**INTENDED PURPOSE**

The ABO reagents are blood grouping reagents intended to be used to qualitatively determine the presence or absence of the A or B antigens on the red cells of blood donors or patients requiring a blood transfusion when tested in accordance with the recommended techniques stated in this IFU.

**PRINCIPLE**

The reagents contain antibodies against the appropriate A and/or B antigen on human red cells and will cause direct agglutination (clumping) of red cells that carry the corresponding ABO antigen. No agglutination generally indicates the absence of the corresponding ABO antigen on human red cells (see Limitations).

**REAGENT**

Lorne Monoclonal IgM ABO blood grouping reagents contain mouse monoclonal antibodies diluted in a phosphate buffer containing sodium chloride, EDTA and bovine albumin. The reagents do not contain or consist of CMR substances, or endocrine disrupting substances or that could result in sensitisation or an allergic reaction by the user. Each reagent is supplied at optimal dilution for use with all the recommended techniques stated below without the need for further dilution or addition. For lot reference number and expiry date see Vial Label.

<table>
<thead>
<tr>
<th>Product</th>
<th>Cell Line/Clone</th>
<th>Colour</th>
<th>Dye Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-A</td>
<td>9113D10</td>
<td>Blue</td>
<td>Patent Blue</td>
</tr>
<tr>
<td>Anti-B</td>
<td>962TA5</td>
<td>Yellow</td>
<td>Tartrazine</td>
</tr>
<tr>
<td>Anti-A,B</td>
<td>525D12 + 52113D10 + ES15</td>
<td>Colourless</td>
<td>None</td>
</tr>
</tbody>
</table>

**STORAGE**

Reagent vials should be stored at 2 - 8°C on receipt. Prolonged storage at temperatures outside this range may result in accelerated loss of reagent reactivity. This reagent has undergone transportation stability studies at 37°C and -25°C as described in document BS EN ISO 23640:2015.

**SAMPLE COLLECTION AND PREPARATION**

Blood samples can be collected into EDTA, citrate, CPDA anticoagulants or as a clotted sample. The samples should be tested as soon as possible following collection. If a delay in testing should occur, store the samples at 2-8°C. Samples displaying gross haemolysis or microbial contamination should not be used for testing. Blood samples showing evidence of lysis may give unreliable results. It is preferable (but not essential) to wash all blood samples with PBS or Isotonic saline before being tested.

**PRECAUTIONS**

1. The reagents are intended for in vitro diagnostic use only.
2. If a reagent vial is cracked or leaking, discard the contents immediately.
3. Do not use the reagents past the expiration date (see Vial Label).
4. Do not use the reagents if a precipitate is present.
5. Protective clothing should be worn when handling the reagents, such as disposable gloves and a laboratory coat.
6. The reagents have been filtered through a 0.2 µm capsule to reduce the bio-burden, but are not supplied sterile. Once a vial has been opened the contents should remain viable up until the expiry date as long as there is no marked turbidity, which can indicate reagent deterioration or contamination.
7. The reagents contain < 0.1% sodium azide. Sodium azide may be toxic if ingested or may react with lead and copper plumbing to form explosive metal azides. On disposal flush away with large volumes of water.
8. No known tests can guarantee that products derived from human or animal sources are free from infectious agents. Care must be taken in the use and disposal of each vial and its contents.

**DISPOSAL OF REAGENT AND DEALING WITH SPILLAGES**

For information on disposal of the reagent and decontamination of a spillage site see Material Safety Data Sheets, available on request.

**CONTROLS AND ADVICE**

1. It is recommended a positive control and a negative control be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected results.

2. Since these reagents do not contain macromolecular potentiators, it is very unlikely that false positive reactions are caused with IgG coated cells.
3. Blood specimens of weak A or B subgroups (e.g. Ax) may give rise to false negative or weak reactions when tested using slides, microtitre plates or gel cards. It is advisable to re-test weak subgroups using tube technique.
4. Individuals older than six months should have their ABO blood-grouping results confirmed by testing their serum or plasma against known group A, B and C cells before their ABO blood group can be confirmed.
5. Before use, let the reagent warm up to room temperature. As soon as the reagent has been used, put the reagent back in storage at 2-8°C.
6. In the Recommended Techniques one volume is approximately 50µl when using the vial dropper provided.
7. The use of the reagents and the interpretation of results must be carried out by properly trained and qualified personnel in accordance with the requirements of the country where the reagents are in use.
8. The user must determine the suitability of the reagents for use in other techniques.

**REAGENTS AND MATERIALS REQUIRED**

- Applicator sticks.
- Automatic plate reader.
- Bio-Rad ID-Cards (NaCl, enzyme test and cold agglutinins).
- Bio-Rad ID-Centrifuge.
- Bio-Rad ID-CellStab or ID-Diluent 2.
- Glass microscope slides or white card tiles.
- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- Microplate centrifuge.
- Ortho BioVue System Cassettes (Neutral).
- Ortho BioVue System Centrifuge.
- Ortho 0.8% Red Cell Diluent.
- Plate shaker.
- PBS solution (pH 6.8–7.2) or Isotonic saline solution (pH 6.5–7.5).
- Positive and negative control red cells:
  - Anti-A: group A (positive control) and group O (negative control).
  - Anti-B: group B (positive control) and group O (negative control).
  - Anti-A,B: group A and group B (positive controls) and group O (negative control).
- Test tube centrifuge.
- Validated "U" well microplates.
- Volumetric pipettes.

**RECOMMENDED TECHNIQUES**

**A. Tube Technique**

1. Prepare a 2-3% suspension of red cells in PBS or Isotonic saline.
2. Place in a labelled test tube: 1 volume of Lorne Anti-ABO reagent and 1 volume of red cell suspension.
3. Mix thoroughly and incubate at room temperature for 1 minute.
4. Centrifuge ID-Card(s) in the Bio-Rad gel card centrifuge.
5. Read macroscopically for agglutination.

**B. Bio-Rad-ID Technique (NaCl, enzyme test and cold agglutinins cards)**

1. Prepare a 0.6% suspension of red cells in ID-CellStab or ID-Diluent 2.
2. Place in a labelled test tube: 1 volume of Lorne Anti-ABO reagent and 1 volume of red cell suspension.
3. Mix thoroughly and incubate at room temperature for 1 minute.
4. Centrifuge all tubes for 10 seconds at 1000 rcf or for a suitable alternative time and force.
5. Gently resuspend red cell button and read macroscopically for agglutination.
6. Any tubes, which show a negative or questionable result, should be incubated for 15 minutes at room temperature.
7. Following incubation, repeat steps 4 and 5.

**C. Ortho BioVue Technique (Neutral cassettes)**

1. Prepare a 0.8% suspension of red cells in 0.8% Ortho Red Cell Diluent.
2. Remove aluminium foil from as many microtubes as needed.
3. Place in appropriate microtube: 50µl of red cell suspension and 25µl of Lorne Anti-ABO reagent.
4. Centrifuge ID-Card(s) in the Bio-Rad gel card centrifuge.
5. Read macroscopically for agglutination.

**D. Microplate Technique, using "U" wells**

1. Prepare a 2-3% suspension of red cells in PBS or Isotonic saline.
2. Place in the appropriate wells: 1 volume Lorne Anti-ABO reagent and 1 volume red cell suspension.
3. Mix thoroughly, preferably using a microplate shaker, taking care to avoid cross-well contamination.
4. Incubate at room temperature for 15 minutes (time dependant on user).
5. Centrifuge the microplate for 1 minute at 140 rcf or for a suitable alternative time and force.
6. Resuspend the cell buttons using carefully controlled agitation on a microplate shaker.


