Blood Grouping Reagent
immuClone® Anti-A IgM, immuClone® Anti B IgM
immuClone® Anti-A,B IgM
For Tube, Slide, Microplate and Automated Microplate Tests

- IVD In Vitro Diagnostic Medical Device
- Consult Instructions for Use
- **Rx ONLY**
- **Warning**
  Preservative: 0.1% Sodium Azide
- 1°C – 10°C Temperature limitation
- Discard if markedly turbid

CAUTIONS: DO NOT PIPETTE BY MOUTH. ALL BLOOD PRODUCTS SHOULD BE TREATED AS POTENTIALLY INFECTIOUS. THIS PRODUCT HAS COMPONENTS (DROPPER BULBS) THAT CONTAIN DRY NATURAL RUBBER.

Manufacturer:IMMUCOR Medizinische Diagnostik GmbH
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Intended Use:

Blood Grouping Reagent	Blood Grouping Reagent
For Tube, Slide, Microplate and Automated Microplate Test	For Tube, Slide, Microplate and Automated Microplate Test
Murine Monoclonal	Murine Monoclonal
Clone	Clone

immuClone® Anti A IgM, immuClone® Anti-B IgM, and immuClone® Anti-A,B IgM blood grouping reagents for ABO cell typing tests are intended for use in slide, tube, microplate and automated microplate tests.

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

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.[4-7]

Adults whose red cells lack A and/or B antigens usually have the corresponding antibody in their serum. Anti-A and Anti-B can cause serious hemolytic transfusion reactions as well as Hemolytic Disease of the Newborn (HDN). The potentially serious consequences of ABO incompatible transfusion requires that both transfusion recipient and donor red cells are reliably tested for the presence of A and B antigens - with subsequent ABO group compatible donor blood selected for transfusion.

ABO grouping of adult patient red cells should always be supplemented by confirmatory serum grouping tests – i.e. testing the individual's serum with known A_1 and B Reagent Red Blood Cells. The red cells of newborn infants do not have full expression of A and B antigens and slightly weaker ABO grouping tests may be encountered. Furthermore, serum from group A, B or O newborn infants may not necessarily contain the expected Anti-A and/or Anti-B. In fact, passively acquired Anti-A and/or Anti-B from the mother's circulation may be present, resulting in unexpected reactions. Therefore, reverse grouping should not be performed on the sera from newborn infants, as it may not provide the expected confirmatory results. Subgroups of A and B are known to exist and may result in weaker than expected or negative direct hemagglutination reactions with Anti-A, Anti-B and/or Anti-A,B reagents. Although not extensively substantiated, it may be important to detect such weak expressions of the A antigen in donor blood units so that such blood is not transfused to group O recipients. Anti-A,B is a useful reagent for confirmation of test results obtained with Anti-A and Anti-B to further ensure accurate and reliable ABO grouping. The use of Anti-A,B can be mandatory according to specific local regulations.

Principle:
Direct agglutination of red 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 cell specimen is determined from the pattern of reactivity obtained with the reagents tested (see RESULTS).

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

BLOOD GROUPING REAGENT
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IMMUCOR

Discrepancies between 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:
immuClone® Anti-A, Anti-B and Anti-A,B IgM murine monoclonal Blood Grouping Reagents are to be used as supplied without further dilution or additions.

Anti-A is derived from the clone Birma-1, and is coloured with FD and C blue #1.

Anti-B is derived from clone LB-2, and is coloured with Napthol Yellow.

Anti-A,B is a blend of antibodies from clones Birma-1, ES4 and ES15, no dye is added to this reagent.

Antibodies are diluted in a buffered saline solution containing bovine albumin (without stabilizers), ethylenediamine tetraacetate (EDTA), and ingredients to facilitate the resuspension of red 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. The reagents have an approximate pH of 7.0.

These reagents are to be used as supplied without further dilution or additions.

Precautions:
For in vitro professional diagnostic use only.

Warning
This reagent contains 0.1% sodium azide, H302: 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.

Discard if markedly turbid — Discard if markedly turbid

Avoid contaminating this product during use. Contamination will adversely affect a product's performance during its shelf life. Marked turbidity may indicate reagent deterioration or contamination. Do not use contaminated reagents. Do not use leaking vials. Do not use unlabeled vials.

Handle and dispose of reagent as if potentially infectious.

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Do not use beyond the expiration date. The format for the expiry date is CCYY-MM-DD, i.e. the date 28th May 2008 would be expressed as 2008-05-28.

Specimen Collection:

Draw a blood specimen using an acceptable phlebotomy technique.

In manual tests, sample drawn into tubes containing EDTA, heparin, ACD, CPD, CPDA-1, CP2D or tubes without anticoagulant may be used.

