a step forward toward the use and implementation of UPOs in biocatalytic synthetic pathways of industrial interest.

MEDLINE/2024222463	The F-box protein gene exo-1 is a target for reverse engineering enzyme hypersecretion in filamentous fungi.	Deconstruction Division, Joint BioEnergy Institute, Emeryville, CA 94608. Biological Systems and Engineering Division, Lawrence Berkeley National Laboratory, Emeryville, CA 94608. Institut fur Genetik, Technische Universitat Braunschweig, 38106 Braunschweig, Germany. a.fleissner@tu-bs.de; benz@hfm.tum.de Technical University of Munich School of Life Sciences Weihenstephan, Technical University of Munich, 85354 Freising, Germany. a.fleissner@tu-bs.de; benz@hfm.tum.de Key Laboratory of Systems Microbial Biotechnology, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin 300308, China. Fungal Genetics Stock Center, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506. Functional and Systems Biology, Environmental Molecular Sciences Division, Pacific Northwest National Laboratory, Richland, WA 99352. Braunschweig Integrated Centre of Systems Biology, Technische Universitat Braunschweig, 38106 Braunschweig, Germany. a.fleissner@tu-bs.de; benz@hfm.tum.de Institute for Advanced Study, Technical University of Munich, 85748 Garching, Germany. a.fleissner@tu-bs.de; benz@hfm.tum.de	Gabriel Raphael Harth Simon Jecmenica Marina Ramamurthy Maya Gorman Jennifer Simmons Blake A Baker Scott E Schuerg Timo Singer Steven W Fleissner Andre Thieme Nils Meyer Lisa T Benz J Philipp Liu Qian Li Fangya Tian Chaoguang McCluskey Kevin	Proceedings of the National Academy of Sciences of the United States of America, (20210629) Vol. 118, No. 26. Journal code: 7505876. E-ISSN: 1091-6490. L-ISSN: 0027-8424. Report No.: PMC-PMC8256007.	20210101	other	Carbohydrate active enzymes (CAZymes) are vital for the lignocellulose-based biorefinery. The development of hypersecreting fungal protein production hosts is therefore a major aim for both academia and industry. However, despite advances in our understanding of their regulation, the number of promising candidate genes for targeted strain engineering remains limited. Here, we resequenced the genome of the classical hypersecreting Neurospora crassa mutant exo-1 and identified the causative point of mutation to reside in the F-box protein-encoding gene, NCU09899. The corresponding deletion strain displayed amylase and invertase activities exceeding those of the carbon catabolite derepressed strain Δcre-1, while glucose repression was still mostly functional in Δexo-1 Surprisingly, RNA sequencing revealed that while plant cell wall degradation genes are broadly misexpressed in Δexo-1, only a small fraction of CAZyme genes and sugar transporters are up-regulated, indicating that EXO-1 affects specific regulatory factors. Aiming to elucidate the underlying mechanism of enzyme hypersecretion, we found the high secretion of amylases and invertase in Δexo-1 to be completely dependent on the transcriptional regulator COL-26. Furthermore, misregulation of COL-26, CRE-1, and cellular carbon and nitrogen metabolism was confirmed by proteomics. Finally, we successfully transferred the hypersecretion trait of the exo-1 disruption by reverse engineering into the industrially deployed fungus Myceliophthora thermophila using CRISPR-Cas9. Our identification of an important F-box protein demonstrates the strength of classical mutants combined with next-generation sequencing to uncover unanticipated candidates for engineering. These data contribute to a more complete understanding of CAZyme regulation and will facilitate targeted engineering of hypersecretion in further organisms of interest.
BIOSIS/2021:641517	Roles and applications of enzymes for resistant pollutants removal in wastewater treatment.	Univ Technol Sydney, Sch Civil and Environm Engn, Sydney, NSW 2007, Australia ngohuuhao121@gmail.com	Feng, Siran [Reprint Author] Ngo, Huu Hao [Reprint Author] Guo, Wenshan Chang, Soon Woong Dinh Duc Nguyen Cheng, Dongle Varjani, Sunita Lei, Zhongfang Liu, Yi	Bioresource Technology, (SEP 2021) Vol. 335, pp. Article No.: 125278. http://www.journals.elsevier.com/bioresource-technology/#description. CODEN: BIRTEB. ISSN: 0960-8524. E-ISSN: 1873-2976.	20210101	other	Resistant pollutants like oil, grease, pharmaceuticals, pesticides, and plastics in wastewater are difficult to be degraded by traditional activated sludge methods. These pollutants are prevalent, posing a great threat to aquatic environments and organisms since they are toxic , resistant to natural biodegradation, and create other serious problems. As a high-efficiency biocatalyst, enzymes are proposed for the treatment of these resistant pollutants. This review focused on the roles and applications of enzymes in wastewater treatment. It discusses the influence of enzyme types and their sources, enzymatic processes in resistant pollutants remediation, identification and ecotoxicity assay of enzymatic transformation products, and typically employed enzymatic wastewater treatment systems. Perspectives on the major challenges and feasible future research directions of enzyme-based wastewater treatment are also proposed.
SCISEARCH/2021:2412374	CRISPR/Cas9-mediated genome editing directed by a 5S rRNA-tRNA(Gly) hybrid promoter in the thermophilic filamentous fungus Humicola insolens	Chinese Acad Agr Sci, Inst Anim Sci, 2 West Yuanmingyuan Rd, Beijing 100193, Peoples R China (Reprint) Chinese Acad Agr Sci, Biotechnol Res Inst, 12 South Zhongguancun St, Beijing 100081, Peoples R China E-mail: xuxinxin@caas.cn E-mail: huanghuoqing@caas.cn Chinese Acad Agr Sci, Biotechnol Res Inst, 12 South Zhongguancun St, Beijing 100081, Peoples R China (Reprint)	Fan, Chao (Reprint) Zhang, Wei Ji, Wangli Zhang, Yuhong Liu, Bo Xu, Xinxin Su, Xiaoyun Luo, Huiying Yao, Bin Huang, Huoqing	BIOTECHNOLOGY FOR BIOFUELS, (23 2021 OCT 2021) Vol. 14, No. 1.	20210101	other	Background Humicola insolens is a filamentous fungus with high potential of producing neutral and heat- and alkali-resistant cellulase. However, the genetic engineering tools, particularly the genome-editing tool, are scarce, hindering the study of cellulase expression regulation in this organism. Results Herein, a CRISPR/Cas9 genome-editing system was established in H. insolens based on a hybrid 5S rRNA-tRNA(Gly) promoter. This system is superior to the HDV (hepatitis delta virus) system in genome editing, allowing highly efficient single gene destruction in H. insolens with rates of deletion up to 84.1% (37/44). With this system, a putative pigment synthesis gene pks and the transcription factor xyr1 gene were disrupted with high efficiency. Moreover, the extracellular protein concentration and cellulase activity largely decreased when xyr1 was deleted, demonstrating for the first time that Xyr1 plays an important role in cellulase expression regulation. Conclusions The established CRISPR/Cas9 system is a powerful genetic operation tool for H. insolens, which will accelerate studies on the regulation mechanism of cellulase expression and engineering of H. insolens for higher cellulase production.
CABA/2021:227009	Prospection of fungal lignocellulolytic enzymes produced from Jatoba (Hymenaea courbaril) and tamarind (Tamarindus indica) seeds: scaling for bioreactor and saccharification profile of sugarcane bagasse.	Departamento de Bioquimica e Imunologia, Faculdade de Medicina de Ribeirao Preto, Universidade de Sao Paulo, Ribeirao Preto 14049-900, Brazil. EMAIL: alexgraca.contato@gmail.com oliveiratb@yahoo.com.br guilhermearanha@usp.br emanuelleneiverthf@gmail.com acvici@gmail.com karolmvnogueira@gmail.com rosymar_lucas@hotmail.com asascarcella@yahoo.com.br msbuck@usp.br rsilvausp@gmail.com polizeli@fflclrp.usp.br	Contato, A. G. Oliveira, T. B. de Aranha, G. M. Freitas, E. N. de Vici, A. C. Nogueira, K. M. V. Lucas, R. C. de Scarcella, A. S. de A. Buckeridge, M. S. Silva, R. N. Polizeli, M. de L. T. de M. de Oliveira, T. B. de Freitas, E. N. de Lucas, R. C.	Microorganisms (2021), Volume 9, Number 3, 44 refs. ISSN: 2076-2607 DOI: https://doi.org/10.3390/microorganisms9030533 Published by: MDPI AG, Basel	20210101	other	The lignocellulosic biomass comprises three main components: cellulose, hemicellulose, and lignin. Degradation and conversion of these three components are attractive to biotechnology. This study aimed to prospect fungal lignocellulolytic enzymes with potential industrial applications, produced through a temporal analysis using Hymenaea courbaril and Tamarindus indica seeds as carbon sources. α-L-arabinofuranosidase, acetyl xylan esterase, endo-1,5-α-L-arabinanase, β-D-galactosidase, β-D-glucosidase, β-glucanase, β-D-xylosidase, cellobiohydrolase, endoglucanase, lichenase, mannanase, polygalacturonase, endo-1,4-β-xylanase, and xyloglucanase activities were determined. The enzymes were produced for eight filamentous fungi: Aspergillus fumigatus, Trametes hirsuta, Lasiodiplodia sp., two strains of Trichoderma longibrachiatum, Neocosmospora perseae, Fusarium sp. and Thermothelomyces thermophilus . The best producers concerning enzymatic activity were T. thermophilus and T. longibrachiatum. The optimal conditions for enzyme production were the media supplemented with tamarind seeds, under agitation, for 72 h. This analysis was essential to demonstrate that cultivation conditions, static and under agitation, exert strong influences on the production of several enzymes produced by different fungi. The kind of sugarcane, pretreatment used, microorganisms, and carbon sources proved limiting sugar profile factors.

MEDLINE/2024207803	Survival of thermophilic fungi in various preservation methods: A comparative study.	Kutahya Health Sciences University, Training and Research Center, 43100, Kutahya, Turkey. derya.berikten@ksbu.edu.tr	Berikten Derya	Cryobiology, (20210800) Vol. 101, pp. 38-43. Electronic Publication Date: 18 Jun 2021 Journal code: 0006252. E-ISSN: 1090-2392. L-ISSN: 0011-2240.	20210101	other	Thermophilic fungi have several biotechnological and industrial applications such as thermostable enzyme production, biodegradation, and tobacco processing, etc. Thermophilic fungi cannot survive at temperatures below 20 °C. Owing to their inability to grow at low temperatures, they are not stable, so stocking is very difficult. Although a large number of different storage methods are available and described, no method can be universally applied to all fungi. Thermophilic fungi present 'heat-loving' characteristics, and therefore a new challenge for its preservation and there is no universal protocol for the preservation of thermophilic fungi. The aim of this study was to evaluate the viability, contamination and stability of thermophilic fungi stored under different preservation methods. In this work, 25 thermophilic fungal isolates of species Thermomyces thermophilus, Rhizomucor pusillus, Trichocladium griseum, Melanocarpus albomyces, Malbranchea cinnamomea, Thermothelomyces thermophilus , Thermothelomyces hinnuleus,Thermothielavioidesterrestris, Mycothermus thermophilus, Humicola insolens maintained constant sub-culturing at room temperature, +4 °C and +20 °C, lyophilization at +4 °C, freezing at -20 °C, freezing block at -20 °C and a new technique liquid preservation at room temperature for the periods ranging 5 years. We evaluated the effect of preservation methods by sub-culturing onto either sabouraud dextrose agar (SDA) or yeast extract soluble starch agar (YpSs) on growth, production and viability of spores and macro- and micromorphology. In this study, preservation methods for thermophilic fungi were investigated extensively for the first time and it is clearly shown that freezing block at -20 °C method and lyophilization were better methods for long-term preservation up to 5 years. Copyright © 2021 Elsevier Inc. All rights reserved.
MEDLINE/2023578274	Strategic aromatic residues in the catalytic cleft of the xyloglucanase MtXgh74 modifying thermostability, mode of enzyme action, and viscosity reduction ability.	National Research Centre 'Kurchatov Institute' - GOSNIIGENETIKA, Kurchatov Genomic Center, 1-st Dorozhniy pr. 1, Moscow, Russian Federation, 117545. mashchenko@yandex.ru National Research Centre 'Kurchatov Institute' 1, Kurchatov Sq, Moscow, Russian Federation, 123182. mashchenko@yandex.ru National Research Centre 'Kurchatov Institute' - GOSNIIGENETIKA, 1-st Dorozhniy pr. 1, Moscow, Russian Federation, 117545. Biological Department, Moscow State University, Leninskie gory 1, Build. 12, Moscow, Russian Federation, 119234. N.N. Semenov Federal Research Center for Chemical Physics, Russian Academy of Sciences, Kosygina Str., Bld. 1, Moscow, Russian Federation, 119991. Moscow Polytechnic University, B. Semenovskaya Str. 38, 107023, Moscow, Russian Federation, 107023. Technical University Munich, Department of Microbiology, Emil-Ramann-Str. 4, 85354, Freising, Germany. vladimir.zverlov@tum.de Aspratis GmbH, Huebnerstr. 11, 80637, Muenchen, Germany. National Research Centre 'Kurchatov Institute' - Institute of Molecular Genetics, Kurchatov Sq. 2, Moscow, Russian Federation, 123182. vladimir.zverlov@tum.de	Berezina Oksana V Rykov Sergey V Yarotsky Sergey V Polyakova Angelina K Bozdaganyan Marine E Sidochenko Anna V Baudrexl Melanie Zverlov Vladimir V Schwarz Wolfgang H	Applied microbiology and biotechnology, (2021 Feb) Vol. 105, No. 4, pp. 1461-1476. Electronic Publication Date: 1 Feb 2021 Journal code: 8406612. E-ISSN: 1432-0614. L-ISSN: 0175-7598.	20210101	other	The thermostable endo-processive xyloglucanase MtXgh74 from Myceliophthora thermophila was used to study the influence of aromatic amino acids in the catalytic cleft on the mode of action and the ability of enzyme to reduce xyloglucan viscosity. The enzyme derivative Mut I with mutations W64A/W67A in the 'negative' subsites of the catalytic cleft resulted in a 5.5-fold increase of the Km value. Mut I produced oligosaccharides of various lengths in addition to xyloglucan building blocks. The W320A/W321A substitutions in the 'positive' subsites of the mutated enzyme Mut II catalytic cleft increased the Km value 54-fold and resulted in an endo-dissociative mode of action. The ability of Mut II to reduce the viscosity of xyloglucan at 50 °C was much better than that of other MtXgh74 variants. Besides, Mut II efficiently reduced viscosity of a natural substrate, the pulp of xyloglucan-containing tamarind seed flour. The Km, Vmax, and kcat values and viscosity reduction ability of the enzyme derivative Mut III (W320A/W321A/G446Y) returned to levels close to that of MtXgh74. The pattern of xyloglucan hydrolysis by Mut III was typical for endo-processive xyloglucanases. The thermostability of Mut I and Mut II at 60 °C decreased significantly compared to the wild type, whereas the thermostability of Mut III at 60 °C restored almost to the MtXgh74-wt value. All mutants lost the ability to cleave the backbone of xyloglucan building blocks which was a characteristic of MtXgh74. Instead they acquired a low branch removing activity. Molecular dynamics simulations revealed the role of mutated amino acids in the complex action mechanism of GH74 enzymes. KEY POINTS: • Endo-processive mode of action of the xyloglucanase MtXgh74 was altered by rational design. • The endo-dissociative mutant Mut II (W320A/W321A) efficiently reduced XyG viscosity. • The substitutions W320A/W321A/G446Y in Mut III recovered the endo-processive mode. • Mut II can be used to reduce the viscosity of biomass slurries containing tamarind seed flour.
EMBASE/20210075034	Protein engineering approaches to enhance fungal laccase production in S. cerevisiae.	Microbial + Plant Biotechnology Department, Centro de Investigaciones Biologicas Margarita Salas (CIB), CSIC, Ramiro de Maeztu 9, Madrid, 28040, Spain. susanacam@cib.csic.es david.rodriguez@cib.csic.es salasdelacuadra@gmail.com pabloaza@cib.csic.es idelafuente011@gmail.com gonzalo.molpeceres@cib.csic.es	Aza, Pablo De Salas, Felipe Molpeceres, Gonzalo Rodriguez-Escribano, David De La Fuente, Inigo Camarero, Susana (correspondence)	International Journal of Molecular Sciences, (1 Feb 2021) Vol. 22, No. 3, pp. 1-19. arn. 1157. Refs: 70 ISSN: 1661-6596; E-ISSN: 1422-0067	20210101	other	Laccases secreted by saprotrophic basidiomycete fungi are versatile biocatalysts able to oxidize a wide range of aromatic compounds using oxygen as the sole requirement. Saccharomyces cerevisiae is a preferred host for engineering fungal laccases. To assist the difficult secretion of active enzymes by yeast, the native signal peptide is usually replaced by the preproleader of S. cerevisiae alfa mating factor (MFα1). However, in most cases, only basal enzyme levels are obtained. During directed evolution in S. cerevisiae of laccases fused to the α-factor preproleader, we demonstrated that mutations accumulated in the signal peptide notably raised enzyme secretion. Here we describe different protein engineering approaches carried out to enhance the laccase activity detected in the liquid extracts of S. cerevisiae cultures. We demonstrate the improved secretion of native and engineered laccases by using the fittest mutated α-factor preproleader obtained through successive laccase evolution campaigns in our lab. Special attention is also paid to the role of protein Nglycosylation in laccase production and properties, and to the introduction of conserved amino acids through consensus design enabling the expression of certain laccases otherwise not produced by the yeast. Finally, we revise the contribution of mutations accumulated in laccase coding sequence (CDS) during previous directed evolution campaigns that facilitate enzyme production.

SCISEARCH/2021:871112	A greener, mild, and efficient bioprocess for the pretreatment and saccharification of rice straw	Cent Univ Haryana, Sch Interdisciplinary + Appl Sci, Dept Biotechnol, Mahendergarh 123031, Haryana, India (Reprint) Maharshi Dayanand Univ, Dept Microbiol, Lab Bioproc Technol, Rohtak 124001, Haryana, India E-mail: ohlanbs@gmail.com Cent Univ Haryana, Dept Chem, Mahendergarh 123031, Haryana, India RPS Degree Coll, Dept Phys, Mahendergarh 123031, Haryana, India E-mail: ohlanbs@gmail.com Maharshi Dayanand Univ, Dept Microbiol, Lab Bioproc Technol, Rohtak 124001, Haryana, India (Reprint)	Anu (Reprint) Singh, Bijender Kumar, Vinod Singh, Davender	BIOMASS CONVERSION AND BIOREFINERY, (30 2021 MAR 2021) . ISSN: 2190-6815.	20210101	duplicates	Open rice straw burning has created hazardous effects on the environment and human health. Biological pretreatment of rice straw has been proven as an environmental-benign and economical feasible process for the production of biofuels and value-added products under milder and greener conditions by avoiding harmful chemicals and toxic reagents. A consolidated bioprocess for the production of cellulolytic enzymes and biological pretreatment was developed to achieve a cost-effective saccharification of rice straw. Biodegradation of biomass was slightly enhanced with increase in cultivation time of microbial pretreatment. Furthermore, the modification in biomass due to biological pretreatment weakened the interaction between hemicellulose and lignin that resulted in significant reduction in lignin using Bacillus subtilis subsp. subtilis JJBS300, Myceliophthora thermophila BJTLRMDU3, and Aspergillus oryzae SBS50 for biological pretreatment of rice straw. Bacterial culture produced maximum FPase, CMCase, and beta-glucosidase of 28, 17, and 20.36 U/g DMR after 2 days during pretreatment process, whereas M. thermophila produced maximum FPase (85.10 U/g DMR), CMCase (96.89 U/g DMR), and beta-glucosidase (91.92 U/g DMR) after 9 days. The mold A.oryzae SBS50 also produced maximum FPase (57.41 U/g DMR), CMCase (55.36 U/g DMR), and beta-glucosidase (48.04 U/g DMR) after 9 days of pretreatment. Maximum amount of reducing sugars of 52.41, 86.74, and 49.59 mg/g substrate were liberated from 6-, 5-, and 3-day-old biological pretreated rice straw by B. subtilis, M. thermophila, and A. oryzae, respectively, after enzymatic hydrolysis for 6 h at 60 C-o and pH 5.0. Laccase-pretreated rice straw followed by enzymastic saccharification resulted in liberation of 162.82 mg/g reducing sugars at pH 5.0 and 60 degrees C after 6h using 20 U/g cellulase. Simultaneous laccase pretreatment and saccharification (SLPS) process further enhanced the liberated reducing sugars, i.e., 179.47 mg/g substrate. FTIR, XRD, and SEM analyses showed marked morphological changes as a result of delignification after biological pretreatment of rice straw. Biological pretreatment being an environmental-benign process causing no harm to the environment in comparison to physical and chemical pretreatments of lignocellulosic biomass could be a better pretreatment strategy for bioremediation of lignocellulosic substrates.
CAPLUS/2020:509855	Thermostabilization of membrane proteins by consensus mutation : A case study for a fungal Δ8-7 sterol isomerase	State Key Laboratory of Molecular Biology, CAS Center for Excellence in Molecular Cell Science, National Center for Protein Science Shanghai, Shanghai Institute of Biochemistry and Cell Biology, University of Chinese Academy of Sciences, Chinese Academy of Sciences, Shanghai, 201210, Peop. Rep. China	Yao, Hebang Cai, Hongmin Li, Dianfan	Journal of Molecular Biology (2020), 432(18), 5162-5183 CODEN: JMOBAK; ISSN: 0022-2836	20200101	other	Membrane proteins are generally challenging to work with because of their notorious instability. Protein engineering has been used increasingly to thermostabilize labile membrane proteins such as G-protein-coupled receptors for structural and functional studies in recent years. Two major strategies exist. Scanning mutagenesis systematically eliminates destabilizing residues, whereas the consensus approach assembles mutants with the most frequent residues among selected homologs, bridging sequence conservation with stability. Here, we applied the consensus concept to stabilize a fungal homolog of the human sterol Δ8-7 isomerase, a 26.4 kDa protein with five transmembrane helixes. The isomerase is also called emopamil-binding protein (EBP), as it binds this anti-ischemic drug with high affinity. The wild-type had an apparent melting temp. (Tm) of 35.9°C as measured by the fluorescence-detection size-exclusion chromatog.-based thermostability assay. A total of 87 consensus mutations sourced from 22 homologs gained expression level and thermostability, increasing the apparent Tm to 69.9°C at the cost of partial function loss. Assessing the stability and activity of several systematic chimeric constructs identified a construct with an apparent Tm of 79.8°C and two regions for function rescue. Further back-mutations of the chimeric construct in the two target regions yielded the final construct with similar apparent activity to the wild-type and an elevated Tm of 88.8°C, totaling an increase of 52.9°C. The consensus approach is effective and efficient because it involves fewer constructs compared with scanning mutagenesis . Our results should encourage more use of the consensus strategy for membrane protein thermostabilization.
MEDLINE/2022362887	A simple approach to mediate genome editing in the filamentous fungus Trichoderma reesei by CRISPR/Cas9-coupled in vivo gRNA transcription.	State Key Lab of Bioreactor Engineering, New World Institute of Biotechnology, East China University of Science and Technology, 130 Meilong Road, P.O.B. 311, Shanghai, 200237, China. wadexp@ecust.edu.cn; myushu@ecust.edu.cn The First Affiliated Middle School of East China Normal University, Shanghai, 200086, China.	Wu Chuan Chen Yumeng Qiu Yifei Niu Xiao Chen Jiehui Yao Hong Wang Wei Ma Yushu Zhu Ningjian	Biotechnology letters, (2020 Jul) Vol. 42, No. 7, pp. 1203-1210. Electronic Publication Date: 16 Apr 2020 Journal code: 8008051. E-ISSN: 1573-6776. L-ISSN: 0141-5492.	20200101	other	OBJECTIVE: To simplify CRISPR/Cas9 genome editing in the industrial filamentous fungus Trichoderma reesei based on in vivo guide RNA (gRNA) transcription. RESULTS: Two putative RNA polymerase III U6 snRNA genes were identified in the genome of T. reesei QM6a by BLASTN using Myceliophthora . thermophila U6 snRNA gene as the template. The regions approximately 500 bp upstream of two U6 genes were efficient promoters for the in vivo expression of gRNA. The CRISPR system consisting of Cas9 and in vivo synthesized gRNA under control of the T. reesei U6 snRNA promoters was sufficient to cause a frameshift mutation in the ura5 gene via non-homologous end-joining-mediated events. CONCLUSIONS: We report a simple gene editing method using a CRISPR/Cas9-coupled in vivo gRNA transcription system in T. reesei.
CAPLUS/2020:1163423	Structure of complete Pol II-DSIF-PAF-SPT6 transcription complex reveals RTF1 allosteric activation	Department of Molecular Biology, Max Planck Institute for Biophysical Chemistry, Goettingen, Germany	Vos, Seychelle M. Farnung, Lucas Linden, Andreas Urlaub, Henning Cramer, Patrick	Nature Structural + Molecular Biology (2020), 27(7), 668-677 CODEN: NSMBCU; ISSN: 1545-9993	20200101	other	Transcription by RNA polymerase II (Pol II) is carried out by an elongation complex. We previously reported an activated porcine Pol II elongation complex, EC*, encompassing the human elongation factors DSIF, PAF1 complex (PAF) and SPT6. Here we report the cryo-EM structure of the complete EC* that contains RTF1, a dissociable PAF subunit crit.for chromatin transcription. The RTF1 Plus3 domain assocs.with Pol II subunit RPB12 and the phosphorylated C-terminal region of DSIF subunit SPT5. RTF1 also forms four α-helixes that extend from the Plus3 domain along the Pol II protrusion and RPB10 to the polymerase funnel. The C-terminal 'fastener' helix retains PAF and is followed by a 'atch' that reaches the end of the bridge helix, a flexible element of the Pol II active site. RTF1 strongly stimulates Pol II elongation, and this requires the latch, possibly suggesting that RTF1 activates transcription allosterically by influencing Pol II translocation.
SCISEARCH/2020:1606502	Laccase Did It again: A Scalable and Clean Regeneration System for NAD(+)and Its Application in the Synthesis of 12-oxo-Hydroxysteroids	E-mail: F.Tonin@tudelft.nl emartisoler@gmail.com I.W.C.E.Arends@tudelft.nl u.hanefeld@tudelft.nl Univ Utrecht, Fac Sci, Budapestlaan 6, NL-3584 CD Utrecht, Netherlands E-mail: I.W.C.E.Arends@tudelft.nl Delft Univ Technol, Dept Biotechnol, Van der Maasweg 9, NL-2629 HZ Delft, Netherlands (Reprint)	Tonin, Fabio (Reprint) Marti, Elisabet Arends, Isabel W. C. E. Hanefeld, Ulf	CATALYSTS, (2020 JUN 2020) Vol. 10, No. 6.	20200101	other	The specific oxidation of 12 alpha-OH group of hydroxysteroids is required for the preparation of cheno- and ursodeoxycholic acid (CDCA and UDCA, respectively). The C(12)oxidation of hydroxysteroids into their 12-oxo derivatives can selectively be performed by employing 12 alpha-hydroxysteroid dehydrogenases. These enzymes use NAD(P)(+)as an electron acceptor, which has to be re-oxidized in a so-called 'regeneration system'. Recently, the enzyme NAD(P)H oxidase (NOX) was applied for the regeneration of NAD(+)in the enzymatic preparation of 12-oxo-CDCA from cholic acid (CA), which allows air to be used as an oxidant. However, the NOX system suffers from low activity and low stability. Moreover, the substrate loading is limited to 10 mM. In this study, the laccase/mediator system was investigated as a possible alternative to NOX, employing air as an oxidant. The laccase/mediator system shows higher productivity and scalability than the NOX system. This was proven with a preparative biotransformation of 20 g of CA into 12-oxo-CDCA (92% isolated yield) by employing a substrate loading of 120 mM (corresponding to 50 g/L). Additionally, the performance of the laccase/mediator system was compared with a classical ADH/acetone regeneration system and with other regeneration systems reported in literature.

