

## Blood Grouping Reagent

Anti-A and Anti-B (Murine Monoclonal) Series 1

Anti-B (Murine Monoclonal) Series 3 • Anti-A,B (Murine Monoclonal Blend) Series 1

For Slide, Tube and Microplate Tests

• IVD

• 10°C  
1°C

• Meets FDA potency requirements



Harmful, Preservative: 0.1% Sodium Azide

Discard if markedly turbid

CAUTION: DO NOT PIPET BY MOUTH THE ABSENCE OF MURINE VIRUSES HAS NOT BEEN DETERMINED. THE PACKAGING OF THIS PRODUCT (DROPPER BULBS) CONTAINS DRY NATURAL RUBBER.



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US LICENSE 886



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## Blood Grouping Reagent

Anti-A (Murine Monoclonal) Series 1

Anti-B (Murine Monoclonal) Series 1

Anti-B (Murine Monoclonal) Series 3

Anti-A,B (Murine Monoclonal Blend)

Series 1

For Slide, Tube and Microplate Tests



### Intended Use:

Immucor Anti-A Series 1 (Murine Monoclonal), Anti-B Series 1 (Murine Monoclonal), Anti-B Series 3 (Murine Monoclonal) and Anti-A,B Series 1 (Murine Monoclonal Blend) are intended for use in slide, tube and microplate tests.

### Summary of the Test:

In 1900, Landsteiner observed that the red blood cells of some of his colleagues were agglutinated by the sera of some of the others.<sup>1</sup> On the basis of observed reactions, Landsteiner divided the bloods of his colleagues into three distinct phenotypes; A, B and O.<sup>2</sup> Decastello and Sturli described the fourth phenotype of this system, AB, in 1902.<sup>3</sup>

The ABO groups of most adults can be determined directly in agglutination tests with Anti-A and Anti-B typing reagents derived from either human serum or supernate of hybridoma cells. Monoclonal antibodies derived from cultured hybridoma cell lines can be used to prepare well-defined, potent, pure Blood Grouping Reagents. That monoclonal antibodies can be used reliably for ABO grouping tests has been shown by several groups of investigators.<sup>4-7</sup>

Immucor Anti-A (Murine Monoclonal) Series 1, Anti-B (Murine Monoclonal) Series 1, Anti-B (Murine Monoclonal) Series 3, and Anti-A,B (Murine Monoclonal Blend) Series 1 are suitable for use in ABO red blood cell typing tests.

### Principle of the Test:

Direct agglutination of red blood cells with a particular reagent indicates the presence of the corresponding antigen. No agglutination generally indicates its absence (see LIMITATIONS). The ABO group of a red blood cell specimen is determined from the pattern of reactivity obtained with the reagents tested (see Interpretation of Results).

Because the sera of most individuals older than 6 months of age consistently and predictably contain antibodies to the ABO antigen(s) they are lacking, serum grouping (reverse grouping) tests, employing reagent A<sub>1</sub> and B red blood cells, are used to confirm results obtained in red blood cell grouping tests of individuals other than newborn infants. Discrepancies between red blood cell and serum grouping must be resolved before the blood group is recorded. The resolution of typing discrepancies is discussed in references 8 and 9.

### Reagents:

Immucor Anti-A (Murine Monoclonal), Anti-B (Murine Monoclonal) and Anti-A,B (Murine Monoclonal) Blood Grouping Reagents are to be used as supplied. Anti-A Series 1, derived from the single clone line Birma-1, has been colored with FD and C blue #1. Anti-B Series 1, from clone ES4, and Anti-B Series 3, from clone LB-2, are colored with Naphthol Yellow. No dye has been added to Anti-A,B Series 1, which is a blend of antibodies from secreting cell lines Birma-1, ES4 and ES15. Antibodies are diluted in a buffered saline solution containing bovine albumin (without stabilizers), ethylenediamine tetraacetate (EDTA), and ingredients to facilitate the resuspension of red blood cell buttons following centrifugation. The Bovine Albumin Solution is sourced from donor animals of United States origin that have been inspected and certified by US Veterinary Service inspectors to be disease-free. This ruminant-based product is deemed to have low-TSE (Transmissible Spongiform Encephalopathy) risk. Sodium azide (0.1% final concentration) has been added to each reagent as a preservative. Series 1 Anti-B has been acidified to approximately pH 6.0. Series 3 Anti-B is not acidified and is of an approximate pH of 7.0, as are Series 1 Anti-A and Anti-A,B reagents.

