

Instructions for Use**INTENDED USE**

The DG Gel 8 Rh Pheno card is for the determination of human C, E, c, and e antigens on the surface of red blood cells of two separate blood samples.

For use with the DG Gel System.

For *in vitro* diagnostic use.

SUMMARY AND EXPLANATION

The determination of Rh is defined by the presence or absence on human red blood cells of the five (D, C, E, c, and e) most important antigens in the Rh system. Antigen D is by far the most immunogenic of the Rh antigens¹. All the antibodies against all Rh antigens should be considered to have the potential to cause hemolytic transfusion reactions (HTRs) and hemolytic disease of the newborn (HDN). The anti-C, anti-E, anti-c, and anti-e reagents contained in the DG Gel 8 Rh Pheno card are used to perform the typing of the antigens C (RH2), E (RH3), c (RH4) and e (RH5) of the Rh blood group system in two separate samples.

PRINCIPLE OF THE TEST

The principle of the test is based on the gel technique described by Yves Lapierre² in 1985 for detecting red blood cell agglutination reactions. The DG Gel 8 plastic cards are composed of eight microtubes. Each microtube is made of a chamber, also known as incubator chamber, at the top of a long and narrow microtube, referred to as the column. Buffered gel solution containing specific antibody (anti-C, anti-E, anti-c, and anti-e) has been pre-filled into the microtube of the plastic card. The agglutination occurs when the red blood cell antigens react with the corresponding antibodies 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 cells 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 Rh Pheno card contains different reagents (monoclonal antibodies) mixed with a gel in buffered medium with preservative. The different microtubes are identified on the front label of the card.

Microtubes **C**: monoclonal antibody anti-C. IgM antibody of human origin, clone MS-24.

Microtubes **E**: monoclonal antibody anti-E. IgM antibody of human origin, clone 906.

Microtubes **c**: monoclonal antibody anti-c. IgM antibody of human origin, clone H-48.

Microtubes **e**: monoclonal antibodies anti-e. Mixture of IgM antibodies of human origin, clones MS-16, MS-21 and MS-63.

Clone 906 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.

Clone H-48 is produced using intermediate products produced for Diagnostic Grifols S.A. in a shared manufacturing agreement with ALBA BIOSCIENCE, Ellen's Glen Road, Edinburgh, EH17 7QT United Kingdom; US License Number 1807.

Clones MS-24, MS-21, MS-63, MS-16 are produced using intermediate products produced for Diagnostic Grifols S.A. in a shared manufacturing agreement with MILLIPORE (UK) LTD, Fleming Road, Livingston EH54 7BN United Kingdom; US License Number 1761.

Reagent preparation

DG Gel 8 Rh Pheno cards are supplied as ready to use. The gel cards and samples to be tested should be brought to room temperature (18-25 °C) before testing.

Material required but not provided

- Automatic pipettes of 10 µL, 50 µL and 1 mL.
- Disposable pipette tips.
- Glass or plastic test tubes.
- Grifols Diluent.
- DG SPIN centrifuge.

STORAGE AND STABILITY

- Do not use beyond the expiration date.
- Store 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.
- The reagents of the DG Gel 8 Rh Pheno card of human monoclonal origin are manufactured using material that have been tested and found non-reactive for the HBs antigen, and for anti-HIV and anti-HCV antibodies. However, there is no known procedure to ensure that products of human origin will not transmit infectious agents. Human blood products and samples should be handled as if they were potentially capable of transmitting infectious diseases.
- 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, consult the authorized distributor.

SPECIMEN COLLECTION AND PREPARATION

Blood samples collected in EDTA, sodium citrate or sodium heparin should be used. The collection, separation and handling of the blood should be performed by qualified technical personnel according to current standards³⁻⁴, and following the instructions of the manufacturer of the materials used for collecting the sample.

Do not use grossly hemolyzed, cloudy or contaminated samples.

Use the red blood cells collected for the determination of the antigens C, E, c and e of the Rh system. If necessary, samples stored at 2-8 °C can be used up to 72 hours after collection.