MEDLINE/2022646667	Creation of Chitinase Producer and Disruption of Micromycete Cell Wall with the Obtained Enzyme Preparation.	Lomonosov Moscow State University, Faculty of Chemistry, Moscow, 119991, Russia. oasinitsyna@gmail.com Fundamentals of Biotechnology Federal Research Center, Russian Academy of Sciences, Moscow, 119071, Russia. Pushchino Scientific Center for Biological Research, Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Moscow Region, 142290, Russia. Russian Research Institute of Food Biotechnology, Moscow, 111033, Russia. All-Russian Research Institute of Phytopathology, Bolshye Vyazemy, Moscow Region, 143050, Russia.	Sinitsyna O A Sinitsyn A P Rubtsova E A Sinelnikov I G Osipov D O Rozhkova A M Matys V Yu Bubnova T V Nemashkalov V A Sereda A S Tcsherbakova L A	Biochemistry. Biokhimiia, (2020 Jun) Vol. 85, No. 6, pp. 717-724. Journal code: 0376536. E-ISSN: 1608-3040. L-ISSN: 0006-2979.	20200101	other	A recombinant strain producing a complex of extracellular enzymes including chitinase from Myceliophtora thermophila was created based on the fungus Penicillium verruculosum. The activity of the enzyme preparations obtained from the cultural fluid of the producer strain was 0.55, 0.53, and 0.66 U/mg protein with chitin and chitosans with the molecular weight of 200 and 1000 kDa, respectively. The temperature optimum for the recombinant chitinase was 52-65°C; the pH optimum was 4.5-6.2, which corresponded to the published data for this class of the enzymes. The content of heterologous chitinase in the obtained enzyme preparations was 47% of total protein content in the cultural fluid. Enzyme preparations produced by the recombinant P. verruculosum XT403 strain and containing heterologous chitinase were able to degrade the mycelium of micromycetes, including phytopathogenic ones, and were very efficient in the bioconversion of microbiological industry waste.
BIOSIS/2020:923648	Advances on Food-Derived Peptidic Antioxidants-A Review.	Tianjin Univ Sci and Technol, State Key Lab Food Nutr and Safety, Tianjin 300457, Peoples R China panmf2012@tust.edu.cn; Liukx2019@163.com; yangjy0823@126.com; lsm20000711@outlook.com; wshan0929niu@163.com; s.wang@tust.edu.cn	Pan, Mingfei [Reprint Author] Liu, Kaixin Yang, Jingying Liu, Shengmiao Wang, Shan Wang, Shuo [Reprint Author]	Antioxidants, (SEP 2020) Vol. 9, No. 9, pp. Article No.: 799. http://www.mdpi.com/journal/antioxidants. E-ISSN: 2076-3921.	20200101	other	The oxidation process is considered to be the main reason behind human aging, human degenerative diseases and food quality degradation. Food-derived peptidic antioxidants (PAs) have wide sources and great activity, and have broad application prospects in removing excess reactive oxygen species in the body, anti-aging and preventing and treating diseases related to oxidative stress. On the other hand, PAs are expected to inhibit the lipid peroxidation of foods and increase the stability of the food system in the food industry. However, the production pathways and action mechanism of food-derived PAs are diverse, which makes it is difficult to evaluate the performance of PAs which is why the commercial application of PAs is still in its infancy. This article focuses on reviewing the preparation, purification, and characterization methods of food-derived PAs, and expounds the latest progress in performance evaluation and potential applications, in order to provide an effective reference for subsequent related research of PAs.
BIOSIS/2020:928241	Current Development in Decolorization of Synthetic Dyes by Immobilized Laccases.	Univ Putra Malaysia, Fac Biotechnol and Biomol Sci, Enzyme and Microbial Technol Res Ctr, Upm Serdang, Selangor, Malaysia fairolniza@upm.edu.my	Morsy, Sherine Ahmed Gamal Zakaria [Reprint Author] Ahmad Tajudin, Asilah Ali, Mohd. Shukuri Mohamad Shariff, Fairolniza Mohd. [Reprint Author]	Frontiers in Microbiology, (SEP 30 2020) Vol. 11, pp. Article No.: 572309. http://www.frontiersin.org/microbiology/. ISSN: 1664-302X. E-ISSN: 1664-302X.	20200101	other	The world today is in a quest for new means of environmental remediation as the methods currently used are not sufficient to halt the damage. Mostly, a global direction is headed toward a shift from traditional chemical-based methods to a more ecofriendly alternative. In this context, biocatalysis is seen as a cost-effective, energy saving, and clean alternative. It is meant to catalyze degradation of recalcitrant chemicals in an easy, rapid, green, and sustainable manner. One already established application of biocatalysis is the removal of dyes from natural water bodies using enzymes, notably oxidoreductases like laccases, due to their wide range of substrate specificity. In order to boost their catalytic activity, various methods of enhancements have been pursued including immobilization of the enzyme on different support materials. Aside from increased catalysis, immobilized laccases have the advantages of higher stability, better durability against harsh environment conditions, longer half-lives, resistance against protease enzymes, and the ability to be recovered for reuse. This review briefly outlines the current methods used for detoxification and decolorization of dye effluents stressing on the importance of laccases as a revolutionary biocatalytic solution to this environmental problem. This work highlights the significance of laccase immobilization and also points out some of the challenges and opportunities of this technology.
MEDLINE/2021866634	Improving cellulases production by Myceliophthora thermophila through disruption of protease genes.	State Key Laboratory of Agrobiotechnology and MOA Key Laboratory of Soil Microbiology, College of Biological Sciences, China Agricultural University, Beijing, 100193, China. qunhe@cau.edu.cn Key Laboratory of Systems Microbial Biotechnology, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin, 300308, China. tian_cg@tib.cas.cn	Li Xiaolin He Qun Liu Qian Sun Wenliang Tian Chaoguang	Biotechnology letters, (2020 Feb) Vol. 42, No. 2, pp. 219-229. Electronic Publication Date: 4 Dec 2019 Journal code: 8008051. E-ISSN: 1573-6776. L-ISSN: 0141-5492.	20200101	other	OBJECTIVE: To identify main protease genes for the proteolytic degradation of cellulases in M. thermophila and generate a lower-proteases fungal host that can be used for further metabolic engineering to increase cellulase production and heterologous protein expression. RESULTS: Systematic transcriptomic analysis were conducted on the expression of proteases genes in M. thermophila genome and five highly expressed genes encoding extracellular proteases were selected for mutation analyses. A series of single- and multi-gene mutants of these five selected genes was constructed using the CRISPR-Cas9 technique. Compared with WT, the ΔMtalp1 and the quintuple mutant showed significantly lower protease activity (decreased 52.7% and 58.4%, respectively) and at least double enhanced cellulase production. CONCLUSIONS: The results indicated that Mtalp1 is a critical protease gene in cellulase degradation in M. thermophila and disruption of protease genes showed significantly decreased protease activity and obviously enhanced cellulase production in the fermentation broth of ΔMtalp1 and the quintuple mutant.
CABA/2020:94195	Endophytic fungi from the medicinal herb Euphorbia geniculata as a potential source for bioactive metabolites.	Botany Department, Faculty of Science, Aswan University, Aswan, Egypt. EMAIL: fatma.fakhry@aswu.edu.eg	Kamel, N. M. Abdel-Motaal, F. F. El-Zayat, S. A.	Archives of Microbiology (2020), Volume 202, Number 2, pp. 247-255, many ref. ISSN: 0302-8933; 1432-072X DOI: https://doi.org/10.1007/s00203-019-01740-x Published by: Springer Berlin, Heidelberg	20200101	other	Many researchers proved that plant endophytes manage successful issues to synthesize active chemicals within plant cells. These bioactive compounds might support a range of plant defense mechanism against many pathogenic microorganisms. In this study, a total of 22 isolates representing 21 fungal species belonging to 15 fungal genera in addition to one variety were isolated and identified for the first time from Euphorbia geniculate plants. The genus Aspergillus was the most common fungus isolated from the studied plant. The fungus Isaria feline was recorded in both leaves and stem, while Aspergillus flavus, A. ochraceus, A. terreus var. terreus, Emercilla nidulans var. acristata, Macrophomina phaseolina colonized both stem and root. The isolated fungi showed antagonistic activities against six strains of plant pathogenic fungi viz., Eupenicillium brefeldianum, Penicillium echinulatum, Alternaria phragmospora, Fusarium oxysporum, Fusarium verticilloid, and Alternaria alternata in dual culture assay. The highest antagonistic activity fungal species (Aspergillus flavus, A. fumigatus, and Fusarium lateritium) and the lowest (Cladosprium herbarum, F. culomrum, and Sporotrichum thermophile) showed twining in their secondary metabolites especially terpens and alkaloids with that of their host E. geniculata. Three concentrations of (0.5, 1.0, and 2.0 mg/ml) of these secondary metabolites extracted by ethyl acetate and n-butanol from the above six endophytic fungal species were tested against three pathogenic fungi isolated from infected tomato plant (E. brefeldianum-EBT-1, P. echinulatum-PET-2, and A. phragmospora-APT-3), whereas these pathogens showed promising sensitivity to these fungal secondary metabolites. In conclusion, this is the first report on the isolation of endophytic fungi from E. geniculata and evaluation of their antifungal activity.
CAPLUS/2020:1608989	In Vivo Reduction of Medium- to Long-Chain Fatty Acids by Carboxylic Acid Reductase (CAR) Enzymes: Limitations and Solutions	acib -Austrian Center of Industrial Biotechnology, Krenngasse 37, Graz, 8010, Austria	Horvat, Melissa Winkler, Margit	ChemCatChem (2020), 12(20), 5076-5090 CODEN: CHEMK3; ISSN: 1867-3880	20200101	other	Fatty aldehyde prodn. by chem. synthesis causes an immense burden to the environment. Within this study, we explored a sustainable, aldehyde-selective and mild alternative approach by utilizing carboxylic acid reductases (CARs). CARs from Neurospora crassa (NcCAR), Thermothelomyces thermophila (TtCAR), Nocardia iowensis (NiCAR), Mycobacterium marinum (MmCAR) and Trametes versicolor (TvCAR) were overexpressed in E. coli K-12 MG1655 RARE (DE3) and screened for medium- to long-chain fatty acid (C6-C18) redn. MmCAR showed the broadest tolerance towards all carbon-chain lengths and was selected for further investigations of fatty aldehyde synthesis in whole cells. To yield relevant product concns., different limitations of CAR whole-cell conversions were elucidated and compensated. We coupled an in vitro cofactor recycling system to a whole-cell biocatalyst to support cofactor supply and achieved 12.36 g L-1 of octanal (STY 0.458 g L-1 h-1) with less than 1.5% of 1-octanol.

MEDLINE/2022925504	Molecular dynamics simulations of two GH74 endo processive xyloglucanases and the mutated variants to understand better the mechanism of the enzyme action.	Department of Chemistry, M. V. Lomonosov Moscow State University, Vorobyovy Gory 1/11, Moscow 119991, Russia. avgusakov@enzyme.chem.msu.ru Department of Chemistry, M. V. Lomonosov Moscow State University, Vorobyovy Gory 1/11, Moscow 119991, Russia.	Gusakov Alexander V Uporov Igor V Sinitsyna Olga A	Biochimica et biophysica acta. General subjects, (20201200) Vol. 1864, No. 12, pp. 129721. Electronic Publication Date: 29 Aug 2020 Journal code: 101731726. E-ISSN: 1872-8006. L-ISSN: 0304-4165.	20200101	other	BACKGROUND: GH74 xyloglucanases are composed of two separate domains connected by two unstructured peptides. Previously, a hypothesis was made that the movement of domains may affect the enzyme mechanism of catalysis. METHODS: The molecular dynamics (MD) simulations of endo-processive xyloglucanases from Paenibacillus odorifer (PoGH74cat) and Myceliophthora thermophila (MtXeg74A) were carried out. RESULTS: MD simulations for both enzymes in complex with XXLG and XGXXLG oligosaccharides confirmed the possibility of domain movement. In the case of MtXeg74A, changes in the distances between Cα atoms of aromatic residues involved in xyloglucan binding in -3 and +3 subsites of the active site cleft and those of selected residues on the opposite side of the cleft reached values up to 10-12 Å. For PoGH74cat the conformational changes were less pronounced. In MtXeg74A variants, the deletion of loop 1, which partially closes the entrance to the cleft, and the additional double mutation of two Trp residues in +3 and +5 subsites caused the enhanced mobility of the XGXXLG and also induced changes in topography of the cleft. CONCLUSIONS: These findings demonstrate the possibility of existence of GH74 xyloglucanases in a more open and more closed enzyme conformation. The enzyme in an open conformation may more easily accommodate the branched polysaccharide, while its transition to the closed conformation, together with loop 1 function, should aid processivity. GENERAL SIGNIFICANCE: Our results provide an insight into a mechanism of action of GH74 xyloglucanases and may be useful for discussing the catalytic mechanisms of glycoside hydrolases from other families. Copyright © 2020 Elsevier B.V. All rights reserved.
BIOSIS/2020:909955	Construction of the R17L mutant of MtC1LPMO for improved lignocellulosic biomass conversion by rational point mutation and investigation of the mechanism by molecular dynamics simulations.	Minist Educ, Key Lab Ind Fermentat Microbiol, Tianjin 300457, Peoples R China fufengliu@tust.edu.cn	Guo, Xiao An, Yajing Chai, Chengcheng Sang, Jingcheng Jiang, Luying Lu, Fuping Dai, Yujie Liu, Fufeng [Reprint Author]	Bioresource Technology, (DEC 2020) Vol. 317, pp. Article No.: 124024. http://www.journals.elsevier.com/bioresource-technology/#description. CODEN: BIRTEB. ISSN: 0960-8524. E-ISSN: 1873-2976.	20200101	other	To enhance the biomass conversion efficiency, the R17L mutant of the lytic polysaccharide monooxygenase (LPMO) MtC1LPMO with improved catalytic efficiency was constructed via rational point mutation based on the HotSpot Wizard 3.0 and dezyme web servers. Compared with the wild-type (WT) MtC1LPMO, R17L exhibited a 1.8-fold increase of specific activity and 1.92-fold increase of catalytic efficiency (k(cat)/K-m). The degree of increase of the reducing sugar yield from microcrystalline cellulose and three plant biomass materials during synergistic hydrolysis using cellulase in combination with R17L was about 2 times higher than with the WT. Molecular dynamics simulations revealed that the R17L mutation reduced the stability of the region R18-136, which then weakened the direct interactions between region N24-V31 and the substrate cellohexaose. Consequently, the deflection time of the cellohexaose conformation in R17L was prolonged compared to the WT, which enhanced its catalytic efficiency.
CAPLUS/2020:1355142	Structural insight into mitochondrial β-barrel outer membrane protein biogenesis	Laboratory of Molecular Biology, National Institute of Diabetes + Digestive + Kidney Diseases, National Institutes of Health, Bethesda, MD, 20892, USA	Diederichs, Kathryn A. Ni, Xiaodan Rollauer, Sarah E. Botos, Istvan Tan, Xiaofeng King, Martin S. Kunji, Edmund R. S. Jiang, Jiansen Buchanan, Susan K.	Nature Communications (2020), 11(1), 3290 CODEN: NCAOBW; ISSN: 2041-1723	20200101	other	Abstr.: In mitochondria, β-barrel outer membrane proteins mediate protein import, metabolite transport, lipid transport, and biogenesis. The Sorting and Assembly Machinery (SAM) complex consists of three proteins that assemble as a 1:1:1 complex to fold β-barrel proteins and insert them into the mitochondrial outer membrane. We report cryoEM structures of the SAM complex from Myceliophthora thermophila , which show that Sam50 forms a 16-stranded transmembrane β-barrel with a single polypeptide-transport-assocd. (POTRA) domain extending into the intermembrane space. Sam35 and Sam37 are located on the cytosolic side of the outer membrane, with Sam35 capping Sam50, and Sam37 interacting extensively with Sam35. Sam35 and Sam37 each adopt a GST-like fold, with no functional, structural, or sequence similarity to their bacterial counterparts. Structural anal. shows how the Sam50 β-barrel opens a lateral gate to accommodate its substrates.
CABA/2020:197686	Mathematical modeling of estrogen degradation in enzymatic membrane reactor.	School of Chemical Engineering, Federal University of Uberlandia, Av. Joao Naves de Avila 2121, Santa Monica - 38408-144, Uberlandia - MG, Brazil. University of Sao Paulo, Lorena, Sao Paulo, Brazil. EMAIL: felixmpereira@usp.br	Carvalho, C. L. S. Gomes, F. M. Pereira, F. M. Editor(s): de Souza Ferreira, J. Batista, F. R. X. Falleiros, L. N. S. S.	Industrial Biotechnology (2020), Volume 16, Number 2, pp. 50-55, 19 refs. ISSN: 1550-9087; 1931-8421 DOI: https://doi.org/10.1089/ind.2020.29200.csc Published by: Mary Ann Liebert, Inc., New Rochelle	20200101	other	Estrogens are endocrine-disrupting chemicals (EDCs) that enter waterbodies via wastewater. Because of this, some countries are creating legislation aimed at developing processes to degrade EDCs in wastewatertreatment plants. Estrone and estradiol are the estrogens found in high concentrations in waterbodies. Some processes in literature suggest using the enzyme laccase to degrade EDCs. This work mathematically models EDCs degradation by laccase in an enzymatic membrane reactor (EMR). The models were built from the mass balances of EDCs and active enzymes in EMR, including Michaelis Menten kinetics and a parameter coupling the enzyme activation, deactivation, and loss by the outflow. Experimental data from literature were used in the models' regressions. The experiments considered three experimental conditions to evaluate two process parameters: EMR residence time and O2 pulse frequency. The models' assessment considered the following statistical tests: Student's t to evaluate the parameters' significance; Akaike Information Criteria to select the best models; and Rsup2 to evaluate the models' fit. Results show the best assessed kinetic models were able to represent degradation in an EMR, and the best operation conditions were a residence time of 4 h and an O2 pulse frequency of 0.5 h. The methods and mathematical models presented in this manuscript can contribute to wastewater plant design for reducing contamination by EDCs in waterbodies. Humans are the main producers of estrogens currently present in the waterbodies. Estrone and estradiol-quoted in literature as EDCs found in high amount in waterbodies -are toxic when found in micro concentrations for both aquatic and nonaquatic animals, including human beings. Laccase, a multicopper polyphenol oxidase enzyme present in many organisms, can degrade these compounds with a low degree of substrate specificity. Operating with free enzymes in a conventional reactor requires high enzyme consumption because they are washed from the reactor by the outflow. To reduce the enzyme loss, an ultrafiltration membrane can be used to recover enzymes back to the reaction vessel. The system is composed of a continuous stirred tank reactor and ultrafiltration using a semipermeable membrane, or EMR. An important step for reactor design and operation is the estimation of kinetic parameters by the regression of phenomenological models to experimental data. This work presents an estimation of kinetic parameters of estrone and estradiol degradation by laccase from Myceliophthora thermophila performed in an EMR.
BIOSIS/2021:139987	Multienzyme Cellulose Films as Sustainable and Self-Degradable Hydrogen Peroxide-Producing Material.	Univ Sao Paulo, Sao Carlos Inst Phys, BR-13566590 Sao Carlos, SP, Brazil marcokadowaki@gmail.com	Califano, Davide Kadowaki, Marco A. S. [Reprint Author] Calabrese, Vincenzo Prade, Rolf Alexander Mattia, Davide Edler, Karen J. Polikarpov, Igor Scott, Janet L.	Biomacromolecules, (DEC 2020) Vol. 21, No. 12, pp. 5315-5322. https://pubs.acs.org/loi/bomaf6?expanded=19. ISSN: 1525-7797. E-ISSN: 1526-4602.	20200101	other	The use of hydrogen peroxide-releasing enzymes as a component to produce alternative and sustainable antimicrobial materials has aroused interest in the scientific community. However, the preparation of such materials requires an effective enzyme binding method that often involves the use of expensive and toxic chemicals. Here, we describe the development of an enzyme-based hydrogen peroxide-producing regenerated cellulose film (RCF) in which a cellobiohydrolase (TrCBHI) and a cellobiose dehydrogenase (MtCDHA) were efficiently adsorbed, 90.38 +/- 2.2 and 82.40 +/- 5.7%, respectively, without making use of cross-linkers. The enzyme adsorption kinetics and binding isotherm experiments showed high affinity of the proteins possessing cellulose-binding modules for RCF, suggesting that binding on regenerated cellulose via specific interactions can be an alternative method for enzyme immobilization. Resistance to compression and porosity at a micrometer scale were found to be tunable by changing cellulose concentration prior to film regeneration. The self-degradation process, triggered by stacking TrCBHI and MtCDHA (previously immobilized onto separate RCF), produced 0.15 nmol/min.cm(2) of H2O2. Moreover, the production of H2O2 was sustained for at least 24 h reaching a concentration of similar to 2 mM. The activity of MtCDHA immobilized on RCF was not affected by reuse for at least 3 days (1 cycle/day), suggesting that no significant enzyme leakage occurred in that timeframe. In the material herein designed, cellulose (regenerated from a 1-ethyl-3-methylimidazolium acetate/dimethyl sulfoxide (DMSO) solution) serves both as support and substrate for the immobilized enzymes. The sequential reaction led to the production of H2O2 at a micromolar-millimolar level revealing the potential use of the material as a self-degradable antimicrobial agent.