Automated methods may require the use of samples drawn into an anticoagulant. For microplate testing with automated instrumentation, refer to instructions provided in the instrument operator manual.

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. Failure to store the specimens at the correct temperature, for example, storage at higher temperature or repeated freezing and thawing may result in false positive or false negative results.

Samples that cannot be tested within 24 hours should be stored at 2-8° 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. Cells drawn into heparin, ACD, CPD, CPDA-1 or CP2D may be tested up to the expiration of the anticoagulant.

Procedure:

Materials Provided

immuClone® Anti-A IgM, immuClone® Anti-B IgM, immuClone® Anti-A,B IgM, in vials ready for use (with dropper for manual use).

Additional Materials Required:

All manual methods:

1. Donor or patient red cells
2. Marking pens
3. Isotonic saline or phosphate-buffered (approximately 15mM) isotonic saline, pH 6.5-7.5

Tube method:

1. Transfer pipettes
2. 10x75mm or 12x75 mm test tubes and a test tube rack
3. Serological centrifuge*
4. Interval timer

Microplate method (manual):

1. Transfer pipettes or pipetting system* (e.g., ABS Precis, Hamilton Microlab AT, Packard Multiprobe 104/204)
2. Microplates*
3. Centrifuge* (eg, Sorvall T6000, IEC Centra-8, Jouan C422, Hettich 30F, Heraeus Labofuge 400) with rotor and carriers capable of accommodating rigid 96-well plates
4. Mechanical microplate shaker* (e.g., Titramax 3101) (optional)
5. Microplate reader* (e.g., I-STAR) (optional)

Automated Microplate method:

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

Slide method:

1. Glass or plastic slides
2. Wax marker (optional)
3. Applicator sticks (for glass slides)
4. Stopwatch or timer
5. Transfer pipettes

* It is the user's responsibility to validate an accessory device for its intended use.

Test Methods:

A. TUBE TEST:

1. Label 1 test tube for each blood grouping reagent to be tested.
2. Add 1 drop (approximately 50 µl) of each blood grouping reagent to the appropriately labeled tube.
3. Using a transfer pipette add 1 drop (approximately 50 µl) of a 2-5% suspension of red cells prepared in saline, plasma or serum to each tube. (Cells may be washed prior to their resuspension in saline). Mix the contents of each tube thoroughly and centrifuge.*
4. Gently agitate each tube to resuspend the red cells buttons. Examine for agglutination.
5. Record results.

*Suggested centrifugation time: 15-30 seconds at 900-1000 x g or a time, appropriate for the centrifuge used, that produces the strongest reaction of antibody with antigen-positive cells, yet allows easy resuspension of antigen-negative red cells. The centrifugal force applied should be the minimum required to produce a clear supernatant and a clearly delineated red cell button that can be easily resuspended.

No single speed or time can be recommended for all types of available centrifuges or test applications. Centrifuges should be calibrated individually to determine the optimal time and speed required to achieve the desired 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.

B. MICROPLATE TEST:

1. Label the microplates to be used in testing.
2. Add 1 drop (approximately 50 µl) of each reagent under test to labeled or identified wells.
3. Prepare a 2-4% approximate suspension of red cells in saline (cells may be washed prior to their resuspension in saline).
4. Using a transfer pipette add 1 drop (approximately 50 µl) of each red cell suspension to the appropriate wells.
5. Mix the contents of each well thoroughly by tapping the plate manually or by using a mechanical microplate shaker.*
6. Centrifuge the plate at 100-250 x g for 40-60 seconds, or for an appropriate time and speed to produce positive results with antigen-positive red cells and negative results with antigen–negative red cells**.
7. Agitate the plate to resuspend each 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.
8. 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.

*Suggested times for mechanical shaker: 1) Mixing: 10-30 seconds on a medium agitation setting. 2) Resuspension: 10-30 seconds on a medium setting or a time and speed appropriate for the shaker used, that allows complete resuspension of the entire cell button without destroying positive reactions.

**Suggested centrifugation time: 40-60 seconds at 100-250 x g or a time, appropriate for the centrifuge used, that produces the strongest reaction of antibody with antigen-positive cells, yet allows easy resuspension of antigen-negative red cells. The centrifugal force applied should be the minimum required to produce a clear supernatant and a clearly delineated red cell button that can be easily resuspended.

No single speed or time can be recommended for all types of available centrifuges or test applications. Centrifuges should be calibrated individually to determine the optimal time and speed required to achieve the desired results.

