BLOOD GROUPING REAGENT
Anti-A (Murine Monoclonal), Anti-B (Murine Monoclonal)
Anti-A,B (Murine Monoclonal Blend)
Gamma-clone®

By Slide, Tube or Microwell Test

IVD

1°C-10°C

Harmful, Preservative: 0.1% Sodium Azide Meets FDA Potency Requirements

Do not use if markedly turbid.

CAUTION: DO NOT PIPETTE THIS PRODUCT BY MOUTH, AS THE ABSENCE OF MURINE VIRUS HAS NOT BEEN DETERMINED. THE PACKAGING OF THIS PRODUCT (DROPPER BULB) MAY CONTAIN DRY NATURAL RUBBER.

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INTENDED USE: Gamma-clone Anti-A (Murine Monoclonal), Anti-B (Murine Monoclonal) and Anti-A,B (Murine Monoclonal Blend) are intended for the detection of ABO blood group antigens on red blood cells by slide, tube or microwell test.

SUMMARY OF THE TEST: The ABO blood group system is unique in that a person lacking the A and/or B antigens from the red blood cells regularly has antibody in the serum directed at the missing antigen or antigens (Table 1).

Blood groups	Antigens present on the Red Blood Cells	Antibodies regularly present in the serum
O	H (neither A nor B)	anti-A and anti-B
A	A	anti-B
B	B	anti-A
AB	A and B	none

Table 1: The principal antigens and antibodies of the ABO system.

Thus, while a person's blood group is determined directly by testing the red blood cells with Anti-A and Anti-B (Cell or Forward Grouping), confirmation of the test results is provided by testing the serum with group A and group B red blood cells (Serum or Reverse Grouping). Additional testing of the red blood cells with Anti-A,B facilitates the recognition of certain uncommon subgroups (such as A_x), and is sometimes used as further confirmation of the reactions obtained with Anti-A and Anti-B.

The interested reader is referred to the appropriate chapter of *Blood Groups in Man* by Race and Sanger for complete details about the ABO blood group system, including information about known subgroups [1].

PRINCIPLE OF THE TEST: A person's blood group is determined by testing the red blood cells with Anti-A and Anti-B. Agglutination of the test red blood cells indicates the presence of the relevant antigen, while no agglutination indicates its absence. Confirmation of the test results is provided by testing the serum of the blood under investigation with group A_1 and group B red blood cells, and by comparing the reaction patterns with those observed in red blood cell testing, see Table 2 under “Interpretation of Test Results”. Any discrepancy between the red blood cell and serum grouping results must be investigated before the blood group is recorded.

If the option is exercised to test the unknown red blood cells with Anti-A,B, agglutination in the test with that product will indicate the presence of the A and/or the B antigens, or may suggest that the blood is of a subgroup (such as A_x).

REAGENT: Blood Grouping Reagent, Anti-A (Murine Monoclonal) and Anti-B (Murine Monoclonal) Gamma-clone are manufactured from antibodies produced by culturing in fluid medium the murine hybridoma cell lines Birma-1 and GAMA110, which secrete anti-A and anti-B, respectively. Anti-A,B (Murine Monoclonal Blend) product is a blend of monoclonal antibodies from the cell lines Birma-1 and ES-4, together with one that reacts with both A and B antigens. This is a product of the hybridoma cell line ES-15. These reagents may include antibodies produced by other licensed manufacturers. The final formulation includes a proprietary buffer to enhance agglutination, with or without bovine albumin, In addition, Anti-A contains Patent Blue and Anti-B contains Naphthol Yellow as coloring agents. Any bovine albumin used in the manufacture of this product is sourced from donor animals of United States origin that have been inspected and certified by USDA Food Safety and Inspection Service inspectors to be disease-free. This ruminant-based product is deemed to have low TSE (Transmissible Spongiform Encephalopathy) risk. Contains 0.1% sodium azide as a preservative.

PRECAUTIONS:

For in vitro diagnostic use. Store at 1° to 10°C when not in use. Do not freeze. Do not dilute. Do not use beyond the expiration date. Effort should be made to minimize contamination during use of the product. Do not use if markedly turbid.

CAUTION: DO NOT PIPETTE THIS PRODUCT BY MOUTH, AS THE ABSENCE OF MURINE VIRUS HAS NOT BEEN DETERMINED. THE PACKAGING OF THIS PRODUCT (DROPPER BULBS) MAY CONTAIN DRY NATURAL RUBBER.