CABA/2020:440915	Characterization of an AA9 LPMO from Thielavia australiensis, TausLPMO9B, under industrially relevant lignocellulose saccharification conditions.	DSM Biotechnology Center, PP 699-0310, Alexander Fleminglaan 1, 2613 AX, Delft, Netherlands. EMAIL: Federica.Calderaro@dsm.com	Calderaro, F. Keser, M. Akeroyd, M. Bevers, L. E. Eijsink, V. G. H. Varnai, A. Berg, M. A. van den van den Berg, M. A.	Biotechnology for Biofuels (2020), Volume 13, Number 195, many ref. ISSN: 1754-6834 DOI: https://doi.org/10.1186/s13068-020-01836-3 Published by: BioMed Central Ltd, London	20200101	other	Background: The discovery of lytic polysaccharide monooxygenases (LPMO) has changed our perspective on enzymatic degradation of plant biomass. Through an oxidative mechanism, these enzymes are able to cleave and depolymerize various polysaccharides, acting not only on crystalline substrates such as chitin and cellulose, but also on other polysaccharides, such as xyloglucan, glucomannan and starch. Despite their widespread use, uncertainties related to substrate specificity and stereospecificity, the nature of the co-substrate, in-process stability, and the nature of the optimal reductant challenge their exploitation in biomass processing applications. Results: In this work, we studied the properties of a novel fungal LPMO from the thermophilic fungus Thielavia australiensis, TausLPMO9B. Heterologous expression of TausLPMO9B in Aspergillus niger yielded a glycosylated protein with a methylated N-terminal histidine showing LPMO activity. High sequence identity of the AA9 domain to that of MtLPMO9B (MYCTH_80312) from Myceliophthora thermophila (84%) indicated strictly C1-oxidizing activity on cellulose, which was confirmed experimentally by the analysis of products released from cellulose using HPAEC. The enzyme was stable and active at a pH ranging from 4 to 6, thus matching the conditions commonly used in industrial biomass processing, where a low pH (between 4 and 5) is used due to the pH-optima of commercial cellulases and a desire to limit microbial contamination . Conclusion: While the oxidative cleavage of phosphoric acid swollen cellulose (PASC) by TausLPMO9B was boosted by the addition of H2O2 as a co-substrate, this effect was not observed during the saccharification of acid pretreated corn stover. This illustrates key differences between the lab-scale tests with artificial, lignin-free substrates and industrial settings with lignocellulosic biomass as substrate.
PQSCITECH/2021:6422	Characterization of an AA9 LPMO from Thielavia australiensis, TausLPMO9B, under industrially relevant lignocellulose saccharification conditions		Calderaro, F Keser, M Akeroyd, M Bevers, L E Eijsink, V G H Varnai, A M. A. van den Berg	Biotechnology for Biofuels, Vol. 13, pp. 1-17, 20200101 E-ISSN: 1754-6834 DOI: 10.1186/s13068-020-01836-3 Published by: BioMed Central, London	20200101	duplicates	Background The discovery of lytic polysaccharide monooxygenases (LPMO) has changed our perspective on enzymatic degradation of plant biomass. Through an oxidative mechanism, these enzymes are able to cleave and depolymerize various polysaccharides, acting not only on crystalline substrates such as chitin and cellulose, but also on other polysaccharides, such as xyloglucan, glucomannan and starch. Despite their widespread use, uncertainties related to substrate specificity and stereospecificity, the nature of the co-substrate, in-process stability, and the nature of the optimal reductant challenge their exploitation in biomass processing applications. Results In this work, we studied the properties of a novel fungal LPMO from the thermophilic fungus Thielavia australiensis, TausLPMO9B. Heterologous expression of TausLPMO9B in Aspergillus niger yielded a glycosylated protein with a methylated N-terminal histidine showing LPMO activity. High sequence identity of the AA9 domain to that of MtLPMO9B (MYCTH_80312) from Myceliophthora thermophila (84%) indicated strictly C1-oxidizing activity on cellulose, which was confirmed experimentally by the analysis of products released from cellulose using HPAEC. The enzyme was stable and active at a pH ranging from 4 to 6, thus matching the conditions commonly used in industrial biomass processing, where a low pH (between 4 and 5) is used due to the pH-optima of commercial cellulases and a desire to limit microbial contamination . Conclusion While the oxidative cleavage of phosphoric acid swollen cellulose (PASC) by TausLPMO9B was boosted by the addition of H2O2 as a co-substrate, this effect was not observed during the saccharification of acid pretreated corn stover. This illustrates key differences between the lab-scale tests with artificial, lignin-free substrates and industrial settings with lignocellulosic biomass as substrate.
MEDLINE/2023349647	Characterization of an AA9 LPMO from Thielavia australiensis, TausLPMO9B, under industrially relevant lignocellulose saccharification conditions.	DSM Biotechnology Center, PP 699-0310, Alexander Fleminglaan 1, 2613 AX, Delft, The Netherlands. Federica.Calderaro@dsm.com Molecular Enzymology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen, The Netherlands. Federica.Calderaro@dsm.com Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences (NMBU), ÅAs, Norway.	Calderaro F Keser M Akeroyd M Bevers L E van den Berg M A Eijsink V G H Varnai A	Biotechnology for biofuels, (2020 Nov 30) Vol. 13, No. 1, pp. 195. Electronic Publication Date: 30 Nov 2020 Journal code: 101316935. ISSN: 1754-6834. L-ISSN: 1754-6834. Report No.: PMC-PMC7706046.	20200101	duplicates	BACKGROUND: The discovery of lytic polysaccharide monooxygenases (LPMO) has changed our perspective on enzymatic degradation of plant biomass. Through an oxidative mechanism, these enzymes are able to cleave and depolymerize various polysaccharides, acting not only on crystalline substrates such as chitin and cellulose, but also on other polysaccharides, such as xyloglucan, glucomannan and starch. Despite their widespread use, uncertainties related to substrate specificity and stereospecificity, the nature of the co-substrate, in-process stability, and the nature of the optimal reductant challenge their exploitation in biomass processing applications. RESULTS: In this work, we studied the properties of a novel fungal LPMO from the thermophilic fungus Thielavia australiensis, TausLPMO9B. Heterologous expression of TausLPMO9B in Aspergillus niger yielded a glycosylated protein with a methylated N-terminal histidine showing LPMO activity. High sequence identity of the AA9 domain to that of MtLPMO9B (MYCTH_80312) from Myceliophthora thermophila (84%) indicated strictly C1-oxidizing activity on cellulose, which was confirmed experimentally by the analysis of products released from cellulose using HPAEC. The enzyme was stable and active at a pH ranging from 4 to 6, thus matching the conditions commonly used in industrial biomass processing, where a low pH (between 4 and 5) is used due to the pH-optima of commercial cellulases and a desire to limit microbial contamination . CONCLUSION: While the oxidative cleavage of phosphoric acid swollen cellulose (PASC) by TausLPMO9B was boosted by the addition of H2O2 as a co-substrate, this effect was not observed during the saccharification of acid pretreated corn stover. This illustrates key differences between the lab-scale tests with artificial, lignin-free substrates and industrial settings with lignocellulosic biomass as substrate.
CAPLUS/2018:1704096	NBR1 is involved in selective pexophagy in filamentous ascomycetes and can be functionally replaced by a tagged version of its human homolog	Department of Genetics of Eukaryotic Microorganisms, Institute of Microbiology and Genetics, University of Goettingen, Goettingen, Germany	Werner, Antonia Herzog, Britta Voigt, Oliver Valerius, Oliver Braus, Gerhard H. Poeggeler, Stefanie	Autophagy (2019), 15(1), 78-97 CODEN: AUTOC9; ISSN: 1554-8635	20190101	other	Macroautophagy/autophagy is a conserved degrdn. process in eukaryotic cells involving the sequestration of proteins and organelles within double-membrane vesicles termed autophagosomes. In filamentous fungi, its main purposes are the regulation of starvation adaptation and developmental processes. In contrast to nonselective bulk autophagy, selective autophagy is characterized by cargo receptors, which bind specific cargos such as superfluous organelles, damaged or harmful proteins, or microbes, and target them for autophagic degrdn. Herein, using the core autophagy protein ATG8 as bait, GFP-Trap anal. followed by liq. chromatog. mass spectrometry (LC/MS) identified a putative homolog of the human autophagy cargo receptor NBR1 (NBR1, autophagy cargo receptor) in the filamentous ascomycete Sordaria macrospora (Sm). Fluorescence microscopy revealed that SmNBR1 colocalizes with SmATG8 at autophagosome-like structures and in the lumen of vacuoles. Delivery of SmNBR1 to the vacuoles requires SmATG8. Both proteins interact in an LC3 interacting region (LIR)-dependent manner. Deletion of Smnbr1 leads to impaired vegetative growth under starvation conditions and reduced sexual spore prodn. under non-starvation conditions. The human NBR1 homolog partially rescues the phenotypic defects of the fungal Smnbr1 deletion mutant. The Smnbr1 mutant can neither use fatty acids as a sole carbon source nor form fruiting bodies under oxidative stress conditions. Fluorescence microscopy revealed that degrdn. of a peroxisomal reporter protein is impaired in the Smnbr1 deletion mutant. Thus, SmNBR1 is a cargo receptor for pexophagy in filamentous ascomycetes.
SCISEARCH/2019:254151	Immobilization of Myceliophthora thermophila laccase on poly(glycidyl methacrylate) microspheres enhances the degradation of azinphos-methyl	E-mail: brivas@udec.cl Univ Nat Resources + Life Sci Vienna, Dept Agrobiotechnol IFA Tulln, Inst Environm Biotechnol, Konrad Lorenz Str 20, A-3430 Tulln, Austria Univ York, Dept Chem, Green Chem Ctr Excellence, York YO10 5DD, N Yorkshire, England Austrian Ctr Ind Biotechnol, Div Polymers + Enzymes, A-3430 Tulln, Austria Univ Concepcion, Fac Chem, Dept Polymer, Casilla 160-C, Edmundo Larenas 129, Concepcion, Chile (Reprint)	Vera, Myleidi (Reprint) Rivas, Bernabe L. Nyanhongo, Gibson S. Pellis, Alessandro Guebitz, Georg M.	JOURNAL OF APPLIED POLYMER SCIENCE, (20 2019 APR 2019) Vol. 136, No. 16. ISSN: 0021-8995.	20190101	other	Laccase enzymes are multicopper oxidases capable of oxidizing different compounds. However, to be able to use this biocatalyst for industrial applications, their immobilization is needed. The present work investigates the immobilization of Myceliophthora thermophila laccase (MtL) on monodisperse microspheres of poly(glycidyl methacrylate) (PGMA) to be used in azinphos-methyl degradation. The immobilization was optimized to achieve the highest activity of the immobilized enzyme. As result, the protein load obtained was 2.5 mg protein g(-1) carrier (35 U g(-1) of carrier). The immobilized enzymes showed a broadened pH and temperature range of optimum activity and significantly improved the storage and operational stability. Finally, the complete degradation of azinphos-methyl using immobilized laccase was achieved after 1 h of reaction. The collected data indicate that the immobilization of MtL on PGMA microspheres is an excellent alternative to improve biochemical properties in the enzyme and to allow their efficient use in pesticides degradation. (c) 2018 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 2019, 136, 47417.

BIOSIS/2019:483196	Metabolic adaptation of fungal strains in response to contamination by polychlorinated biphenyls.	Univ Grenoble Alpes, CNRS, UMR 5553, Lab Ecol Alpine,USMB, F-38058 Grenoble 9, France bello.mouhamadou@univ-grenoble-alpes.fr	Perigon, Sophie Massier, Martin Germain, Joaquim Binet, Marie-Noelle Legay, Nicolas Mouhamadou, Bello [Reprint Author]	Environmental Science and Pollution Research International, (MAY 2019) Vol. 26, No. 15, Sp. Iss. SI, pp. 14943-14950. https://www.springer.com/journal/11356. ISSN: 0944-1344. E-ISSN: 1614-7499.	20190101	other	Polychlorinated biphenyls (PCBs) represent a large group of recalcitrant environmental pollutants. Up to now, many studies have focused on bioremediation of PCBs by fungal strains; however, the mechanisms of adaptation of these strains towards PCBs remain unknown despite their importance in developing effective bioremediation processes. We studied five species, each consisting of two strains isolated either from PCB-polluted or PCB-unpolluted substrates (control strains). We investigated their responses to PCB contamination by studying their tolerance to PCBs, their ability to reduce these pollutants, and their expression level of Laccase genes. In Thermothelomyces thermophila , Thermothelomyces heterothallica, Thermoascus crustaceus, and Fusarium solani, all the studied strains showed a similar tolerance and PCB degradation regardless of their origin. In Schizophyllum commune, while both strains showed similar resistance to PCBs, i.e., PCBs and their degradation products presented no toxicity for these strains, the rate of PCB degradation of the strain from a PCB-polluted environment was significantly slightly higher. The PCB degradation did not correlate with the expression level of genes encoding Laccases. These results demonstrate that the tolerance and PCB degradation by the fungal strains, which did not involve Laccase genes, required different adaptation systems which seem to be constitutive or rapidly inducible by PCB according to the fungal species.
PQSCITECH/2019:405006	Practical guidance for the implementation of the CRISPR genome editing tool in filamentous fungi		Min Jin Kwon Schuetze, Tabea Spohner, Sebastian Haefner, Stefan Meyer, Vera	Fungal Biology and Biotechnology, Vol. 6, 20190101 E-ISSN: 2054-3085 DOI: https://doi.org/10.1186/s40694-019-0079-4 Published by: BioMed Central, London	20190101	duplicates	Background Within the last years, numerous reports described successful application of the CRISPR nucleases Cas9 and Cpf1 for genome editing in filamentous fungi. However, still a lot of efforts are invested to develop and improve protocols for the fungus and genes of interest with respect to applicability, scalability and targeting efficiencies. These efforts are often hampered by the fact that-although many different protocols are available-none have systematically analysed and compared different CRISPR nucleases and different application procedures thereof for the efficiency of single- and multiplex-targeting approaches in the same fungus. Results We present here data for successful genome editing in the cell factory Thermothelomyces thermophilus , formerly known as Myceliophthora thermophila , using the three different nucleases SpCas9, FnCpf1, AsCpf1 guided to four different gene targets of our interest. These included a polyketide synthase (pks4.2), an alkaline protease (alp1), a SNARE protein (snc1) and a potential transcription factor (ptf1). For all four genes, guide RNAs were developed which enabled successful single-targeting and multiplex-targeting. CRISPR nucleases were either delivered to T. thermophilus on plasmids or preassembled with in vitro transcribed gRNA to form ribonucleoproteins (RNPs). We also evaluated the efficiency of single oligonucleotides for site-directed mutagenesis . Finally, we were able to scale down the transformation protocol to microtiter plate format which generated high numbers of positive transformants and will thus pave the way for future high-throughput investigations. Conclusion We provide here the first comprehensive analysis and evaluation of different CRISPR approaches for a filamentous fungus. All approaches followed enabled successful genome editing in T. thermophilus; however, with different success rates. In addition, we show that the success rate depends on the respective nuclease and on the targeted gene locus. We finally present a practical guidance for experimental considerations aiming to guide the reader for successful implementation of CRISPR technology for other fungi.
CAPLUS/2019:1391097	Physical and chemical mutagens improved Sporotrichum thermophile , strain ST20 for enhanced Phytase activity	Department of Biosciences, COMSATS University Islamabad, Sahiwal, Pak.	Mehmood, Asim Baneen, Umal Zaheer, Ahmad Wasim Sajid, Muhammad Hussain, Abrar Saleem, Shahzad Yousafi, Qudsia Rashid, Hamid Riaz, Hassan Ihsan, Awais Jamil, Farrukh Sajjad, Yasar Zahid, Nageena Shahzad Anjam, Muhammad Arshad, Muhammad Mirza, Zeenat Karim, Sajjad Rasool, Mahmood	Saudi Journal of Biological Sciences (2019), 26(7), 1485-1491 CODEN: SJBSAG; ISSN: 1319-562X	20190101	other	Phosphorus is an essential micronutrient of plants and involved in crit. biol. functions. In nature, phosphorus is mostly present in immobilized inorg. mineral and in the fixed org. form including phytic acid and phosphoesteric compds. However, the bioavailability of bound phosphorus could be enhanced by the use of phosphate solubilizing microorganisms such as bacteria and fungi. The phytases are widespread in an environment and have been isolated from different sources comprising bacteria and fungi. In current studies, we show the successful use of gamma rays and EMS (Et Methane Sulfonate) mutagenesis for enhanced activity of phytases in a fungal strain Sporotrichum thermophile . We report an improved strain ST2 that could produce a clear halo zone around the colony, up to 24 mm. The max. enzymic activity was found of 382 U/mL on pH 5.5. However, the phytase activity was improved to 387 U/mL at 45°C. We also report that the mutants produced through EMS showed the greater potential for phytase prodn. The current study highlights the potential of EMS mutagenesis for strain improvement over phys. mutagens .
CABA/2020:48543	CLR-4, a novel conserved transcription factor for cellulase gene expression in ascomycete fungi.	Key Laboratory of Systems Microbial Biotechnology, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin 300308, China. EMAIL: tian_cg@tib.cas.cn	Liu Qian Li JinGen Gao RanRan Li JinYang Ma GuoLi Tian ChaoGuang Liu, Q. Li, J. G. Gao, R. R. Li, J. Y. Ma, G. L. Tian, C. G.	Molecular Microbiology (2019), Volume 111, Number 2, pp. 373-394 ISSN: 0950-382X; 1365-2958 Published by: Wiley, Oxford	20190101	other	Fungal degradation of lignocellulosic biomass requires various (hemi-)cellulases and is an important part of the natural carbon cycle. Although induction of cellulases has been described for some saprobic filamentous fungi, the regulation of cellulase transcription is complex and many aspects are still poorly understood. Here, we identified and characterized the novel cellulase regulation factor NcCLR-4 in Neurospora crassa and its ortholog MtCLR-4 in Myceliophthora thermophila . Deletion of CLR-4 resulted in similarly defective cellulolytic enzyme production and activities. Transcriptome analyses of ΔNcclr-4/ΔMtclr-4 revealed the down-regulation of genes encoding (hemi-)cellulases and pivotal regulators (clr-1, clr-2 and xyr-1) and key genes in the cAMP signaling pathway such as adenylate cyclase Nccr-1. Intracellular cAMP levels were markedly lower in ΔNcclr-4/ΔMtclr-4 than in wild-type during cellulose utilization. In electrophoretic mobility shift (EMSA) and DNase I footprinting assays, NcCLR-4/MtCLR-4 can directly bound to the promoters of Nccr-1/Mtcr-1 (encoding adenylyl cyclase). EMSAs also demonstrated that NcCLR-4/MtCLR-4 could directly bound to clr-1 (encoding a key cellulase regulator), Mtclr-2 and Mtxyr-1 (encoding biomass deconstruction regulators). These findings about the novel cellulase expression regulators NcCLR-4 and MtCLR-4 enrich our understanding of how cellulose degradation is regulated and provide new targets for engineering fungi to deconstruct plant biomass in biorefineries.

TOXCENTER/2019:546412	Practical guidance for the implementation of the CRISPR genome editing tool in filamentous fungi	1Chair of Applied and Molecular Microbiology, Institute of Biotechnology, Technische Universitat Berlin, 10263 Berlin, Germany 2BASF SE, Carl-Bosch-Strasse 38, 67056 Ludwigshafen, Germany	Kwon Min Jin Schutze Tabea Meyer Vera Spohner Sebastian Haefner Stefan	Fungal biology and biotechnology, (2019) Vol. 6, pp. 15. Electronic Publication Date: 17 Oct 2019. Journal code: 101655873. E-ISSN: 2054-3085. L-ISSN: 2054-3085. Report No.: PMC-PMC6796461.	20190101	other	BACKGROUND: Within the last years, numerous reports described successful application of the CRISPR nucleases Cas9 and Cpf1 for genome editing in filamentous fungi. However, still a lot of efforts are invested to develop and improve protocols for the fungus and genes of interest with respect to applicability, scalability and targeting efficiencies. These efforts are often hampered by the fact that-although many different protocols are available-none have systematically analysed and compared different CRISPR nucleases and different application procedures thereof for the efficiency of single- and multiplex-targeting approaches in the same fungus. RESULTS: We present here data for successful genome editing in the cell factory Thermothelomyces thermophilus , formerly known as Myceliophthora thermophila , using the three different nucleases SpCas9, FnCpf1, AsCpf1 guided to four different gene targets of our interest. These included a polyketide synthase (pks4.2), an alkaline protease (alp1), a SNARE protein (snc1) and a potential transcription factor (ptf1). For all four genes, guide RNAs were developed which enabled successful single-targeting and multiplex-targeting. CRISPR nucleases were either delivered to T. thermophilus on plasmids or preassembled with in vitro transcribed gRNA to form ribonucleoproteins (RNPs). We also evaluated the efficiency of single oligonucleotides for site-directed mutagenesis . Finally, we were able to scale down the transformation protocol to microtiter plate format which generated high numbers of positive transformants and will thus pave the way for future high-throughput investigations. CONCLUSION: We provide here the first comprehensive analysis and evaluation of different CRISPR approaches for a filamentous fungus. All approaches followed enabled successful genome editing in T. thermophilus; however, with different success rates. In addition, we show that the success rate depends on the respective nuclease and on the targeted gene locus. We finally present a practical guidance for experimental considerations aiming to guide the reader for successful implementation of CRISPR technology for other fungi.
CAPLUS/2020:826892	Crosstalk of cellulose and mannan perception pathways leads to inhibition of cellulase production in several filamentous fungi	TUM School of Life Sciences Weihenstephan, Technical University of Munich, Freising, Germany	Hassan, Lara Lin, Liangcai Sorek, Hagit Sperl, Laura E. Goudoulas, Thomas Hagn, Franz Germann, Natalie Tian, Chaoguang Benz, J. Philipp	mBio (2019), 10(4), e00277-19/1-e00277-19/14 CODEN: MBIOCL; ISSN: 2150-7511 URL: http://mbio.asm.org/	20190101	other	In present work, we provide evidence of crosstalk between the perception pathways for cellulose and hemicellulose mannan being conserved in several filamentous fungi and leading to inhibition of cellulase expression. We used functional genomics tools available for Neurospora crassa to investigate this overlap at mol. level. Crosstalk and competitive inhibition could be identified both during uptake by cellodextrin transporters and intracellularly. Importantly, overlap is independent of CRE-1-mediated catabolite repression. These results provide novel insights into regulatory networks of lignocellulolytic fungi and will contribute to rational optimization of fungal enzyme prodn. for efficient plant biomass depolymn. and utilization. In fungi, prodn. of enzymes for polysaccharide degrdn. is controlled by complex signaling networks. We tackled for the first time mol. interplay between two seemingly unrelated perception pathways: those for cellulose and the hemicellulose (gluco)mannan. We identified a so far unknown competitive inhibition between resp. degrdn. products acting as signaling mols. Competition was detected both at level of uptake and intracellularly, upstream of main transcriptional regulator CLR-2. Our findings provide novel insights into mol. communication between perception pathways. Also, they present possible targets for improvement of industrial strains for higher cellulase prodn. through engineering of mannan insensitivity.
BIOSIS/2019:738883	Peptides with antioxidant properties identified from casein, whey, and egg albumin hydrolysates generated by two novel fungal proteases.	Univ Sao Paulo, Sch Pharmaceut Sci Ribeirao Preto, Cafe Ave S-N, Sao Paulo, Brazil hamilton@fcfrp.usb.br	Hamin Neto, Youssef A. A. Rosa, Jose Cesar Cabral, Hamilton [Reprint Author]	Preparative Biochemistry + Biotechnology, (AUG 9 2019) Vol. 49, No. 7, pp. 639-648. http://www.tandfonline.com/loi/lpbb20. ISSN: 1082-6068. E-ISSN: 1532-2297.	20190101	other	There are many diseases linked to oxidative stress, including cancer . Importantly, endogenous antioxidants are insufficient to protect against this process. Peptides derived from food proteins produced by hydrolysis have been investigated as exogenous antioxidants. The present study aimed to identify novel peptides with antioxidant potential produced from egg and milk proteins hydrolysis with two new fungal proteases isolated from Eupenicillium javanicum and Myceliophthora thermophila . The degree of hydrolysis at several time points was calculated and correlated to DPPH scavenging and metal chelating assays, all hydrolysates presented antioxidant activity. Casein hydrolyzed by the M. thermophila protease showed the best antioxidant activity. The identified sequences showed that the proportions of amino acids that influence antioxidant activity support the antioxidant assay. Our data reveal the conditions necessary for the successful generation of antioxidant peptides using two novel fungal proteases. This opens a potential new avenue for the design and manufacture of antioxidant molecules.
CABA/2020:124310	Characteristics and applications of a thermostable and acidic exochitinase of the thermophilic mould Myceliophthora thermophila .	Department of Microbiology, University of Delhi South campus, Benito Juarez Road, New Delhi-110021, India. EMAIL: tsnarayana@gmail.com	Bharti Rohatgi Satyanarayana, T. Rohatgi, B.	Kavaka (2019), Volume 52, pp. 52-61, many ref. ISSN: 0379-5179 Published by: Mycological Society of India, Chennai	20190101	other	The thermophilic mould Myceliophthora thermophila (Apinis) van Oorschot produces an extracellular thermo-acid-stable chitinase, which has been purified to apparent homogeneity by affinity adsorption followed by hydrophobic interaction chromatography. The pure enzyme is a monomer with a molecular mass of 43.0 kD on SDS-PAGE and pI of 4.0. Based on MALDI-ToF-MS/MS and LC-MS analysis of peptides, it was identified as glycosyl hydrolase family 18 protein. The chitinase is optimally active at pH 4.0 and 55°C with T1/2 values of 9 and 3 h at 55 and at 70°C, respectively. The Km and Vmax values (for colloidal chitin hydrolysis) are 0.396 mg mlsup-1 and 25.25 nkats mgsup-1 Ssup-1. It is strongly inhibited by Hgsup2+, Alsup3+ and Fesup3+, but stimulated by Mnsup2+, Casup2+ and Basup2+. The chitinase is tolerant to organic solvents and detergents. It liberates N-acetylglucosamine and chitobiose from colloidal chitin degradation and has a potential to degrade powdered chitin and chitosan. The high temperature optimum and thermostability makes it a suitable candidate for the production of pharmacologically important N-acetylglucosamine and chitobiose from chitin. The cell walls of spores and hyphae are disrupted in Fusarium oxysporum, Curvularia sp. and Stachybotrys sp. due to the degradation of chitin in the cell walls. The enzyme arrests hatching of eggs in the nematode, Meloidogyne incognita and induces mortality in the insect larvae of Aedes aegypti as well as mealy bug (Maconellicoccus hirsutus).
BIOSIS/2020:561558	Itaconic acid: an effective sorbent for removal of pollutants from dye industry effluents.	Vel Tech High Tech Dr Rangarajan Dr Sakunthala En, Chennai 600062, Tamil Nadu, India btrbio@gmail.com; drsvs18@gmail.com	Bharathiraja, B. [Reprint Author] Selvakumari, I. Aberna Ebenezer Iyyappan, J. Varjani, Sunita [Reprint Author]	Current Opinion in Environmental Science + Health, (DEC 2019) Vol. 12, No. Sp. Iss. SI, pp. 6-17. https://www.journals.elsevier.com/current-opinion- in-environmental-science-and-health. ISSN: 2468-5844. E-ISSN: 2468-5844.	20190101	other	This review aimed to introduce about mechanism of itaconic acid hydrogels for removal of metal ions and dyes from wastewater. Industrial discharge consists of toxic carcinogenic pollutants in dye effluents, and so far, conventional treatment techniques used for the treatment of harmful contaminants present in the industrial effluent are not adequate for complete elimination of pollutants. Therefore, developing and using significant cost-efficient control measures and treatment processes to safeguard the ecosystem and natural resources is the need of research. In this regard, a recent trend in the development of hydrogels that function as an excellent adsorbent in the removal of organic pollutants in dye industrial effluents has attracted researchers+apos; attention. This is due to simplicity and high adsorptive nature of the hydrogels compared with other existing conventional adsorbents. This review has been organized to address itaconic acid- based hydrogels as a practical solution in treatment of industrial dye effluents, and it further highlights the preparation, performance, and future perspectives of using this technique in dye removal. Thus, itaconic acid-based hydrogels could be used as an efficient sorbent for eco-friendly environmental applications.
SCISEARCH/2019:1286029	Highly Efficient Degradation of Xylan into Xylose by a Single Enzyme	E-mail: lou@cau.edu.cn jiangwei01@cau.edu.cn China Agr Univ, Coll Biol Sci, State Key Lab Agrobiotechnol, Beijing 100193, Peoples R China (Reprint)	Basit, Abdul (Reprint) Miao, Ting Liu, Junquan Wen, Jiaqi Song, Lina Zheng, Fengzhen Lou, Huiqiang Jiang, Wei	ACS SUSTAINABLE CHEMISTRY + ENGINEERING, (1 2019 JUL 2019) Vol. 7, No. 13, pp. 11360-11368. ISSN: 2168-0485.	20190101	other	Here, we report highly efficient degradation of xylan into xylose by a single multifunctional xylanolytic enzyme from the filamentous fungus Thermothelomyces thermophila (termed Ttxy43). Ttxy43 shows three different enzyme activities toward carbohydrates, beta-xylosidase (80.8 U/mg), endoxylanase (105.42 U/mg), and alpha-L-arabinofuranosidase enzyme activities (15.81 U/mg). Analysis of the catalytic mode of action of Ttxy43 for birchwood-xylan (BWX) reveals that endoxylanase initially degrades xylan to unbranched xylooligosaccharides (XOSs) (xylobiose, xylotriose, xylotetraose) as intermediates, which are then quickly hydrolyzed into single xylose by beta-xylosidase. Site-directed mutagenesis studies indicate that Ttxy43 residues Asp(134) and Glu(228) are essential catalytic sites, while Glu(176), Asp(38), and Asp(85) play an accessory role. More importantly, Ttxy43 displays higher degradation efficiency in comparison with a commercial beta-xylosidase and endoxylanase 'cocktail'. These findings elucidate an efficient integrated degradation mechanism of xylan under industrial reaction conditions, which provides a novel strategy to design techniques for biomass energy production.

