Monoclonal Blood Grouping Reagents

Anti-A
Anti-B
Anti-A,B

CATALOGUE NUMBER
Anti-A: BG-A5, BG-A10, BG-A1000
Anti-B: BG-B5, BG-B10, BG-B1000
Anti-A,B: BG-ABS. BG-AB10, BG-AB1000

INTENDED USE
These reagents are suitable for use by the slide, tube and microplate techniques and are designed for use by operators trained in serological techniques.

INTRODUCTION
The ABO Blood Group System
In 1900, Landsteiner discovered that the serum of some individuals would agglutinate the red cells of others and that this phenomenon could be used to classify individuals into different blood group phenotypes. Four common phenotypes are recognised – O, A, B and AB. Subgroups of the A and B antigens have since been identified. The ABO phenotype of an individual is usually determined by the agglutination reactions of the individual's red cells with Anti-A, Anti-B and Anti-A,B antisera (forward grouping). In testing blood samples from adults, confirmation of the ABO blood group can be provided by the reactions of the individual’s serum with standard A and B red cell suspensions (reverse grouping).

PRINCIPLE
Anti-A monoclonal murine IgM blood grouping reagent contains antibody from the cell line BIRMA-1, Anti-B monoclonal murine IgM blood grouping reagent contains antibody from LB-2 and Anti-A,B monoclonal murine IgM blood grouping reagent contains antibodies from cell lines ES-4/ES-15. When used by the recommended techniques these reagents will cause agglutination (clumping) of red cells carrying the specific antigen (positive test). Lack of agglutination of the red cells demonstrates the absence of the specific antigen (negative test). These reagents have been optimised for use by the recommended techniques without further dilution or additions.

REAGENTS AND MATERIALS
Blood grouping reagents contain monoclonal murine IgM antibodies in a buffer solution. The solution is a phosphate buffer containing sodium chloride, EDTA and bovine material. This reagent contains <0.1% sodium azide and the following colourants:

<table>
<thead>
<tr>
<th>Blood Grouping Reagent</th>
<th>Reagent Colour</th>
<th>Dye</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-A</td>
<td>Blue</td>
<td>Patent Blue</td>
</tr>
<tr>
<td>Anti-B</td>
<td>Yellow</td>
<td>Tartrazine</td>
</tr>
<tr>
<td>Anti-A,B</td>
<td>Colourless</td>
<td>N/A</td>
</tr>
</tbody>
</table>

MATERIALS required but not provided

<table>
<thead>
<tr>
<th>Slide Technique:</th>
<th>Tube Technique:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscope slide</td>
<td>Test tube</td>
</tr>
<tr>
<td>Timer</td>
<td>Centrifuge (1000 rcf)</td>
</tr>
<tr>
<td>Isotonic saline</td>
<td>Isotonic saline</td>
</tr>
<tr>
<td>Compatible plasma/serum</td>
<td>Timer</td>
</tr>
</tbody>
</table>

PRECAUTIONS
1. The cell lines used to produce these reagents are of murine origin and have been tested and found to be negative for Mouse Antibody Production (MAP) viruses. Care must be taken in the use and disposal of each container and its contents.
2. These reagents contain <0.1% (w/v) sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form highly explosive salts. On disposal, flush with large quantities of water.
3. These products should be clear. Turbidity may indicate bacterial contamination. These reagents should not be used if a precipitate, fibrin gel or particles are present.
4. These reagents are for professional in vitro diagnostic use only.
5. The bovine materials are obtained from USDA approved sources or from sources for which origin information is available. The donor animals for bovine material have been inspected and certified disease free and are deemed to have low TSE (Transmissible Spongiform Encephalopathy) risk.
6. These products should be disposed of either by overnight immersion in disinfectants at appropriate concentrations or by autoclaving.

ADVICE TO USERS
It is recommended that a positive control and a negative control should be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show the expected reactions. It is not required to use a reagent control in parallel with all tests using these reagents. Only in typing the red cells of patients known to have auto antibodies or protein abnormalities is the use of a reagent control recommended. This should be tested in parallel with the reagents. These reagents have been characterised by the procedures recommended in this package insert, their suitability for use in other techniques must be determined by the user.
Monoclonal Blood Grouping Reagents
Anti-A
Anti-B
Anti-A,B

STORAGE AND STABILITY
Store the opened/unopened products at 2-8°C until the expiry date detailed on the product label. Failure to store the products at the correct temperature, for example, storage at higher temperature or repeated freezing and thawing may result in accelerated loss of reagent activity.

