

ANTI-HUMAN GLOBULIN

DG GEL 8 ANTI-IgG (Rabbit)

REF 210394

Instructions for Use

Rx Only

INTENDED USE

The DG Gel 8 Anti-IgG (Rabbit) card is for the Direct and Indirect Antiglobulin Test of human blood samples. This test does not contain antibodies to complement components.

For use with the DG Gel System.

For *in vitro* diagnostic use.

SUMMARY AND EXPLANATION

Carlo Moreschi described the principle of antiglobulin technique in 1908¹. In 1945, Coombs and his co-workers Mourant and Race, unaware of this previous description, published and introduced the use of anti-human globulin for the detection of red blood cells coated with non-agglutinating antibodies². After Coombs' publication the antiglobulin test was rapidly applied in regular clinical laboratory practice and must rank as almost as important as the discovery of the ABO groups¹. The monospecific anti-IgG Antiglobulin Test is based on the use of anti-human globulin that allows the detection of red blood cells coated with immunoglobulin (IgG).

The Direct Antiglobulin Test allows the detection of red blood cells sensitized *in vivo* by immunoglobulin.

The Indirect Antiglobulin Test allows the detection of red blood cell antibodies present in the patient's serum or plasma by *in vitro* sensitization of red blood cells and the determination of red blood cells antigens. The goal of screening for unexpected antibodies is detection of clinically significant antibodies present in the donor's or patient's sample. In a positive screening of unexpected antibodies, the autocontrol will indicate whether it is due to the presence of an autoantibody, an alloantibody or both. In the antiglobulin crossmatch test the donor's red blood cells combined with the recipient's serum or plasma will show the presence or absence of unexpected antibodies in the recipient's blood that are specific to the antigens of the donor's red blood cells.

The Indirect Antiglobulin Test is also used for investigation purposes such as titration of antibodies against red blood cell antigens. In this case the serum or plasma sample should be diluted in Grifols Diluent in order to prepare the set of dilutions before performing the Indirect Antiglobulin Test.

PRINCIPLE OF THE TEST

The principle of the test is based on the gel technique described by Yves Lapierre³ in 1985 for detecting red blood cell agglutination reactions. The DG Gel 8 cards are composed of eight microtubes. Each microtube is made of a chamber, also known as incubation chamber, at the top of a long and narrow microtube, referred to as the column. Buffered gel solutions containing polyclonal anti-human globulin have been prefilled into the microtubes of the plastic card. The agglutination occurs when the red blood cell sensitized *in vivo* or *in vitro* by human IgG antibodies react with the anti-human globulin present in the gel solution. The gel column acts as a filter that traps agglutinated red blood cells as they pass through the gel column during the centrifugation of the card. The gel column separates agglutinated red blood cells from non- agglutinated red blood cells based on size. Any agglutinated red blood cells are captured at the top of or along the gel column and non-agglutinated red blood reach the bottom of the microtube forming a pellet.

REAGENTS

Observable indications

Note: Inspect the condition of the cards before use (see Warnings and Precautions).

Cards with an alteration or change in color, trapped bubbles in the gel, cracked gel or gel with fissures, drying gel, presence of other artifacts, and opened or damaged seals may indicate an alteration of the product.

Material provided

Note: All microtubes contain sodium azide (NaN₃) as a preservative at a final concentration of 0.09%.

Each microtube of the DG Gel 8 Anti-IgG (Rabbit) card contains reagent (polyclonal antibodies) mixed with a gel in buffered medium with preservative. The microtubes are identified on the front label of the card.

Microtubes **IgG**: buffered low ionic strength solution (LISS) with rabbit polyclonal anti-human globulin, is produced using intermediate products produced for Diagnostic Grifols S.A. in a shared manufacturing agreement with DIAGAST, Parc Eurasante, 251 av. Eugene Avinee-BP9, 59374 Loos Cedex France; US License Number 1744.

Reagent preparation

DG Gel 8 Anti-IgG (Rabbit) cards are supplied as ready to use. The gel cards and samples to be tested should be brought to room temperature (20-25 °C) before testing.