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

C. SLIDE TEST:

1. Label slide to be used in testing
2. Place one drop (approximately 50 µl) of each blood grouping reagent to be tested on separate clean glass or plastic slide. Do not place the slides on a heated illuminated surface.
3. Add one drop (approximately 50 µl) of whole blood (or 35-45% suspension of red cells in saline or group-compatible plasma or serum) from the sample to each reagent on glass or plastic slide using a transfer pipette or applicator stick.
4. Mix the blood and reagent. On glass slides, use a separate clean applicator stick to mix each reagent/cell mixture over and oval area approximately 20 x 40 mm. On plastic slides follow the manufacture's insert.
5. Observe for macroscopic agglutination. On glass slides this is achieved by slow rotation over a period up to a maximum of 2 minutes. On plastic slides follow the manufacture's inset. Do not place slides on a heated illuminated surface.
6. Record results.

D. Automated Microplate 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 cells or dissociation of red cell agglutinates.

Quality Control:

To confirm the reactivity of immuClone® Anti-A, Anti-B or Anti-A,B it is recommended that these reagents be tested each day of use with antigen positive cells, such as A_2B cells. For QC frequency minimum requirements refer to national guidelines. These reagents can be considered to be satisfactory if the antigen-positive cells are agglutinated.

Interpretation of Results:

Positive Test: agglutination of red cells.

Negative Test: no agglutination of red cells.

EXPECTED CELL TYPING RESULTS

Blood Group	Reagent			Frequency in US-population (%)[10]	
	Anti-A	Anti-B	Anti-A,B	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 cells of most random donors. Depending on the subgroup involved, some may appear non-reactive in direct agglutination tube, microtitration plate or slide tests.

immuClone® Anti-A may not react with all examples of red cells classified as A_x and has been shown not to react with red cells that have been characterised as B(A).

immuClone® Anti-B does not recognize the acquired B antigen.

immuClone® Anti-AB does detect A_x. Part of lot release testing includes testing each lot with 3 examples of A_x cells.

The red cells of people with some disease states may give falsely positive or falsely negative reactions with anti-A or anti-B.[9] Some cord blood specimens may give weakened reactions with these reagents. Cord 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 cells of known ABO groups, are used to confirm the results of cell typing procedures. However, discrepancies may occur between serum and 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.

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_1 and B cells in serum (reverse) grouping tests. They can also produce nonspecific agglutination in cell (forward) tests with Anti-A, and -B and Anti-A,B when unwashed, plasma-suspended or serum –suspended 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 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. ImmuClone® Anti-A, Anti-B and Anti-A,B are prepared in a diluent at approximately pH 7.0. Nonspecific agglutination produced by autoagglutinins can range in strength from weak to strong. When unwashed red cells are used and an ABO discrepancy persists on repeat testing, evaluating the cells with other blood grouping reagents (prepared at pH 7.0) or testing the serum or plasma with additional reagent blood cells may be indicated.

With reference to the microplate method, new, unused plastic microplates are capable of passively adsorbing cells and serum proteins to their surfaces. This nonspecific adsorption can lead to erroneous test results.[11] Each batch of microplates should be evaluated in the user's system prior to acceptance for routine use.

Where necessary, microplates can be treated prior to use to block nonspecific adsorption. Bovine albumin (1-2%) or 1% gelatin can be used as a blocking agent. Incubate the solution in the wells for 10 minutes at 18-30°C. Plates should then be thoroughly rinsed (approximately 10 times) in distilled or deionized water. Decant the water from the wells as thoroughly as possible following each rinse. Allow plates to dry before their use in testing.

Deviation from the Recommended Instructions for Use may result in less than optimal product performance. Slide test procedures may not be sufficiently sensitive for reliable detection of weakened antigen expression. User-defined modifications to test procedures may require validation.

Specific Performance Characteristics:

immuClone® Anti-A, Anti-B and Anti-A,B meet the requirements of the Common Technical Specifications for products defined in Annex II, List A of directive 98/79/EC on in vitro Diagnostic Medical Devices. The reagents show same or better performance characteristics compared to established and approved devices.

Prior to release, each lot of immuClone® Anti-A, Anti-B and Anti-A,B is tested by insert methods against a panel of antigen–positive red cells to ensure suitable reactivity. The performance of this product is dependent on adhering to 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(g) and Wr(a), have been excluded either in direct tests employing ABO compatible red cells or in tests employing reagents previously adsorbed to remove Anti-A or anti-B. Antibodies to the antigens Le(c) and Le(d) are not necessarily excluded. Additional information regarding specificity testing performed at the time of the manufacture or as performed subsequent to product release may be furnished upon request by consulting Immucor's Technical Service at (+49) 6074 8420-50 or via e-mail:- tech.support.eu@immucor.com

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REF	Description
0066001; 0066080	immuClone® Anti-A IgM
0066002; 0066081	immuClone® Anti-B IgM
0066003; 0066082	immuClone® Anti-A,B IgM

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