SPECIMEN COLLECTION AND PREPARATION
No special preparation of the patient is required prior to specimen collection. Blood should be collected by an approved phlebotomy technique. The specimen should be tested as soon as possible following collection. If a delay in testing should occur, store the specimen at 2-8°C. Specimens displaying gross haemolysis or microbial contamination should not be tested with this reagent. Failure to store the specimens at the correct temperature, for example, storage at higher temperature or repeated freezing and thawing may result in false positive or false negative results.

INSTRUCTIONS FOR USE
1. SLIDE TECHNIQUE
1. Prepare a 35-50% suspension of test red cells in autologous (or compatible) plasma, serum or in isotonic saline.
2. Add one drop (40-50μl) of either Anti-A, Anti-B or Anti-A,B reagent to a clean, labelled microscope slide.
3. Add one drop (40-50μl) of the suspension of test red cells.
4. Mix the antiserum and cells over an area about 2cm in diameter by gently and continuously rocking the slide.
5. Read macroscopically after 3 minutes. Do not confuse any drying of the mixture with agglutination.

2. TUBE TECHNIQUE
1. 2.1 Prepare a 3-5% suspension of test red cells in isotonic saline.
2. 2.2 Add 1 drop (40-50μl) of either Anti-A, Anti-B or Anti-A,B reagent to an appropriately labelled test tube.
3. 2.3 Add 1 drop (40-50μl) of the suspension of test red cells.
4. 2.4 Mix and centrifuge at 1000 rcf for 20 seconds.
5. 2.5 Gently agitate the tube to dislodge the red cells and examine macroscopically for agglutination.
6. 2.6 Incubate weaker than expected reactions for 1 minute at room temperature and then re-spin.

LIMITATIONS
The results of red cell grouping should be confirmed by reverse grouping the individual’s serum with known A1 and B red cells. No recipient should be given AB blood unless the cells of the recipient are clearly positive with Anti-A and Anti-B and the recipient’s serum shown to give negative reactions with A1 and B cells (unless the recipient has been shown to be a subgroup of AB with Anti-A1 in the serum). Anti-A blood grouping reagent is not validated to detect all examples of Ax cells. False positive or false negative results may occur through contamination of test materials or any deviation from the recommended technique.

BIBLIOGRAPHY
**Monoclonal Blood Grouping Reagents**

**Anti-A**

**Anti-B**

**Anti-A,B**

---

**INTENDED USE**

These reagents are suitable for use by the slide, tube and microplate techniques and are designed for use by operators trained in serological techniques.

---

**INTRODUCTION**

**The ABO Blood Group System**

In 1900, Landsteiner discovered that the serum of some individuals would agglutinate the red cells of others and that this phenomenon could be used to classify individuals into different blood group phenotypes. Four common phenotypes are recognised – O, A, B and AB. Subgroups of the A and B antigens have since been identified. The ABO phenotype of an individual is usually determined by the agglutination reactions of the individual's red cells with Anti-A, Anti-B and Anti-A,B antisera (forward grouping). In testing blood samples from adults, confirmation of the ABO blood group can be provided by the reactions of the individual's serum with standard A and B red cell suspensions (reverse grouping).

---

**PRINCIPLE**

Anti-A monoclonal murine IgM blood grouping reagent contains antibody from the cell line BIRMA-1, Anti-B monoclonal murine IgM blood grouping reagent contains antibody from LB-2 and Anti-A,B monoclonal murine IgM blood grouping reagent contains antibodies from cell lines ES-4/ES-15. When used by the recommended techniques these reagents will cause agglutination (clumping) of red cells carrying the specific antigen (positive test). Lack of agglutination of the red cells demonstrates the absence of the specific antigen (negative test). These reagents have been optimised for use by the recommended techniques without further dilution or additions.