Material required but not provided

For Manual Method

- Automatic pipettes of 10 µL, 25 µL, 50 µL and 1 mL.
- Disposable pipette tips.
- Glass or plastic test tubes.
- Grifols Diluent.
- DG THERM incubator.
- DG SPIN centrifuge.
- Reagent Red Blood Cells at 0.8% from Medion Grifols Diagnostics AG or other validated RBCs at 0.8%.
- Anti-D (RH1) IgM/IgG Blood Grouping Reagent.
- DG Reader Net or DG Reader (optional).

For Fully Automated Methods

- Grifols Diluent.
- Reagent Red Blood Cells at 0.8% from Medion Grifols Diagnostics AG or other validated RBCs at 0.8%.
- Anti-D (RH1) IgM/IgG Blood Grouping Reagent.
- Grifols Wash Solution A and Grifols Wash Solution B.
- Erytra Eflexis, Erytra or WADiana Compact.

STORAGE AND STABILITY

- Do not use beyond the expiration date.
- Stored upright (as indicated by the two arrows on the outer packaging) with seal intact at 2-25 °C.
- Do not freeze.
- Do not expose cards to excessive heat.

WARNINGS AND PRECAUTIONS

- The results by themselves alone are not a clinical diagnosis. Evaluate the results together with the patient's clinical information and other data.
- If you observe microbiological contamination, alterations or changes in color, or other artifacts do not use the card.
- If you observe trapped bubbles in the gel, cracked gel or gel with fissures, drying gel, or gel without a visible fine line of supernatant do not use the card.
- Do not use the card if opened or if the aluminum film seal is damaged.
- If you identify incorrect temperature conditions during storage or shipment, do not use the cards.
- If you identify improper storage or shipping conditions that results in dispersed drops observed at the top of the microtube, the card should be centrifuged with the DG SPIN before use. If after one centrifugation with the DG SPIN the drops do not descend, do not use the card.
- The product should only be used by qualified personnel.
- The use of volumes and/or red blood cell suspension in concentrations other than those indicated in the method may modify the reaction and lead to incorrect test results, i.e., false positive or false negative results.
- The use of diluents other than Grifols Diluent for the red blood cell suspension may modify the reaction and lead to incorrect test results.
- Do not use a centrifuge other than the DG SPIN centrifuge.
- Do not use enzyme treated red blood cells.
- All products with animal derived material, and human blood products and samples should be handled as if they were potentially capable of transmitting infectious diseases.
- Once used, dispose the product in containers for biological waste, according to local, state and national regulations.
- If you have any questions or need further information on the use of this product, please contact your local Grifols service representative.

SPECIMEN COLLECTION AND PREPARATION

Blood samples collected in EDTA or sodium citrate should be used. The collection, separation and handling the blood should be performed by qualified technical personnel according to current standards⁴⁻⁵, and following the instructions of the manufacturer of the material used for collecting the sample. The recommended centrifugation range is 1000 - 2000 x g for 5 minutes.

Do not use grossly hemolyzed, cloudy or contaminated samples.

Use serum or plasma collected for screening and/or identification of unexpected antibodies, crossmatch tests, autocontrol and titration. The samples should be tested as soon as possible. Frozen samples stored up to 5 years at -20 °C or colder may be used after thawing. If the recipient has been pregnant or transfused within the previous three months samples stored at 2-8 °C should be used within 72 hours after collection. Additionally, for titration, plasma from bags collected in CPD can be used until the expiration date indicated on the bag's label.

Use the red blood cells collected for Direct Antiglobulin Test, crossmatch tests and autocontrol. If necessary, samples stored at 2-8 °C can be used up to 72 hours after their collection, except for Direct Antiglobulin Test where sample storage less than 24 hours is recommended.

Red blood cells from bags collected in ACD, CPD, CPDA-1, CP2D or AS-1 (Adsol) or AS-3 can also be used up to 7 days after the expiration date indicated on the label of the bag. If red blood cells from a bag segment are used, it is suggested that these be washed with physiological saline solution before preparing the suspension. Do not use if clots or hemolysis are observed.

For red blood cell typing, follow the Instructions for Use of the Anti-D (RH1) IgM/IgG Blood Grouping Reagent.

PROCEDURE

• For **Direct Antiglobulin** test:

1. Allow DG Gel 8 Anti-IgG (Rabbit) cards, additional reagents and the samples to reach room temperature (20-25 °C).

Note: For fully automated instruments, skip the next steps and refer to the Instructions for Use of the related instruments.