Key:

Underline = Addition or significant change; ▲ = Deletion of text

These reagents contain antibodies derived from cell lines produced by other licensed manufacturers.

Meets FDA potency requirements.

### Precautions:

For in vitro diagnostic use.



This reagent contains 0.1% sodium azide and is classified as Harmful (Xn). R22 Harmful if swallowed

Sodium azide may react with lead and copper plumbing to form explosive compounds. If discarded into the sink, flush with a large volume of water to prevent azide build-up.

Store at 1-10 C when not in use. Do not freeze or expose to elevated temperatures.

Turbidity may indicate reagent deterioration or contamination. Do not use contaminated reagents. Do not use beyond expiration date. Do not use leaking vials. Avoid contamination of reagent.

Handle and dispose of reagent as if potentially infectious.

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The format for the expiration date is expressed as CCYY-MM-DD (year-month-day).

Certain precautions and limitations apply to this reagent when it is used with automated instrumentation. These conditions are described in detail in the instrument operator manual. ▲

### Specimen Collection and Preparation:

Draw a blood specimen using an acceptable phlebotomy technique. Samples may be drawn into EDTA, heparin, ACD, CPD, CPDA-1, CP2D or may be drawn without anticoagulant. Semi automated methods may require the use of samples drawn into an anticoagulant. Consult the instrument's operator manual for specific anticoagulants. ▲ All testing should be performed as soon as possible following collection to minimize the chance that falsely positive or falsely negative reactions will occur due to improper storage or contamination of the specimen. Samples that cannot be tested within 24 hours should be stored at 1-10 C. Do not use samples drawn into tubes with neutral gel separators. False positive results may occur with such samples. EDTA samples can be tested up to 10 days, clotted samples up to 21 days. Red blood cells drawn into heparin, ACD, CPD, CPDA-1 or CP2D may be tested up to the expiration of the anticoagulant.

### Procedure:

#### Materials Provided:

Immucor Series 1 Anti-A (Murine Monoclonal), Anti-B (Murine Monoclonal), Anti-A,B Murine Monoclonal Blend), or Immucor Series 3 Anti-B (Murine Monoclonal).

#### Additional Materials Required:

##### All methods:

1. Donor patient red blood cells
2. Marking pens

- Isotonic saline or phosphate-buffered (approximately 15mM) isotonic saline, pH 6.5-7.5

**Slide method:**

- Glass slides
- Wax marker (optional)
- Applicator sticks
- Stopwatch or timer
- Transfer pipettes

**Tube method:**

- Transfer pipettes
- 10x75mm or 12x75 mm test tubes and a test tube rack
- Serological centrifuge
- Interval timer

**Microplate or microwell methods:**

- Transfer pipettes or pipetting system\*
- Microplates, microwells, Immucor Hemagglutination/Dilution Strips
- Centrifuge\* with rotor and carriers capable of accommodating rigid 96-well plates or rigid 1x8 strips of wells
- Mechanical microplate shaker\* (optional)
- Microplate reader\* (optional)

\*It is the users responsibility to validate an accessory device (either listed or otherwise) for its intended use. Validation results should be maintained as part of the laboratory's records for review by regulatory agencies.

**Automated Method:**

For microplate testing with automated instrumentation, refer to instructions provided in the instrument operator manual.

**Test Methods:**

**Tube Test**

- Label 1 test tube for each blood grouping reagent to be tested.
- Add 1 drop of each blood grouping reagent to the appropriately labeled tube.
- Using a transfer pipette add 1 drop of a 2-5% suspension of red blood cells prepared in saline, plasma or serum to each tube. (Red blood cells may be washed prior to their resuspension in saline.) Alternatively, applicator sticks may be used to transfer red blood cells from clotted or anticoagulated specimens sufficient to make a 2-5% suspension in each tube containing a reagent.
- Mix the contents of each tube thoroughly and centrifuge.
- Gently agitate each tube to resuspend the red blood cells buttons. Examine for agglutination. Record results.

NOTE: Incubation for 5-60 minutes at 18-30 C may be necessary to enhance the reactivity of the blood grouping reagents with some of the weak subgroups of A and B.

