matrix™
GEL • SYSTEM

Cat. No.	102790005
Presentation	5 ml

Anti-D IgG for Weak D testing on Matrix™ Gel System

SUMMARY

Human red blood cells are classified as Rho (D) positive or Rho (D) negative depending upon the presence or absence of Rho (D) antigen on them. Most Rho (D) positive red cells show clear positive results with Anti-D reagent. For some D positive red cells, demonstration of Rho (D) antigen requires incubation with Anti-D reagent followed by antiglobulin phase. The red cells giving negative or weaker reactions in direct Anti-D test and positive by IAT after incubation are considered as weak D.
In Matrix™ Gel System using direct Anti-D test, reactions greater than or equal to 3+ indicates the presence of D antigen, and reactions weaker than 3+ indicates weak D. Weak positive reactions may vary depending upon the monoclonal cell lines used. In Matrix™ Gel System weak D testing can be done by using monoclonal Matrix™ Anti-D IgG reagent on Matrix™ Gel Card containing Coombs Anti-IgG.

REAGENT

Matrix™ Anti-D IgG contains monoclonal Anti-D IgG (Clone- MCAD6). The concentration of Anti-D IgG is standardized for application on Matrix™ Gel System.

REAGENT STORAGE AND STABILITY

Store the reagent at 2-8°C. DO NOT FREEZE.
The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label. Once opened, the shelf life of reagent is as per the expiry date mentioned on the reagent vial label, provided it is not contaminated.

ADDITIONAL REAGENT AND MATERIALS REQUIRED

Matrix™ Diluent-2 LISS for preparation of red cell suspension. Matrix™ Coombs Anti-IgG Card (Refer package insert before use). Gel card centrifuge (85g), Incubator (37°C), Work station, Micropipette capable of delivering 5-50µl of specimen and Bottle top dispenser.

PRINCIPLE

IgG antibodies are also known as incomplete antibodies. These antibodies can sensitize red cells when allowed to incubate at 37°C in presence of corresponding antigen. Matrix™ Anti-D IgG contains monoclonal Anti-D IgG in a standardized concentration. Red blood cells when allowed to incubate with Matrix™ Anti-D IgG, red blood cells will get sensitized if Rho (D) antigen or weaker expression is present. This reaction can be detected when Matrix™ gel card containing Coombs Anti-IgG is centrifuged under controlled conditions. Cells sensitized with Anti-D IgG will be trapped within the gel matrix in presence of Coombs Anti-IgG. The red blood cells, which do not react are not trapped in the gel column and get settled at the bottom of the microtube.

SAMPLE COLLECTION

No special preparation of patient is required prior to sample collection by approved techniques. For optimal results, freshly collected samples should be used. Anticoagulants like EDTA, CPD-A and Citrate can be used.
Samples should be centrifuged at 1500g for 10 minutes to avoid fibrin residue which may interfere with results.

SAMPLE PREPARATION

Prepare 0.8% red blood cell suspension in Matrix™ Diluent- 2 LISS as follows:

1. Bring the Matrix™ Diluent -2 LISS to room temperature before use.
2. Dispense 1.0 ml of Matrix™ Diluent -2 LISS into a clean test tube.
3. Add 10µl of packed red cells to Matrix™ Diluent -2 LISS collected in test tube and mix gently.
4. Red blood cell suspension so obtained should be used for testing.

TEST PROCEDURE

1. Label the appropriate microtube of the Matrix™ Coombs Anti-IgG card with patient's / donor's name or identification number. Remove the aluminium foil of required number of microtubes carefully by pulling it backwards.
2. Pipette 50µl of 0.8% patient's / donor's red blood cell suspension to the microtube, taking care to ensure that the micropipette tip does not touches the microtube.

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3. Add 25µl of Matrix™ Anti-D IgG to the above microtube.
4. Incubate the Matrix™ gel card for 15 minutes at 37°C in an incubator.
5. After incubation, centrifuge the Matrix™ gel card for 10 minutes in the gel card centrifuge.
6. Retrieve the card from centrifuge, read and record the results.

INTERPRETATION OF RESULTS

Positive reaction: Agglutinated red blood cells forming a clear line at the top of the gel column or agglutinates dispersed in the gel column.

Negative reaction: Non-agglutinated red blood cells settle at the bottom of the microtube forming a compact button.

WEAK D CONFIRMATION BY IAT

Reaction for Rho (D)

Rho (D) positive	Weak D	Rho (D) Negative
++++	± to +++	-

Interpretation of Rho (D) and Weak D	Rho (D) positive	Weak D
Direct test using a Matrix™ card with Anti-D	++++	+++ to neg.
Confirmatory test with the Matrix™ Coombs Anti-IgG card	++++	+++ to ±

The reaction strength may be recorded as follows:

Strength of reaction	Comments
4+	Agglutinated red blood cells form a line at the top of the gel microtube.
3+	Most agglutinated red blood cells remain in the upper half of the gel microtube.
2+	Agglutinated red blood cells are observed throughout the length of the microtube. A small button of red blood cells may also be visible at the bottom of the gel microtube.
1+	Most agglutinated red blood cells remain in the lower half of the microtube. A button of cells may also be visible at the bottom of the gel microtube.
±	Most agglutinated red blood cells are in the lower third part of the gel microtube.
Negative	All the red blood cells pass through and form a compact button at the bottom of the gel microtube.
Mixed field agglutination	Agglutinated red blood cells form a line at the top of the gel and non-agglutinated red blood cells form a compact button at the bottom of the gel microtube.
H	Hemolysis of red blood cells

NOTE

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. The Matrix™ Anti-D IgG contains sodium azide <0.1% as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantity of water.
3. Use of red blood cells concentration/ volume and reagents other than those described may lead to erroneous results. Follow the instructions carefully.
4. Do not use hemolysed, lipemic, icteric and hyperproteic samples.
5. Extreme turbidity or discoloration may indicate microbial contamination or denaturation of protein due to thermal damage. Such reagents should be discarded.
6. Contamination of reagents during usage may cause false positive or negative results.
7. To avoid contamination always use fresh tips to pipette reagent from reagent vial.

REMARKS

1. Known positive and negative controls should be tested as per Good Laboratory Practices.

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