PQSCITECH/2019:464973	Optimization of Transesterification Reactions with CLEA-Immobilized Feruloyl Esterases from Thermothelomyces thermophila and Talaromyces wortmannii		Zerva, Anastasia Antonopoulou, Io Enman, Josefine Iancu, Laura Juetten, Peter Rova, Ulrika Christakopoulos, Paul	Molecules, Vol. 23, No. 9, 20180101 E-ISSN: 1420-3049 DOI: 10.3390/molecules23092403 Published by: MDPI AG, Basel	20180101	other	Feruloyl esterases (FAEs, E.C. 3.1.1.73) are biotechnologically important enzymes with several applications in ferulic acid production from biomass, but also in synthesis of hydroxycinnamic acid derivatives. The use of such biocatalysts in commercial processes can become feasible by their immobilization, providing the advantages of isolation and recycling. In this work, eight feruloyl esterases, immobilized in cross-linked enzyme aggregates (CLEAs) were tested in regard to their transesterification performance, towards the production of prenyl ferulate (PFA) and arabinose ferulate (AFA). After solvent screening, comparison with the activity of respective soluble enzymes, and operational stability tests, FAE125 was selected as the most promising biocatalyst. A central composite design revealed the optimum conditions for each transesterification product, in terms of water content, time, and substrate ratio for both products, and temperature and enzyme load additionally for prenyl ferulate. The optimum product yields obtained were 83.7% for PFA and 58.1% for AFA. FAE125 CLEAs are stable in the optimum conditions of transesterification reactions, maintaining 70% residual activity after five consecutive reactions. Overall, FAE125 CLEAs seem to be able to perform as a robust biocatalyst, offering satisfactory yields and stability, and thus showing significant potential for industrial applications.
SCISEARCH/2018:395929	Secretory expression of recombinant small laccase from Streptomyces coelicolor A3(2) in Pichia pastoris	E-mail: tukayik@dut.ac.za Cape Peninsula Univ Technol, Inst Biomed + Microbial Biotechnol, Biocatalysis + Tech Biol Res Grp, Bellville Campus, Symphony Way, POB 1906, ZA-7535 Bellville, South Africa Durban Univ Technol, Fac Appl Sci, Dept Biotechnol + Food Technol, POB 1334, ZA-4000 Durban, South Africa (Reprint)	Yadav, Deepti (Reprint) Ranjan, Bibhuti Mchunu, Nokuthula Kudanga, Tukayi Le Roes-Hill, Marilize	INTERNATIONAL JOURNAL OF BIOLOGICAL MACROMOLECULES, (2018 MAR 2018) Vol. 108, pp. 642-649. ISSN: 0141-8130.	20180101	other	(b) (4)
BIOSIS/2018:1076666	Versatile Fungal Polyphenol Oxidase with Chlorophenol Bioremediation Potential: Characterization and Protein Engineering.	Natl Tech Univ Athens, Sch Chem Engn, Biotechnol Lab, Athens, Greece vtopakas@chemeng.ntua.gr	Nikolaivits, Efstratios [Reprint Author] Dimarogona, Maria Karagiannaki, Ioanna Chalima, Angelina Fishman, Ayelet Topakas, Evangelos [Reprint Author]	Applied and Environmental Microbiology, (DEC 2018) Vol. 84, No. 23, pp. Article No.: e01628-18. https://journals.asm.org/. CODEN: AEMIDF. ISSN: 0099-2240. E-ISSN: 1098-5336.	20180101	other	Polyphenol oxidases (PPOs) have been mostly associated with the undesirable postharvest browning in fruits and vegetables and have implications in human melanogenesis. Nonetheless, they are considered useful biocatalysts in the food, pharmaceutical, and cosmetic industries. The aim of the present work was to characterize a novel PPO and explore its potential as a bioremediation agent. A gene encoding an extracellular tyrosinase-like enzyme was amplified from the genome of Thermothelomyces thermophila and expressed in Pichia pastoris. The recombinant enzyme (TtPPO) was purified and biochemically characterized. Its production reached 40 mg/liter, and it appeared to be a glycosylated and N-terminally processed protein. TtPPO showed broad substrate specificity, as it could oxidize 28/30 compounds tested, including polyphenols, substituted phenols, catechols, and methoxyphenols. Its optimum temperature was 65 degrees C, with a half-life of 18.3 h at 50 degrees C, while its optimum pH was 7.5. The homology model of TtPPO was constructed, and site-directed mutagenesis was performed in order to increase its activity on mono- and dichlorophenols (di-CPs). The G292N/Y296V variant of TtPPO 5.3-fold increased activity on 3,5-dichlorophenol (3,5-diCP) compared to the wild type.IMPORTANCE A novel fungal PPO was heterologously expressed and biochemically characterized. Construction of single and double mutants led to the generation of variants with altered specificity against CPs. Through this work, knowledge is gained regarding the effect of mutations on the substrate specificity of PPOs. This work also demonstrates that more potent biocatalysts for the bioremediation of harmful CPs can be developed by applying site-directed mutagenesis .
SCISEARCH/2018:2094460	Versatile Fungal Polyphenol Oxidase with Chlorophenol Bioremediation Potential: Characterization and Protein Engineering	Lulea Univ Technol, Biochem + Chem Proc Engn, Div Sustainable Proc Engn, Dept Civil Environm + Nat Resources Engn, Lulea, Sweden (Reprint) Natl Tech Univ Athens, Sch Chem Engn, Biotechnol Lab, Athens, Greece E-mail: vtopakas@chemeng.ntua.gr Univ Patras, Dept Chem Engn, Patras, Greece Technion Israel Inst Technol, Dept Biotechnol + Food Engn, Haifa, Israel E-mail: vtopakas@chemeng.ntua.gr Natl Tech Univ Athens, Sch Chem Engn, Biotechnol Lab, Athens, Greece (Reprint)	Nikolaivits, Efstratios (Reprint) Karagiannaki, Ioanna Chalima, Angelina Topakas, Evangelos Dimarogona, Maria Fishman, Ayelet	APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (2018 DEC 2018) Vol. 84, No. 23. ISSN: 0099-2240.	20180101	duplicates	Polyphenol oxidases (PPOs) have been mostly associated with the undesirable postharvest browning in fruits and vegetables and have implications in human melanogenesis. Nonetheless, they are considered useful biocatalysts in the food, pharmaceutical, and cosmetic industries. The aim of the present work was to characterize a novel PPO and explore its potential as a bioremediation agent. A gene encoding an extracellular tyrosinase-like enzyme was amplified from the genome of Thermothelomyces thermophila and expressed in Pichia pastoris. The recombinant enzyme (TtPPO) was purified and biochemically characterized. Its production reached 40 mg/liter, and it appeared to be a glycosylated and N-terminally processed protein. TtPPO showed broad substrate specificity, as it could oxidize 28/30 compounds tested, including polyphenols, substituted phenols, catechols, and methoxyphenols. Its optimum temperature was 65 degrees C, with a half-life of 18.3 h at 50 degrees C, while its optimum pH was 7.5. The homology model of TtPPO was constructed, and site-directed mutagenesis was performed in order to increase its activity on mono- and dichlorophenols (di-CPs). The G292N/Y296V variant of TtPPO 5.3-fold increased activity on 3,5-dichlorophenol (3,5-diCP) compared to the wild type. IMPORTANCE A novel fungal PPO was heterologously expressed and biochemically characterized. Construction of single and double mutants led to the generation of variants with altered specificity against CPs. Through this work, knowledge is gained regarding the effect of mutations on the substrate specificity of PPOs. This work also demonstrates that more potent biocatalysts for the bioremediation of harmful CPs can be developed by applying site-directed mutagenesis .
BIOSIS/2018:987096	Functional characterization of a lytic polysaccharide monooxygenase from the thermophilic fungus Myceliophthora thermophila .	Univ Sao Paulo, Dept Phys and Interdisciplinary Sci, Sao Carlos Inst Phys, Sao Carlos, SP, Brazil ipolikarpov@ifsc.usp.br; vincent.eijsink@nmbu.no	Kadowaki, Marco A. S. [Reprint Author] Varnai, Aniko Jameson, John-Kristian Leite, Ana E. T. Costa-Filho, Antonio J. Kumagai, Patricia S. Prade, Rolf A. Polikarpov, Igor [Reprint Author] Eijsink, Vincent G. H. [Reprint Author]	PLoS One, (AUG 20 2018) Vol. 13, No. 8, pp. Article No.: e0202148. http://journals.plos.org/plosone/. ISSN: 1932-6203. E-ISSN: 1932-6203.	20180101	other	Thermophilic fungi are a promising source of thermostable enzymes able to hydrolytically or oxidatively degrade plant cell wall components. Among these enzymes are lytic polysaccharide monooxygenases (LPMOs), enzymes capable of enhancing biomass hydrolysis through an oxidative mechanism. Myceliophthora thermophila (synonym Sporotrichum thermophile), an Ascomycete fungus, expresses and secretes over a dozen different LPMOs. In this study, we report the overexpression and biochemical study of a previously uncharacterized LPMO (MtLPMO9J) from M. thermophila M77 in Aspergillus nidulans. MtLPMO9J is a single-domain LPMO and has 63% sequence similarity with the catalytic domain of NcLPMO9C from Neurospora crassa. Biochemical characterization of MtLPMO9J revealed that it performs C4-oxidation and is active against cellulose, soluble cello-oligosaccharides and xyloglucan. Moreover, biophysical studies showed that MtLPMO9J is structurally stable at pH above 5 and at temperatures up to 50 degrees C. Importantly, LC-MS analysis of the peptides after tryptic digestion of the recombinantly produced protein revealed not only the correct processing of the signal peptide and methylation of the N-terminal histidine, but also partial autoxidation of the catalytic center. This shows that redox conditions need to be controlled, not only during LPMO reactions but also during protein production, to protect LPMOs from oxidative damage.
BIOSIS/2018:267138	Anti-inflammatory and anti-oxidant properties of laccase-synthesized phenolic-O-carboxymethyl chitosan hydrogels.	Univ Nat Resources and Life Sci Vienna, Inst Environm Biotechnol, Konrad Lorenz Str 20, A-3430 Tulln, Austria g.nyanhongo@boku.ac.at	Huber, Daniela Grzelak, Adrianna Baumann, Martina Borth, Nicole Schleining, Gerhard Nyanhongo, Gibson S. [Reprint Author] Guebitz, Georg M.	New Biotechnology, (JAN 25 2018) Vol. 40, No. Part B, pp. 236-244. http://www.journals.elsevier.com/new-biotechnology/#description. ISSN: 1871-6784. E-ISSN: 1876-4347.	20180101	other	A bioactive O-carboxymethyl chitosan (CMCS) hydrogel crosslinked with natural phenolics with potential application in wound dressings was synthesized using a laccase from Myceliophthora thermophila (MTL). The highest degree of cross-linking (49.7%) was achieved with catechol. All the phenolic-CMCS hydrogels synthesized showed excellent anti-oxidant properties with a free radical scavenging activity up to 4-fold higher than in the absence of the phenolics, as quantified by the di(phenyl)-(2,4,6-trinitrophenyl) iminoazanium (DPPH) assay. In addition, the hydrogels produced showed an anti-inflammatory effect as evidenced by the inhibition of enzymes [myeloperoxidase (MPO), matrix-metalloproteinase-1 (MMP-1) and human neutrophil elastase (HNE)] over-expressed in chronic wounds. Sinapyl-CMCS hydrogels showed an MMP-1 inhibition of 37%. Further, the phenolic-CMCS hydrogels did not affect the viability of the NIH 3T3 mouse fibroblast cell line and were also able to slowly release human fibroblast growth factor 2, reaching 48.3% over a period of 28 days. This study thus shows the possibility of synthesizing multifunctional bioactive chitosan based hydrogels with anti-oxidant and anti-inflammatory properties using natural occurring phenolics as crosslinkers.

CABA/2018:114113	Laccase catalyzed elimination of morphine from aqueous systems.	Institute of Environmental Biotechnology, University of Natural Resources and Life Sciences, Vienna, Konrad Lorenz Strasse 20, 3430 Tulln, Austria. EMAIL: katrin.greimel@acib.at	Huber, D. Bleymaier, K. Pellis, A. Vielnascher, R. Daxbacher, A. Greimel, K. J. Guebitz, G. M.	New Biotechnology (2018), Volume 42, pp. 19-25 ISSN: 1871-6784 DOI: https://doi.org/10.1016/j.nbt.2018.01.003 Published by: Elsevier Ltd, Oxford	20180101	other	Pharmaceuticals contaminate the environment for several reasons, including metabolic excretion after intake, industrial waste and improper disposal. The narcotic drug morphine is commonly utilized for chronic pain management, and the distribution of morphine in aqueous systems and in waste waters is of high concern. Here, the removal of morphine by a laccase from Myceliophthora thermophila both in its free form as well as immobilized on Accurel MP1000 beads was investigated. Complete morphine elimination was achieved within 30 min for the free and the immobilized enzyme (70% bound protein) for concentrations between 1 and 1,000 mg Lsup-1 according to LC-TOF mass spectrometry analysis. Higher morphine concentrations up to 60 g Lsup-1 were also tested and total elimination was achieved within 6 h. Therefore, laccases are ideal candidates for removing morphine from aqueous systems.
CABA/2019:365537	Decolorization and detoxification of textile wastewaters by recombinant Myceliophthora thermophila and Trametes trogii laccases.	Laboratory of Viral and Microbial Proteins, Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic. EMAIL: iva.pichova@uochb.cas.cz	Herkommerova, K. Dostal, J. Pichova, I.	3 Biotech (2018), Volume 8, Number 12, pp. 505, 67 refs. ISSN: 2190-572X; 2190-5738 DOI: https://doi.org/10.1007/s13205-018-1525-3 Published by: Springer Berlin, Heidelberg	20180101	other	Laccases are multi-copper oxidoreductases with broad biotechnological applications. Here, we report detailed biochemical characterization of purified recombinant laccases originating from Myceliophthora thermophila (MtL) and Trametes trogii (TtL). We identified optimal conditions for decolorization of commercial dyes and textile wastewater samples. We also tested the toxicity of decolorized wastewater samples using human peripheral blood mononuclear cells. MtL and TtL were expressed in Saccharomyces cerevisiae, and secreted enzymes were purified by consecutive hydrophobic and gel chromatography. The molecular masses of TtL (~65 kDa) and MtL (>100 kDa) suggested glycosylation of the recombinant enzymes. Deglycosylation of MtL and TtL led to 25% and 10% decreases in activity, respectively. In a thermal stability assay, TtL retained 61% and MtL 86% of the initial activity at 40°C. While TtL retained roughly 50% activity at 60°C, MtL lost stability at temperatures higher than 40°C. MtL and TtL preferred syringaldazine as a substrate, and the catalytic efficiencies for ABTS oxidation were 7.5 times lower than for syringaldazine oxidation. In the presence of the mediator HBT, purified TtL almost completely decolorized dyes within 30 min and substantially decolorized wastewater samples from a textile factory (up to 74%) within 20 h. However, products of TtL-catalyzed decolorization were more toxic than MtL-decolorized products, which were almost completely detoxified .
CAPLUS/2018:239953	Structural Architecture of the Nucleosome Remodeler ISWI Determined from crosslinking, Mass Spectrometry, SAXS, and Modeling	Molecular Biology, Biomedical Center, Faculty of Medicine, Martinsried, 82152, Germany	Harrer, Nadine Schindler, Christina E. M. Bruetzel, Linda K. Forne, Ignasi Ludwigsen, Johanna Imhof, Axel Zacharias, Martin Lipfert, Jan Mueller-Planitz, Felix	Structure (Oxford, United Kingdom) (2018), 26(2), 282-294.e6 CODEN: STRUE6; ISSN: 0969-2126	20180101	other	Chromatin remodeling factors assume crit. roles by regulating access to nucleosomal DNA. To det. the architecture of the Drosophila ISWI remodeling enzyme, we developed an integrative structural approach that combines protein crosslinking, mass spectrometry, small-angle X-ray scattering, and computational modeling. The resulting structural model shows the ATPase module in a resting state with both ATPase lobes twisted against each other, providing support for a conformation that was recently trapped by crystallog. The autoinhibiting NegC region does not protrude from the ATPase module as suggested previously. The regulatory NTR domain is located near both ATPase lobes. The full-length enzyme is flexible and can adopt a compact structure in soln. with the C-terminal HSS domain packing against the ATPase module. Our data imply a series of conformational changes upon activation of the enzyme and illustrate how the NTR, NegC, and HSS domains contribute to regulation of the ATPase module.
SCISEARCH/2018:2212219	Degradation of tetracyclines and sulfonamides by stevensite- and biochar-immobilized laccase systems and impact on residual antibiotic activity	E-mail: carlos.garciadelgado@uam.es Spanish Natl Res Council IRNASA CSIC, Inst Nat Resources + Agrobiol Salamanca, Salamanca, Spain E-mail: carlos.garciadelgado@uam.es Univ Tuscia, Dept Innovat Biol Agrofood + Forest Syst DIBAF, Viterbo, Italy Autonomous Univ Madrid, Dept Agr Chem + Food Sci, E-28049 Madrid, Spain (Reprint)	Garcia-Delgado, Carlos (Reprint) Eymar, Enrique Camacho-Arevalo, Raquel Petruccioli, Maurizio Crognale, Silvia D'Annibale, Alessandro	JOURNAL OF CHEMICAL TECHNOLOGY AND BIOTECHNOLOGY, (2018 DEC 2018) Vol. 93, No. 12, pp. 3394-3409. ISSN: 0268-2575.	20180101	other	BACKGROUND Stevensite and biochar were investigated to covalently immobilize laccase from Myceliophthora thermophila (MtL) and Pleurotus eryngii (PeL) through the sequential application of aminopropyltriethoxysilane and glutaraldehyde. The immobilized preparations were tested to remove three tetracyclines and six sulfonamides at 0.1 mmol L-1 of each antibiotic. Degradation experiments were conducted both in the absence and in the presence (0.2 mmol L-1) of ABTS, 1-hydroxybenzotriazol (HBT), syringaldehyde or violuric acid. The residual antibiotic activity was tested towards five bacterial species and a bacterial consortium from wastewater. RESULTS Higher values of activity yields (74% and 70.3%) and catalytic capabilities (1426 and 1405 IU g(-1)) were obtained with PeL on stevensite and biochar than with MtL. Stevensite enabled higher reusability and storage stability than biochar. Best removals of tetracyclines and sulfonamides were obtained with immobilized-laccase systems coupled to ABTS or syringaldehyde. Immobilized-laccase/ABTS systems removed 100% of tetracyclines while only chlortetracycline was completely removed in the presence of syringaldehyde. With ABTS, the most effectively removed sulfonamides were sulfathiazole and sulfadiazine (up to 100% and 54%), while syringaldehyde best supported the removal of sulfanilamide, sulfamethazine and sulfamethoxazole (up to 42%, 45% and 46%, respectively). In some cases, an effective antibiotics removal led to either low or no residual antibiotic activity. CONCLUSION MtL and PeL were immobilized successfully on biochar and stevensite. The addition of either ABTS or syringaldehyde enhanced significant removals, up to 100%, of tetracyclines and sulfonamides by the immobilized laccase systems. Noteworthy, biochar-immobilized laccases/ABTS led to complete suppression of the antibiotic activity of tetracyclines. (c) 2018 Society of Chemical Industry
BIOSIS/2018:564617	Distinct Substrate Specificities and Electron-Donating Systems of Fungal Lytic Polysaccharide Monooxygenases.	Wageningen Univ and Res, Biochem Lab, Wageningen, Netherlands willem.vanberkel@wur.nl	Frommhagen, Matthias Westphal, Adrie H. van Berkel, Willem J. H. [Reprint Author] Kabel, Mirjam A.	Frontiers in Microbiology, (MAY 29 2018) Vol. 9, pp. Article No.: 1080. http://www.frontiersin.org/microbiology/. E-ISSN: 1664-302X.	20180101	other	Lytic polysaccharide monooxygenases (LPMOs) are powerful enzymes that oxidatively cleave glycosidic bonds in polysaccharides. The ability of these copper enzymes to boost the degradation of lignocellulose has greatly stimulated research efforts and biocatalytic applications within the biorefinery field. Initially found as oxidizing recalcitrant substrates, such as chitin and cellulose, it is now clear that LPMOs cleave a broad range of oligo- and poly-saccharides and make use of various electron-donating systems. Herein, substrate specificities and electron-donating systems of fungal LPMOs are summarized. A closer look at LPMOs as part of the fungal enzyme machinery might provide insights into their role in fungal growth and plant-pathogen interactions to further stimulate the search for novel LPMO applications.
SCISEARCH/2018:369672	Optimization of enzymatic synthesis of L-arabinose ferulate catalyzed by feruloyl esterases from Myceliophthora thermophila in detergentless microemulsions and assessment of its antioxidant and cytotoxicity activities	E-mail: paul.christakopoulos@ltu.se NCSR Demokritos, Inst Biosci + Applicat, Lab Cell Proliferat + Ageing, Athens 15310, Greece DuPont Ind Biosci, Nieuwe Kanaal 7-S, NL-6709 PA Wageningen, Netherlands Univ Naples Federico II, Dept Chem Sci, Via Cintia 4, I-80126 Naples, Italy Korres Nat Prod, 57 Km Natl Rd, Athens Lamia 32011, Greece Taros Chem GmbH + Co KG, Emil Figge Str 76a, D-44227 Dortmund, Germany Lulea Univ Technol, Dept Civil Environm + Nat Resources Engn, Div Chem Engn, Biochem Proc Engn, S-97187 Lulea, Sweden (Reprint)	Antonopoulou, Io (Reprint) Rova, Ulrika Christakopoulos, Paul Papadopoulou, Adamantia Kletsas, Dimitris Iancu, Laura Cerullo, Gabriella Faraco, Vincenza Ralli, Marianna Juetten, Peter Piechot, Alexander	PROCESS BIOCHEMISTRY, (2018 FEB 2018) Vol. 65, pp. 100-108. ISSN: 1359-5113.	20180101	other	The feruloyl esterases FaeA1, FaeA2, FaeB1, FaeB2 from Myceliophthora thermophila CI and MtFae1a from M. thermophila ATCC 42464 were used as biocatalysts for the transesterification of vinyl ferulate (VFA) with L-arabinose in detergentless microemulsions. The effect of parameters such as the microemulsion composition, the substrate concentration, the enzyme load, the pH, the temperature and the agitation was investigated. FaeA1 offered the highest transesterification yield (52.2 +/- 4.3%) after 8 h of incubation at 50 degrees C using 80 mM VFA, 55 mM L-arabinose and 0.02 mg FAE mL(-1) in a mixture comprising of 19.8: 74.7: 5.5 v/v/v n-hexane: t-butanol: 100 mM MOPS-NaOH pH 8.0. The ability of L-arabinose ferulate (AFA) to scavenge 2,2-diphenyl-1-picrylhy-drazyl (DPPH) radicals was significant (IC50 386.5 mu M). AFA was not cytotoxic even at high concentrations (1 mM) however was found to be pro-oxidant at concentrations higher than 20 mu M when the antioxidant activity was determined with the dichloro-dihydro-fluorescein diacetate (DCFH-DA) assay in human skin fibroblasts.

SCISEARCH/2017:1826246	Ultrasound-assisted swelling of bacterial cellulose	Univ Minho, Ctr Biol Engn, Campus Gualtar, P-4710057 Braga, Portugal (Reprint) Sookmyung Womens Univ, Dept Clothing + Text, Seoul 04310, South Korea E-mail: khyerim@sookmyung.ac.kr Jiangnan Univ, Coll Text, Wuxi, Jiangsu, Peoples R China E-mail: carla.silva@ceb.uminho.pt Sookmyung Womens Univ, Dept Clothing + Text, Seoul 04310, South Korea (Reprint)	Song, Ji Eun (Reprint) Kim, Hye Rim Su, Jing Cavaco-Paulo, Artur Loureiro, Ana Martins, Madalena Silva, Carla	ENGINEERING IN LIFE SCIENCES, (2017 OCT 2017) Vol. 17, No. 10, pp. 1108-1117. ISSN: 1618-0240.	20170101	other	Bacterial cellulose (BC) was obtained by static cultivation using commercial BC gel from scoby. BC membranes (oven dried and freeze-dried) were swelled with 8% NaOH, in the absence and in the presence of ultrasound (US), for 30, 60, and 90 min. The influence of swelling conditions on both physico-chemical properties and molecules entrapment was evaluated. Considering the highest levels of entrapment, an optimum swelling procedure was established: 8% NaOH for 30min at room temperature in the presence of US. Native and PEGylated laccase from Myceliophthora thermophila was immobilized on BC membranes and a different catalytic behaviour was observed after immobilization. Native laccase presented activity values similar to published reports (5-7U/gBC) after immobilization whereas PEGylated enzymes showed much lower activity (1-2U/gBC). BC swelled membranes are presented herein as a potential support for the preparation of immobilized enzymes for industrial applications, like phenolics polymerization.
SCISEARCH/2017:1127134	Expression of a novel recombinant cyanate hydratase (rTI-Cyn) in Pichia pastoris, characteristics and applicability in the detoxification of cyanate	E-mail: singhs@dut.ac.za Durban Univ Technol, Fac Appl Sci, Dept Biotechnol + Food Technol, ZA-4000 Durban, South Africa (Reprint)	Ranjan, Bibhuti (Reprint) Pillai, Santhosh Permaul, Kugenthiren Singh, Suren	BIORESOURCE TECHNOLOGY, (2017 AUG 2017) Vol. 238, pp. 582-588. ISSN: 0960-8524.	20170101	other	A recombinant Pichia pastoris harbouring the cyanate hydratase gene (rTI-Cyn) from the thermophilic fungus Thermomyces lanuginosus SSBP yielded a high titre of extracellular cyanate hydratase (100 +/- 13 U mL(-1)) which was similar to 10-fold higher than the native fungal strain. The purified rTI-Cyn had a molecular mass of similar to 20 kDa on SDS-PAGE, with K-m, V-max, k(cat) and k(cat)/K-m values of 0.34 mM, 2857.14 mu moles mg(-1) min(-1), 2.14 x 10(4) s(-1) and 6.3 x 10(7) M-1 s(-1), respectively. Its properties of thermostability, pH stability, and heavy metals insensitivity, make it a suitable candidate for bioremediation in extreme environments. The rTI-Cyn was able to degrade toxic cyanate completely with the liberation of ammonia, which was confirmed by FTIR analysis. This is the first report of any known cyanate hydratase that has been expressed in P. pastoris, characterized and effectively evaluated for cyanate detoxification . (C) 2017 Elsevier Ltd. All rights reserved.
CABA/2017:250153	Thermothelomyces thermophila human infections .	Laboratoire de Parasitologie-Mycologie, CHU Clermont-Ferrand, Clermont-Ferrand, France. EMAIL: olivier.lortholary@aphp.fr olivier.lortholary@pasteur.fr	Nourrisson, C. Garcia-Hermoso, D. Morio, F. Kauffmann-Lacroix, C. Berrette, N. Bonhomme, J. Poirier, P. Lortholary, O.	Clinical Microbiology and Infection (2017), Volume 23, Number 5, pp. 338-341 ISSN: 1198-743X; 1469-0691 Published by: Elsevier, Oxford	20170101	other	
SCISEARCH/2017:1253048	Thermothelomyces thermophila human infections	Univ Paris 05, Sorbonne Paris Cite, Paris, France (Reprint) CHU Clermont Ferrand, Lab Parasitol Mycol, Clermont Ferrand, France Clermont Univ, Univ Blaise Pascal, Lab Microorganismes Genome + Environm, Clermont Ferrand, France Inst Pasteur, Ctr Natl Reference Mycoses Invas + Antifong, Unite Mycol Mol, Paris, France E-mail: olivier.lortholary@aphp.fr CHU Nantes, Lab Parasitol Mycol, Nantes, France Univ Nantes, Atlantique Univ, Dept Parasitol + Mycol Med, Fac Pharm, IICiMed EA1155, Nantes, France CHU Poitiers, Lab Parasitol Mycol, 2 Rue Miletrie, F-86021 Poitiers, France Ctr Hosp Le Mans, Lab Microbiol, Le Mans, France CHU Caen, Lab Microbiol, Caen, France CNRS URA3012, Paris, France E-mail: olivier.lortholary@aphp.fr Hop Univ Necker Enfants Malades, AP HP, IHU Imagine, Ctr Infectiol Necker Pasteur, Paris, France E-mail: olivier.lortholary@aphp.fr E-mail: olivier.lortholary@aphp.fr Inst Pasteur, Ctr Natl Reference Mycoses Invas + Antifong, Unite Mycol Mol, Paris, France (Reprint) CNRS URA3012, Paris, France (Reprint) Hop Univ Necker Enfants Malades, AP HP, IHU Imagine, Ctr Infectiol Necker Pasteur, Paris, France (Reprint)	Nourrisson, C. (Reprint) Poirier, P. Garcia-Hermoso, D. Lortholary, O. Morio, F. Kauffmann-Lacroix, C. Berrette, N. Bonhomme, J.	CLINICAL MICROBIOLOGY AND INFECTION, (2017 MAY 2017) Vol. 23, No. 5, pp. 338-341. ISSN: 1198-743X.	20170101	duplicates	
CABA/2017:33128	Bioprocess for the production of recombinant HAP phytase of the thermophilic mold Sporotrichum thermophile and its structural and biochemical characteristics.	Department of Microbiology, University of Delhi South Campus, New Delhi 110 021, India. EMAIL: tsnarayana@gmail.com	Maurya, A. K. Deepak Parashar Satyanarayana, T. Parashar, D.	International Journal of Biological Macromolecules (2017), Volume 94, Number Part A, pp. 36-44, 43 refs. ISSN: 0141-8130 DOI: https://doi.org/10.1016/j.ijbiomac.2016.09.102 Published by: Elsevier Ltd, Oxford	20170101	other	Thermophilic mold Sporotrichum thermophile secretes an acidstable and thermostable phytase, which finds application as a food and feed additive because of its adequate thermostability, acid stability, protease insensitivity and broad substrate spectrum. Low extracellular phytase production by the mold is a major bottleneck for its application on a commercial scale. We have successfully overcome this problem by constitutive secretary expression of codon optimized rStPhy under glyceraldehyde phosphate dehydrogenase (GAP) promoter in Pichia pastoris. A 4~1-fold improvement in rStPhy production has been achieved. Circular Dichroism (CD) spectra revealed that rStPhy is composed of 26.65% α-helices, 5.26% β-sheets and 68.09% random coils at pH 5.0 and 60°C, the optima for the enzyme activity. The melting temperature (Tm) of the enzyme is ~73°C. The 3D structure of rStPhy displayed characteristic signature sequences (RHGXRXP and HD) of HAP phytase. The catalytically important amino acids (Arg74, His75, Arg78, His368 and Asp369) were identified by docking and site directed mutagenesis . Fluorescence quenching by N-bromosuccinimide (NBS) and CsCl exposed tryptophan residues surrounded by negative charges, which play a key role in maintaining structural integrity of rStPhy.