**Slide Test**

- Place one drop of each blood grouping reagent to be tested on separate clean glass slides or on opposite ends of clean slides. Do not place the slides on a heated illuminated surface.
- Using a transfer pipette or applicator sticks, add 1 drop of a 35-45% suspension of red blood cells prepared in saline, or group-compatible plasma or serum to each drop of reagent.
- Using separate clean applicator sticks, mix each red blood cell reagent mixture over an oval area of approximately 20 mm x 40 mm.
- Slowly rotate each slide and observe for macroscopic agglutination for a period not to exceed 2 minutes. (NOTE: Do not place slides on a heated illuminated surface.) Record results.

**Microplate/Microwell Test**

- Label the plates of strips of wells to be used in testing.
- Add 1 drop of each reagent under test to labeled or identified wells.
- Prepare a 2% approximate suspension of red blood cells in saline, serum or plasma. (Red blood cells may be washed prior to their resuspension in saline.) Alternatively, applicator stick can be used to

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transfer red blood cells from clotted or anticoagulated samples to prepare the suspension in each well containing reagent. (NOTE: Centrifugation or agitation speeds needed with serum- or plasma-suspended red blood cells may differ significantly from those used with saline suspended red blood cells.)

- Using a transfer pipette add 1 drop of each red blood cells suspension to the appropriate wells.
- Mix the contents of each well thoroughly by tapping the plate manually or by using a mechanical microplate shaker.
- Centrifuge the plate at 150-250 x g for 60 seconds, or for an appropriate time and speed to produce positive results with antigen-positive red blood cells and negative results with antigen-negative red blood cells.
- Agitate the plate to resuspend each red blood cell button by manually tapping the plate or placing the plate on a plate agitator. Examine each well for agglutination. If desired, a mirror or reader may be used to examine the reaction in each well. Record results.

NOTE: Incubation for 5-60 minutes at 18-30 C may be necessary to enhance the reactivity of weak subgroups of A and B.

**Automated Method**

For microplate testing with automated instrumentation, refer to instructions provided in the instrument operator manual.

**Stability of the Reaction:**

Following centrifugation, all tube tests should be read immediately and results interpreted without delay. Delays may result in dissociation of antigen-antibody complexes leading to falsely negative or, at most, weakly positive reactions. Slide tests should be completed within the time period specified to avoid the possibility that a negative result may be incorrectly interpreted as positive due to drying of the reagents. Microplate tests should be interpreted immediately following resuspension to avoid erroneous test results due to settling of red blood cells or dissociation of red blood cell agglutinates.

Automated instrumentation reads results at test completion and stores results for reporting at the completion of the batch operation.

**Quality Control:**

To confirm the reactivity of Immucor Anti-A, Anti-B or Anti-A,B it is recommended that these reagents be tested each day of use with antigen positive red blood cells, such as A<sub>2</sub>B red blood cells. These reagents can be considered to be satisfactory if the antigen-positive red blood cells are agglutinated.

For microplate testing with automated instrumentation, refer to instructions provided in the instrument operator manual.



**Interpretation of Results:**

**Positive Test:** agglutination of red blood cells.

**Negative Test:** no agglutination of red blood cells.

Instrumentation automatically interprets test results.

**EXPECTED RED BLOOD CELL TYPING RESULTS**

Blood Group	Anti-A	Reagent Anti-B	Anti-A, B	Frequency (%) <sup>10</sup>	
				Whites	Blacks
A	+	0	+	40	27
B	0	+	+	11	20
O	0	0	0	45	49
AB	+	+	+	4	4

**Limitations:**

Falsely positive or falsely negative test results can occur from bacterial or chemical contamination of test materials, inadequate incubation time or temperature, improper centrifugation, improper storage of materials, or omission of test reagents. Under centrifugation or over centrifugation may result in the occurrence of numerous false-negative or false positives.

Certain subgroups of A and B may produce reactions that are weaker than those obtained with A or B red blood cells of most random donors. Depending on the subgroup involved, some may appear nonreactive in direct agglutination tube, microtitration plate or slide tests.

The red blood cells of people with some disease states may give falsely positive or falsely negative reactions with anti-A or anti-B.<sup>9</sup> Some cord blood specimens may give weakened reactions with these reagents. Cord red blood cells contaminated with Wharton's jelly may give falsely positive reactions.

