U.S. FOOD & DRUG ADMINISTRATION

Human Foods Program

Carissa Lannon
Novozymes North America, Inc.
77 Perrys Chapel Church Road
P.O. Box 576
Franklinton, NC 27525

Re: GRAS Notice No. GRN 001289

Dear Ms. Lannon:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001289. We received Novozymes North America, Inc.'s (Novozymes) notice on July 23, 2025 and filed it on January 7, 2026. Novozymes submitted an amendment to the notice on May 5, 2026, that provided clarifications on the manufacturing, specifications, and safety narrative.

The subject of the notice is protein-glutamine glutaminase enzyme preparation produced by *Bacillus licheniformis* expressing a gene encoding protein-glutamine glutaminase from *Chryseobacterium viscerum* (protein-glutamine glutaminase enzyme preparation) for use as an enzyme at up 315 mg total organic solids (TOS)/ kg raw material in processing of yogurt and plant-based dairy analogues, processed fish (non-Siluriformes only), plant-based meat and fish analogues, processed yeast and yeast products, and plant-based analogues, and up to 168 mg TOS/ kg raw material in processing of dairy beverages and plant-based dairy beverage analogues. The notice informs us of Novozymes's view that this use of protein-glutamine glutaminase enzyme preparation is GRAS through scientific procedures.

Commercial enzyme preparations that are used in food processing typically contain an enzyme component that catalyzes the chemical reaction, as well as substances used as stabilizers, preservatives, or diluents. Enzyme preparations may also contain components derived from the production organism and from the manufacturing process, e.g., constituents of the fermentation media or the residues of processing aids. Novozymes' notice provides information about the components in the protein-glutamine glutaminase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, protein-glutamine glutaminase is identified by the Chemical Abstracts Service number 62213-11-0 and the Enzyme Commission Number 3.5.1.44.[1] Novozymes states that the primary sequence of protein-glutamine glutaminase consists of 188 amino acids with molecular weight of 20 kDa.

[1] https://iubmb.qmul.ac.uk/enzyme/EC3/5/1/44.html

Novozymes states that the *B. licheniformis* production organism is non-pathogenic and non-toxigenic and is a well-characterized production organism with history of safe use in the food industry. Novozymes states that the production strain, "BT13285," was constructed through transformation with an expression cassette carrying a modified gene for protein-glutamine glutaminase from *C. viscerum* and deletion of a native background protein. Novozymes states that they confirmed sequence integration by polymerase chain reaction and DNA sequencing. Novozymes evaluated the genetic stability of the production strain by measuring production of protein-glutamine glutaminase enzyme. Novozymes verified the absence of functional or transferable antibiotic resistance genes in the final production strain genome.

Novozymes states that the protein-glutamine glutaminase enzyme preparation is produced by submerged, fed-batch fermentation of a pure culture of the *B. licheniformis* production strain under controlled conditions. The protein-glutamine glutaminase enzyme is secreted into the fermentation medium and then recovered by pretreatment via pH adjustment and/or flocculation, filtration or centrifugation, and concentration via ultrafiltration and/or evaporation. The enzyme preparation is then stabilized with sodium chloride and sucrose, and formulated with water, resulting in a light brown/ yellow cloudy liquid. Novozymes states that the entire process is performed in accordance with current good manufacturing practices using food grade raw materials and processing aids that are approved for their respective uses via a regulation in 21 CFR or are GRAS for their intended use.

Novozymes has established food grade specifications including limit for lead (< 0.5 mg/kg) and states that the protein-glutamine glutaminase enzyme preparation conforms to the specifications set in the Food Chemicals Codex (FCC, 14th edition, 2024) and to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing established by the FAO/WHO Joint Expert Committee on Food Additives (JECFA, 2006). Novozymes provides results from analyses of three non-consecutive batches of protein-glutamine glutaminase enzyme concentrate to demonstrate that the manufacturing acceptance criteria can be met, including the absence of the production organism and antibiotic activity.