Red blood cells from bags collected in ACD, CPD, CPDA-1, CP2D, 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 stored at 2-8 °C. If red blood cells from the bag segment are used, it is recommended that these be washed with physiological saline solution before preparing the suspension. Do not use if clots or hemolysis are observed.

PROCEDURE

1. Allow DG Gel 8 Rh Pheno cards, additional reagents and the samples to reach room temperature (18-25 °C).
2. Inspect the condition of the cards before use (see Warnings and Precautions).
3. Identify the cards to be used and the samples to be tested.
4. Prepare a 5% red blood cell suspension in Grifols Diluent (50 µL of packed red blood cells in 1 mL of Grifols Diluent).
5. 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.

6. Ensure the re-suspension of the red blood cells before use.
7. Add 10 µL of the 5% red blood cell suspension into each of the microtubes indicated.

Note: Carefully dispense the red blood cell suspension avoiding contact of the pipette tip with the wall or the contents of the microtubes to prevent carryover.
8. Centrifuge the gel card in the DG SPIN centrifuge.
9. After centrifugation, remove the gel card from the centrifuge and read the results.

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 a 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 very weak reaction may indicate a very weak or partial expression of Rh antigens.

Notes:

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 6).
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	0	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.
	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.
	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.
	3+	Medium-sized clumps of agglutinated cells in the upper half of the gel column.
	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 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 results is obtained, a complete assessment of the instrument, reagents and material used should be made.

Interpretation of the results

A positive result in the corresponding microtube indicates the presence of antigen **C, E, c, and e (Rh system)**.

Notes:

1. Precaution should be taken in the interpretation of mixed-field events. Not all mixed-cell situations are detected. 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-field⁵. Mixed-field is also observed in some ABO subgroups (A₃), in blood group chimerism in fraternal twins, and in the very rare case of mosaicism arising from dispermy⁵.
2. 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 collection and/or handling of the sample.

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. Antigen expression may be weakened in the red blood cells of persons with leukemia or other malignant diseases⁵.

4. Abnormal concentrations of serum proteins, the presence of infused macromolecular solutions in the serum or plasma may cause non-specific agglutination of the red blood cells⁵.
5. Samples with high-potency antibodies may coat the red blood cells completely, causing spontaneous agglutination⁵.
6. 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 test⁵.
7. A very weak expression or variants of the C, E, c, and e antigens may not be detected.
8. On occasions, unagglutinated red blood cells may be retained somewhere in the gel column with the appearance of very minute red dot or fleck. However, this nonspecific retention should not interfere with the interpretation of the result.

SPECIFIC PERFORMANCE CHARACTERISTICS

Grifols DG Gel 8 Blood Grouping Reagents Anti-C, Anti-E, Anti-c, Anti-e meet FDA potency requirements for Blood Grouping Reagents.

Every lot has been tested against a panel of positive and negative samples for the relevant antigens to assure reactivity and specificity in accordance with FDA requirements. Details of specificity test results submitted to the FDA for release of the product will be furnished upon request.

BIBLIOGRAPHY

1. Klein HG and Anstee DJ. Mollison's Blood Transfusion in Clinical Medicine, 11th edition, Blackwell Scientific Publications, Oxford, 2005.
2. Lapierre Y, *et al.* The gel test: a new way to detect red cells antigen-antibody reactions. *Transfusion*, 30: 109-113, 1990.
3. CLSI H3-A6: Procedures for the collection of diagnostic blood specimens by venipuncture; Approved Standard, 6th edition, 2007.
4. CLSI H18-A3: Procedures for the handling and processing of blood specimens; Approved Guideline, 3rd edition, 2004.
5. Technical Manual, 17th edition, American Association of Blood Banks, Bethesda, Maryland, 2011.

PRESENTATION

210387 DG Gel 8 Rh Pheno 50 Cards

Manufactured by:

Diagnostic Grifols, S.A.

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







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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
	Batch code
	Use by YYYY-MM-DD or YYYY-MM
	Temperature limitation
	Consult instructions for use
	Catalog number
	This way up
	Fragile, handle with care
	Keep dry