

MONOCLONAL BLOOD GROUPING REAGENTS.



DIRECTIONS FOR USE

Anti-A, Anti-B and Anti-A,B Monoclonal:

For Tube, DiaMed-ID, Ortho BioVue, Microplate and Slide Techniques.

SUMMARY

In 1900, Landsteiner discovered the serum of some people would agglutinate the red cells of others. Four common phenotypes are now recognised: O, A, B and AB. Subgroups of A and B have since been identified.

Forward Group			Reverse Group			ABO Phenotype	Caucasians %
A	B	A,B	A ₁	A ₂	B	O	
+	0	+	0	0	+	0	A 42
0	+	+	+	+	0	0	B 10
0	0	0	+	+	+	0	O 44
+	+	+	0	0	0	0	AB 4

PRINCIPLE

The reagents will cause direct agglutination (clumping) of test red cells that carry the corresponding ABO antigen. No agglutination generally indicates absence of the corresponding ABO antigen (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. 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.

Product	Cell Line/Clone	Colour	Dye Used
Anti-A	9113D10	Blue	Patent Blue
Anti-B	9621A8	Yellow	Tartrazine
Anti-A,B	152D12 + 9113D10	Colourless	None

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 EN13640:2002.

SAMPLE COLLECTION AND PREPARATION

Blood samples drawn with or without anticoagulant may be used for antigen typing. If testing is delayed, then store specimens at 2-8°C. EDTA and citrate samples should be typed within 7 days after collection. Samples collected into ACD, CPD or CPDA-1 may be tested up to 35 days from the date of withdrawal. All blood samples should be washed at least twice with PBS or Isotonic saline before being tested. Blood samples showing evidence of lysis may give unreliable results.

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. 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 and 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 A₁ and B cells before their ABO blood group can be confirmed.
5. In the Recommended Techniques one volume is approximately 50µl when using the vial dropper provided.
6. The use of the reagents and the interpretation of results must be carried out by properly trained and qualified personnel in accordance with the

7. requirements of the country where the reagents are in use.
7. The user must determine the suitability of the reagents for use in other techniques.

REAGENTS AND MATERIALS REQUIRED

- Applicator sticks.
- Automatic plate reader.
- DiaMed ID-Cards (Neutral).
- DiaMed ID-Centrifuge.
- DiaMed ID-CellStab or ID-Diluent 2.
- Glass microscope slides.
- 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 A2B (positive control) and group O (negative control).
- Anti-B: group A1B (positive control) and group O (negative control).
- Anti-A,B: group A1B (positive control) 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 washed test 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 test 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.

B. DiaMed-ID Micro Typing Technique

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

C. Ortho BioVue Typing Technique

1. Prepare a 0.8% suspension of washed test red cells in 0.8% Ortho Red Cell Diluent.
2. Remove aluminium foil from as many reaction chambers as needed.
3. Place in appropriate reaction chamber: 50µl of test red cell suspension and 40µl of Lorne Anti-ABO reagent.
4. Centrifuge cassette(s) in an Ortho BioVue System Centrifuge.
5. Read macroscopically for agglutination.

D. Microplate Technique, using "U" wells

1. Prepare a 2-3% suspension of washed test red cells in PBS or Isotonic saline.
2. Place in the appropriate well: 1 volume Lorne Anti-ABO reagent and 1 volume test 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
7. Read macroscopically or with a validated automatic reader.
8. Any weak reactions should be repeated by the tube technique.

E. Slide Technique

1. Prepare a 35-45% suspension of test red cells in serum, plasma or PBS or Isotonic saline.
2. Place on a labelled glass slide: 1 volume of Lorne Anti-ABO reagent and 1 volume of test red cell suspension.
3. Using a clean applicator stick, mix reagent and cells over an area of about 20 x 40 mm.
4. Slowly tilt the slide back and forth for 30 seconds, with occasional further mixing during the 2-minute period, maintaining slide at room temperature.
5. Read macroscopically after 2 minutes over a diffuse light and do not



mistake fibrin strands as agglutination.