Novozymes intends to use protein-glutamine glutaminase enzyme preparation at a maximum use level of 315 mg total organic solids (TOS)/ kg raw material in food processing and up to 168 mg TOS/ kg raw material in beverage processing. Novozymes states that protein-glutamine glutaminase catalyzes the deamidation of glutamine residues in substrate proteins or polypeptides into glutamic acid, which releases ammonia. Novozymes notes that the final enzyme is expected to be inactivated during production of the final food. Novozymes estimates a maximum dietary exposure to protein-glutamine glutaminase enzyme preparation to be 1.63 mg TOS/kg body weight per day (mg TOS/kg bw/d) from the intended uses, and assumes that all of the protein-

glutamine glutaminase enzyme preparation will be active and remain in the final food.[2]

In support of the safety of the protein-glutamine glutaminase enzyme preparation, Novozymes discusses published information that supports the history of safe use of protein-glutamine glutaminase in food including the similarities between the subject of this notice and the wild-type. Novozymes conducted a literature search through May 2026 and states that they did not identify any information that would indicate safety concerns for the intended uses of protein-glutamine glutaminase enzyme preparation. Novozymes also discusses, as supportive evidence of the safety of the notified enzyme, a published 90-day oral toxicological study on a similar protein-glutamine glutaminase derived from a different production organism and noted that no adverse effects were observed up to highest dose tested. Novozymes further relies on published information that discusses the safety of the *B. licheniformis* production organism and use of the parent strain for production of other food ingredients.

Novozymes discusses publicly available literature, as well as the conclusions of several organizations and working groups, about the low risk of allergenicity posed by enzymes from their intended use, to address potential allergenicity of the protein-glutamine glutaminase. Based on bioinformatic analyses, using criteria recommended by FAO/WHO (FAO/WHO, 2001; Codex Alimentarius, 2009; JECFA, 2016), Novozymes reports that no significant sequence homology of protein-glutamine glutaminase, the subject of this notice, to known allergens was identified that would raise allergenicity concerns. Based on the totality of information available, Novozymes concludes that it is unlikely that oral exposure of the protein-glutamine glutaminase enzyme preparation from the intended uses will result in allergic responses.

Based on the data and information summarized above, Novozymes concludes that protein-glutamine glutaminase enzyme preparation is GRAS for its intended use.

Standards of Identity

In the notice, Novozymes states its intention to use protein-glutamine glutaminase enzyme preparation in several food categories, including foods for which standards of identity exist, located in Title 21 of the CFR. We note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity.

Section 301(ll) of the Federal Food, Drug, and Cosmetic Act (FD&C Act)

Section 301(ll) of the FD&C Act prohibits the introduction or delivery for introduction into interstate commerce of any food that contains a drug approved under section 505 of the FD&C Act, a biological product licensed under section 351 of the Public Health Service Act, or a drug or a biological product for which substantial clinical investigations

[2] Novozymes uses the Budget method to estimate the dietary exposure to protein-glutamine glutaminase enzyme preparation based on the consumption of 12.5 g solid foods per kg bw/d and 25 mL of non-milk beverages per kg bw/d (worst case scenario) containing protein-glutamine glutaminase enzyme preparation at the recommended use level.

have been instituted and their existence made public, unless one of the exemptions in section 301(ll)(1)-(4) applies. In our evaluation of Novozymes's notice concluding that protein-glutamine glutaminase enzyme preparation is GRAS under its intended conditions of use, we did not consider whether section 301(ll) or any of its exemptions apply to foods containing protein-glutamine glutaminase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing protein-glutamine glutaminase enzyme preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that Novozymes provided, as well as other information available to FDA, we have no questions at this time regarding Novozymes's conclusion that protein-glutamine glutaminase enzyme preparation is GRAS under its intended conditions of use. This letter is not an affirmation that protein-glutamine glutaminase enzyme preparation is GRAS under 21 CFR 170.35. Unless noted above, our review did not address other provisions of the FD&C Act. Food ingredient manufacturers and food producers are responsible for ensuring that marketed products are safe and compliant with all applicable legal and regulatory requirements.

In accordance with 21 CFR 170.275(b)(2), the text of this letter responding to GRN 001289 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

SUSAN J. CARLSON -S

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