



TriClone Anti-A, Anti-B, Anti-AB, Anti-D (IgM/ IgG)

Monoclonal Blood Grouping Reagents

For Tube, Microplate and Slide Techniques

For professional in vitro diagnostic use only.

INTENDED USE

This reagent is designated for professional use in laboratory qualitative determination the presence or absence of the A and/or B antigens and D (RH1) on human red blood cells.

GENERALITIES

In 1900, Landsteiner discovered the serum of some people would agglutinate the red cells of others. Four common phenotypes are now recognized: O, A, B and AB. Subgroups of A and B have since been identified. The Rh blood group system was discovered in 1940. The D antigen is the most clinically significant non-ABO red blood cell antigen and has been implicated in causing Hemolytic Transfusion Reactions and Hemolytic Disease of the Newborn.

TEST PRINCIPLE

The reagents will cause direct agglutination (clumping) of test red cells that carry the corresponding ABO antigen. No agglutination generally indicates the absence of the corresponding ABO antigen. The Anti D reagent contains antibodies against the D antigen on human red cells and will cause direct agglutination (clumping) of human red cells that carry the D antigen, and indirect agglutination of human red cells that are Category D^{VI} in the antiglobulin phase of testing.

REAGENT COMPOSITION

Anti-A, Anti-B and Anti-AB blood grouping (Monoclonal IgM) 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.

Anti-D (IgM/IgG), monoclonal blood grouping reagent is a low protein, blended reagent containing a human monoclonal IgM and IgG anti-D,

Reagent	Clone	Color
Anti-A	A-11H5	Blue
Anti-B	B-6F9	Yellow
Anti-AB	A-5E10 + B-2D7	Colorless
Anti-D	IgM: BS225	Colorless
	IgG: MS-26	

STORAGE AND SHELF LIFE

Reagent vials should be stored at 2-8°C. Prolonged storage at temperatures outside this range may result in accelerated loss of reagent reactivity. If stored at 2-8 °C, the test reagents can be used until the date indicated on the label. Avoid bacterial contamination. Do not use reagents if markedly turbid. Do not freeze.

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 occurs, store the samples at 2-8°C. Samples displaying gross y give unreliable results. It is preferable (but not essential) to wash all blood samples with PBS or Isotonic saline before being tested.

MATERIALS REQUIRED

- Applicator sticks
- Glass microscope slides
- Glass test tubes (10 x 75 mm or 12 x 75 mm)
- Microplate centrifuge
- Red Cell Diluent
- Plate shaker
- Phosphate Buffered Saline (PBS): NaCl 0.9%, pH 7.0 ± 0.2 at 22°C ± 1°C
- Positive and negative (group O) control red cells.
- Test tube centrifuge.
- Validated “U” well microplates
- Volumetric pipettes.

TEST PROCEDURE

A. Slide Technique

1. Prepare a 35-45% suspension of test red cells in serum, plasma or PBS.
2. Place on a labelled glass slide: 1 volume (40 µL) of Anti-ABO reagent and 1 volume (Drop 40 µL) of test red cell suspension.
3. Using a clean applicator stick, mix reagent and cells over an area of about 20x40 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.
6. Any weak reactions should be repeated by the tube technique.

B. 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 ABO reagent, monoclonal 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. Microplate Technique:

1. Prepare a 2-3% suspension of washed test red cells in PBS.
2. Place in the appropriate well: 1 volume 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 dependent on user). Centrifuge the microplate for 1 minute at 140 rcf or for a suitable alternative time and force.
5. Resuspend the cell buttons using carefully controlled agitation on a microplate shaker.
6. Read macroscopically or with a validated automatic reader.
7. Any weak reactions should be repeated by the tube technique.

INTERNAL QUALITY CONTROL

For each test, positive and negative control red blood cells have to be tested in parallel.

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.

Test results of cells that are agglutinated using the reagent negative control shall be excluded, as the agglutination is most probably caused by the effect of the macromolecular potentiators in the reagent on sensitized cells.

PERFORMANCE

1. The reagents have been characterized by all the procedures mentioned in the recommended techniques and tested against a panel of antigen-positive red cells to ensure suitable reactivity.
2. The quality Control of the reagents was performed using red cells that had been washed twice with PBS prior to use.

Reagent	Positive	False Negative	Sensitivity
Anti-A	12359	0	100 %
Anti-B	5535	0	100 %
Anti-AB	1215	0	100 %
Anti-D	1507	4 (*)	99.7 %

(*) Samples with a less pronounced D feature

Reagent	Negative	False positive	Specificity
Anti-A	13326	0	100 %
Anti-B	20150	0	100 %
Anti-AB	462	0	100 %
Anti-D	466	0	100 %

LIMITATIONS















- Concentrations of red blood cells that are lower than 3 % may cause weaker reactions on plates or slides.
- 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.
- The interpretation of reactions obtained when testing infant blood may be complicated by the fact that the infant's serum does not necessarily contain antibody for any antigen absent from the cells, and passive anti-A and/or anti-B antibodies from the mother's circulation may yield conflicting results when tests are performed on umbilical cord blood specimens. Umbilical cord blood specimens may also produce weaker than normal reactions in the cell grouping, as the ABH antigens are imperfectly developed at birth.
- Weakly expressed D antigen (weak D) is recognized by the plate test with only insufficient sensitivity. Therefore, the tube test with post-incubation in the case of negative, doubtful or weak positive results is recommended.
- A weakly positive reaction indicates the possible presence of weak D or a category (partial D).
- Older blood specimens may show weaker reactions than fresh specimens.

- The monoclonal test reagents agglutinate weakly expressed antigens with normal or weaker agglutination strength (A_3 , B^{weak}) or weaker to negative reaction (A_x). To determine weakly expressed antigens the tube test should be used because of its higher sensitivity, if necessary, with incubation for 30 minutes.
- Monoclonal test reagent Anti-B does not react with acquired B.
- Monoclonal test reagent Anti-A reacts with Tn. Tn positive persons must be excluded from donating blood as the occurrence of Tn is a symptom of a preleukaemic state and the red blood cells are poly agglutinable.
- The concentrations of red blood cell suspensions and the reaction times and conditions specified above must be observed to obtain correct results.

LITERATURE

- Messeter, et al. Mouse monoclonal antibodies with Anti-A, anti-B and Anti-AB specificities, some superior to human polyclonal ABO reagents. Vox Sang 1984; 46, 185-194.
- Race RR, Sanger R. Blood Groups in Man, 6th Edition. Blackwell Scientific, Oxford 1975; Chapter 2.
- Richtlinie zur Gewinnung von Blut und Blutbestandteilen und zur Anwendung von Blutprodukten (Richtlinie Hämotherapie), current version.
- Technical Manual, 17th ed. Bethesda, American Assoc. of Blood Banks, 2011 (a) method 1.5 b) method 1.6).

USED SYMBOLS

	In Vitro Diagnostic Medical Device
	Manufacturer
	Date of Manufacture
	Catalogue Number
	Batch Code
	Use by YYYY-MM (MM = end of month)
	Operator's Manual; Operating Instructions
	Keep away from Sunlight
	Keep away from Rain
	Temperature Limit
	Caution
	Do not use if Package is Damaged
	Do Not Re-Use
	Contains Sufficient for <N> Tests