2. Identify the cards to be used and the samples to be tested.
3. Prepare a 1% red blood cell suspension in Grifols Diluent (10 µL of packed red blood cells in 1 mL of Grifols Diluent).
4. Remove the foil seal from the complete DG Gel 8 card or from the individual microtubes to be used for testing. Carefully peel off the aluminum film to prevent cross-contamination of the microtube contents among them.
5. Ensure the re-suspension of the red blood cells before use.
6. Dispense 50 µL of the 1% red blood cell suspension into the corresponding microtube.
Note: Carefully dispense the red blood cell suspension and the serum or plasma, avoiding contact of the pipette tip with the wall or the contents of the microtubes to prevent carryover.
7. Centrifuge the gel card in the DG SPIN centrifuge.
8. After centrifugation, remove the gel card from the centrifuge and read the results. Alternatively, use the DG Reader or DG Reader Net to read and to interpret the results.

• For **Indirect Antiglobulin** test:

Screening and identification of unexpected antibodies

1. Allow DG Gel 8 Anti-IgG (Rabbit) cards, additional reagents and the samples to reach room temperature (20-25 °C).

Note: For fully automated instruments, skip the next steps and refer to the Instructions for Use of the related instruments.

2. Identify the cards to be used and the samples to be tested.
3. Remove the foil seal from the complete DG Gel 8 card or from the individual microtubes to be used for testing. Carefully peel off the aluminum film to prevent cross-contamination of the microtube contents among them.
4. Thoroughly mix the vials of Reagent Red Blood Cells for the screening and/or identification of unexpected antibodies to ensure homogeneous suspension of the red blood cells before use.
5. Dispense 50 µL of the Reagent Red Blood Cells (as supplied) into the microtubes.
6. Add 25 µL of serum or plasma into the microtubes.

Note: Carefully dispense the red blood cell suspension and the serum or plasma, avoiding contact of the pipette tip with the wall or the contents of the microtubes to prevent carryover.

Note: If a very low concentrated antibody or low affinity antibody is suspected, increasing the volume of serum or plasma from 25 µL to 50 µL may improve the sensitivity of the standard technique.

7. Incubate 15 minutes at 37 °C using DG THERM incubator.

- Note:** If a very low concentrated antibody or low affinity antibody is suspected, increasing the incubation time from 15 minutes to 30 minutes may improve the sensitivity of the standard technique.
8. Centrifuge the gel card in the DG SPIN centrifuge.
 9. After centrifugation, remove the gel card from the centrifuge and read the results. Alternatively, use the DG Reader or DG Reader Net to read and to interpret the results.

Antiglobulin Crossmatch tests

1. Allow DG Gel 8 Anti-IgG (Rabbit) cards, additional reagents and the samples to reach room temperature (20-25 °C).

Note: For fully automated instruments, skip the next steps and refer to the Instructions for Use of the related instruments.
2. Identify the cards to be used and the samples to be tested.
3. Prepare a 1% donor's red blood cell suspension in Grifols Diluent (10 µL of packed red blood cells in 1 mL of Grifols Diluent).
4. Remove the foil seal from the complete DG Gel 8 card or from the individual microtubes to be used for testing. Carefully peel off the aluminum film to prevent cross-contamination of the microtube contents among them.
5. Ensure the re-suspension of the red blood cells before use.
6. Dispense 50 µL of the donor's 1% red blood cell suspension into the corresponding microtube.
7. Add 25 µL of the recipient's serum or plasma into the microtubes.

Note: Carefully dispense the red blood cell suspension and the serum or plasma, avoiding contact of the pipette tip with the wall or the contents of the microtubes to prevent carryover.

Note: If a very low concentrated antibody or low affinity antibody is suspected, increasing the volume of serum or plasma from 25 µL to 50 µL may improve the sensitivity of the standard technique.
8. Incubate 15 minutes at 37 °C using DG THERM incubator.

Note: If a very low concentrated antibody or low affinity antibody is suspected, increasing the incubation time from 15 minutes to 30 minutes may improve the sensitivity of the standard technique.
9. Centrifuge the gel card in the DG SPIN centrifuge.
10. After centrifugation, remove the gel card from the centrifuge and read the results. Alternatively, use the DG Reader or DG Reader Net to read and to interpret the results.