This reagent contains 0.1% sodium azide. Warning: H302 Harmful if swallowed.

Warning: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. If discarded into sinks, flush with a large volume of water to prevent azide build-up.

Handle and dispose of reagent as potentially infectious.

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

SPECIMEN COLLECTION AND PREPARATION: No special preparation of the patient is required prior to specimen collection. Blood should be drawn by aseptic technique, with or without an anticoagulant. The specimen should be tested as soon as possible after collection. If delay in testing should occur, the specimen must be stored at 1° to 10°C. Blood drawn into EDTA should not be stored for longer than seven days. It is best to test oxalated or heparinized blood samples within two days of being drawn. Clotted specimens may be tested up to 14 days after collection, and donor blood may be tested up to the expiration date. Storage may result in weaker-than-normal reactions.

PROCEDURE:

Materials Provided: Gamma-clone Anti-A (Murine Monoclonal), Anti-B (Murine Monoclonal) or Anti-A,B (Murine Monoclonal Blend)

Additional Materials Required: Test tubes (12×75 mm or 10×75 mm), microwell plates with U-bottom wells (those of rigid or flexible construction are equally suitable), pipettes, slides, applicator sticks, isotonic saline or phosphate-buffered (approximately 15 mM) isotonic saline pH 6.5-7.5, timer, centrifuge, microwell plate carriers, a mechanical device for the resuspension of tests in microplates, and an optical aid such as a hand lens or a concave mirror. Red blood cells known to be positive for the A and B antigens, as controls.

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TEST METHODS:

Slide Method

1. Place 1 drop of Gamma-clone Anti-A and Anti-B on opposite ends of a clean properly labeled slide. Place 1 drop of Gamma-clone Anti-A,B on a separate slide for confirmation of Group O.
2. Add 1 drop of an approximate 35-45% suspension of the red blood cells to be tested to each drop of reagent. The red blood cells may be suspended in saline, in their own serum or plasma, or the proper amount of whole blood may be transferred to the slide by the use of applicator sticks or a dropper.
3. Mix each drop thoroughly (using separate applicator sticks) over a circular area approximately 20mm in diameter. Rock the slides (slowly) for a period not exceeding two minutes. Caution: Extending the period before interpretation beyond two minutes may result in drying of the reactants on the slide, and may cause false positive results.
4. Read macroscopically for agglutination and record test results.

Tube Method

1. Place 1 drop of Gamma-clone Anti-A, Anti-B and Anti-A,B (if used), respectively, in three properly labeled test tubes.
2. Add 1 drop of an approximate 3-4% suspension of the red blood cells to be tested (washed or unwashed) to each tube. The red blood cells may be transferred directly from the clot with applicator sticks or may be a prepared suspension in saline, or in their own serum or plasma.
3. Mix thoroughly by shaking the tube and centrifuge for:
   (a) 1 minute at 1,000 rpm (rcf 100 to 125) or
   (b) 15 seconds at 3,400 rpm (rcf 900 to 1,000) or
   (c) a time appropriate to the calibration of the centrifuge.
4. Examine for the absence of hemolysis. *NOTE: Hemolysis may be the consequence of bacterial contamination and should not be interpreted as a positive result.*
5. Resuspend the red blood cells by gentle shaking and examine for macroscopic agglutination, an optical aid may be used. Record results.
6. Most red blood cells of the A_X phenotype may be expected to show macroscopic agglutination with Anti-A,B at the immediate-spin phase of the Tube Test Method. However, weak agglutination associated with weak subgroups may develop greater strength with incubation (e.g. 5 minutes) at room temperature (23°±3°C). Incubation should not be extended beyond 30 minutes.

Stability of Reaction: Test results must be interpreted immediately upon completion of the test.

Microwell Method

(in conventional microplates)

NOTE: In some cases, plastic microplates may require pretreatment before use, such as rinsing in distilled water, or may need to stand on a damp towel when being filled in order to dissipate static electricity. It is the laboratory's responsibility to develop its own procedures for the pretreatment of microplates, if needed.