PQSCITECH/2018:205614	Development of a genome-editing CRISPR/Cas9 system in thermophilic fungal Myceliophthora species and its application to hyper-cellulase production strain engineering		Liu, Qian Gao, Ranran Li, Jingen Lin, Liangcai Zhao, Junqi Sun, Wenliang Tian, Chaoguang Liu, Qian Gao, Ranran Li, Jingen Lin, Liangcai Zhao, Junqi Sun, Wenliang Tian, Chaoguang	Biotechnology for Biofuels, Vol. 10, 20170101 E-ISSN: 1754-6834 DOI: https://doi.org/10.1186/s13068-016-0693-9 Published by: BioMed Central, London	20170101	duplicates	Background Over the past 3 years, the CRISPR/Cas9 system has revolutionized the field of genome engineering. However, its application has not yet been validated in thermophilic fungi. Myceliophthora thermophila , an important thermophilic biomass-degrading fungus, has attracted industrial interest for the production of efficient thermostable enzymes. Genetic manipulation of Myceliophthora is crucial for metabolic engineering and to unravel the mechanism of lignocellulose deconstruction. The lack of a powerful, versatile genome-editing tool has impeded the broader exploitation of M. thermophila in biotechnology. Results In this study, a CRISPR/Cas9 system for efficient multiplexed genome engineering was successfully developed in the thermophilic species M. thermophila and M. heterothallica. This CRISPR/Cas9 system could efficiently mutate the imported amdS gene in the genome via NHEJ-mediated events. As a proof of principle, the genes of the cellulase production pathway, including cre-1, res-1, gh1-1, and alp-1, were chosen as editing targets. Simultaneous multigene disruptions of up to four of these different loci were accomplished with neomycin selection marker integration via a single transformation using the CRISPR/Cas9 system. Using this genome-engineering tool, multiple strains exhibiting pronounced hyper-cellulase production were generated, in which the extracellular secreted protein and lignocellulase activities were significantly increased (up to 5- and 13-fold, respectively) compared with the parental strain. Conclusions A genome-wide engineering system for thermophilic fungi was established based on CRISPR/Cas9. Successful expansion of this system without modification to M. heterothallica indicates it has wide adaptability and flexibility for use in other Myceliophthora species. This system could greatly accelerate strain engineering of thermophilic fungi for production of industrial enzymes, such as cellulases as shown in this study and possibly bio-based fuels and chemicals in the future.
SCISEARCH/2017:415527	Development of a genome-editing CRISPR/Cas9 system in thermophilic fungal Myceliophthora species and its application to hyper-cellulase production strain engineering	E-mail: tian_cg@tib.cas.cn Chinese Acad Sci, Tianjin Inst Ind Biotechnol, Key Lab Syst Microbial Biotechnol, Tianjin 300308, Peoples R China (Reprint)	Liu, Qian (Reprint) Gao, Ranran Li, Jingen Lin, Liangcai Zhao, Junqi Sun, Wenliang Tian, Chaoguang	BIOTECHNOLOGY FOR BIOFUELS, (3 2017 JAN 2017) Vol. 10.	20170101	duplicates	Background: Over the past 3 years, the CRISPR/Cas9 system has revolutionized the field of genome engineering. However, its application has not yet been validated in thermophilic fungi. Myceliophthora thermophila , an important thermophilic biomass-degrading fungus, has attracted industrial interest for the production of efficient thermostable enzymes. Genetic manipulation of Myceliophthora is crucial for metabolic engineering and to unravel the mechanism of lignocellulose deconstruction. The lack of a powerful, versatile genome-editing tool has impeded the broader exploitation of M. thermophila in biotechnology. Results: In this study, a CRISPR/Cas9 system for efficient multiplexed genome engineering was successfully developed in the thermophilic species M. thermophila and M. heterothallica. This CRISPR/Cas9 system could efficiently mutate the imported amdS gene in the genome via NHEJ-mediated events. As a proof of principle, the genes of the cellulase production pathway, including cre-1, res-1, gh1-1, and alp-1, were chosen as editing targets. Simultaneous multigene disruptions of up to four of these different loci were accomplished with neomycin selection marker integration via a single transformation using the CRISPR/Cas9 system. Using this genome-engineering tool, multiple strains exhibiting pronounced hyper-cellulase production were generated, in which the extracellular secreted protein and lignocellulase activities were significantly increased (up to 5-and 13-fold, respectively) compared with the parental strain. Conclusions: A genome-wide engineering system for thermophilic fungi was established based on CRISPR/Cas9. Successful expansion of this system without modification to M. heterothallica indicates it has wide adaptability and flexibility for use in other Myceliophthora species. This system could greatly accelerate strain engineering of thermophilic fungi for production of industrial enzymes, such as cellulases as shown in this study and possibly bio-based fuels and chemicals in the future.
CABA/2017:54439	Development of a genome-editing CRISPR/Cas9 system in thermophilic fungal Myceliophthora species and its application to hyper-cellulase production strain engineering.	Key Laboratory of Systems Microbial Biotechnology, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin 300308, China. EMAIL: tian_cg@tib.cas.cn	Liu Qian Gao RanRan Li JinGen Lin LiangCai Zhao JunQi Sun WenLiang Tian ChaoGuang Liu, Q. Gao, R. R. Li, J. G. Lin, L. C. Zhao, J. Q. Sun, W. L. Tian, C. G.	Biotechnology for Biofuels (2017), Volume 10, Number 1, pp. (3 January 2017), 60 refs. ISSN: 1754-6834 DOI: https://doi.org/10.1186/s13068-016-0693-9 Published by: BioMed Central Ltd, London	20170101	other	Background: Over the past 3 years, the CRISPR/Cas9 system has revolutionized the field of genome engineering. However, its application has not yet been validated in thermophilic fungi. Myceliophthora thermophila , an important thermophilic biomass-degrading fungus, has attracted industrial interest for the production of efficient thermostable enzymes. Genetic manipulation of Myceliophthora is crucial for metabolic engineering and to unravel the mechanism of lignocellulose deconstruction. The lack of a powerful, versatile genome-editing tool has impeded the broader exploitation of M. thermophila in biotechnology. Results: In this study, a CRISPR/Cas9 system for efficient multiplexed genome engineering was successfully developed in the thermophilic species M. thermophila and M. heterothallica. This CRISPR/Cas9 system could efficiently mutate the imported amdS gene in the genome via NHEJ-mediated events. As a proof of principle, the genes of the cellulase production pathway, including cre-1, res-1, gh1-1, and alp-1, were chosen as editing targets. Simultaneous multigene disruptions of up to four of these different loci were accomplished with neomycin selection marker integration via a single transformation using the CRISPR/Cas9 system. Using this genome-engineering tool, multiple strains exhibiting pronounced hyper-cellulase production were generated, in which the extracellular secreted protein and lignocellulase activities were significantly increased (up to 5- and 13-fold, respectively) compared with the parental strain. Conclusions: A genome-wide engineering system for thermophilic fungi was established based on CRISPR/Cas9. Successful expansion of this system without modification to M. heterothallica indicates it has wide adaptability and flexibility for use in other Myceliophthora species. This system could greatly accelerate strain engineering of thermophilic fungi for production of industrial enzymes, such as cellulases as shown in this study and possibly bio-based fuels and chemicals in the future.
PQSCITECH/2018:199811	Combined genome and transcriptome sequencing to investigate the plant cell wall degrading enzyme system in the thermophilic fungus Malbranchea cinnamomea		Huttner, Silvia Nguyen, Thanh Thuy Granchi, Zoraide Chin-A-Woeng, Thomas Ahren, Dag Larsbrink, Johan Vu Nguyen Thanh Olsson, Lisbeth	Biotechnology for Biofuels, Vol. 10, 20170101 E-ISSN: 1754-6834 DOI: https://doi.org/10.1186/s13068-017-0956-0 Published by: BioMed Central, London	20170101	other	(b) (4)

SCISEARCH/2017:813909	Optimized synthesis of novel prenyl ferulate performed by feruloyl esterases from Myceliophthora thermophila in microemulsions	E-mail: paul.christakopoulos@ltu.se DuPont Ind Biosci, Nieuwe Kanaal 7-S, NL-6709 PA Wageningen, Netherlands Taros Chem GmbH + Co KG, Emil Figge Str 76a, D-44227 Dortmund, Germany Univ Naples Federico II, Dept Chem Sci, Via Cintia 4, I-80126 Naples, Italy Inst Biosci + Applicat NCSR Demokritos, Lab Cell Proliferat + Aging, Athens 15310, Greece Korres Nat Prod, 57 Km Natl Rd, Athens 32011, Greece Lulea Univ Technol, Dept Civil Environm + Nat Resources Engn, Div Chem Engn, S-97187 Lulea, Sweden (Reprint)	Antonopoulou, Io (Reprint) Rova, Ulrika Christakopoulos, Paul Leonov, Laura Juetten, Peter Cerullo, Gabriella Faraco, Vincenza Papadopoulou, Adamantia Kletsas, Dimitris Ralli, Marianna	APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, (2017 APR 2017) Vol. 101, No. 8, pp. 3213-3226. ISSN: 0175-7598.	20170101	other	Five feruloyl esterases (FAEs; EC 3.1.1.73), FaeA1, FaeA2, FaeB1, and FaeB2 from Myceliophthora thermophila C1 and MtFae1a from M. thermophila ATCC 42464, were tested for their ability to catalyze the transesterification of vinyl ferulate (VFA) with prenol in detergentless microemulsions. Reaction conditions were optimized investigating parameters such as the medium composition, the substrate concentration, the enzyme load, the pH, the temperature, and agitation. FaeB2 offered the highest transesterification yield (71.5 +/- 0.2%) after 24 h of incubation at 30 A degrees C using 60 mM VFA, 1 M prenol, and 0.02 mg FAE/mL in a mixture comprising of 53.4:43.4:3.2 v/v/v n-hexane:t-butanol:100 mM MOPS-NaOH, pH 6.0. At these conditions, the competitive side hydrolysis of VFA was 4.7-fold minimized. The ability of prenyl ferulate (PFA) and its corresponding ferulic acid (FA) to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals was significant and similar (IC50 423.39 mu M for PFA, 329.9 mu M for FA). PFA was not cytotoxic at 0.8-100 mu M (IC50 220.23 mu M) and reduced intracellular reactive oxygen species (ROS) in human skin fibroblasts at concentrations ranging between 4 and 20 mu M as determined with the dichloro-dihydro-fluorescein diacetate (DCFH-DA) assay.
CAPLUS/2016:435153	Effect of Chinese medicinal herbal residues on microbial community succession and anti-pathogenic properties during co-composting with food waste	Sino-Forest Applied Research Centre for Pearl River Delta Environment and Department of Biology, Hong Kong Baptist University, Hong Kong	Zhou, Ying Selvam, Ammaiyappan Wong, Jonathan W. C.	Bioresource Technology (2016), 217, 190-199 CODEN: BIRTEB; ISSN: 0960-8524	20160101	other	This study investigated the antimicrobial properties of Chinese medicinal herbal residues during its co-composting with food waste in two different ratios along with a control. Inhibition on total microbial population were assessed while the numerically dominant microbes were isolated and their antagonistic effects were assessed. Results indicate that the active ingredients persist in the composting mass did not affect the microbes unspecifically as revealed from almost similar bacterial and fungal populations. Rather specific inhibitory activities against Alternaria solani and Fusarium oxysporum were obsd. Apart from the CMHR-born active compds., CMHR-induced changes in the antagonistic and mycoparasitic abilities of the bacteria and fungi also contribute to the specific inhibition against the tested pathogens . Therefore use of CMHRs during the composting of CMHRs enhances its antipathogenic property resulting in an anti-pathogenic compost.
CABA/2016:109360	Bioethanol production from NAOH+H2O2 pretreated Populus deltoides wood using cocktail of inhouse and commercial enzymes under four different modes of separate hydrolysis and fermentation.	Microbiology Laboratory, Department of Basis Sciences, Dr. Y S Parmar University of Horticulture and Forestry, Nauni, Solan 173 230 (HP), India.	Nisha Sharma Nivedita Sharma Sharma, N. Sharma, N.	World Journal of Pharmaceutical Research (2016), Volume 5, Number 2, pp. 661-676, 26 refs. ISSN: 2277-7105 Published by: World Journal of Pharmaceutical Research, Sofia	20160101	other	In the present study the bioconversion of alkaline hydrogen peroxide pretreated P. deltoides wood by using cocktail of inhouse enzymes from Bacillus stratospericus N12 (M), Myceliophthora thermophila SH1 and Bacillus altitudinis Kd1 (M) under submerged as well as solidstate fermentation was done which was compared with commercial enzymes. Comparative study of bioethanol production from P. deltoides wood was carried out using different methods of non-detoxification /detoxification , enzymatic hydrolysis and fermentation. In method I and II supernatant of alkaline hydrogen peroxide pretreated P. deltoides wood was pooled together with enzyme saccharified residue fraction, while in method III and IV supernatant after pretreatment was discarded and only enzymatic hydrolysate containing simple sugars was used for the fermentation. Different ethanologenic yeasts and bacteria were employed under monoculture and co-culture combinations for fermentation. The highest ethanol yield of 18.47 g/l was obtained in method IV as compared to others and 21.41 g/l in case of commercial enzymes. The co-culture of Saccharomyces cerevisiae II + Pichia stipitis was found best combination of ethanologens in both cases i.e. in inhouse enzyme saccharified hydrolysate as well as by using commercial enzymes.
SCISEARCH/2016:1773599	Efficacy and safety of itraconazole use in infants	E-mail: ranyuping@vip.sina.com Sichuan Univ, West China Hosp, Dept Dermatovenereol, Chengdu 610041, Peoples R China (Reprint)	Chen, Shuang (Reprint) Sun, Kai-Yi Feng, Xiao-Wei Ran, Xin Lama, Jebina Ran, Yu-Ping	WORLD JOURNAL OF PEDIATRICS, (2016 NOV 2016) Vol. 12, No. 4, pp. 399-407. ISSN: 1708-8569.	20160101	other	Background: Itraconazole has been used to treat fungal infections , in particular invasive fungal infections in infants or neonates in many countries. Data sources: Literature search was conducted through Ovid EMBASE, PubMed, ISI Web of Science, CNKI and Google scholarship using the following key words: 'pediatric' or 'infant' or 'neonate' and 'fungal infection ' in combination with 'itraconazole'. Based on the literature and our clinical experience, we outline the administration of itraconazole in infants in order to develop evidence-based pharmacotherapy. Results: Of 45 articles on the use of itraconazole in infancy, 13 are related to superficial fungal infections including tinea capitis, sporotrichosis, mucosal fungal infections and opportunistic infections . The other 32 articles are related to systemic fungal infections including candidiasis, aspergillosis, histoplasmosis, zygomycosis, trichosporonosis and opportunistic infections as caused by Myceliophthora thermophila . Conclusions: Itraconazole is safe and effective at a dose of 5 mg/kg per day in a short duration of therapy for superficial fungal infections and 10 mg/kg per day for systemic fungal infections in infants. With a good compliance, it is cost-effective in treating infantile fungal infections . The profiles of adverse events induced by itraconazole in infants are similar to those in adults and children.
EMBASE/20151000553	EFFECT OF MUTATIONS ON THE THERMOSTABILITY OF ASPERGILLUS ACULEATUS β-1,4-GALACTANASE.	Department of Chemistry, University of Copenhagen, Universitetsparken 5, Copenhagen, 2100, Denmark. leila@chem.ku.dk Novozymes A/S, Smormosevej 25, Bagsvaerd, 2880, Denmark. NSM, Research Unit for Functional Biomaterials, University of Roskilde, Universitetsvej 1, Roskilde, 4000, Denmark.	Torpenholt, Sos Olsson, Mats H.M. Jensen, Jan H. Lo Leggio, Leila (correspondence) De Maria, Leonardo Christensen, Lars H. Skjot, Michael Westh, Peter	Computational and Structural Biotechnology Journal, (2015) Vol. 13, pp. 256-264. Refs: 39 E-ISSN: 2001-0370	20150101	other	New variants of β-1,4-galactanase from the mesophilic organism Aspergillus aculeatus were designed using the structure of β-1,4-galactanase from the thermophile organism Myceliophthora thermophila as a template. Some of the variants were generated using PROPKA 3.0, a validated pKa prediction tool, to test its usefulness as an enzyme design tool. The PROPKA designed variants were D182N and S185D/Q188T, G104D/A156R. Variants Y295F and G306A were designed by a consensus approach, as a complementary and validated design method. D58N was a stabilizing mutation predicted by both methods. The predictions were experimentally validated by measurements of the melting temperature (Tm) by differential scanning calorimetry. We found that the Tm is elevated by 1.1 °C for G306A, slightly increased (in the range of 0.34 to 0.65 °C) for D182N, D58N, Y295F and unchanged or decreased for S185D/Q188T and G104D/A156R. The Tm changes were in the range predicted by PROPKA. Given the experimental errors, only the D58N and G306A show significant increase in thermodynamic stability. Given the practical importance of kinetic stability, the kinetics of the irreversible enzyme inactivation process were also investigated for the wild-type and three variants and found to be biphasic. The half-lives of thermal inactivation were approximately doubled in G306A, unchanged for D182N and, disappointingly, a lot lower for D58N. In conclusion, this study tests a new method for estimating Tm changes for mutants, adds to the available data on the effect of substitutions on protein thermostability and identifies an interesting thermostabilizing mutation , which may be beneficial also in other galactanases.
BIOSIS/2016:333394	Enzymatic synthesis of poly(catechin)-antibiotic conjugates: an antimicrobial approach for indwelling catheters.	Univ Minho, European Inst Excellence Tissue Engn and Regenerat, 3Bs Res Grp, AvePk, P-4806909 Taipas, Guimaraes, Portugal cmpsilva@ceb.uminho.pt; artur@deb.uminho.pt	Goncalves, Idalina Abreu, Ana S. Matama, Teresa Ribeiro, Artur Gomes, Andreia C. Silva, Carla [Reprint Author] Cavaco-Paulo, Artur	Applied Microbiology and Biotechnology, (JAN 2015) Vol. 99, No. 2, pp. 637-651. http://www.springerlink.com/content/100457/. CODEN: AMBIDG. ISSN: 0175-7598. E-ISSN: 1432-0614.	20150101	other	Biofilm formation in urinary indwelling catheters is one of the most critical issues that patients face. Catheters were coated with poly(catechin)-antibiotic conjugates with enhanced antimicrobial properties. Catechin was conjugated with two antibiotics, namely trimethoprim (TMP) and sulfamethoxazole (SMZ) via activation with N,N+apos;-disuccinimidyl carbonate (DSC) and subsequent coupling to molecules containing alpha-amine moieties. Silicone and polyurethane catheters were functionalized in situ through laccase oxidation of catechin-antibiotic conjugates. Four antimicrobial coatings were produced, namely with poly(catechin), poly(catechin)-TMP, poly(catechin)-SMZ and poly(catechin)-TMP-SMZ. The bacterial adhesion reduction was tested on the functionalized devices using gram-negative and gram-positive strains. The most significant reduction in adhesion was observed with poly(catechin)-TMP (gram-negative -85 % and gram-positive -87 %) and with poly(catechin)-TMP-SMZ (gram-negative -85 % and gram-positive -91 %). The cytotoxicity to mammalian cells was tested by indirect contact for 5 days and revealed that all the tested coatings supported more than 90 % of viable cells. A promising approach for the increase of the indwelling catheter lifespan was developed aiming to reduce catheter-associated chronic infections .

BIOSIS/2014:536754	Autochthonous ascomycetes in depollution of polychlorinated biphenyls contaminated soil and sediment.	Univ Grenoble 1, Lab Ecol Alpine UMR UJF CNRS 5553, BP 53, F-38041 Grenoble 9, France bello.mouhamadou@ujf-grenoble.fr	Sage, Lucile Perigon, Sophie Faure, Mathieu Gaignaire, Carole Abdelghafour, Mohamed Mehu, Jacques Geremia, Roberto A. Mouhamadou, Bello [Reprint Author]	Chemosphere, (SEP 2014) Vol. 110, pp. 62-69. https://www.journals.elsevier.com/chemosphere. CODEN: CMSHAF. ISSN: 0045-6535. E-ISSN: 1879-1298.	20140101	other	We investigated the capacity of a consortium of ascomycetous strains, Doratomyces nanus, Doratomyces purpureofuscus, Doratomyces verrucisporus, Myceliophthora thermophila , Phoma eupyrena and Thermoascus crustaceus in the mycoremediation of historically contaminated soil and sediment by polychlorinated biphenyls (PCBs). Analyses of 15 PCB concentrations in three mesocosms containing soil from which the fungal strains had previously been isolated, revealed significant PCB depletions of 16.9% for the 6 indicator PCBs (i-PCBs) and 18.7% for the total 15 PCBs analyzed after 6 months treatment. The degradation rate did not statistically vary whether the soil had been treated with non-inoculated straw or colonized straw or without straw and inoculated with the consortium of the six strains. Concerning the sediment, we evidenced significant depletions of 31.8% for the 6 i-PCBs and 33.3% for the 15 PCB congeners. The PCB depletions affected most of the 15 PCBs analyzed without preference for lower chlorinated congeners. Bioaugmented strains were evidenced in different mesocosms, but their reintroduction, after six months treatment, did not improve the rate of PCB degradation, suggesting that the biodegradation could affect the bioavailable PCB fraction. Our results demonstrate that the ascomycetous strains potentially adapted to PCBs may be propitious to the remediation of PCB contaminated sites. (C) 2014 Elsevier Ltd. All rights reserved.
BIOSIS/2015:19863	Transcriptome and exoproteome analysis of utilization of plant-derived biomass by Myceliophthora themiophila.	Concordia Univ, Ctr Struct and Funct Genom, L GE-120-11,7141 Sherbrooke St West, Montreal, PQ H4B 1R6, Canada magdalena.kolbusz@concordia.ca; marcos.difalco@concordia.ca; nadeeza.ishmael@concordia.ca; sandrine.marqueteau@concordia.ca; marie-claude.moisan@concordia.ca; Cassio.Baptista@nrc-cnrc.gc.ca; justin.powlowski@concordia.ca; adrian.tsang@concordia.ca	Kolbusz, Magdalena Anna [Reprint Author] Di Falco, Marcos Ishmael, Nadeeza Marqueteau, Sandrine Moisan, Marie-Claude Baptista, Cassio da Silva Powlowski, Justin Tsang, Adrian	Fungal Genetics and Biology, (NOV 2014) Vol. 72, No. Sp. Iss. SI, pp. 10-20. http://www.journals.elsevier.com/fungal-genetics-and-biology/#description. ISSN: 1087-1845. E-ISSN: 1096-0937.	20140101	other	Myceliophthora thermophila is a thermophilic fungus whose genome encodes a wide range of carbohydrate-active enzymes (CAZymes) involved in plant biomass degradation. Such enzymes have potential applications in turning different kinds of lignocellulosic feedstock into sugar precursors for biofuels and chemicals. The present study examined and compared the transcriptomes and exoproteomes of M. thermophila during cultivation on different types of complex biomass to gain insight into how its secreted enzymatic machinery varies with different sources of lignocellulose. In the transcriptome analysis three monocot (barley, oat, triticale) and three dicot (alfalfa, canola, flax) plants were used whereas in the proteome analysis additional substrates, i.e. wood and corn stover pulps, were included. A core set of 59 genes encoding CAZymes was up-regulated in response to both monocot and dicot straws, including nine polysaccharide monooxygenases and GH10, but not GH11, xylanases. Genes encoding additional xylanolytic enzymes were up-regulated during growth on monocot straws, while genes encoding additional pectinolytic enzymes were up-regulated in response to dicot biomass. Exoproteome analysis was generally consistent with the conclusions drawn from transcriptome analysis, but additional CAZymes that accumulated to high levels were identified. Despite the wide variety of biomass sources tested some CAZy family members were not expressed under any condition. The results of this study provide a comprehensive view from both transcriptome and exoproteome levels, of how M. thermophila responds to a wide range of biomass sources using its genomic resources. (C) 2014 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).
CABA/2014:275576	Production of cellobiose dehydrogenase from a newly isolated white rot fungus Termitomyces sp. OE147.	Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology Delhi, New Delhi, 110 016, India. EMAIL: saroj@dbeb.iitd.ac.in	Gupteshwar Gupta Rishabh Gangwar Ashwani Gautam Lalit Kumar Anuj Dhariwal Vikram Sahai Saroj Mishra Gupta, G. Gangwar, R. Gautam, A. Kumar, L. Dhariwal, A. Sahai, V. Mishra, S.	Applied Biochemistry and Biotechnology (2014), Volume 173, Number 8, pp. 2099-2115, 41 refs. ISSN: 0273-2289; 1559-0291 DOI: https://doi.org/10.1007/s12010-014-1010-3 Published by: Springer US, Philadelphia	20140101	other	Class I cellobiose dehydrogenases (CDHs) are extracellular hemoflavo enzymes produced at low levels by the Basidiomycetes (white rot fungi). In presence of suitable electron acceptors, e.g., cytochrome c, 2,6-dichlorophenol-indophenol, or metal ions, it oxidizes cellobiose to cellobionolactone. A stringent requirement for disaccharides makes CDH also useful for conversion of lactose to lactobionic acid, an important ingredient in pharma and detergent industry. In this work, class I CDH was produced using a newly identified white rot fungus Termitomyces sp. OE147. Four media were evaluated for CDH production, and maximum enzyme activity of 0.92 international unit (IU)/ml was obtained on Ludwig medium under submerged conditions. Statistical optimization of N source, which had significant effect on CDH production, using Box-Behnken design followed by optimization of inoculum size and age resulted in an increase in activity to 2.9 IU/ml and a productivity of ~25 IU/l/h. The nearly purified CDH exhibited high activity of 26.4 IU/mg protein on lactose indicating this enzyme to be useful for lactobionic acid synthesis. Some of the internal peptide sequences bore 100% homology to the CDH produced in Myceliophthora thermophila . The fungal isolate was amenable to scale up, and an overall productivity of ~18 IU/l/h was obtained at 14-l level.
CABA/2013:327153	Laccase-catalysed oxidation of ferulic acid and ethyl ferulate in aqueous medium: a green procedure for the synthesis of new compounds.	Laboratoire d'Ingenierie des Biomolecules (LIBio), Universite de Lorraine, 2 avenue de la Foret de Haye, TSA 40602, 54518 Vandoeuvre-les-Nancy, France. EMAIL: lionel.muniglia@univ-lorraine.fr	Aljawish, A. Chevalot, I. Jasniewski, J. Paris, C. Scher, J. Muniglia, L.	Food Chemistry (2014), Volume 145, pp. 1046-1054 ISSN: 0308-8146 DOI: https://doi.org/10.1016/j.foodchem.2013.07.119 Published by: Elsevier Ltd, Oxford	20140101	other	The enzymatic oxidation of ferulic acid (FA) and ethyl ferulate (EF) with Myceliophthora thermophila laccase, as biocatalyst, was performed in aqueous medium using an eco-friendly procedure to synthesize new active molecules. First, the commercial laccase was ultrafiltrated allowing for the elimination of phenolic contaminants and increasing the specific activity by a factor of 2. Then, kinetic parameters of this laccase were determined for both substrates (FA, EF), indicating a higher substrate affinity for ethyl ferulate. Additionally, enzymatic oxidation led to the synthesis of a FA-major product, exhibiting a molecular mass of 386 g/mol and a EF-major product with a molecular mass of 442 g/mol. Structural analyses by mass spectrometry allowed the identification of dimeric derivatives. The optical properties of the oxidation products showed the increase of red and yellow colours, with FA products compared to EF-products. Additionally, enzymatic oxidation led to a decrease of antioxidant and cytotoxic activities compared to initial substrates. Consequently, this enzymatic procedure in aqueous medium could provide new compounds presenting optical, antioxidant and cytotoxic interest.
SCISEARCH/2013:786744	Widening the pH Activity Profile of a Fungal Laccase by Directed Evolution	CSIC, Inst Catalysis, Dept Biocatalysis, Marie Curie St 2, Madrid 28049, Spain (Reprint) CSIC, Inst Catalysis, Dept Biocatalysis, Madrid 28049, Spain E-mail: malcalde@icp.csic.es	Torres-Salas, Pamela (Reprint) Mate, Diana M. Ghazi, Iraj Plou, Francisco J. Ballesteros, Antonio O. Alcalde, Miguel	CHEMBIOCHEM, (27 2013 MAY 2013) Vol. 14, No. 8, pp. 934-937. ISSN: 1439-4227.	20130101	other	
SCISEARCH/2013:1680481	Corynascus verrucosus, second record for science and new for India	E-mail: rohit@nccs.res.in Natl Ctr Cell Sci, Mol Biol Unit, Pune, Maharashtra, India Natl Ctr Cell Sci, Pune, Maharashtra, India (Reprint)	Sharma, Rohit (Reprint) Shouche, Yogesh S. Kulkarni, Girish	NOVA HEDWIGIA, (2013 2013) Vol. 97, No. 3-4, pp. 485-494. ISSN: 0029-5035.	20130101	other	Colynascus verrucosus, a member of Chaetomiaceae, is described and illustrated as second record for science and new to India. It was isolated as endophyte from flower bud of Jatropha podagrica. It is characterized by non-ostiolate ascomata, peridium with brown appendages and ellipsoidal ascospores with acute ends and two sub-terminal to oblique germ pores. Its anamorph was also produced on all media tested. The ascomata and ascospores are somewhat smaller than those described for the holotype of this species. The morphological identification is supported by ITS DNA sequence data.