Autocontrol

1. Allow DG Gel 8 Anti-IgG (Rabbit) cards, additional reagents and the samples to reach room temperature (20-25 °C).

Note: For fully automated instruments, skip the next steps and refer to the Instructions for Use of the related instruments.
2. Identify the cards to be used and the samples to be tested.
3. Prepare a patient 1% red blood cell suspension in Grifols Diluent (10 µL of packed red blood cells in 1 mL of Grifols Diluent).
4. Remove the foil seal from the complete DG Gel 8 card or from the individual microtubes to be used for testing. Carefully peel off the aluminum film to prevent cross-contamination of the microtube contents among them.
5. Ensure the re-suspension of the red blood cells before use.
6. Dispense 50 µL of the patient's 1% red blood cell suspension into the corresponding microtube.
7. Add 25 µL of the patient's serum or plasma into the corresponding microtube.

- Note:** Carefully dispense the red blood cell suspension and the serum or plasma, avoiding contact of the pipette tip with the wall or the contents of the microtubes to prevent carryover.
- Note:** If a very low concentrated antibody or low affinity antibody is suspected, increasing the volume of serum or plasma from 25 μ L to 50 μ L may improve the sensitivity of the standard technique.
8. Incubate 15 minutes at 37 °C using DG THERM incubator.

Note: If a very low concentrated antibody or low affinity antibody is suspected, increasing the incubation time from 15 minutes to 30 minutes may improve the sensitivity of the standard technique.
 9. Centrifuge the gel card in the DG SPIN centrifuge.
 10. After centrifugation, remove the gel card from the centrifuge and read the results. Alternatively, use the DG Reader or DG Reader Net to read and to interpret the results.

Antibody titration test

1. Allow DG Gel 8 Anti-IgG (Rabbit) cards, additional reagents and the samples to reach room temperature (20-25 °C).

Note: For fully automated instruments, skip the next steps and refer to the Instructions for Use of the related instruments.
2. Identify the cards to be used and the samples to be tested.
3. Use Grifols Diluent to perform serial dilution of the antibody in a test tube (100 μ L of plasma or serum in 100 μ L of Grifols Diluent).

Note: Dilution set should be used immediately. If necessary, it may be used up to 1 hour after preparation.
4. Select the Reagent Red Blood Cells with the same specificity as the antibody presented in the sample. Thoroughly mix the vial of Reagent Red Blood Cells to ensure a homogeneous suspension of the red blood cells before use.

Note: If it is not possible to use Reagent Red Blood Cells, prepare a 1% red blood cell suspension in Grifols Diluent.
5. Carefully remove the aluminum foil from the entire gel card or the microtubes to be used for testing.

Note: Use the microtubes immediately once the foil has been removed.
6. Dispense 50 μ L of Reagent Red Blood Cells into the microtubes.
7. Add 25 μ L (for unexpected antibodies) or 50 μ L (for anti-A and anti-B antibodies) of each serum/plasma dilution to the same microtubes.

Note: The antibody titration test could be executed pipetting only one dilution (single dilution titration test).
8. Incubate for 15 minutes at 37 °C Δ using DG THERM incubator.
9. Centrifuge the gel card in the DG SPIN centrifuge.
10. After centrifugation, remove the gel card from the centrifuge and read the results. Alternatively, use the DG Reader or DG Reader Net to read and to interpret the results.

Red blood cell typing

Follow the Instructions for Use of the Anti-D (RH1) IgM/IgG Blood Grouping Reagent.

RESULTS

Report results as an agglutination grade, absence of agglutination or hemolysis.

Negative results: no agglutination and no hemolysis of red blood cells is visible in the microtube. In a negative result the red blood cells are located in the bottom of the gel column.

Positive results: agglutination and/or hemolysis of the red blood cells is visible in the microtube. In a positive result the agglutinated red blood cells may remain throughout the gel column showing different reaction grades (see Reaction Grades and Figure 1 for a picture of example of reaction grades). Some positive reactions may also form a pellet in the bottom of the microtube. A positive result indicates the presence of IgG antibodies in the serum or plasma sample or coated on the red blood cells.

Note:

1. Some fibrin, particulates or other artifacts may trap red blood cells at the top of the gel columns erroneously leading to an abnormal result (see limitation number 5).
2. Occasionally red blood cell retention in the incubation chamber may occur with positive 4+ samples, without interfering in the result interpretation.

