INTRODUCTION
ABO blood grouping is generally performed by testing red cells with anti-A and anti-B. In order to generate confirmatory blood group information and exclude misgrouping of weak A variants as group O, etc, many laboratories also test with anti-A and anti-B. Reverse or serum grouping of the patient’s serum by testing with ABO variants as group O, B, AB and A,B. Reverse or serum grouping of the patient’s serum by testing with ABO blood grouping is generally performed by testing red cells with B cells (A− cells may be additionally included) should be performed to provide a further check of the accuracy of observed ABO blood groups.

Monoclonal antibodies exhibit a high degree of potency, avidity and specificity. When using such antibodies, great care should be taken to ensure that appropriate serum: cell ratios are maintained in all test systems.


STORAGE CONDITIONS
The reagent should be stored at 2°C – 8°C. Do not use if turbid. Do not dilute. The reagent is stable until the expiry date stated on the product label.

INTERPRETATION OF LABEL SYMBOLS

INTENDED PURPOSE
The Anti-A,B reagent is for the in vitro detection and identification of human ABO blood groups by direct agglutination.

REAGENT DESCRIPTION
The main components of this reagent are derived from the in vitro culture of the immunoglobulin secreting mouse hybridomas:-

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Product Code</th>
<th>Cell Line</th>
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<tbody>
<tr>
<td>Anti-A,B</td>
<td>Z021</td>
<td>LA2, LB2, ES15</td>
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</table>

The formulation also contains sodium chloride and EDTA and 1g/l sodium azide. The volume delivered by the reagent dropper bottle is approximately 40µl; bearing this in mind, care should be taken to ensure that appropriate serum: cell ratios are maintained in all test systems.

This reagent contains 0.1% sodium azide (EC No.247-852-1) and is classified as harmful. R22 - Harmful if swallowed. Sodium azide may react with lead and copper plumbing to form explosive compounds. If discarded into sink, flush with a large volume of water to prevent azide build-up.

As this reagent is of animal origin care must be taken during use and disposal as there is a potential infection risk.

Additional Materials and Reagents Required

- PBS pH 7.0 ± 0.2
- LISS
- Reagent red cells for use in ABO grouping
- 12 x 75mm glass test tubes
- Glass slides
- Pipettes
- Optical aid
- Centrifuge

RECOMMENDED TECHNIQUES

Tube Technique - Immediate Spin

- Add 1 volume of blood grouping reagent to a test tube.
- Add 1 volume of red cells suspended to 2-3% in PBS pH 7.0 ± 0.2 or 1.5 - 2% in LISS.
- Mix the test well.
- Centrifuge immediately at 1000g for 10 seconds or at a suitable alternative g force and time.
- Gently shake the tube to dislodge the cell button from the bottom and observe macroscopically for agglutination.

Tube Technique - LIS

- Add 1 volume of blood grouping reagent to a test tube.
- Add 1 volume of red cells suspended to 1.5 - 2% in LISS.
- Mix the test well and incubate for 15-20 minutes at approximately 20°C.
- Centrifuge at 1000g for 10 seconds or at a suitable alternative g force and time.
- Gently shake the tube to dislodge the cell button from the bottom and observe macroscopically for agglutination.

Slide Technique

- Add 1 volume of blood grouping reagent to an appropriately prepared area of a glass slide eg a wax pencil oval.

This reagent is for in vitro professional use only.

SPECIMEN COLLECTION AND PREPARATION

Specimens should be collected by aseptic technique with or without an anticoagulant. The specimen should be tested as soon as possible after collection. If testing is delayed, the specimen should be stored at 2°C - 8°C. Blood specimens exhibiting gross haemolysis or contamination should not be used. Clotted samples or those collected in EDTA should be tested within seven days from collection. Donor blood stored in citrate anticoagulant may be tested until the expiry date of the donation.

TEST PROCEDURES

General Information

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

PRECAUTIONS FOR USE AND DISPOSAL

This reagent contains 0.1% sodium azide (EC No.247-852-1) and is classified as harmful. R22 - Harmful if swallowed. Sodium azide may react with lead and copper plumbing to form explosive compounds. If discarded into sink, flush with a large volume of water to prevent azide build-up.

As this reagent is of animal origin care must be taken during use and disposal as there is a potential infection risk.
Add 1 volume of red cells suspended to 30-45% in PBS pH 7.0 ± 0.2 or in group homologous plasma/serum.

Mix well by rocking the slide for approximately 30 seconds and incubate the test for 5 minutes at room temperature with occasional mixing.

Observe macroscopically for agglutination. This may be facilitated by reading over a diffuse light source.

**INTERPRETATION OF RESULTS**

The reaction patterns of the most common ABO phenotypes are shown below.

<table>
<thead>
<tr>
<th>Anti-A</th>
<th>Anti-B</th>
<th>Anti-A,B</th>
<th>Blood Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>O</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>A</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>+</td>
<td>B</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>AB</td>
</tr>
</tbody>
</table>

All cell (forward) grouping tests, except those on cells of infant blood, should be confirmed by serum (reverse) grouping tests using known A_1 and B cells.

**QUALITY CONTROL**

Quality control of reagents is essential and should be performed at the start of each day’s testing, with each series of groups performed and with single tests, eg an emergency compatibility test ie:

- Anti-A
- Anti-B
- Anti-A,B

should be tested with A_1, A_2, B and O cells

**PERFORMANCE LIMITATIONS**

ABO antigens are not fully expressed at birth and, therefore, tests involving cord/neonatal red cells should be interpreted with particular care.

Slide tests are not recommended for detection of weak subgroups. All slide tests should be confirmed by tube grouping.

Tests should be read by a ‘tip and roll’ procedure. Excessive agitation may disrupt weak agglutination and produce false negative results.

It is important to use the recommended g force during centrifugation as excessive centrifugation can lead to difficulty in resuspending the cell button, while inadequate centrifugation may result in agglutinates that are easily dispersed.

The expression of certain red cell antigens may diminish in strength during storage, particularly in EDTA and clotted samples. Better results will be obtained with fresh samples.

False positive or false negative results can occur due to contamination of test materials, improper reaction temperature, improper storage of materials, omission of test reagents and certain disease states.

**SPECIFIC PERFORMANCE CHARACTERISTICS**


This Anti-A,B reagent will detect most weak subgroups of A, especially A_x.

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For further information or advice please contact your local distributor.

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