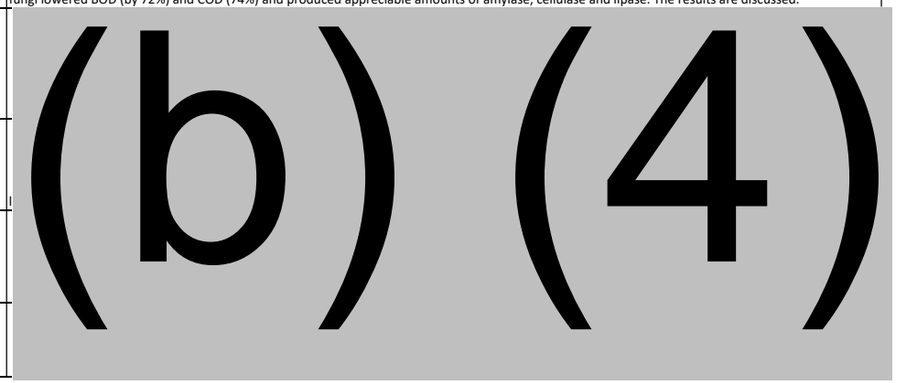
BIOSIS/2013:451326	Potential of autochthonous fungal strains isolated from contaminated soils for degradation of polychlorinated biphenyls.	Univ Grenoble 1, UMR CNRS 5553, Lab Ecol Alpine, BP 53, F-38041 Grenoble 9, France bello.mouhamadou@ujf-grenoble.fr	Mouhamadou, Bello [Reprint Author] Faure, Mathieu Sage, Lucile Marcais, Johanna Souard, Florence Geremia, Roberto A.	Fungal Biology, (APR 2013) Vol. 117, No. 4, pp. 268-274. http://www.journals.elsevier.com/fungal-biology/#description. ISSN: 1878-6146. E-ISSN: 1878-6162.	20130101	other	Up to now, most studies on polychlorinated biphenyl (PCB) bioremediation have examined the ability of model fungal strains to biodegrade PCBs. Yet, there is limited information concerning the potential of autochthonous filamentous fungal strains in the biodegradation of PCBs and their possible use in the environmental technologies. In this study, we investigated the capacity of autochthonous fungal strains in the biodegradation of PCBs by isolating 24 taxa from former industrial sites highly contaminated by PCBs. Microscopic and molecular analyses using the internal transcribed spacer (ITS) region revealed that the fungal strains belonged to the phyla Ascomycota (19 strains) and Zygomycota (five strains). The chromatography gas analysis revealed evidence of degradation of seven PCB congeners. With the exception of Circinella rnuscae which presented no degradation potential, the other fungal strains exhibited a rate of biodegradation ranging from 29 to 85 % after 7 d of incubation in liquid medium. Among these strains, Doratomyces nanus, Doratomyces purpureofuscus, Doratomyces verrucisporus, Myceliophthora thermophila , Phoma eupyrena, and Thermoascus crustaceus showed remarkable degradation ability (>70 %) regardless of the number of chlorine substituents on the biphenyl nucleus and a high tolerance towards PCBs. To our knowledge, this is the first study that demonstrates the ability of PCB degradation by these species and indicates the potential effectiveness of some autochthonous fungal strains in bioremediation systems. (c) 2013 The British Mycological Society. Published by Elsevier Ltd. All rights reserved.
BIOSIS/2013:161279	The structure of a novel glucuronoyl esterase from Myceliophthora thermophila gives new insights into its role as a potential biocatalyst.	Natl Hellen Res Fdn, Inst Biol Med Chem and Biotechnol, 48 Vassileos Constantinou Ave, Athens 11635, Greece echrysina@eie.gr	Charavgi, Maria-Despoina Dimarogona, Maria Topakas, Evangelos Christakopoulos, Paul Chrysina, Evangelia D. [Reprint Author]	Acta Crystallographica Section D Structural Biology, (JAN 2013) Vol. 69, No. Part 1, pp. 63-73. http://journals.iucr.org/d/. ISSN: 2059-7983. E-ISSN: 2059-7983.	20130101	other	The increasing demand for the development of efficient biocatalysts is a consequence of their broad industrial applications. Typical difficulties that are encountered during their exploitation in a variety of processes are interconnected with factors such as temperature, pH, product inhibitors etc. To eliminate these, research has been directed towards the identification of new enzymes that would comply with the required standards. To this end, the recently discovered glucuronoyl esterases (GEs) are an enigmatic family within the carbohydrate esterase (CE) family. Structures of the thermophilic StGE2 esterase from Myceliophthora thermophila (synonym Sporotrichum thermophile), a member of the CE15 family, and its S213A mutant were determined at 1.55 and 1.9 angstrom resolution, respectively. The first crystal structure of the S213A mutant in complex with a substrate analogue, methyl 4-O-methyl-beta-D-glucopyranuronate, was determined at 2.35 angstrom resolution. All of the three-dimensional protein structures have an alpha/beta-hydrolase fold with a three-layer alpha beta alpha-sandwich architecture and a Rossmann topology and comprise one molecule per asymmetric unit. These are the first crystal structures of a thermophilic GE both in an unliganded form and bound to a substrate analogue, thus unravelling the organization of the catalytic triad residues and their neighbours lining the active site. The knowledge derived offers novel insights into the key structural elements that drive the hydrolysis of glucuronic acid esters.
SCISEARCH/2013:193567	Combinatorial approach of statistical optimization and mutagenesis for improved production of acidic phytase by Aspergillus niger NCIM 563 under submerged fermentation condition	E-mail: jmkhire@yahoo.com Natl Chem Lab, Chem Engn + Proc Dev Div, Pune 411008, Maharashtra, India Natl Chem Lab, NCIM Resource Ctr, Pune 411008, Maharashtra, India (Reprint)	Bhavsar, K. (Reprint) Gujar, P. Shah, P. Khire, J. M. Kumar, V. Ravi	APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, (2013 JAN 2013) Vol. 97, No. 2, pp. 673-679. ISSN: 0175-7598.	20130101	other	Combination of statistical optimization and mutagenesis to isolate hypersecretory strains is studied to maximize phytase production from Aspergillus niger NCIM 563 under submerged fermentation. The overall results obtained show a remarkable 5.98-fold improvement in phytase production rates when compared to that using basal medium. Optimization of culture conditions from parent strain is studied first by the Plackett-Burman technique to evaluate the effects of 11 variables for phytase production. The results showed that glucose, MgSO4, KCl, incubation period, and MnSO4 are the most significant variables affecting enzyme production. Further optimization in these variables, using a central composite design technique, resulted in 3.74-fold increase in the yield of phytase production to 254,500 U/l when compared with the activity observed with basal media (68,000 U/l) in shake flask. Our experiments show that the phytase from A. niger NCIM 563 exhibits desirable activity in simulated gastric fluid conditions with low pH and also improved thermostability when compared to commercial phytase. The improved yield demonstrates the potential applicability of phytase enzyme as a source of phytase supplement for phosphorus nutrition and environmental protection in animal feed industry. Physical and chemical mutagenesis experiments were carried out in parallel to isolate hypersecretory mutants that could possibly further enhance the enzyme production. Using optimized media conditions of the parent strain, our results show that mutant strain A. niger NCIM 1359 increased the phytase activity by another 1.6-fold to 407,200 U/l.
CABA/2012:180180	Isolation and identification of actinomycetes from termite's gut against human pathogen .	Faculty of Science and Technology, Phranakhon Rajabhat University, Bangkok 10220, Thailand.	Khucharoenphaisan, K. Sripairoj, N. Sinma, K.	Asian Journal of Animal and Veterinary Advances (2012), Volume 7, Number 1, pp. 68-73, 25 refs. ISSN: 1683-9919; 1996-3289 DOI: https://doi.org/10.3923/ajava.2012.68.73 Published by: Academic Journals Inc., New York	20120101	other	Actinomycetes are a group of prokaryotic organisms belonging to Gram-positive bacteria and play an important ecological role in recycling substances in the nature. The objective of this study was to isolate and identify actinomycetes from termite's gut against human pathogen . Total isolates were examined for antimicrobial activity and selected isolate was identified using morphological characters and molecular technique. The results showed that eighty-three strains of actinomycetes were isolated from guts of Termes sp. Among these, 66, 67, 7, 9 and 3 isolated strains were active against the tested pathogenic microorganism of Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Candida utilis, respectively. Furthermore, some isolate of actinomycetes was able to inhibit both Gram-positive and Gram-negative pathogen . The isolated actinomycetes strain FSPNRU 102 having broad spectrum of inhibition was selected. The morphological character of this strain showed aerial mycelium with longitudinal spirales-type spore chain and light black soluble pigment. The aerial spore color varied from white to gray. Moreover, this strain contained LL-diaminopimelic acid of the peptidoglycan in the whole-cell hydrolysate of chemotaxonomical characteristic. These results assigned strain FSPNRU 102 to genus Streptomyces. Based on its 16S rDNA sequence and phylogenetic tree analysis, this new isolate belong to the Streptomyces niveoruber.
CABA/2011:348419	Invasive Myceliophthora thermophila infection mimicking invasive aspergillosis in a neutropenic patient : a new cause of cross-reactivity with the Aspergillus galactomannan serum antigen assay.	Laboratoire de Parasitologie-Mycologie, CHU de Nantes, Hotel Dieu, 9 Quai Moncousu, 44093 Nantes Cedex 1, France. EMAIL: florent.morio@chu-nantes.fr	Morio, F. Fraissinet, F. Gastinne, T. Pape, P. le Delaunay, J. Sigler, L. Gibas, C. F. E. C. Miegeville, M. le Pape, P.	Medical Mycology (2011), Volume 49, Number 8, pp. 883-886, 16 refs. ISSN: 1369-3786; 1460-2709 Published by: Informa Healthcare, Stockholm	20110101	other	Myceliophthora thermophila is a thermophilic mould widely found in the environment but rarely responsible for human infections . We describe a case of invasive Myceliophthora thermophila infection mimicking invasive aspergillosis in a neutropenic patient with haematological malignancy. Cross-reactivity with Aspergillus galactomannan assay (GM) was demonstrated by repeated positive results and confirmed by cross-reaction between the fungal isolate and the GM assay. The patient was successfully treated with voriconazole. Potential GM cross-reactivity must be considered in future studies including patients categorized as having probable invasive aspergillosis using the GM as the only mycological criterion.
EMBASE/2010430631	Laccase-catalyzed degradation of anti-inflammatories and estrogens.	Dept. of Chemical Engineering, School of Engineering, University of Santiago de Compostela, Santiago de Compostela E-15782, Spain. maite.morei ra@usc.es	Lloret, L. Eibes, G. Lu-Chau, T.A. Moreira, M.T. (correspondence) Feijoo, G. Lema, J.M.	Biochemical Engineering Journal, (September 2010) Vol. 51, No. 3, pp. 124-131. Refs: 32 ISSN: 1369-703X CODEN: BEJOFV	20100101	other	Pharmaceuticals are regarded as emerging environmental pollutants since many of them are ubiquitous, recalcitrant and biologically active. In this paper, the degradation of several pharmaceuticals such as anti-inflammatory drugs (diclofenac and naproxen) and estrogen hormones (estrone, 17β-estradiol, 17α-ethinylestradiol) was assessed by means of the commercial laccase (Lac) from Myceliophthora thermophila . The influence of different mediators (synthetic and natural) and their concentration on the Lac-based oxidation system were evaluated. Estrogens were completely degraded after only 15. min while the other types of pharmaceuticals presented higher persistence since 1. h of incubation was required for total removal of diclofenac and 8. h to attain up to 60% of naproxen degradation. Among the different natural mediators, syringaldehyde greatly enhanced the action of the Lac, similarly to the synthetic mediator 1-hydroxibenzotriazole (HBT) in the case of estrogens and diclofenac. The other natural mediators presented significantly high efficiency, obtaining removal percentages ranging from 80% to 100% after 24. h of enzymatic reaction. © 2010 Elsevier B.V.

BIOSIS/2010:327299	Oxidative dehalogenation of chlorinated hydroxybiphenyls by laccases of white-rot fungi.	Ernst Moritz Arndt Univ Greifswald, Inst Microbiol, Friedrich Ludwig Jahn Str 15, D-17487 Greifswald, Germany annett.mikolasch@uni-greifswald.de	Kordon, Korinna Mikolasch, Annett [Reprint Author] Schauer, Frieder	International Biodeterioration + Biodegradation, (JUN 2010) Vol. 64, No. 3, pp. 203-209. http://www.journals.elsevier.com/international- biodeterioration-and-biodegradation/#description. CODEN: IBBIES. ISSN: 0964-8305. E-ISSN: 1879-0208.	20100101	other	Chlorinated biphenyls have been widely used in industry because of their advantageous chemical and physical properties. But the transformation products of chlorinated biphenyls derived from hydroxylation, the chlorinated hydroxybiphenyls, have oestrogenic, immunosuppressive, and carcinogenic activities. Because of the risks to humans and animals, the ability to transform chlorinated hydroxybiphenyls is a major goal of environmental research. We here investigated transformations of the chlorinated hydroxybiphenyls 5-chloro-2-hydroxybiphenyl, 3,5-dichloro-2-hydroxybiphenyl, and 2+apos;,5,5+apos;-trichloro-2-hydroxybiphenyl by laccases from Pycnoporus cinnabarinus and Myceliophthora thermophila , because very little is known about the transformation pathways of the named substances by filamentous fungi though these organisms play important roles in soil ecosystems and in contaminated sludges and wastewater. Oxidative dechlorination and formation of quinoid and hydroquinoid derivatives were observed in all three cases. Furthermore, dimer formation was detected. Therefore, laccase leads not only to dechlorination of the initial chlorinated hydroxybiphenyls but also to dechlorinated di- and oligomeric products. In addition 4-chloro-2-hydroxydibenzofuran was found and purified as a product of the laccase reaction on 3,5-dichloro-2-hydroxybiphenyl. (c) 2010 Elsevier Ltd. All rights reserved.
CABA/2010:235544	Derivatization of the azole 1-aminobenzotriazole using laccase of Pycnoporus cinnabarinus and Myceliophthora thermophila : influence of methanol on the reaction and biological evaluation of the derivatives.	Institute of Microbiology, Ernst-Moritz-Arndt-University Greifswald, F.-L.-Jahnstr. 15, 17487 Greifswald, Germany. EMAIL: veronikahahn@gmx.at	Hahn, V. Mikolasch, A. Wende, K. Bartrow, H. Lindequist, U. Schauer, F.	Biotechnology and Applied Biochemistry (2010), Volume 56, Number 2, pp. 43-48, 34 refs. ISSN: 0885-4513; 1470-8744 DOI: https://doi.org/10.1042/BA20100078 Published by: Portland Press Ltd, London	20100101	other	The laccases of Pycnoporus cinnabarinus and Myceliophthora thermophila are extracellular enzymes with high protein stability. They were used for the 'one pot' synthesis of azole derivatives from 1-aminobenzotriazole together with the p-dihydroxylated laccase substrates 2,5-dihydroxybenzoic acid methyl ester and 2,5-dihydroxybenzoic acid ethyl ester. The reactions yielded heteromolecular dimers (in yields of up to 34%). Methanol was used as the co-solvent to determine the influence of solvent concentration on the course of reaction. The resulting products were isolated, structurally characterized and tested for their antibacterial, antifungal and cytotoxic activities. The products showed low antimicrobial activity and low cytotoxicity compared with commercial available standard compounds but these variables exceeded those of the initial reactants used for the synthesis. In addition to the synthesis of heteromolecular dimers, oligomers were formed and structurally characterized by LC/MS (liquid chromatography/MS).
SCISEARCH/2010:317171	Polymerization study of the aromatic amines generated by the biodegradation of azo dyes using the laccase enzyme	E-mail: elisfran@fea.unicamp.br Univ Minho, Dept Text Engn, P-4800058 Guimaraes, Portugal Univ Estadual Campinas, Chem Biol + Agr Pluridisciplinary Res Ctr CPQBA, BR-13083970 Campinas, SP, Brazil BioSage, Lawrenceville, NJ 08648 USA Univ Estadual Campinas, Dept Food Sci, BR-13083970 Campinas, SP, Brazil (Reprint)	Franciscon, Elisangela (Reprint) Piubeli, Francine de Menezes, Cristiano Ragagnin Silva, Isis Serrano Durrant, Lucia Regina Cavaco-Paulo, Artur Fantinatti-Garboggini, Fabiana Grossman, Matthew James	ENZYME AND MICROBIAL TECHNOLOGY, (5 2010 APR 2010) Vol. 46, No. 5, pp. 360-365. ISSN: 0141-0229.	20100101	other	Four different azo dyes were decolorized (color reduction >90%) by bacteria isolated from a textile wastewater effluent. Dye decolorizing was carried out under microaerobic conditions until completion, after which the aromatic amine concentration was determined. A laccase from Myceliophthora thermophila was used to catalyze coupling reactions of the aromatic amines produced from decolorizing the dyes. The reaction was carried out with stirring (100 rpm) in a weak acidic buffer solution (pH 5.0) at 45 degrees C for 3 days. The presence of aromatic amines in the samples after bacterial decolorizing confirmed the azo bond was reduced in the process. In addition, the UV-vis spectrum was shifted significantly after the sequential bacterial-laccase treatment also indicating a chemical transformation of the dyes. After laccase treatment the solutions formed colored soluble and precipitated products. The particles sizes making up the precipitates formed after laccase treatment varied between 105 and 483 nm as determined by Photon Correlation Spectroscopy (PCS). The laccase treatment also reduced the COD of the dye solutions by similar to 20%. We show that successive bacterial-laccase treatment is effective in decolorized azo dyes by reduction of the azo bonds, and promoting coupling reactions between the aromatic amines formed. Promoting coupling reactions between the aromatic amines using enzymes may prove useful for the physical removal and reuse of these amines. (C) 2010 Published by Elsevier Inc.
CABA/2009:87638	Left ventricular assist device outflow cannula obstruction by the rare environmental fungus Myceliophthora thermophila .	Department of Anesthesia, University of Colorado Denver and Health Sciences Center, Aurora, Colorado, USA. EMAIL: nathaen.weitzel@uchsc.edu	Weitzel, N. Puskas, F. Cleveland, J. Levi, M. E. Seres, T.	Anesthesia and Analgesia (2009), Volume 108, Number 1, pp. 73-75 ISSN: 1526-7598 DOI: https://doi.org/10.1213/ane.0b013e318187b8fc Published by: Lippincott Williams + Wilkins, Hagerstown	20090101	other	Left ventricular assist devices are used to provide mechanical circulatory support during end-stage heart failure either as a destination therapy or as a bridge to heart transplantation. Perioperative transesophageal echocardiography is becoming an invaluable tool to investigate device function during implantation and in case of mechanical malfunction. Most malfunctions are due to inflow graft occlusion, or device malfunction, while outflow graft dysfunction is rare. Here, we present a case of severe outflow conduit obstruction by a rare environmental fungus, Myceliophthora thermophila . After replacement of the infected device and intensive antifungal treatment, heart transplantation was performed 2 yr later.
BIOSIS/2009:470478	Thermotolerant and thermostable laccases.	Univ Nottingham, Sch Biol, Univ Pk, Nottingham NG7 2RD, UK kristiina.s.hilden@helsinki.fi	Hilden, Kristiina [Reprint Author] Hakala, Terhi K. Lundell, Taina	Biotechnology Letters, (AUG 2009) Vol. 31, No. 8, pp. 1117-1128. http://www.springerlink.com/content/100138/. CODEN: BILED3. ISSN: 0141-5492. E-ISSN: 1573-6776.	20090101	other	Laccases are phenol-oxidizing, usually four-copper containing metalloenzymes. For industrial and biotechnological purposes, laccases were among the first fungal oxidoreductases providing larger-scale applications such as removal of polyphenols in wine and beverages, conversion of toxic compounds and textile dyes in waste waters, and in bleaching and removal of lignin from wood and non-wood fibres. In order to facilitate novel and more efficient bio-catalytic process applications, there is a need for laccases with improved biochemical properties, such as thermostability and thermotolerance. This review gives a current overview on the sources and characteristics of such laccases, with particular emphasis on the fungal enzymes.
CABA/2010:59548	Synthesis of model morpholine derivatives with biological activities by laccase-catalysed reactions.	Institute of Microbiology, Ernst-Moritz-Arndt-University Greifswald, Friedrich-Ludwig-Jahnstrasse 15, 17487 Greifswald, Germany. EMAIL: veronikahahn@gmx.at	Hahn, V. Mikolasch, A. Wende, K. Bartrow, H. Lindequist, U. Schauer, F.	Biotechnology and Applied Biochemistry (2009), Volume 54, Number 4, pp. 187-195, 27 refs. ISSN: 0885-4513; 1470-8744 DOI: https://doi.org/10.1042/BA20090219 Published by: Portland Press, Colchester	20090101	other	The efficient enzyme-catalysed reaction of morpholines as model structures for bioactive compounds with para-dihydroxylated aromatic systems was carried out using the oxidoreductase laccase and atmospheric oxygen to produce eight novel morpholine-substituted aromatics. The laccase of Myceliophthora thermophila was used for cross-linking morpholines containing primary or secondary amino groups with para-dihydroxylated laccase substrates. We demonstrate that not only primary amino groups, but also secondary amino groups, are able to couple with para-dihydroxylated aromatic systems in laccase-catalysed reactions. The resulting model products (yields up to 80%) were isolated, structurally characterized and tested for their antibacterial, antifungal and cytotoxic activities. Four of the eight products showed low to moderate growth inhibition against several Gram-positive and -negative bacterial strains and against the yeasts Candida maltosa and Candida albicans. The antibacterial and antifungal activities were determined by an agar disc diffusion test and a modified method according to the EUCAST discussion document E.Dis 7.1 [Rodriguez-Tudela et al. (2003) Clin. Microbiol. Infect . 9, i-viii] for the evaluation of MIC (minimal inhibitory concentration). Differences in cytotoxicity against the human urinary bladder carcinoma cell line 5637 are discussed.
CABA/2010:228683	Combinatorial saturation mutagenesis of the Myceliophthora thermophila laccase T2 mutant: the connection between the C-terminal plug and the conserved.	Department of Biocatalysis, Institute of Catalysis, CSIC, Cantoblanco, 28049 Madrid, Spain.	Zumarraga, M. Dominguez, C. V. Camarero, S. Shleev, S. Polaina, J. Martinez-Arias, A. Ferrer, M. Lacey, A. L. de Fernandez, V. M. Ballesteros, A. Plou, F. J. Alcalde, M. de Lacey, A. L.	Combinatorial Chemistry + High Throughput Screening (2008), Volume 11, Number 10, pp. 807-816 ISSN: 1386-2073; 1875-5402 DOI: https://doi.org/10.2174/138620708786734235 Published by: Bentham Science Publishers BV, Hilversum	20080101	other	A mutant laccase from the Ascomycete Myceliophthora thermophila has been submitted to iterative cycles of combinatorial saturation mutagenesis through in vivo overlap extension in Saccharomyces cerevisiae. Over 180,000 clones were explored, among which the S510G mutant revealed a direct interaction between the conserved 509VSG511 tripeptide, located in the neighborhood of the T1 site, and the C-terminal plug. The Km O2 value of the mutant increased 1.5-fold, and the electron transfer pathway between the reducing substrate and the T1 copper ion was altered, improving the catalytic efficiency towards non-phenolic and phenolic substrates by about 3- and 8-fold. Although the geometry at the T1 site was perturbed by the mutation , paradoxically the laccase redox potential was not significantly altered. Together, the results obtained in this study suggest that the 509VSG511 tripeptide may play a hitherto unrecognized role in regulating the traffic of oxygen through the C-terminal plug, the latter blocking access to the T2/T3 copper cluster in the native enzyme.