Reaction Grades:

Negative -	Well-defined pellet of non-agglutinated red blood cells at the bottom of the gel column and no visible agglutinated cells in the rest of the gel column.
Positive W+	Barely visible small-sized clumps of agglutinated cells in the lower part of the gel column and a pellet of unagglutinated cells at the bottom.
Positive 1+	Some small-sized clumps of agglutinated cells most frequently in the lower half of the gel column. A small pellet may also be observed at the bottom of the gel column.
Positive 2+	Small or medium-sized clumps of agglutinated cells throughout the gel column. A few unagglutinated cells may be visible at the bottom of the gel column.
Positive 3+	Medium-sized clumps of agglutinated cells in the upper half of the gel column.
Positive 4+	A well-defined band of agglutinated red blood cells in the top part gel column. A few agglutinated cells may be visible below the band.
mf	Mixed-field. A band of red blood cells at the top part of the gel or dispersed throughout the gel column, and a pellet in the bottom as a negative result.
H	Hemolysis in the microtube with very few or no red blood cells in the gel column. Report if hemolysis is present in the microtube but not in the sample.

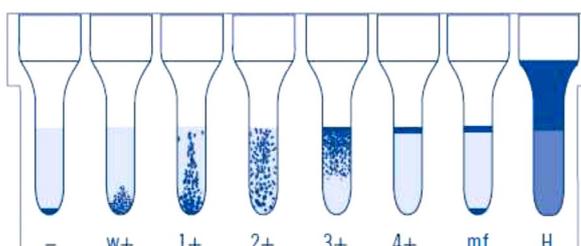


Figure 1. Picture of an example of reaction grades

Stability of the results

After centrifuging the cards it is recommended that the results be read immediately. Do not leave processed cards in a horizontal position. If necessary, a delayed reading can be performed up to 24 hours after processing the cards if they are kept in an upright position, refrigerated (2 - 8 °C) and sealed with a laboratory covering film to avoid evaporation of the supernatant.

QUALITY CONTROL

Include positive and negative controls with testing on each day of use. If an unexpected control result is obtained, a complete assessment of the instrument, reagents and material used should be made.

Interpretation of the results

- **Direct Antiglobulin Test (DAT):**

- Negative reaction: Indicates the absence of detectable IgG antibodies on the red blood cells.
- Positive reaction: Indicates that the patient's red blood cells are sensitized (red blood cells coated with IgG antibodies).

- **Antibody screening and antibody identification tests (IAT):**

- Negative screening: Indicates the absence of detectable unexpected antibodies in the patient's or donor's serum or plasma.
- Positive screening: Indicates the presence of unexpected antibodies in the patient's or donor's serum or plasma against one or more antigens present on the Reagent Red Blood Cells.
In case of positive screening, an identification panel should be performed to identify the antibody present in the plasma or serum sample.

Note: The autocontrol should be negative. If the autocontrol is positive, it may indicate the presence of autoantibody in the sample or a non-specific reaction.

The antigen table provided with the Reagent Red Blood Cells should be used to identify the antibody present in the sample.

- **Crossmatch test (IAT):**

- Negative reaction: Indicates compatibility of the donor blood with the recipient.
- Positive reaction: Indicates incompatibility of the donor blood with the recipient due to the presence of antibodies directed against antigens on the donor's red blood cells. Further investigation to identify the antibody specificity should be performed.

Note: The autocontrol should be negative. If the autocontrol is positive, it may indicate the presence of autoantibody in the sample or a non-specific reaction.

- **Antibody titration test (IAT):**

The titer is the reciprocal of the highest dilution that produces a positive reaction ($\geq 1+$); i.e., in a 1:16 dilution, the titer = 16.⁶ If there is agglutination in the microtube containing the most diluted serum or plasma, the endpoint has not been reached.

- **Red blood cell typing test (IAT):**

Follow the Instructions for Use for the antisera reagents used.