---

**REAGENTS AND MATERIALS**

Blood grouping reagents contain monoclonal murine IgM antibodies in a buffer solution. The solution is a phosphate buffer containing sodium chloride, EDTA and bovine material. This reagent contains <0.1% sodium azide and the following colourants:

<table>
<thead>
<tr>
<th>Blood Grouping Reagent</th>
<th>Reagent Colour</th>
<th>Dye</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-A</td>
<td>Blue</td>
<td>Patent Blue</td>
</tr>
<tr>
<td>Anti-B</td>
<td>Yellow</td>
<td>Tartrazine</td>
</tr>
<tr>
<td>Anti-A,B</td>
<td>Colourless</td>
<td>N/A</td>
</tr>
</tbody>
</table>

---

**Materials required but not provided**

<table>
<thead>
<tr>
<th>Slide Technique:</th>
<th>Tube Technique:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscope slide</td>
<td>Test tube</td>
</tr>
<tr>
<td>Timer</td>
<td>Centrifuge (1000 rcf)</td>
</tr>
<tr>
<td>Isotonic saline</td>
<td>Isotonic saline</td>
</tr>
<tr>
<td>Compatible plasma/serum</td>
<td>Timer</td>
</tr>
</tbody>
</table>

---

**PRECAUTIONS**

7. The cell lines used to produce these reagents are of murine origin and have been tested and found to be negative for Mouse Antibody Production (MAP) viruses. Care must be taken in the use and disposal of each container and its contents.

8. These reagents contain <0.1% (w/v) sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form highly explosive salts. On disposal, flush with large quantities of water.

9. These products should be clear. Turbidity may indicate bacterial contamination. These reagents should not be used if a precipitate, fibrin gel or particles are present.

10. These reagents are for professional *in vitro* diagnostic use only.

11. The bovine materials are obtained from USDA approved sources or from sources for which origin information is available. The donor animals for bovine material have been inspected and certified disease free and are deemed to have low TSE (Transmissible Spongiform Encephalopathy) risk.

12. These products should be disposed of either by overnight immersion in disinfectants at appropriate concentrations or by autoclaving.

---

**ADVICE TO USERS**

It is recommended that a positive control and a negative control should be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show the expected reactions. It is not required to use a reagent control in parallel with all tests using these reagents. Only in typing the red cells of patients known to have auto antibodies or protein abnormalities is the use of a reagent control recommended. This should be tested in parallel with the reagents. These reagents have been characterised by the procedures recommended in this package insert, their suitability for use in other techniques must be determined by the user.
Monoclonal Blood Grouping Reagents

Anti-A
Anti-B
Anti-A,B

STORAGE AND STABILITY
Store the opened /unopened products at 2-8°C until the expiry date detailed on the product label. Failure to store the products at the correct temperature, for example, storage at higher temperature or repeated freezing and thawing may result in accelerated loss of reagent activity.

SPECIMEN COLLECTION AND PREPARATION
No special preparation of the patient is required prior to specimen collection. Blood should be collected by an approved phlebotomy technique. The specimen should be tested as soon as possible following collection. If a delay in testing should occur, store the specimen at 2-8°C. Specimens displaying gross haemolysis or microbial contamination should not be tested with this reagent. Failure to store the specimens at the correct temperature, for example, storage at higher temperature or repeated freezing and thawing may result in false positive or false negative results.

INSTRUCTIONS FOR USE
1. SLIDE TECHNIQUE
6 Prepare a 35-50% suspension of test red cells in autologous (or compatible) plasma, serum or in isotonic saline.
7 Add one drop (40-50μl) of either Anti-A, Anti-B or Anti-A,B reagent to a clean, labelled microscope slide.
8 Add one drop (40-50μl) of the suspension of test red cells.
9 Mix the antiserum and cells over an area about 2cm in diameter by gently and continuously rocking the slide.
10 Read macroscopically after 3 minutes. Do not confuse any drying of the mixture with agglutination.

2. TUBE TECHNIQUE
7 2.1 Prepare a 3-5% suspension of test red cells in isotonic saline.
8 2.2 Add 1 drop (40-50μl) of either Anti-A, Anti-B or Anti-A,B reagent to an appropriately labelled test tube.
9 2.3 Add 1 drop (40-50μl) of the suspension of test red cells.
10 2.4 Mix and centrifuge at 1000 rcf for 20 seconds.
11 2.5 Gently agitate the tube to dislodge the red cells and examine macroscopically for agglutination.
12 2.6 Incubate weaker than expected reactions for 1 minute at room temperature and then re-spin.

LIMITATIONS
The results of red cell grouping should be confirmed by reverse grouping the individual's serum with known A1 and B red cells. No recipient should be given AB blood unless the cells of the recipient are clearly positive with Anti-A and Anti-B and the recipient’s serum shown to give negative reactions with A1 and B cells (unless the recipient has been shown to be a subgroup of AB with Anti-A1 in the serum). Anti-A blood grouping reagent is not validated to detect all examples of Ax cells. False positive or false negative results may occur through contamination of test materials or any deviation from the recommended technique.

BIBLIOGRAPHY