BIOSIS/2008:9846	Bioremediation of polycyclic aromatic hydrocarbons by fungal laccases engineered by directed evolution.	CSIC, Inst Catalisis and Petroleoquim, E-28049 Madrid, Spain malcalde@icp.csic.es	Zumarraga, Miren Plou, Francisco J. Garcia-Arellano, Humberto Ballesteros, Antonio Alcalde, Miguel [Reprint Author]	Biocatalysis and Biotransformation, (2007) Vol. 25, No. 2-4, pp. 219-228. http://www.tandfonline.com/toc/ibab20/. ISSN: 1024-2422. E-ISSN: 1029-2446.	20070101	other	Fungal laccases are useful for several remarkable transformations, such as bioremediation of polycyclic aromatic hydrocarbons (PAHs), synthesis of phenolic-based resins, oxidation of lignin derivatives and others. Most of these substrates are barely water-soluble, and although polar organic co-solvents may be added to enhance their solubility, transformation rates dramatically decrease due to the negative effect of organic solvents on the protein structure. Laccase from Myceliophthora thermophila variant T2 (MtLT2) has been submitted to laboratory evolution in Saccharomyces cerevisiae with the aim of improving activity and stability in organic co-solvents. Some 4500 clones created by random mutagenesis were screened in two rounds of directed evolution. Libraries were explored under increasing concentrations of acetonitrile and ethanol, and several mutants with improved features were purified and further characterised. Turnover rates of MtLT2 in 30% (v/v) acetonitrile and 50% (v/v) ethanol were increased up to 6.5- and 7.5-fold, respectively. The best variants showed similar rates in 20% (v/v) acetonitrile or 30% (v/v) ethanol as the parent type in aqueous media. Mutant laccases were also tested for the oxidation of anthracene in the presence of 20% (v/v) acetonitrile.
CABA/2008:41831	Biodegradation of indole at high concentration by persolvent fermentation with the thermophilic fungus Sporotrichum thermophile .	Biotechnology Laboratory, School of Chemical Engineering, National Technical University of Athens, 5 Iroon Polytechniou Str, Zografou Campus, 15700 Athens, Greece. EMAIL: hristako@orfeas.chemeng.ntua.gr	Katapodis, P. Moukouli, M. Christakopoulos, P.	International Biodeterioration + Biodegradation (2007), Volume 60, Number 4, pp. 267-272 ISSN: 0964-8305 DOI: https://doi.org/10.1016/j.ibiod.2007.04.002 Published by: Elsevier, Oxford	20070101	other	Indole and its derivatives form a class of toxic recalcitrant environmental pollutants. Sporotrichum thermophile was grown in a persolvent fermentation system containing a large amount of indole. The medium contained up to 20% by volume soybean oil and up to 2 g Lsup-1 indole. Most of the indole was partitioned in the organic solvent layer. When the organism was grown in the medium containing indole at 1 g Lsup-1, indole was totally consumed after 6 days. Under a fed-batch fermentation process where daily batches of indole (1 g Lsup-1) supplemented the microbial culture for 4 days, the biodegradation level was 3.0 g Lsup-1. These values make this process promising and worthy of further investigation for the microbial degradation of other toxic compounds.
CABA/2007:185684	Comparative spore germination of filamentous fungi on solid state fermentation under different culture conditions.	Institut Agronomique et Veterinaire (IAV)-Hassan II, Laboratoire des Bioconversions, BP. 6202-Instituts, 10101 Rabat, Morocco. EMAIL: s.roussos@univ.u-3mrs.fr s.roussos@univ-cezanne.fr	Hassouni, H. Ismaili-Alaoui, M. Lamrani, K. Gaime-Perraud, I. Augur, C. Roussos, S.	Micologia Aplicada International (2007), Volume 19, Number 1, pp. 7-14, 18 refs. ISSN: 1534-2581 Published by: Micologia Aplicada International, Berkeley	20070101	other	Environmental factors influencing spore germination of three filamentous fungi (Aspergillus niger, Myceliophthora thermophila , Rhizopus microsporus [Rhizopus microsporus var. microsporus]) were studied. Four substrates, all containing sugarcane bagasse as support, were tested for spore germination. Different temperature, moisture, aeration and humidity conditions were evaluated for each species. Spore germination was assessed by observing emergence of the germ tube. Germination rates in A. niger, M. thermophila, and R. microsporus cultivated in solid state fermentation were affected by the substrate, incubation temperature, and initial moisture. A mixture of sugarcane bagasse and wheat bran (80:20 w/w) resulted in high germination rates for all species tested, and was selected for additional experiments. The optimal temperature for spore germination and initial growth was 30°C for A. niger and 45°C for M. thermophila and R. microsporus. Higher germination rates were recorded in all species at 85% (aw 0.99) initial moisture after 6 h of incubation at optimal temperature. Forced humid aeration increased germination rates and decreased the time required for complete spore germination in all species studied.
CABA/2007:183845	Severe osteomyelitis caused by Myceliophthora thermophila after a pitchfork injury.	Department of Pediatrics, Medical College of Wisconsin, Children's Hospital of Wisconsin, Wisconsin, USA. EMAIL: ldestino@mcw.edu suttond@UTHSCSA.edu anna.helon@gmail.com phavens@mcw.edu jthometz@mcw.edu rewillou@mcw.edu mchusid@mcw.edu	Destino, L. Sutton, D. A. Helon, A. L. Havens, P. L. Thometz, J. G. Willoughby, R. E., Jr. Chusid, M. J.	Annals of Clinical Microbiology and Antimicrobials (2006), Volume 5, Number 21, pp. (08 September 2006), 18 refs. ISSN: 1476-0711 Published by: BioMed Central Ltd, London	20060101	other	Background: Traumatic injuries occurring in agricultural settings are often associated with infections caused by unusual organisms. Such agents may be difficult to isolate, identify, and treat effectively. Case report: A 4-year-old boy developed an extensive infection of his knee and distal femur following a barnyard pitchfork injury. Ultimately the primary infecting agent was determined to be Myceliophthora thermophila , a thermophilic melanized hyphomycete, rarely associated with human infection , found in animal excreta. Because of resistance to standard antifungal agents including amphotericin B and caspofungin, therapy was instituted with a prolonged course of terbinafine and voriconazole. Voriconazole blood levels demonstrated that the patient required a drug dosage (13.4 mg/kg) several fold greater than that recommended for adults in order to attain therapeutic blood levels. Conclusion: Unusual pathogens should be sought following traumatic farm injuries. Pharmacokinetic studies may be of critical importance when utilizing antifungal therapy with agents for which little information exists regarding drug metabolism in children.
CABA/2005:153021	Xylanases as a tool for the production of novel phytopharmaceuticals.	Biotechnology Laboratory, Chemical Engineering Department, National Technical University of Athens, 5 Iroon Polytechniou Str, Zografou Campus, 15700, Athens, Greece. EMAIL: hristako@orfeas.chemeng.ntua.gr	Katapodis, P. Christakopoulos, P.	NutraCos (2005), Volume 4, Number 2, pp. 17-21, 10 refs. ISSN: 1720-4011 Published by: B5 srl, Milano	20050101	other	Results are presented of a study on the production and biological activities of acidic and feruloyl oligosaccharides from xylan by family 10 and 11 endoxylanases [endo-1,4-β-xylanase] from Thermoascus aurantiacus and Sporotrichum thermophile [Myceliophthora thermophila]. Both family 10 and 11 endoxylanases were able to release branched xylooligosaccharides from xylan. Feruloyl oligosaccharides acted as antioxidants and showed protective effects against copper-catalysed peroxidation of low-density lipoproteins. Acidic xylooligosaccharides, products of 4-O-methyl-D-glucurono-D-xylan hydrolysis, functioned as plant growth regulators and antibacterial agents. Aldotetrauronic acid induced callus and somatic embryogenesis in culture explants of common mallow (Malva sylvestris) and cotton (Gossypium hirsutum). Aldopentauronic acid caused an increase in both the number of regenerants and their fresh weight to cucumber (cultivars Stella and Gilda) liquid culture in plant airlift bioreactors. Both acidic xylooligosaccharides were active against Bacillus cereus, Micrococcus flavus and Helicobacter pylori.
CABA/2005:39802	Two new records of thermophilic Myceliophthora in China.	College of Plant Protection, Shandong Agricultural University, Taian, Shandong 271018, China. EMAIL: lidc20@sdau.edu.cn	Wang DongMei Li DuoChuan Zhao ChunQing Wang, D. M. Li, D. C. Zhao, C. Q.	Journal of Fungal Research (2004), Volume 2, Number 2, pp. 47-49, 6 refs. ISSN: 1672-3538 Published by: Jilin Agricultural University, Changchun	20040101	other	(b) (4)
TOXCENTER/2004:218312	Evaluation of certain food additives and contaminants		Anonymous	World Health Organization technical report series, (2004) Vol. 922, pp. 1-176. Journal code: 7903212. ISSN: 0512-3054. L-ISSN: 0512-3054.	20040101	duplicates	This report represents the conclusions of a Joint FAO/WHO Expert Committee convened to evaluate the safety of various food additives, with a view to recommending acceptable daily intakes (ADIs) and to prepare specifications for the identity and purity of food additives. The first part of the report contains a general discussion of the principles governing the toxicological evaluation of food additives (including flavouring agents) and contaminants, assessments of intake, and the establishment and revision of specifications for food additives. A summary follows of the Committee's evaluations of toxicological and intake data on various specific food additives (alpha-amylase from Bacillus lichenformis containing a genetically engineered alpha-amylase gene from B. licheniformis, annatto extracts, curcumin, diacetyl and fatty acid esters of glycerol, D-tagatose, laccase from Myceliophthora thermophila expressed in Aspergillus oryzae, mixed xylanase, beta-glucanase enzyme preparation produced by a strain of Humicola insolens, neotame, polyvinyl alcohol, quillaia extracts and xylanase from Thermomyces lanuginosus expressed in Fusarium venenatum), flavouring agents, a nutritional source of iron (ferrous glycinate, processed with citric acid), a disinfectant for drinking-water (sodium dichloroisocyanurate) and contaminants (cadmium and methylmercury). Annexed to the report are tables summarizing the Committee's recommendations for ADIs of the food additives, recommendations on the flavouring agents considered, and tolerable intakes of the contaminants considered, changes in the status of specifications and further information requested or desired.

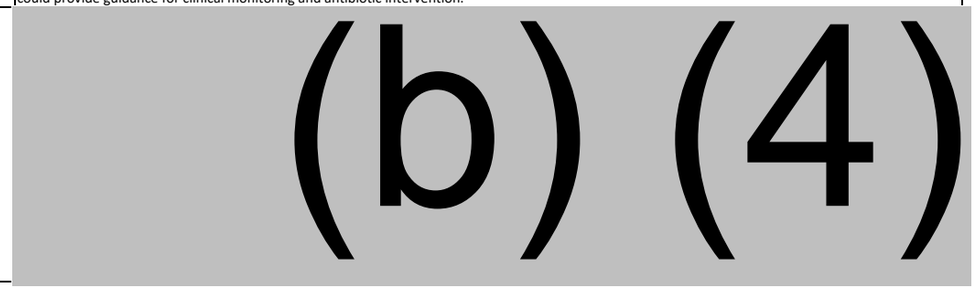
CABA/2005:78293	Safety evaluation of certain food additives and contaminants.	Viale delle Terme di Caracalla, 00100 Rome, Italy.		WHO Food Additives Series (2004), Number No.52, pp. vi + 667 pp., many ref. ISSN: 0300-0923 Published by: World Health Organization, Geneva	20040101	other	This volume contains monographs prepared at the 61st meeting of the Joint FAO/WHO Expert Committee on Food Additives, which met in Rome, Italy, from 10 to 19 June 2003. The monographs in this volume summarize data on the safety of a number of food additives, including α-amylase from Bacillus licheniformis containing a genetically engineered α-amylase gene from B. licheniformis, annatto extracts, curcumin, diacetyltartaric and fatty acid esters of glycerol, laccase from Myceliophthora thermophila expressed in Aspergillus oryzae, mixed xylanase, β-glucanase enzyme preparation, produced by a strain of Humicola insolens, neotame, polyvinyl alcohol, D-tagatose and xylanase from Thermomyces lanuginosus expressed in Fusarium venenatum. Monographs on 6 groups of related flavouring agents evaluated by the Procedure for the Safety Evaluation of Flavouring Agents are also included, together with a monograph on the safety evaluation of natural flavouring complexes. This volume also contains monographs summarizing the toxicological and intake data for a nutritional source of iron, ferrous glycinate, and a disinfectant for drinking-water, sodium dichloroisocyanurate, and for the contaminants cadmium and methylmercury. This volume and others in the WHO Food Additives Series contain information that is useful to those produce and use food additives and veterinary drugs and those involved with controlling contaminants in food, government and food regulatory officers, industrial testing laboratories, toxicological laboratories, and universities.
CABA/2004:163708	Evaluation of certain food additives and contaminants.	Viale delle Terme di Caracalla, 00100 Rome, Italy.		WHO Technical Report Series (2004), Number No.922, pp. x + 176 pp., 24 refs. ISSN: 0512-3054 Published by: World Health Organization, Geneva	20040101	other	This report discusses the principles governing the toxicological evaluation of food additives and contaminants, assessments of intake, and the establishment and revision of specifications for food additives. A summary follows of the Committee's evaluations of toxicological and intake data on various specific food additives (α-amylase from Bacillus licheniformis containing a genetically engineered α-amylase gene from B. licheniformis, annatto extracts, curcumin, diacetyl and fatty acid esters of glycerol, D-tagatose, laccase from Myceliophthora thermophila expressed in Aspergillus oryzae, mixed xylanase, β-glucanase enzyme preparation produced by a strain of Humicola insolens, neotame, polyvinyl alcohol, quillaia extracts, and xylanase from Thermomyces lanuginosus expressed in Fusarium venenatum), flavouring agents, a nutritional source of iron (ferrous glycinate, processed with citric acid), a disinfectant for drinking water (sodium dichloroisocyanurate), and contaminants (cadmium and methylmercury). The Committee's recommendations for ADIs (acceptable daily intakes) of the food additives, recommendations on the flavouring agents considered, tolerable intakes of the contaminants considered, and changes in the status of specifications and further information requested or desired are summarized in tables.
CABA/2003:182363	Purification and characterization of a Fusarium oxysporum feruloyl esterase (FoFAE-I) catalysing transesterification of phenolic acid esters.	Biotechnology Laboratory, Chemical Engineering Department, National Technical University of Athens, 5 Iroon Polytechniou Str, Zografou Campus, Athens 15700, Greece. EMAIL: pc@biocentrum.dtu.dk	Topakas, E. Stamatis, H. Mastihubova, M. Biely, P. Kekos, D. Macris, B. J. Christakopoulos, P.	Enzyme and Microbial Technology (2003), Volume 33, Number 5, pp. 729-737, 61 refs. ISSN: 0141-0229 DOI: https://doi.org/10.1016/S0141-0229(03)00213-8 Published by: Elsevier Science Inc., New York	20030101	other	An extracellular feruloyl esterase (FoFAE-I) from the culture filtrates of Fusarium oxysporum F3 was purified to homogeneity by ion-exchange, hydrophobic interaction and gel filtration chromatographies. The protein corresponded to molecular mass and pI values of 31 kDa and 9.5, respectively. The enzyme was optimally active at pH 7.0 and 55 °C. The purified esterase was fully stable at pH 7.0-9.0 and temperature up to 30 °C. Determination of kcat/Km revealed that the enzyme hydrolysed methyl p-coumarate (MpCA) 4.5, 9, and 239 times more efficiently than methyl caffeate (MCA), methyl ferulate (MFA) and methyl sinapinate (MSA), respectively. The enzyme was active on substrates containing ferulic acid ester linked to the C-5 and C-2 linkages of arabinofuranose but showed preference for the ester at position 2. 4-Nitrophenyl-2-O-trans-feruloyl-α-L-arabinofuranoside (NPh-5-Fe-Araf) was hydrolysed 100 times more efficiently than 4-nitrophenyl-5-O-trans-feruloyl-α-L-arabinofuranoside (NPh-2-Fe-Araf). Ferulic acid (FA) was efficiently released from destarched wheat bran (DSWB) when the esterase was incubated together with xylanase from Sporotrichum thermophile (a maximum of 92% total ferulic acid released after 4 h incubation). FoFAE-I by itself could release FA but at a level almost five-fold lower than that obtained in the presence of xylanase. The potential of FAE-I for the synthesis of various phenolic acid esters was tested using as a reaction system a surfactantless microemulsions formed in ternary mixture consisting of n-hexane, 1-butanol and water.
CABA/2003:96345	Fungal biota associated with bats in Western France.	Laboratoire de Parasitologie-Mycologie, Groupe d'Etude des Interactions Hote-Parasite, UPRES-EA 3142, Centre Hospitalier Universitaire, 4, rue Larrey, 49033 Angers, France. EMAIL: gerald.larcher@univ-angers.fr	Larcher, G. Bouchara, J. P. Pailley, P. Montfort, D. Beguin, H. Bievre, C. de Chabasse, D. de Bievre, C.	Journal de Mycologie Medicale (2003), Volume 13, Number 1, pp. 29-34, 58 refs. ISSN: 1156-5233 Published by: Editions Masson, Paris	20030101	other	Although the association between tropical bats and pathogenic fungi has been reported long ago, no study has been performed in Europe to our knowledge. The fungal biota associated with European Chiroptera in the region of Pays de la Loire (Western France) was therefore analysed to determine if these mammals harbour fungi pathogenic for humans or household animals. One hundred and seven samples of guano or hair/skin coming from six bat species were collected from caverns of Western France during the national annual counting of Chiroptera performed in the course of the hibernation period. They consisted of 82 samples of guano and 25 samples of hair/skin swabs. Mycological analysis of these samples revealed the great diversity of the fungal biota, more pronounced for Rhinolophidae Chiroptera than for Vespertilionidae. Among the pathogenic fungi, yeasts of Candida genus (C. glabrata and C. guilliermondii) and eight species of keratinophilic fungi (Trichophyton terrestre, Scopulariopsis brevicaulis, Aphanoascus fulvescens, Myceliophthora thermophila , Chrysosporium lobatum, Chrysosporium merdarium, Chrysosporium pseudomerdarium and Chrysosporium pruinosum) were identified. In addition, an Onygenaceae highly resembling Histoplasma capsulatum was isolated. Our results demonstrate that several human or animal pathogenic fungi may be associated with bats. Moreover, their habit to gather, sometimes in vast colonies in certain caverns, creates an environmental medium suitable for the proliferation of these fungi by enrichment of the soil with hair, skin, faeces and bats corpses. However, due to the particular habitat of these animals, one may consider from this study that bats do not constitute a real danger for human or animal health.
CABA/2003:145678	Studies on thermophilic fungi of Agaricus bisporus compost.	National Research Centre for Mushroom, Chambaghat, Solan 173 213, India.	Vijay, B. Sharma, S. R. Lakhanpal, T. N.	Journal of Mycology and Plant Pathology (2002), Volume 32, Number 2, pp. 240-243, 15 refs. ISSN: 0971-9393; 0975-4180 Published by: Indian Society of Mycology and Plant Pathology, Udaipur	20020101	other	An in vitro study was conducted to determine whether thermophilic fungi as well as their culture filtrates could hamper or increase the growth of Agaricus bisporus and its fungal competitors/pathogenic fungi. The thermophilic fungi used in the study include Chaetomium thermophilium, Humicola grisea, H. insolens, Scytalidium thermophilium, Sporotrichum thermophilum [Myceliophthora thermophila] and Thermomyces lanuginosus. The culture filtrates used were C. indicum, Cladobotryum dendroides [Hypomyces rosellus], Sepedonium maheshwarianum, Trichoderma viride and Verticillium fungicola. Most of the thermophilic fungi reduced the growth of A. bisporus and its competitors, whereas H. insolens and Scytalidium thermophilium increased the growth of A. bisporus by 4.3 and 22.6%, respectively. A marked reduction in the growth of T. viride was observed with all thermophilic fungi, wherein the maximum was by C. thermophilium and the least was by S. thermophilium. Almost all the culture filtrates reduced the growth of A. bisporus and its competitors. However, a marginal increase in the growth of A. bisporus, Chaetomium indicum and V. fungicola was observed in case of H. insolens, C. thermophilium and H. grisea, respectively. Cladobotryum dendroides was the most sensitive fungus towards different culture filtrates and H. grisea reduced its growth by 86.1%. The least inhibition in this case was recorded with Chaetomium thermophilium. A maximum reduction in the growth due to culture filtrates was recorded in V. fungicola compared with the other test fungi.
CABA/2003:14805	Miscellaneous opportunistic fungi: Microascaceae and other Ascomycetes, Hyphomycetes, Coelomycetes, and Basidiomycetes. Mycology Series Volume 16	Microfungus Collection and Herbarium, Devonian Botanic Garden and Medical Microbiology and Immunology, University of Alberta, Edmonton, Alberta, Canada. Microfungus Collection and Herbarium, Devonian Botanic Garden and Medical Microbiology and Immunology, University of Alberta, Edmonton, Alberta, Canada.	Sigler, L. Editor(s): Howard, D. H.	Pathogenic fungi in humans and animals (2002), Number Ed. 2, pp. 637-676, 119 refs. Published by: Marcel Dekker, Inc., New York	20020101	other	(b) (4)

SCISEARCH/2002:88161	Disseminated phaeohyphomycosis: Review of an emerging mycosis	Vet Adm Med Ctr, Div Infect Dis 111D, 4500 S Lancaster Rd, Dallas, TX 75216 USA (Reprint) E-mail: sanjay.revankar@med.va.gov E-mail: sanjay.revankar@med.va.gov Vet Adm Med Ctr, Div Infect Dis 111D, Dallas, TX 75216 USA Univ Texas, SW Med Ctr, Dept Med, Dallas, TX USA Univ Texas, Hlth Sci Ctr, Dept Med, San Antonio, TX 78284 USA Univ Texas, Hlth Sci Ctr, Dept Pathol, San Antonio, TX 78284 USA Audie L Murphy Mem Vet Adm Med Ctr, San Antonio, TX 78284 USA	Revankar, SG (Reprint) Patterson, JE Sutton, DA Pullen, R Rinaldi, MG	CLINICAL INFECTIOUS DISEASES, (15 2002 FEB 2002) Vol. 34, No. 4, pp. 467-476. ISSN: 1058-4838.	20020101	other	Disseminated phaeohyphomycosis is an uncommon infection caused by dematiaceous fungi, although the number of case reports about this infection has been increasing in recent years. A total of 72 cases are reviewed. Scedosporium prolificans is by far the most common cause. The presence of melanin in their cell walls may be a virulence factor for these fungi. The primary risk factor is decreased host immunity, although cases in apparently immunocompetent patients have been reported. Eosinophilia was seen in 11% of cases. Endocarditis is mostly reported on bioprosthetic valves, particularly those of porcine origin. The outcome of antifungal therapy remains poor, with an overall mortality rate of 79%. Special precautions taken for immunocompromised patients may help prevent exposure to fungi during the patients ' period of greatest risk. The development of newer antifungal agents and combination therapy may hold promise in improving the management of these devastating infections in the future.
CABA/2003:8313	Toxicological studies on Laccase from Myceliophthora thermophila expressed in Aspergillus oryzae.	Novozymes A/S, Krogshoejvej 36, Bagsvaerd, DK-2880, Denmark. EMAIL: dsbr@novozymes.com	Brinch, D. S. Pedersen, P. B.	Regulatory Toxicology and Pharmacology (2002), Volume 35, Number 3, pp. 296-307 ISSN: 0273-2300 DOI: https://doi.org/10.1006/rtph.2002.1538 Published by: Academic Press, Orlando	20020101	other	The bioindustrially produced enzyme Laccase can be used in different technical and food applications to facilitate processes. It can be added to different oral care products such as mouthwash, toothpaste, mints, and gums to prevent halitosis. Laccase, produced by submerged fermentation of Aspergillus oryzae, containing a gene originating from Myceliophthora thermophila , was subject to a series of toxicological tests to document its safety in use. It was not found to be mutagenic in the Salmonella typhimurium reverse mutation assay, nor did it cause chromosomal aberrations in cultured human lymphocytes. No evidence of inhalation toxicity or skin and eye irritation was found. There was no evidence of possible skin sensitization in a human skin sensitization test when Laccase was tested at 10% (w/v): thus Laccase would appear to have a low skin sensitization potential. Oral administration to rats of up to 10.0 mL/kg body wt/day (equivalent to a total organic solids dosage of 1.72 g/kg body wt/day) for 13 weeks did not cause any adverse effect .
CABA/1999:135711	Biophysical characterization of fungal phytases (myo-inositol hexakisphosphate phosphohydrolases): molecular size, glycosylation pattern, and engineering of proteolytic resistance.	VFB Department, F. Hoffmann-La Roche Ltd., CH-4070 Basel, Switzerland.	Wyss, M. Pasamontes, L. Friedlein, A. Remy, R. Tessier, M. Kronenberger, A. Middendorf, A. Lehmann, M. Schnoebelen, L. Roethlisberger, U. Kusznir, E. Wahl, G. Mueller, F. Lahm, H. W. Vogel, K. Loon, A. P. G. M. van van Loon, A. P. G. M.	Applied and Environmental Microbiology (1999), Volume 65, Number 2, pp. 359-366, 32 refs. ISSN: 0099-2240; 1098-5336 Published by: ,	19990101	other	Phytases (myo-inositol hexakisphosphate phosphohydrolases) are found naturally in plants and microorganisms, particularly fungi. Interest in these enzymes has been stimulated by the fact that phytase supplements increase the availability of phosphorus in pig and poultry feed and thereby reduce environmental pollution due to excess phosphate excretion in areas where there is intensive livestock production. The wild-type phytases from 6 different fungi, Aspergillus niger, A. terreus, A. fumigatus, Emericella nidulans, Myceliophthora thermophila and Talaromyces thermophilus, were overexpressed in filamentous fungi or yeasts and purified, and their biophysical properties were compared with those of a phytase from Escherichia coli. All of the phytases examined are monomeric proteins. While E. coli phytase is a nonglycosylated enzyme, the glycosylation patterns of the fungal phytases proved to be highly variable, differing for individual phytases, for a given phytase produced in different expression systems, and for individual batches of a given phytase produced in a particular expression system. Whereas the extents of glycosylation were moderate when the fungal phytases were expressed in filamentous fungi, they were excessive when the phytases were expressed in yeasts. However, the different extents of glycosylation had no effect on the specific activity, the thermostability, or the refolding properties of individual phytases. When expressed in A. niger, several fungal phytases were susceptible to limited proteolysis by proteases present in the culture supernatant. N-terminal sequencing of the fragments revealed that cleavage invariably occurred at exposed loops on the surface of the molecule. Site-directed mutagenesis of A. fumigatus and E. nidulans phytases at the cleavage sites yielded mutants that were considerably more resistant to proteolytic attack. Therefore, engineering of exposed surface loops may be a strategy for improving phytase stability during feed processing and in the digestive tract.
CABA/1998:151803	Site-directed mutations in fungal laccase: effect on redox potential, activity and pH profile.	Novo Nordisk Biotech, 1445 Drew Avenue, Davis, CA 95616, USA.	Xu Feng Berka, R. M. Wahleithner, J. A. Nelson, B. A. Shuster, J. R. Brown, S. H. Palmer, A. E. Solomon, E. I. Xu, F.	Biochemical Journal (London) (1998), Volume 334, Number 1, pp. 63-70, 42 refs. ISSN: 0264-6021; 1470-8728 Published by: ,	19980101	other	A Myceliophthora thermophila laccase and a Rhizoctonia solani laccase were mutated on a pentapeptide segment believed to be near the type-1 Cu site. The mutation L513F in Myceliophthora laccase and the mutation L470F in Rhizoctonia laccase took place at a position corresponding to the type-1 Cu axial methionine (M517) ligand in Zucchini ascorbate oxidase. The triple mutations V509L, S510E, G511A in Myceliophthora laccase and L466V, E467S, A468G in Rhizoctonia laccase involved a sequence segment whose homologue in ascorbate oxidase is flanked by the M517 and a type-1 Cu-ligating histidine (H512). The single mutation did not yield significant changes in the enzymic properties (including any significant increase in the redox potential of the type-1 Cu). In contrast, the triple mutation resulted in several significant changes. In comparison with the wild type, the Rhizoctonia and Myceliophthora laccase triple mutants had a phenol-oxidase activity whose pH optimum shifted 1 unit lower and higher, respectively. Although the redox potentials were not significantly altered, the Km, kcat and fluoride inhibition of the laccases were greatly changed by the mutations . The observed effects were interpreted as possible mutation -induced structural perturbations on the molecular recognition between the reducing substrate and laccase and on the electron transfer from the substrate to the type-1 Cu centre.
CABA/1998:121655	Fatal aortic Myceliophthora thermophila infection in a patient affected by cystic medial necrosis.	Microbiology Institute, Azienda Ospedaliera 'Ospedali Riuniti di Bergamo', 24128 Bergamo, Italy.	Farina, C. Gamba, A. Tambini, R. Beguin, H. Trouillet, J. L.	Medical Mycology (1998), Volume 36, Number 2, pp. 113-118, 31 refs. Published by: ,	19980101	other	(b) (4)
CABA/1995:158521	Effects of an organophosphorus insecticide on the growth and cellulolytic activity of fungi.	Department of Botany, Faculty of Science, University of Assiut, Assiut, Egypt.	Omar, S. A. Moharram, A. M. Abd-Alla, M. H.	International Biodeterioration + Biodegradation (1993), Volume 31, Number 4, pp. 305-310, 13 refs. ISSN: 0964-8305 DOI: https://doi.org/10.1016/0964-8305(93)90025-W Published by: ,	19930101	other	(b) (4)