Notes:

1. A Direct Antiglobulin Test with a negative result does not mean absence of hemolytic disease of the newborn, especially in cases where ABO incompatibility is suspected.
2. Drug-induced antibodies can cause a positive Direct Antiglobulin Test⁶.
3. Precaution should be taken in the interpretation of mixed-field events. Not all mixed-cell situations are detected.
4. Additional information on patient history and additional testing will be necessary for resolution. Transfused patients or those subjected to bone marrow transplant may present images of mixed-field⁶.
5. The observation of complete or partial hemolysis (pinkish supernatant and/or gel column) in microtubes should be interpreted as a positive result, after verifying that it is not due to a problem of cell collection and/or handling of the sample.
6. The prozone phenomenon may occur so the first microtubes may have a weaker reaction than the more diluted serum on titration techniques. In order to avoid misinterpretation of results, it may be preferable to examine first the microtube containing the most diluted serum and then proceed through the more concentrated ones.

LIMITATIONS OF THE PROCEDURE

1. Grossly hemolyzed, cloudy or contaminated samples or samples with presence of a clot may cause false positive or false negative results.
2. Aged or hemolyzed specimens may cause weaker reactions compared to those obtained with fresh sample.
3. Abnormal concentrations of serum proteins, the presence of infused macromolecular solutions in the serum or plasma or the presence of Wharton's jelly in cord blood samples may cause non-specific agglutination of the red blood cells. It is suggested that red blood cells be washed before performing the test⁶.
4. Samples with high-potency antibodies may coat the red blood cells completely, causing spontaneous agglutination⁶.
5. If poorly anti-coagulated plasma or incompletely clotted serum is used, fibrin residues may trap non-agglutinated red blood cells at the top of the gel, appearing as a pinkish or reddish layer. Although the results could be correctly interpreted, in a negative reaction the false appearance of a mixed-field could lead to a misinterpretation. In case of incompletely clotted serum samples, it is recommended to re-clot the serum and repeat the test⁶.
6. Each laboratory should establish the antibody titer threshold using the titration procedure, taking into account clinical findings and laboratory data to ensure meaningful interpretation based on its own titration values.
7. No single method is able to detect all unexpected antibodies. The optimum reaction conditions (e.g., sample volume, incubation time) may vary for different antibody specificities. For screening and identification of unexpected antibodies, crossmatch and autocontrol tests, increasing the volume of serum or plasma from 25 μ L to 50 μ L as well as extending the incubation time from 15 to 30 minutes is acceptable. This variation in the concentration of antibodies brings the antigen/antibody ratio down and may improve the detection of antibodies at very low concentrations.⁶
8. If antibody detection test is used for antibody single dilution titration studies, the laboratory should establish the antibody titer threshold using the procedure with clinical findings and laboratory data to ensure meaningful interpretation based on its own titration values.
9. Samples with low or high titers processed automatically may encounter titration results variability and additional testing may be needed.

10. The presence of a high concentration of IgG paraproteins in the sample can neutralize the polyspecific anti-human globulin and lead to a false negative result in the antiglobulin test⁶.
11. Rare antibodies, notably some anti-Jka or anti-Jkb, may be detected only when polyspecific AHG is used and when active complement is present⁶.
12. The Indirect Antiglobulin Test at 37 °C in gel or glass sphere techniques have been reported to show a lower level of sensitivity than results obtained with the tube technique, in the detection of weak agglutination reactions of the ABO system⁷.
13. A false positive result in the Direct Antiglobulin Test can be due to the complement attached to red blood cells in specimens collected from infusion lines used to administer dextrose-containing solutions⁶ or in specimens collected in tubes containing silicone gel⁶.
14. In the Direct Antiglobulin Test not all positive reactions infer that clinically significant antibodies are present. Specific anti-IgG reagent and elution techniques may be used for additional investigation of positive results.
15. Nonspecifically adsorbed proteins (e.g., high-dose intravenous immune globulin, multiple myeloma, autoimmune disorders and other diseases associated with elevated serum globulin) and modification of red cell membrane by some drug, can cause positive Direct Antiglobulin test⁶.
16. Red blood cell samples with a positive Direct Antiglobulin Test should not be used for Indirect Antiglobulin testing.
17. Antibody activity may decrease in the elderly, infants or persons with disease.
18. On occasions, unagglutinated red blood cells may be retained somewhere in the gel column with the appearance of very minute red dots or flecks. However, this nonspecific retention should not interfere with the interpretation of the result.