The ABO system is the only blood group system known where individuals, older than 6 months of age, consistently and predictably produce antibodies to antigens they lack. Serum grouping tests, employing red blood cells of known ABO groups, are used to confirm the results of red blood cell typing procedures. However, discrepancies may occur between serum and red blood cell grouping if the specimen under test possesses unexpected antigens or agglutinins, or if the specimen lacks expected antigens or agglutinins. See reference 9 for a more detailed discussion of ABO grouping discrepancies. Any discrepancies that occur should be resolved before an ABO group is assigned.

Do not use murine monoclonal reagents in indirect antiglobulin tests using antihuman globulin reagents.

In certain clinical situations, group A red blood cells may acquire a B-like antigen *in vivo* due to the action of bacterial enzymes called deacetylases. The enzymes change acetyl galactosamine (the blood group A immunodominant sugar) to galactosamine. Some Anti-B reagents of monoclonal or polyclonal origin can react with galactosamine because it is similar in structure to galactose, the B blood group immunodominant sugar. Depending on the degree of transformation in the particular red blood cells being tested, Series 1 Anti-B (from clone ES4) may react strongly with acquired B red blood cells unless the antibody is prepared in an acidified diluent. Series 1 Anti-B reagent has been formulated to a pH of approximately 6.0 to diminish the frequency and strength of reaction with acquired B red blood cells. It should produce reactions with acquired B red blood cells that are more comparable to those observed with human polyclonal Anti-B. However, in some instances, Series 1 Anti-B may still react more strongly than human Anti-B. In cases where the results with Series 1 Anti-B reagent are questionable, further testing of red blood cells should be carried out using human polyclonal Anti-B or monoclonal Anti-B derived from a hybridoma cell line other than ES4 which is known to be nonreactive with acquired B red blood cells, such as Series 3 Anti-B (from clone LB-2). Further acidification of Series 1 Anti-B should not be attempted.

Autoagglutinins reactive at room temperature are a potential source of error in ABO grouping tests. The presence of these antibodies cannot be predicted. When sufficiently strong they can cause the nonspecific agglutination of reagent A<sub>1</sub> and B cells in serum (reverse) grouping tests. They can also produce nonspecific agglutination in red blood cell (forward) tests with Anti-A, and -B and Anti-A,B when unwashed, plasma-suspended or serum-suspended red blood cells are used. It is for this reason that both forward and reverse grouping tests are performed and the results are compared before ABO interpretations are made. All ABO tests should be read carefully. Discrepancies between forward and reverse results should be investigated thoroughly before an ABO group is assigned, regardless of the strength of the reactions obtained in any red blood cell or serum test. The strong reactions obtained in forward tests cannot be assumed to be more correct than weaker reactions seen in reverse tests with the same sample and vice-versa. Some autoagglutinins reactive at room temperature react best when the test environment is below pH 6.5. Immucor's Series 1 Anti-A, Series 3 Anti-B and Series 1 Anti-A,B are prepared in a diluent at approximately pH 7.0. In contrast, Series 1 Anti-B derived from the clone ES4 is prepared at an approximate pH 6.0 to inhibit the detection of the acquired B antigen. Thus, when unwashed or insufficiently washed red blood cells are being used for testing, acid-dependent autoagglutinins are a potential source of false-positive agglutination in tests with Series 1 Anti-B. The same nonspecific reactivity may not be seen in tests with Series 1 Anti-A, Series 1 Anti-A,B or Series 3 Anti-B reagents, or may be perceptibly weaker than in the test with Series 1 Anti-B. Nonspecific agglutination produced by autoagglutinins can range in strength from weak to strong. When unwashed red blood cells are used and an ABO discrepancy persists on repeat testing, evaluating the red blood cells with other blood grouping reagents (prepared at pH 7.0) or testing the serum or plasma with additional reagent red blood cells may be indicated.

#### ▲ Specific Performance Characteristics:

Prior to release, each lot of Immucor Anti-A, Anti-B and Anti-A,B (Murine Monoclonal) is tested by insert methods against a panel of antigen-positive red blood cells to insure suitable reactivity. The performance of this product is dependent on adhering to Key:

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the recommended methods found in this insert. The presence of contaminating antibodies to antigens with an incidence of 1% or greater in the random population and including M<sup>s</sup> and W<sup>r</sup>s, have been excluded either in direct tests employing ABO compatible red blood cells or in tests employing reagents previously adsorbed to remove anti-A or anti-B. Antibodies to the antigens Le<sup>c</sup> and Le<sup>d</sup> are not necessarily excluded. For additional information or for technical support, contact Technical Service at 800-492-BLUD (2583) or 770-441-2051.

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