1. Place 1 drop of Gamma-clone Anti-A, Anti-B and Anti-A,B, respectively, into three identified microwells. *NOTE: if reactions are to be interpreted from the streaming patterns of the test red blood cells, the use of Gamma-clone Control is recommended, in which case 1 drop of Gamma-clone Control should be placed into a fourth microwell.*
2. To each microwell, add 1 drop of an approximate 3-4% suspension of the red blood cells to be tested, previously prepared in saline. For optimal reactions when the test is to be read by streaming, the drop of red blood cell suspension should be equal in volume to that of the serum added at step 1. The red blood cells may be used washed or unwashed.
3. Mix the contents of the microwells either manually or by using a mechanical device.
4. Centrifuge microplate at an appropriate speed and time for the centrifuge being used. As a guide, a speed of 1,000 rpm (rcf 280-300) for 15 seconds is suggested for the Sorvall GLC-2B centrifuge. *NOTE: Centrifugation is critical for proper test results. Each laboratory should calibrate its own centrifuges to determine the optimum time and speed of centrifugation required to achieve acceptable reaction patterns by the microwell method with red blood cell suspensions of known phenotypes. The speed of centrifugation will vary with flexible or rigid plates, and with different centrifuges. Correct interpretation of test reactions is dependent on the application of appropriate centrifugal force to the microplate to produce distinct red blood cell buttons with clear supernatant backgrounds. Streaming of the red blood cells in negative tests should commence within 15 to 60 seconds of tilting the microwell plate to a 60 to 90° angle, and streaming patterns should be interpretable within 2 to 4 minutes.*
5. Examine for the absence of hemolysis. *NOTE: Hemolysis may be caused by bacterial contamination and should not be interpreted as a positive result.*
6. Read by tilting the microwell plate at a 60 to 90° angle to the bench top and examining the streaming pattern of the red blood cells in each microwell. Distinct red blood cell streaming patterns will be observed within 2 to 4 minutes, although streaming may commence in as little time as 15 to 60 seconds. It is recommended that test results should not be interpreted after four minutes have elapsed. In a negative reaction, the button of red blood cells will stream downwards. In a positive reaction, the red blood cells remain as a distinct button in the bottom of the microwell, as deposited during centrifugation, but may become dislodged if disturbed and may then fall as a large clump, without streaming, or may fold down to present a "half-moon" appearance. Doubtful results may be confirmed by gently resuspending the red blood cells by hand or with a mechanical device, while observing the resuspension to detect weak agglutination. Each laboratory should determine the optimum speed and time for its own mechanical resuspension device. In general, only sufficient force should be applied to bring the red blood cell buttons off the base of the wells. On resuspending, positive reactions are interpreted based on the normal appearance of agglutinated red blood cells in a conventional tube test, while a negative reaction will appear as a smooth red blood cell suspension in the microwell. An optical aid may be used if desired.
7. Record test results.
8. Most red blood cells of the A_X phenotype may be expected to show macroscopic agglutination with Anti-A,B at the immediate-spin phase of the test. However, weak agglutination associated with weak subgroups may develop greater strength with incubation (e.g. 5 minutes) at room temperature (23°±3°C). Incubation should not be extended beyond 30 minutes.

Stability of Reaction: Test results must be interpreted immediately upon completion of the test.

QUALITY CONTROL: Confirmation of results obtained in red blood cell (forward) grouping must be obtained by performing the serum (reverse) grouping test, in which the serum or plasma of the blood being examined is tested for expected antibodies with known group A_1 and group B red blood cells [2]. In addition, the reactivity of ABO blood grouping reagents should be confirmed on each day of use by testing with known positive red blood cells.

INTERPRETATION OF TEST RESULTS: Agglutination in the slide method, tube method, microwell method (when read by resuspension), and no streaming in the microwell method (when read by streaming) is a positive (+) result and indicates the presence of the corresponding antigen. No agglutination in the slide method, tube method, microwell method (when read by resuspension), and streaming in the microwell method (when read by streaming) is a negative (0) result and indicates the absence of the corresponding antigen. The reaction patterns of the most common ABO phenotypes are shown in Table 2.

Cell Grouping			Serum Grouping			ABO
Anti-A	Anti-B	Anti-A,B	A cells	B cells	O cells*	Group
+	0	+	0	+	0	A
0	+	+	+	0	0	B
0	0	0	+	+	0	O
+	+	+	0	0	0	AB

Table 2: The common reaction patterns obtained in red blood cell (forward) grouping and serum (reverse) grouping procedures.

Group	% Frequency		
	Whites	African Americans	Asians
A	40	27	27
B	11	20	25
O	45	49	43
AB	4	4	5

Table 3 Approximate frequencies among Whites, African Americans and Asians.