- Any weak reactions should be repeated by the tube technique.

INTERPRETATION OF TEST RESULTS

- Positive: Agglutination of the test red cells constitutes a positive test result and within accepted limitations of test procedure, indicates the presence of the appropriate ABO antigen on the test red cells.
- Negative: No agglutination of the test red cells constitutes a negative result and within the accepted limitations of the test procedure, indicates the absence of the appropriate ABO antigen on the test red cells.
- Discrepancies: If the results obtained with reverse group don't correlate with forward group, further investigation is required.

STABILITY OF THE REACTIONS

- Read all tube and microplate tests 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.
- Caution should be exercised in the interpretation of results of tests performed at temperatures other than those recommended.

LIMITATIONS

- ABO antigens are not fully developed at birth and so weaker reactions may therefore occur with cord or neonatal specimens.
- When using Monoclonal Anti-A,B, 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 the tube technique.
- Lorne monoclonal Anti-A and monoclonal Anti-B are not validated to detect Ax and A3 or Bx and B3 antigens resp and we therefore do not claim reactivity of the monoclonal Anti-A or Anti-B reagent against these weak A and B sub-groups.
- Stored blood may give weaker reactions than fresh blood.
- False positive or false negative results may also occur due to:
 - Contamination of test materials
 - Improper storage, cell concentration, incubation time or temperature
 - Improper or excessive centrifugation
 - Deviation from the recommended techniques
 - Cord samples contaminated with Wharton's jelly

SPECIFIC PERFORMANCE CHARACTERISTICS

- The reagents have been characterised by all the procedures mentioned in the Recommended Techniques.
- Prior to release, each lot of Lorne Monoclonal Anti-A, Anti-B and Anti-A,B is tested by the Recommended Techniques against a panel of antigen-positive red cells to ensure suitable reactivity.
- Specificity of source monoclonal antibodies is demonstrated using a panel of antigen-negative cells.
- The potency of the reagents has been tested against the following minimum potency reference standards obtained from National Institute of Biological Standards and Controls (NIBSC): Anti-A reference standard 03/188 And / Or Anti-B reference standard 03/164
- Lorne Anti-B does not react with "Acquired-B" red cells.
- Lorne Monoclonal ABO reagents do not detect crypt antigens such as T, Tn or Cad.
- The Quality Control of the reagents was performed using red cells that had been washed at least twice with PBS or Isotonic saline prior to use.
- The reagents comply with the recommendations contained in the latest issue of the Guidelines for the UK Blood Transfusion Services.

DISCLAIMER

- The user is responsible for the performance of the reagents by any method other than those mentioned in the Recommended Techniques.
- Any deviations from the Recommended Techniques should be validated prior to use⁹.

BIBLIOGRAPHY

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- BSCH Blood Transfusion Task Force. Guidelines for microplate techniques in liquid-phase blood grouping and antibody screening. *Clinical Laboratory Haematology* 1990; 12, 437-460.
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new techniques for blood grouping, antibody screening and cross matching. *Transfusion Medicine*, 1995, 5, 145-150.

AVAILABLE REAGENT SIZES

	Vial Size	Catalogue Number
Anti-A Monoclonal	10 ml	600010
	1000 ml	600000*
	5000 ml	600000X5*
Anti-B Monoclonal	10 ml	610010
	1000 ml	610000*
	5000 ml	610000X5*
Anti-A,B Monoclonal	10 ml	620010
	1000 ml	620000*
	5000 ml	620000X5*

*This size is For Further Manufacturing Use (FFMU) only and is therefore not CE marked.

TABLE OF SYMBOLS

	Batch Number		In-vitro Diagnostic
	Catalogue Reference		Store At
	Expiry Date		Manufacturer
	Read Pack Insert		



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