

# BLOOD GROUPING REAGENT

## DG GEL 8 ANTI-K

REF 210119

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### Instructions for Use

#### INTENDED USE

The DG Gel 8 Anti-K card is for the determination of K antigen on the surface of red blood cells in eight human blood samples.

For use with the DG Gel System.

For *in vitro* diagnostic use.

#### SUMMARY AND EXPLANATION

The antigen K or KEL1 is the antigen of the Kell system most important from a clinical point of view, as the corresponding antibody is involved in hemolytic transfusion reactions (HTRs) and in hemolytic disease of the newborn (HDN)<sup>1</sup>. The anti-K reagent contained in the DG Gel 8 Anti-K card is used to perform the typing of the K antigen.

#### PRINCIPLE OF THE TEST

The principle of the test is based on the gel technique described by Yves Lapierre<sup>1</sup> 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 solution containing specific antibody (anti-K) has been prefilled 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 ( $\text{NaN}_3$ ) as a preservative at a final concentration of 0.09%.

Each microtube of the DG Gel 8 Anti-K cards contains monoclonal antibodies mixed with a gel in buffered medium with preservative. The DG Gel 8 Anti-K contains 8 microtubes of anti-K.

The microtubes are identified on the front label of the card.

Microtube **K**: monoclonal antibody anti-K. IgM antibody of human origin, clone MS-56.

Clone MS-56 is 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 Anti-K 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

### For Manual Method

- Automatic pipettes of 10  $\mu\text{L}$ , 50  $\mu\text{L}$  and 1 mL.
- Disposable pipette tips.
- Glass or plastic test tubes.
- Grifols Diluent.
- DG Gel 8 Neutral (if a control microtube is needed).
- DG SPIN centrifuge.
- DG Reader Net or DG Reader (optional).

### For Fully Automated Methods

- Grifols Diluent.
- DG Gel 8 Neutral (if a control microtube is needed).
- 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.
- 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 suspensions 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.
- Samples collected in sodium citrate or heparin should be tested by manual method.
- The reagent of the DG Gel 8 Anti-K card of human monoclonal origin is manufactured using materials 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, please contact your local Grifols service representative.

## **SPECIMEN COLLECTION AND PREPARATION**

Blood samples collected in EDTA, sodium citrate or heparin should be used. The collection, separation and handling of the blood should be performed by qualified technical personnel according to current

standards<sup>2-3</sup>, 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 antigen K of the Kell system. Samples should be tested as soon as possible. If necessary, samples collected in EDTA and stored at 2 - 8 °C can be used up to 7 days after collection. Samples collected in sodium citrate or heparin and stored at 2 - 8 °C can be used up to 3 days 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 red blood cells from the 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.

## PROCEDURE

1. Allow DG Gel 8 Anti-K cards, additional reagents and the samples to reach room temperature (18-25 °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 5% red blood cell suspension in Grifols Diluent (50 µL of packed red blood cells in 1 mL of Grifols Diluent).
4. Remove the foil seal from the complete DG Gel 8 Anti-K card or from the individual microtubes to be used for testing. Carefully peel off the aluminum film.
5. Ensure the re-suspension of the red blood cells before use.
6. Add 10 µL of the 5% red blood cell suspension into the microtube to be used.

**Note:** Carefully dispense the red blood cell suspension avoiding contact of the pipette tip with the wall or the contents of the microtubes.

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 to read and to interpret the results.

**Note:** If a control microtube (a gel in a buffered medium without antibodies) has not been processed in another DG Gel 8 card for the sample tested, a microtube of the DG Gel 8 Neutral card can be used for this purpose. To process a control microtube, add 10 µL of the same 5% red blood cell suspension sample into one microtube of the DG Gel 8 Neutral card, centrifuge the card in the DG SPIN, 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 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. Samples with normal expression of K antigen provide strong positive reaction grades. Weaker reactions may indicate a weak or partial expression of K antigen.

### 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:

<b>Negative:</b>	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.
<b>Positive:</b>	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.
<b>mf</b>		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.
<b>H</b>		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.

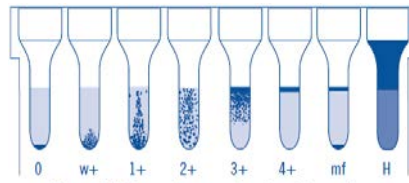


Figure 1. Picture of an example of reaction grades.

### 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

A positive result indicates the presence of antigen K or KEL1 (Kell system).

### Notes:

1. In the control microtube (buffered gel solution without antibodies), the result should be negative. If it is positive, due to the formation of rouleaux, to strong cold autoagglutinins or other causes, invalidate the test. Repeat the determination after washing the red blood cells with physiological saline solution and preparing a new suspension of the washed red blood cells. If the control microtube of the repeated test is negative, the results of the test can be interpreted; if it is positive, invalidate the test.
2. 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-field<sup>4</sup>.
3. 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 diseases<sup>4</sup>.
4. 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 test<sup>4</sup>.
5. Samples with high-potency antibodies may coat the red blood cells completely, causing spontaneous agglutination<sup>4</sup>.
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, it is recommended to re-clot the serum and repeat the test<sup>4</sup>.
7. A very weak expression or variants of K antigen 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 Reagent Anti-K meets 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.
- 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.

**Table “Overall Statistical Analysis Results of the comparison study”**

	Nº of samples	Negative Percent Agreement (Lower 95% CI)	Nº of samples	Positive Percent Agreement (Lower 95% CI)
Anti-K	3942	99.90% (99.77%)	322	100.00% (99.07%)

- 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 manual method using DG Reader or DG Reader Net and for automated method, please refer to the Instructions for Use of the related instrument.

#### **BIBLIOGRAPHY**

1. Lapierre Y, et al. The gel test: a new way to detect red cells antigen-antibody reactions. *Transfusion*, 30: 109-113, 1990.
2. CLSI H3-A6: Procedures for the collection of diagnostic blood specimens by venipuncture; Approved Standard, 6th edition, 2007.
3. CLSI H18-A3: Procedures for the handling and processing of blood specimens; Approved Guideline, 3rd edition, 2004.
4. Technical Manual, 19th edition, American Association of Blood Banks, Bethesda, Maryland, 2017.

#### **PRESENTATION**

210119 DG Gel 8 Anti-K            50 Cards

#### **Manufactured by:**

Diagnostic Grifols, S.A.

Passeig Fluvial 24 08150 Parets del Vallès (Barcelona), Spain

U.S License No. 1887

#### **Technical Service:**

Emeryville, CA, USA 94608

Telephone (in U.S.): (800)452-6877

Or: (510)923-3757

Fax: (800)462-3938










Email: [service.americas@grifols.com](mailto:service.americas@grifols.com)



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## SYMBOLS KEY

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

 IVD	<i>In vitro</i> diagnostic medical device
 LOT	Batch code
	Use by YYYY-MM-DD or YYYY-MM
	Temperature limitation
	Consult instructions for use
 REF	Catalog number
	This way up
	Fragile, handle with care
	Keep dry