*Group O red blood cells are used in antibody screening tests. Since antibody screening is usually employed in processing both patient and donor blood specimens, all serums are tested with one or more group O red blood cells. This serves as a control for the serum grouping test, and facilitates the recognition of factors other than anti-A and anti-B that may cause cellular aggregation in the serum grouping test (e.g. autoagglutination, unexpected antibodies, rouleaux, etc.)

The interpretation of reactions obtained when testing infant bloods may be complicated by the fact that the infant's serum does not necessarily contain antibody for any antigen absent from the red blood cells, and passive anti-A and/or anti-B from the mother's circulation may yield conflicting reactions when tests are

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performed on cord blood specimens. Cord blood specimens may also give weaker-than-normal reactions in the red blood cell grouping test, as the ABH antigens often are imperfectly developed at birth. This may lead to false-negative results, particularly with Anti-A.

In all other cases, any discrepancy between red blood cell and serum grouping must be investigated and resolved before the blood group is recorded. The preliminary investigation of such a discrepancy will include repeating the red blood cell grouping on washed red blood cells. If the discrepancy persists on repeating the test, testing of the red blood cells with other blood grouping reagents, and of the serum with additional Reagent Red Blood Cells, may be indicated. The possible causes of discrepancies between red blood cell and serum grouping tests are many and are beyond the scope of this direction insert. They are described in detail in the *Technical Manual* of the American Association of Blood Banks [3].

LIMITATIONS:

Factors that may cause false test results include the following:

1. Contamination of blood specimens, reagent and/or supplementary materials.
2. Aged blood specimens, which may yield weaker reactions than those obtained with fresh red blood cells. Bloods older than the time limits stated under "Specimen Collection and Preparation" may be tested with these reagents; however, agglutination may be weaker with older red blood cells than with those from freshly drawn blood.
3. Too light or too heavy a red blood cell suspension.
4. Improper incubation time or temperature.
5. Calibration of the centrifuge is critical. Excessive centrifugation may lead to difficulty in resuspending the red blood cell button in the tube test. At the same time, inadequate centrifugation may yield unclear red blood cell button patterns and agglutinates that are too readily dispersed.
6. Improper examination for agglutination (usually too vigorous shaking). The resuspension of reactions in the tube test procedure must be carried out by gentle shaking. Shaking too vigorously may cause agglutinates to be dispersed.
7. Deviation from the recommended test procedure.
8. Very weak subgroups (of both A and B) may not be detected by these reagents.
9. The Slide Method is not considered suitable for the detection of the A_x phenotype.

SPECIFIC PERFORMANCE CHARACTERISTICS: Gamma-clone Anti-A (Murine Monoclonal), Anti-B (Murine Monoclonal) and Anti-A,B (Murine Monoclonal Blend) meet FDA potency requirements. Each lot is tested against at least 10 red blood cell samples positive for the relevant antigen, in order to assure adequate reactivity in use. The test red blood cells include at least three examples of the phenotype A_2B in the case of Gamma-clone Anti-A, and the reactivity of Gamma-clone Anti-A,B with A_x red blood cells in manual testing is proved by including at least three examples of that phenotype among the test red blood cells. All lots are further tested against a panel of at least 10 selected red blood cell samples negative for the appropriate antigen, in order to assure true specificity when used by the recommended test procedures. The panel includes red blood cells positive (or presumptively positive) for antigens having a frequency of 1% or more in the general population of the U.S. The absence of antibodies to V, VS and Js^a is confirmed. However, antibodies to Le^c and Le^d are not necessarily excluded, nor are antibodies to such low-incidence antigens as Wr^a, M^g, Di^a and Vw, although testing for such contaminants may be undertaken if suitable test red blood cells are available. The performance of this product is dependent on adhering to the recommended methods found in this insert. For additional information or for technical support, contact Immucor at 855-IMMUCOR (466-8267).

BIBLIOGRAPHY:

1. Race RR, Sanger R. Blood groups in man. 6th ed. Oxford, Blackwell Scientific Publications; 1975: 8-91.
2. Standards for blood banks and transfusion services. 24th ed. Bethesda 2006; American Association of Blood Banks: 44, 5.13.1.
3. Technical manual. 15th ed. Bethesda, MD, American Association of Blood Banks; 2005:296-303,469.

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Revised: 02/2013

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