CABA/1995:43213	Effect of elevated amendments of urea on soil ureolytic activity and mycofloral count.	Botany Department, Faculty of Science, Cairo University, Cairo, Egypt.	Salama, A. M. Abdel-Rahman, T. M. A. Abo-Ellil, A. H. Aly, M. I. A. El-Selhdar, A. A. H.	Egyptian Journal of Microbiology (1992), Volume 27, Number 2, pp. 197-214, 38 refs. ISSN: 0022-2704; 2357-0881 Published by: ,	19920101	other	Increasing concn of urea decreased total fungal counts in a clay soil (pH 6.5). No fungi were isolated in soil amended with 12.5% urea. Urea concn of >4.5% inhibited ammonification, accelerated the oxidation of ammonia to nitrates, inhibited oxidation of nitrites to nitrates and accumulation of nitrites resulting in a reduced fungal count. Fungal counts were increased by application of lower concn of urea by promotion of ammonification, reinitiation of nitrate accumulation and accumulation of nitrites. Soil urease activity was max. at urea concn of 2.5, 3.5 and 4.5% but was inhibited by urea levels above and below these concn. A total of 36 species of fungi was isolated including Aspergillus fumigatus, A. terreus, Mucor pusillus [Rhizomucor pusillus], Humicola grisea and Sporotrichum thermophilum [Myceliophthora thermophila]. No fungal species was isolated at a density of >50% of the total count at all urea concn. An increase in urea concn led to the rapid elimination of fungal species from soil. A. fumigatus, A. flavus, A. niger and Penicillium notatum were the fungi most tolerant of increasing urea concn.
CABA/1992:130923	Fatal disseminated infection caused by Myceliophthora thermophila , a new agent of mycosis : case history and laboratory characteristics.	Department of Laboratory Medicine, Geisinger Medical Center, Danville, Pennsylvania 17822, USA.	Bourbeau, P. McGough, D. A. Fraser, H. Shah, N. Rinaldi, M. G.	Journal of Clinical Microbiology (1992), Volume 30, Number 11, pp. 3019-3023, 14 refs. ISSN: 0095-1137; 1098-660X Published by: ,	19920101	other	
CABA/1990:143695	Studies on the mycoflora of Aswan High Dam Lake, Egypt: monthly variations.	Botany Department, Faculty of Science, Assiut University, Assiut, Egypt.	El-Hissy, F. T. Moharram, A. M. El-Zayat, S. A.	Journal of Basic Microbiology (1990), Volume 30, Number 2, pp. 81-94, 23 refs. ISSN: 0233-111X; 1521-4028 DOI: https://doi.org/10.1002/jobm.3620300207 Published by: ,	19900101	other	var. and 18 genera) and submerged mud (78 spp., 1 var. and 30 genera) of Aswan High Dam Lake during July 1985-Dec. 1986. The most common species were Aspergillus fumigatus, A. flavus, A. terreus, A. niger and Penicillium funiculosum. The highest fungal populations were detected in Oct., in Dec. 1985 or in Feb. 1986. Of the 12 thermophilic and thermotolerant fungal species, A. fumigatus and A. nidulans were the most common. Paecilomyces variotii, Rhizomucor pusillus, Thermomyces lanuginosus, Thermoascus thermophilus and Sporotrichum thermophilum [Myceliophthora thermophila] were fairly common in one or more localities. The physico-chemical characteristics of water and mud samples were also studied.
CABA/1993:103964	Fungal flora associated with combine harvester wheat and sorghum dusts from Egypt.		Abdel-Hafez, S. I. I. Moubasher, A. H. Shoreit, A. A. M. Ismail, M. A.	Journal of Basic Microbiology (1990), Volume 30, Number 7, pp. 467-479, 47 refs. ISSN: 0233-111X; 1521-4028 DOI: https://doi.org/10.1002/jobm.3620300702 Published by: ,	19900101	other	A total of 107 species and 8 species varieties belonging to 44 genera were collected from combine harvester wheat and sorghum dusts (35 genera and 91 species + 4 varieties) and from the atmosphere of their hay sites (26 genera and 69 species + 4 varieties) on glucose- and cellulose-Czapek's Dox agar at 28°C and 45°C. The mycoflora of wheat and sorghum dusts were basically similar on the 2 types of media and the most common fungi were: Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, A. ochraceous, A. sydowii, A. terreus, Cochliobolus spicifer, Emericella nidulans, Fusarium moniliforme [Gibberella fujikuroi], Penicillium chrysogenum, P. duclauxii, P. funiculosum and P. oxalicum. Thermophilic species were frequently encountered from the 2 substrates: Chaetomium thermophilum, Humicola grisea var. thermoidea, H. insolens, Malbranchea pulchella var. sulphurea, Rhizomucor pusillus, Sporotrichum thermophilum [Myceliophthora thermophila], Talaromyces thermophilus, Thermoascus thermophilus and Thermomyces lanuginosus. The airborne fungi in the 2 atmospheres were basically similar and the most prevalent species were members of Alternaria (3 spp.), Aspergillus (19 spp. + 2 var.), Cladosporium (2 spp.), Cochliobolus (3 spp.), Emericella (2 spp. + 2 var.), Fusarium (5 spp.), Mucor (1 sp.), Penicillium (14 spp.) and Stachybotrys (1 sp.).
CABA/1989:35840	Utilization and treatment of palm oil mill effluent by selected fungi.		Vikineswary Sabaratnam Sabaratnam, V.	Wallaceana (1988), Volume 52-53, pp. 26 ISSN: 0126-8236 Published by: ,	19880101	other	Mesophilic (28 spp.) and thermophilic (8 spp.) fungi were isolated from the effluent (POME). Aspergillus niger and Trichoderma harzianum were the most frequently occurring mesophilic while Chaetomium thermophila var. dissitum, Myceliophthora thermophila , Rhizomucor pusillus, Thermoascus aurantiacus and Thermomyces lanuginosus the most frequently isolated thermophilic spp. Mycelial extracts of M. thermophila and T. harzianum were non-toxic to mice while that of C. thermophila var. dissitum caused 50% mortality of the mice within 48 h and that of R. pusillus caused weight loss. In residues after harvesting the fungal biomass (M. thermophila and T. harzianum) did not inhibit germination of Brassica and maize seeds. The latter 2 fungi lowered BOD (by 72%) and COD (74%) and produced appreciable amounts of amylase, cellulase and lipase. The results are discussed.
CABA/1988:93800	Production of protein by thermophilic fungi from sugar-beet pulp in solid-state fermentation.	Acad. Agric., 60-624 Poznan, Poland.	Grajek, W.	Biotechnology and Bioengineering (1988), Volume 32, Number 1, pp. 255-260, 5 refs. ISSN: 0006-3592; 1097-0290 DOI: https://doi.org/10.1002/bit.260320218 Published by: ,	19880101	other	
CABA/1988:93553	Thermophilic and thermotolerant fungi associated with seeds of five members of Umbelliferae from Egypt.	Dep. Bot., Fac. Sci., Sohag, Univ. Assiut, Egypt.	Abdel-Hafez, A. I. I. Moharram, A. M. M. Abdel-Mallek, A. Y.	Cryptogamie, Mycologie (1987), Volume 8, Number 4, pp. 315-320, 22 refs. ISSN: 0181-1584; 1776-100X Published by: ,	19870101	other	
CABA/1976:61719	Experimentally induced mycetoma: species of Sporotrichum and Sporothrix.	Dep. Chem., Loughborough Univ. Technol., Loughborough, UK.	Stretton, R. J. 13 fig.	Mycopathologia (1975), Volume 55, Number 2, pp. 83-90, 39 refs. ISSN: 0301-486X; 1573-0832 DOI: https://doi.org/10.1007/BF00444276 Published by: ,	19750101	other	
CABA/1975:62942	Influence of antibiotics on the swelling phase and spore germination of thermophilic fungi.	Univ. Saugar, Sagar, MP, India.	Thakre, R. P. Johri, B. N. 7 graphs, 3 tab.	Hindustan Antibiotics Bulletin (1973), Volume 16, Number 2/3, pp. 109-114, 7 refs. ISSN: 0018-1935 Published by: ,	19730101	other	

Annex 2 - Part B: 2023 Update Search List -Toxicological Relevant

IDFromSource	Title	Company/Institute	Author	Source	Publ. Date	Category	Abstracts
MEDLINE/2026784371	Isolation of the Anti-Inflammatory Agent Myceliostatin from a Methionine-Enriched Culture of Myceliophthora thermophila ATCC 42464.	Department of Molecular Target Medicine, Aichi Medical University School of Medicine, Nagakute 480-1195, Nagoya, Aichi, Japan. Department of Pharmacology, Aichi Medical University School of Medicine, Nagakute 480-1195, Nagoya, Aichi, Japan. Shenzhen Key Laboratory of Marine Bioresource and Eco-environmental Science, College of Life Sciences and Oceanography, Shenzhen University, Shenzhen 518060, People's Republic of China.	Wu Yanhua Lin Yinzhi Kojima Shiori Umezawa Kazuo Okada Shoshiro Shen Junfeng Wang Liyan	Journal of natural products, (20230224) Vol. 86, No. 2, pp. 239-245. Electronic Publication Date: 3 Feb 2023 Journal code: 7906882. E-ISSN: 1520-6025. L-ISSN: 0163-3864.	20230101	Tox	Safe and effective nonsteroidal anti-inflammatory drugs are needed. Meanwhile, addition of amino acids to cultures of microorganisms is likely to increase the possibility of novel secondary metabolite isolation. In the course of screening for anti-inflammatory agents using cellular lipopolysaccharide (LPS)-induced nitric oxide (NO) production, two new related compounds with the myceliothermophin structure from a methionine-enriched culture of Myceliophthora thermophila ATCC 42464 were isolated. The new compounds have an additional methylthio group on the myceliothermophin structure and were named myceliostatins A and B. Both compounds inhibited LPS-induced NO production at nontoxic concentrations in macrophage-like mouse monocytic leukemia RAW264.7 cells. Myceliostatin B inhibited the expression of LPS-induced iNOS, IL-6, and IL-1β and the upstream NF-κB activity in situ and in vitro. Finally, it was found to inhibit NF-κB binding to DNA in the reconstruction system with purified p65. Myceliostatin B also inhibited LPS-induced reactive oxygen species (ROS) production. Thus, myceliostatin B, a novel compound derived from M. thermophila, was found to be a new anti-inflammatory and antioxidant compound directly inhibiting NF-κB.
MEDLINE/2026213902	A fatal case of pneumonia caused by Thermothelomyces thermophila.	Department of Laboratory Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, South Korea. Department of Pediatrics, College of Medicine, The Catholic University of Korea, Seoul, South Korea. Department of Radiology, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, South Korea. Department of Laboratory Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, South Korea. yjpk@catholic.ac.kr	Yoo In Young Kim Kyunghun Lee Jae Wook Kang Hyun Mi Im Soo-Ah Park Yeon-Joon	Journal of infection and chemotherapy : official journal of the Japan Society of Chemotherapy, (2023 Jan) Vol. 29, No. 1, pp. 87-89. Electronic Publication Date: 24 Sep 2022 Journal code: 9608375. E-ISSN: 1437-7780. L-ISSN: 1341-321X.	20230101	Tox	Thermothelomyces thermophila (formerly Myceliophthora thermophila) is usually found in soil and specifically compost as an environmental dematiaceous fungus. Here, we report the first case of invasive pulmonary infection caused by T. thermophila in a pediatric patient with acute lymphoblastic leukemia. T. thermophila was serially cultured from bronchoalveolar lavage (BAL) fluid and sputum samples obtained from this patient with respiratory symptoms. The patient received antifungal treatment with liposomal amphotericin B (160 mg daily) and itraconazole (200 mg daily) combination therapy, but she died. By the antifungal susceptibility testing, low minimum inhibitory concentrations (MIC) were observed for itraconazole (MIC 0.06 μg/mL), voriconazole (MIC 0.12 μg/mL), and posaconazole (MIC 0.03 μg/mL) but high MIC was observed with amphotericin B (MIC 4.0 μg/mL). Since T. thermophila is usually found in the environment, it can be considered as a contaminant and may cause difficulties in diagnosis. Therefore, it is necessary to confirm the potential of pathogen through repeated culture and to conduct an antifungal susceptibility testing to find a suitable antifungal agent. Copyright © 2022 Japanese Society of Chemotherapy and The Japanese Association for Infectious Diseases. Published by Elsevier Ltd. All rights reserved.
MEDLINE/2021377838	Isolation and characterization of a new cytotoxic polyketide-amino acid hybrid from Thermothelomyces thermophilus ATCC 42464.	Engineering Research Center of Industrial Microbiology, College of Life Sciences, Fujian Normal University, Fuzhou, China. State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China.	Gao Yang-Le Zhang Ming-Liang Wang Xue Zhang Huai-Dong Huang Jian-Zhong Li Li	Natural product research, (2021 Jun) Vol. 35, No. 11, pp. 1792-1798. Electronic Publication Date: 16 Jul 2019 Journal code: 101167924. E-ISSN: 1478-6427. L-ISSN: 1478-6419.	20210101	Tox	Fungi are a rich source of novel anticancer compounds. Bioassay-guided isolation has led to the isolation of four polyketide-amino acid hybrid compounds with trans-fused decalin system from the fungus Thermothelomyces thermophilus ATCC 42464 (=Myceliophthora thermophila ATCC 42464): myceliothermophins A, B, E and F (1-4). The structure of the new compound (myceliothermophin F, compound 4) was clearly determined by a combination of nuclear magnetic resonance (NMR) analysis and high-resolution electrospray ionisation mass spectroscopy (HRESIMS). The new compound exhibited promising cytotoxicity against some cell lines derived from colorectal carcinoma , hepatic carcinoma and gastric carcinoma , indicating that compounds with trans-fused decalin system would be promising in the course of developing novel anticancer drugs.
MEDLINE/2023358726	Safety evaluation of a β-mannanase enzyme preparation produced with Thermothelomyces thermophilus expressing a protein-engineered β-mannanase gene.	BASF Corporation, San Diego, California, United States of America. Experimental Toxicology and Ecology, BASF SE, Ludwigshafen am Rhein, Germany. Global Service Cluster Safety, BASF SE, Ludwigshafen am Rhein, Germany.	Kern Andreas Shanahan Diane Buesen Roland Geiger Dominik	PloS one, (2020) Vol. 15, No. 12, pp. e0243647. Electronic Publication Date: 10 Dec 2020 Journal code: 101285081. E-ISSN: 1932-6203. L-ISSN: 1932-6203. Report No.: PMC-PMC7728267.	20200101	Tox	Mannanase 19287 enzyme is an engineered β-mannanase that can be added to diets for animals raised for human consumption to hydrolyze β-mannans. Established toxicological analyses were conducted with the enzyme preparation to ensure the safety of this product for the intended use. The mannanase 19287 preparation was produced with Thermothelomyces thermophilus strain DSM 33149. In vitro toxicity studies presented here used dosages of the mannanase 19287 test articles up to 5000 μg/plate. For in vivo toxicity studies in Wistar rats, test articles were administered at 5.1 mg/L for inhalation toxicity and up to 15,000 mg/kg rat feed for oral toxicity , based on the Total Organic Solids (TOS) content in each test article. No treatment related adverse effects were reported in any study. The No Observed Adverse Effect Levels in the high dose group of the subchronic oral toxicity study were calculated as 1117-1298 mg TOS/kg bw/day in rats. Comparing these values to an Estimated Daily Intake for poultry demonstrated safety factors larger than 5000. Our results confirm that T. thermophilus fulfills the recognized safety criteria for the manufacture of food enzyme preparations and represent the first peer-reviewed safety evaluation of an enzyme preparation by T. thermophilus. The results of the toxicity studies presented herein attest to the safety of the mannanase 19287 enzyme for its intended use.

MEDLINE/2026915300	Safety and efficacy of a feed additive consisting of endo-1,4-β-d-mannanase produced by Thermothelomyces thermophilusDSM 33149 (Natupulse® TS/TS L) for chickens and turkeys for fattening, minor poultry species for fattening and ornamental birds (BASF SE).		Bampidis Vasileios Azimonti Giovanna Bastos Maria de Lourdes Christensen Henrik Durjava Mojca Dusemund Birgit Kouba Maryline Lopez-Alonso Marta Lopez Puente Secundino Marcon Francesca Mayo Baltasar Pechova Alena Petkova Mariana Ramos Fernando Sanz Yolanda Villa Roberto Edoardo Woutersen Ruud Brantom Paul Herman Lieve Louro Henriqueta Anguita Montserrat Brozzi Rosella Galobart Jaume Innocenti Matteo Lorenzo Ortuno Jordi Pettenati Elisa Pizzo Fabiola	EFSA journal. European Food Safety Authority, (2023 Feb) Vol. 21, No. 2, pp. e07873. Electronic Publication Date: 28 Feb 2023 Journal code: 101642076. E-ISSN: 1831-4732. L-ISSN: 1831-4732. Report No.: PMC-PMC9972018.	20230101	Tox	Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of endo-1,4-β-d-mannanase produced by Thermothelomyces thermophilus DSM 33149 (Natupulse® TS/TS L) as a zootechnical feed additive for chickens and turkeys for fattening, minor poultry species for fattening and ornamental birds. The additive under assessment, Natupulse® TS/TS L, does not pose any safety concern regarding the production strain. The FEEDAP Panel concluded that the additive is tolerated by chickens for fattening, and this conclusion can be extrapolated to all poultry for fattening. Due to the lack of reliable data regarding the potential of the additive to induce chromosomal damage, the FEEDAP Panel cannot conclude on the safety of the additive for the target species and on the safety for consumer. The use of the additive in animal nutrition is considered safe for the environment. The additive is considered not to be irritant to the skin and eyes but is considered a respiratory sensitiser, although exposure by inhalation is unlikely. The Panel could not conclude on the potential of the additive to be a skin sensitiser. Due to the lack of reliable data, the FEEDAP Panel considered that a potential of the additive to induce chromosomal damage in exposed unprotected users cannot be excluded. Consequently, exposure of users should be minimised. The Panel concluded that the additive Natupulse® TS/TS L has the potential to be efficacious in chickens for fattening at the proposed conditions of use and this conclusion can be extrapolated to turkeys for fattening, minor poultry species for fattening and ornamental birds. © 2023 European Food Safety Authority. EFSA Journal published by Wiley-VCH GmbH on behalf of European Food Safety Authority.
MEDLINE/2024155211	Characterization of the Blood and Cerebrospinal Fluid Microbiome in Children with Bacterial Meningitis and Its Potential Correlation with Inflammation.	Key Laboratory of Molecular Genetics, Shanghai Institute of Nutrition and Health, University of Chinese Academy of Sciences, Chinese Academy of Sciences (CAS), Shanghai, China. Key Laboratory of Major Diseases in Children, Ministry of Education, Department of Infectious Diseases, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing, China. Research Unit of Critical Infection in Children, Chinese Academy of Medical Sciences, Beijing, China.	Liao Huiping Zhang Yuchao Guo Wei Wang Hailong Ye Haocheng Wu Kai Zhang Yu-Hang Zhu Yufei Hu Landian Kong Xiangyin Wang Xi Guo Lingyun Guo Yongli Liu Gang	mSystems, (2021 Jun 29) Vol. 6, No. 3, pp. e0004921. Electronic Publication Date: 8 Jun 2021 Journal code: 101680636. ISSN: 2379-5077. L-ISSN: 2379-5077. Report No.: PMC-PMC8269202.	20210101	Tox	Bacterial meningitis shows a higher incidence in children than adults, but signs may be scarce. Although some pathogenic microorganisms of meningitis from cerebrospinal fluid (CSF) have been reported, the signature of the representative microbiota in CSF and blood samples from patients remains incompletely revealed. To extend the understanding of the microbiome in patients , we recruited 32 children with bacterial meningitis, 30 undiagnosed infectious children, and 10 matched healthy individuals, which was followed by untargeted metagenomic next-generation sequencing (mNGS) and bioinformatic analysis. Our results showed that children with bacterial meningitis exhibited different microbiome signatures in their CSF and blood compared with undiagnosed and healthy children, and patients could be divided into varied subsets according to these signatures, including Escherichia coli, Klebsiella pneumoniae, Thermothelomyces thermophila , Lactobacillus acidophilus, and Staphylococcus haemolyticus. To further explore their potential role in patients ' conditions, we examined their correlation with clinical parameters. Importantly, microbiome signatures with compositional changes were correlated with the C-reactive protein (CRP) level in blood and granulocyte percentage in CSF. Moreover, the blood in subsets of patients with a predominance of Klebsiella pneumoniae could replace CSF as the main specimen for clinical monitoring. IMPORTANCE This study revealed the microbial compositions in children with bacterial meningitis who were treated with antibiotics and made a comprehensive comparison between blood and CSF specimens for the risk and prognosis assessment. We found that microbiome signatures could distinguish patient subsets in the children and were correlated with the CRP level in blood and granulocyte percentage in CSF. The compositional changes in representative microbiota constituents could provide guidance for clinical monitoring and antibiotic intervention.
BIOSIS/2022:691942	DISCOVERY AND CHARACTERIZATION OF A SPECIFIC HUMAN GUT FUNGI WITH ANTI-OBESITY EFFECTS.		Zhan, Hui Zhang, Fen Wan, Yating ZHU, Wenyi Xu, Zhilu Tang, Whitney Ng, Enders K. Wong, Simon K. Yeoh, Yun Kit Sun, Yang Miao, Yinglei Zuo, Tao Chan, Francis K. Ng, Siew C.	Gastroenterology, (MAY 2022) Vol. 162, No. 7, Suppl. SUPPL, pp. S1103. http://www.gastrojournal.org/. Meeting Info.: Digestive Disease Week (DDW). San Diego, CA, USA. May 21 - 24, 2022. CODEN: GASTAB. ISSN: 0016-5085. E-ISSN: 1528-0012.	20220101	Tox	

CAPLUS/2021:1481423	Mining the capacity of human-associated microorganisms to trigger rheumatoid arthritis-A systematic immunoinformatics analysis of T cell epitopes	Institute of Physiology and Biochemistry 'Ivan Djaja', Faculty of Biology, University of Belgrade, Serbia	Repac, Jelena Mandic, Marija Lunic, Tanja Bozic, Bojan Bozic Nedeljkovic, Biljana	PLoS One (2021), 16(6), e0253918 CODEN: POLNCL; ISSN: 1932-6203 URL: https://journals.plos.org/plosone/	20210101	Tox	Autoimmune diseases , often triggered by infection , affect ~5% of the worldwide population. Rheumatoid Arthritis (RA)-a painful condition characterized by the chronic inflammation of joints-comprises up to 20% of known autoimmune pathologies, with the tendency of increasing prevalence. Mol. mimicry is recognized as the leading mechanism underlying infection -mediated autoimmunity, which assumes sequence similarity between microbial and self-peptides driving the activation of autoreactive lymphocytes. T lymphocytes are leading immune cells in the RA-development. Therefore, deeper understanding of the capacity of microorganisms (both pathogens and commensals) to trigger autoreactive T cells is needed, calling for more systematic approaches. In the present study, we address this problem through a comprehensive immunoinformatics anal. of exptl. detd. RA-related T cell epitopes against the proteomes of Bacteria, Fungi, and Viruses, to identify the scope of organisms providing homologous antigenic peptide determinants. By this, initial homol. screening was complemented with de novo T cell epitope prediction and another round of homol. search, to enable: (i) the confirmation of homologous microbial peptides as T cell epitopes based on the predicted binding affinity to RA-related HLA polymorphisms; (ii) sequence similarity inference for top de novo T cell epitope predictions to the RA-related autoantigens to reveal the robustness of RA-triggering capacity for identified (micro/myco)organisms. Our study reveals a much larger repertoire of candidate RA-triggering organisms, than previously recognized, providing insights into the underestimated role of Fungi in autoimmunity and the possibility of a more direct involvement of bacterial commensals in RA-pathol. Finally, our study pinpoints Endoplasmic reticulum chaperone BiP as the most potent (most likely mimicked) RA-related autoantigen, opening an avenue for identifying the most potent autoantigens in a variety of different autoimmune pathologies, with possible implications in the design of next-generation therapeutics aiming to induce self-tolerance by affecting highly reactive autoantigens.