ODAK Blood Grouping System

**OBG Anti-D**

Blood Grouping Reagent for Slide and Tube Tests

**INTENDED USE**

The Anti-D reagent is for the in vitro detection and identification of the D antigen on human red blood cells by direct agglutination.

**INTRODUCTION**

The ABO system is defined by the presence or absence of the A and/or B antigens in the red blood cells. The ABO blood group system is determined directly by testing the red blood cells with Anti-A and Anti-B reagents. The determination of Rh D is defined by the presence or absence of the D antigen in the red blood cells.

**REAGENT**

Ready to use: 10 ml bottle

ODAK OBG Anti-D blood grouping reagent contains mouse monoclonal IgM antibodies (Anti-D IgM Clone BS 225). The antibody is diluted in a phosphate buffer containing sodium chloride, EDTA and bovine albumin.

This monoclonal Anti-D IgM will directly agglutinate red cells from most weak D and partial RhD except DVI and, therefore, is suitable for RhD grouping of patient samples.

**MATERIAL REQUIRED BUT NOT PROVIDED**

- Pipettes.
- Disposable pipette tips.
- Test tubes.
- LiSS Diluent
- Phosphate Buffered Saline (PBS): NaCl 0.9%, pH 7.0±0.2
- Glass slides

**STORAGE CONDITIONS AND STABILITY**

The reagent should be stored at 2 - 8°C. The reagent is stable until the expiry date stated on the product label.

Once opened the shelf life of the reagent vial is as described on the reagent vial label if stored 2-8°C. Prolonged storage at temperatures outside may result in accelerated loss of reagent reactivity.

**PRECAUTIONS AND WARNINGS**

- This reagent kit is for in vitro diagnosis only.
- Do not use if turbid.
- Do not dilute.
- This reagent kit is for professional use only.
- The reagent contains sodium azide 0.1% as preservative.
- Avoid contact with skin and mucosa.
- Do not use damaged or leaking reagents.
- Do not use the reagents past the expiration date.
- Do not use the reagents if a precipitate is present.
- All kit components and specimens should be regarded as potential hazards to health. It should be used and discarded according to your own laboratory’s safety procedures.

**SAMPLE COLLECTION**

Fresh red blood cells are preferred for testing. Use the red blood cells collected with anticoagulants for determination of the antigens of the ABO/Rh system. If necessary, samples stored at 2-8 °C can be used up to 48 hours. Red blood cells collected in ACD, CPD, SAGM and PAGGSM can also be used until the expiry date indicated on the label of the bag at 2-8 °C. Fibrin residues may interfere with the reaction pattern.

**PREPARATION OF BLOOD SAMPLE**

( for tube method)

2-3 % red blood cell suspension:

Do not use haemolyzed, cloudy or contaminated samples or those containing clots

1- Dispense 0.5 ml of LiSS Diluent or PBS into a clean tube.
2- Add 25 μl of whole blood to the diluent and mix gently.

**RECOMMENDED TEST PROCEDURES**

This reagent has been standardised for use by the techniques described below and therefore its suitability for use in other techniques cannot be guaranteed.

*Slide techniques are not recommended for the detection of weak D or partial RhD samples.*

Bring reagents and samples to room temperature before testing.

**Tube Method - Immediate Spin**

1- Place 1 drop of Anti-D reagent into a test tube.
2- Add 50 μl of 2-3% suspension of the red cells and mix well.
3- Centrifuge the tubes for 20 seconds at 1000 g
4- Gently shake the tube to dislodge the cell button from the bottom and observe macroscopically for agglutination.

**Tube Method - LiSS**

1- Place 1 drop of Anti-D reagent into a test tube.
2- Add 50 μl of of red cells suspended to 2 - 3% in LiSS.
3- Mix the test well and incubate for 15 minutes at 37°C.
4- Centrifuge the tubes for 20 seconds at 1000 g
5- Gently shake the tube to dislodge the cell button from the bottom and observe macroscopically for agglutination.

**Slide Method**

1- Place 1 drop of Anti-D reagent on a glass slide.
2- Add 1 small drop (20-30 μl) of whole blood.
3- Using a clean applicator stick, mix reagent and cells over an area of approximately 2.5 cm. Mix well by rocking the slide for approximately 30 seconds.
4- Read macroscopically after 2 minutes over a diffuse light.

**Interpretation of the results:**

1. Positive: Agglutination of the test red cells indicates the presence of D antigen.
2. Negative: No agglutination of the test red cells indicates the absence of D antigen.
3. Discordance: If there is discordance, do not report the result, further investigation is required

<table>
<thead>
<tr>
<th>Rh system</th>
<th>D</th>
<th>+</th>
<th>RhD Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td></td>
<td></td>
<td>RhD Negative</td>
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</table>

**Stability of the results:**

- Read the tube test straight after centrifugation.
- Slide tests should be interpreted within two minutes to ensure specificity and to avoid the possibility a negative result may be incorrectly interpreted as positive due to drying of the reagent.

**LIMITATIONS**

- Some very weak D and/or partial RhD samples may not react with monoclonal anti-D reagents.
- Slide techniques are not recommended for the detection of weak D or partial RhD samples.
- Stored blood may give weaker reactions than fresh blood.
- Excessive centrifugation and inadequate centrifugation could lead to erroneous results.
- False positive or false negative results can occur due to contamination of test materials, improper reaction temperature, improper storage of materials.
PERFORMANCE CHARACTERISTICS

Diagnostic sensitivity and specificity of ABO/Rh system:

The diagnostic sensitivity and specificity of the antibodies present in OBG ABO for the determination of the antigens of the ABO and Rh systems have been studied in a representative number of positive and negative samples. In the study using 1230 blood samples the sensitivity and specificity of ODAK Blood Grouping System was as follows:

<table>
<thead>
<tr>
<th>Number of samples</th>
<th>Sensitivity</th>
<th>Specificity</th>
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</thead>
<tbody>
<tr>
<td>Anti-A</td>
<td>1230</td>
<td>% 100</td>
</tr>
<tr>
<td>Anti-B</td>
<td>1230</td>
<td>% 100</td>
</tr>
<tr>
<td>Anti-D</td>
<td>1230</td>
<td>% 100</td>
</tr>
</tbody>
</table>

Precision:

Inter-assay and intra-assay reproducibility tests; no false positive or false negative results were obtained. Variability between agglutination view in positive samples were 1 agglutination rating or less in all assays.

Label symbols

- **LOT** Batch code
- **Expiry date**
- **Storage temperature**
- **Consult instructions for use**
- **IVD** In vitro diagnostic medical device
- **REF** Product code
- **Manufacturer**
- **Harmful**

BIBLIOGRAPHY