SPECIFIC PERFORMANCE CHARACTERISTICS

- Contains Anti-IgG with no anti-complement activity.
- Every lot has been tested against a panel of positive and negative samples for the relevant antigens to assure Anti-IgG sensitivity and absence of contaminating antibodies. Details of specificity test results submitted to the FDA for release of the product will be furnished upon request.
- The potency of Anti-IgG is verified by tests with red blood cells sensitized with Anti-D and Anti-Fya according to methods approved by FDA. The Anti-IgG meets FDA potency requirements.
- For the manual method, the performance of the reagents was confirmed against FDA-licensed reagents in a comparison study where reagents were tested in parallel at different clinical sites. The estimated percent agreements and their lower limits of 95% one-side confidence interval for all sites combined are indicated on the table below.

Overall Statistical Analysis Results of the comparison study:

Technique	Nº of samples	Negative Percent Agreement (Lower 95% CI)	Nº of samples	Positive Percent Agreement (Lower 95% CI)
Ab. Screening Anti-IgG (polyclonal)	998	99.50% (98.95%)	221	99.55% (97.87%)
Ab. Identification Anti-IgG (polyclonal)	613	99.67% (98.98%)	553	94.03% (92.10%)
IgG-Crossmatch Anti-IgG (polyclonal)	187	100.00% (98.41%)	191	98.95% (96.74%)
DAT Anti-IgG Anti-IgG (polyclonal)	202	100.00% (98.53%)	73	100.00% (95.98%)

- Percent of Agreement only indicates agreement between the Diagnostic Grifols reagents and the FDA-licensed reagents and does not indicate which reagent gave the correct result(s).
- For further information about the performance data for the manual method using DG Reader or DG Reader Net and for automated method, please refer to the Instructions for Use of the related instrument.
- For further information about the performance data for the Anti-D (RH1) IgM/IgG Blood Grouping Reagent, please refer to this reagent's Instructions for Use.
- For the antibody titration test, the performance of the reagents in the automatic technique was confirmed against the manual method in a comparison study. For further information about the performance of the automated method, please refer to the instruction for use of the related instrument.

BIBLIOGRAPHY

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PRESENTATION

210394 DG Gel 8 Anti-IgG (Rabbit) 50 Cards

Manufactured by:

Diagnostic Grifols, S.A.

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SYMBOLS KEY

One or more of these symbols may have been used in the labeling/packaging of this product.

 <i>In vitro</i> diagnostic medical device	 Temperature limitation	 This way up
 Batch code	 Consult instructions for use	 Fragile, handle with care
 Use by YYYY-MM-DD or YYYY-MM	 Catalog number	 Keep dry
 Box raw material (paperboard)	 Recycled and recyclable packaging	

REVISION HISTORY

Revision Date	Revisions *
2022-11	<ul style="list-style-type: none">• Include the possibility to perform titration tests.• Include the DG Reader Net in "Procedure" Section.• Include further information on how to interpret the results of each technique in "Interpretation of the results" Section.• The negative result has been changed to "-" in the Reaction Grades Table and Figure 1, in the "Results" Section.• Added recommended centrifugation range in "Specimen collection and preparation" Section.• Modify the statement "Date of last version" to "Date of Issue".• "Revision History" Section has been created.
2023-09	<ul style="list-style-type: none">• Add new notes in items 6 and 7 of the "Screening and identification of unexpected antibodies" procedure described in the "Procedure" section.• Add new notes in items 7 and 8 of the "Antiglobulin Crossmatch tests" and "Autocontrol" procedures described in the "Procedure" section.• Modify item 7 of the "Limitations of the procedure" section to include the possibility of increasing the sensitivity of the standard technique in the Crossmatch and Autocontrol tests and specify the incubation time extension.• Include information on how to interpret the results obtained in the "Direct Antiglobulin Test (DAT)" in the "Interpretation of the results" Section.• Update the information related to the Technical Service.
2025-05	<ul style="list-style-type: none">• Correction of a typographical error in step 8 of Antibody Titration Test Procedure• Include the possibility to perform single dilution titration tests.

Revision Date	Revisions *
	<ul style="list-style-type: none">• Add two limitations of the procedure related to titration tests.• Update the Bibliography reference <i>Klein HG and Anstee DJ. Mollison's Blood Transfusion in Clinical Medicine</i> to 12th Edition.• Add "Rx only" mention

***Note:** Changes with respect to the previous revision are shaded in grey. Deletion of text is indicated as Δ.