

MONOCLONAL BLOOD GROUPING REAGENTS ANTI-A, ANTI-B AND ANTI-A,B

TUBE, SLIDE AND MICROPLATE TESTS

STORE AT 2 – 8°C

FOR IN-VITRO DIAGNOSTIC USE ONLY

Principle:

The test is based on the principle of agglutination. Red cells which the antigen will agglutinate when tested against the corresponding antibody.

Presentation:

Reagent	Code	Size
Anti-A Monoclonal	BGA00010	10 ml
Anti-B Monoclonal	BGB00010	10 ml
Anti-A,B Monoclonal	BGAB0010	10 ml

Composition: Monoclonal ABO Grouping Reagents are prepared using in vitro culture supernatants from hybridised immunoglobulin-secreting mouse cell lines. Each antibody is then diluted in a phosphate buffer which contains sodium chloride, EDTA and bovine albumin resulting in a reagent which is optimised for use in slide, tube and microplate test methods.

- Anti-A is coloured with acid blue dye.
- Anti-B is coloured with acid yellow dye.
- Anti-AB is uncoloured.

Although all our components which have been derived from human origin have been tested and found to be negative for the presence of anti-HIV, anti-HCV as well as HbsAg, it is recommended that they be handled cautiously and treated potentially infectious.

Storage:

- Store components at 2-8°C.
- Do not freeze or expose to elevated temperatures.
- Do not use beyond the expiry date.
- Marked turbidity may indicate reagent contamination or deterioration.

Samples:

- Blood samples which have been drawn with or without anti-coagulant may be used.
- Testing should be performed as soon as possible to avoid false reactions occurring due to contamination or incorrect storage.
- Samples may be stored at between 2 and 8°C and tested within two days provided there is no evidence of haemolysis.

Test Procedures:

Tube Technique:

1. Prepare a 2-3% red cell suspension using isotonic buffered saline with a pH of 6.9.
2. Place in a glass test tube 1 volume of ABO Grouping Reagent and 1 volume of the Red Cell Suspension.
3. Mix well and incubate at room temperature (18 – 25°C) for 5 – 15 minutes.
4. Centrifuge at 900 to 1000 rcf for 15 seconds.
5. Gently resuspend the cell button and examine macroscopically for signs of agglutination.
6. Record the results.

Microplate Technique:

1. Prepare a 2 – 3 % Red Cell Suspension using isotonic buffered saline with a pH of 6.9.
2. Place in the appropriate well of a U-bottom microplate 1 volume (30 - 50µl) of ABO Grouping Reagent and 1 volume of the 2 – 3 % (30 - 50µl) Red Cell Suspension.
3. Mix well, preferably using a microplate shaker, taking care to avoid cross-well contamination.
4. Incubate at room temperature (18 – 25°C) for 5 – 15 minutes.
5. Centrifuge the microplate at 140rcf for 1 minute.
6. Tilt the plate at an angle of 60 – 90° to the bench top and observe over the next three minutes for signs of streaming. Negative reactions allow the cells to flow downwards in a uniform stream. Positive reactions remain as distinct buttons either on the bottom of the well or occasionally sliding down the side.
7. As an alternative to tilting the plate, the cell buttons can be resuspended using carefully controlled agitation on a

microplate shaker, then examined for agglutination either visually or by means of a validated automated reader.

Slide Technique:

1. Prepare a 35 – 45% red cell suspension using either their own group or group compatible plasma or serum.
2. Onto a slide which is at room temperature (18 – 25°C) place 1 volume of ABO Grouping Reagent and 1 volume of the 35 – 45% Red Cell Suspension.
3. Using a clean applicator stick, mix the reagent and cell suspension over an area of approximately 20 x 40 mm.
4. Slowly tilt the slide back and forth for no longer than two minutes and observe for signs of agglutination.
5. Record the results.

Reaction Stability: Following centrifugation all tube and microplate tests should be read immediately and results interpreted without delay. Slide tests should be interpreted within two minutes to avoid drying of the reagents which might result in negative reactions being called positive.

Quality Control:

- It is recommended that appropriate antigen-positive and antigen-negative cells be tested with the reagents on each day of use in order to confirm the reactivity and specificity of the blood grouping reagents.
- Reverse grouping should be carried out on individuals who are older than 6 months of age.

Reactions:

Reagent			Cell Suspension				ABO
Anti-A	Anti-B	Anti-AB	A ₁	A ₂	B	O	Group
+	-	+	-	-	+	-	A
-	+	+	+	+	-	-	B
+	+	+	-	-	-	-	AB
-	-	-	+	+	+	-	O

If the results obtained with the serum do not correlate with the red cell test, further investigation is required.

Limitations of the Procedure:

- False positive and negative results may occur due to contamination of test materials, improper cell concentrations, incorrect centrifugation, incubation and temperature times.
- Any deviation from the test procedure could result in inaccurate results.
- Weaker reactions may be observed with stored blood.
- Weaker reactions may be observed with cord blood or neonatal red cells as ABO Antigens are not fully developed at birth.
- Cord samples contaminated with Whartons Jelly may give false positive results.
- Blood samples of weak A or B subgroups may result in false negative or weak reactions. Extending the incubation times to 30 minutes might improve the reaction strength.

Bibliography:

BCSH Blood Transfusion Task Force Guidelines for microplate techniques in liquid phase blood grouping. Clin. Lab. Haem, 1990: 12, 437 – 460
 Voak D., et al. (1982) Monoclonal anti-A and anti-B development as cost effective reagents. Med. Lab. Sci. 39, 109 – 122.
 Kholer G., Milstein C., (1975) Continuous culture of fused cells secreting ab predefined specificity. Nature 256, 495 – 497
 Mollison P.L. Blood Transfusion in Clinical Medicine, 8th Ed, Oxford: Blackwell Scientific, 1987, Chapter 7.
 Issitt P.D. Applied Blood Group Serology, 3rd ed. Miami: Montgomery Scientific, 198 Chapter 6
 Race RR., Sanger R., Blood Grps in Man 6th Ed. Oxford, Blackwell Sci. 1987: Chapter 7.