US

Blood Grouping Reagents

Anti-A (ABO1)

Seraclone® Murine Monoclonal
(A003)

Anti-B (ABO2)

Seraclone® Murine Monoclonal
(B005)

Anti-A,B (ABO3)

Seraclone® Murine Monoclonal Blend
(BS63/85)

FOR IN-VITRO DIAGNOSTIC USE
For Tube Testing
MEETS FDA POTENCY REQUIREMENTS
U.S. License Number: 1798

Package size

REF	801325100	VOL	10 x 10 mL	Seraclone® Anti-A (ABO1)
REF	801350100	VOL	10 x 10 mL	Seraclone® Anti-B (ABO2)
REF	801375100	VOL	10 x 10 mL	Seraclone® Anti-A,B (ABO3)

Intended Use
For the determination of the A (ABO1), B (ABO2), A,B (ABO2) antigens of red blood cells using the tube test.

Summary
Between 1900 and 1902, Landsteiner and associates discovered the ABO system of red blood cell antigens. The importance of this discovery is the recognition that antibodies are present when the corresponding antigens are lacking. The ABO system is the only blood group system in which the reciprocal antibodies are consistently and predictably present in most people.[1] Due to this reciprocity, an ABO blood type determination is considered valid if serum typing corresponds with the red blood cell antigen grouping.

Incidence (%) in US population[1]		
ABO group	Whites	Blacks
0	45	49
A	40	27
B	11	20
AB	4	4

Biotest Anti-A, Anti-B and Anti-A,B Blood Group Reagents are used to test for the presence or absence of the corresponding antigens. Routine pretransfusion studies always include tests for the ABO antigens.

Phenotype Frequency (%)[2]

	Caucasians	Blacks	Asians	Mexican
A_1	33	19	27	22
A_2	10	8	Rare	6
B	9	20	25	13
0	44	49	43	55
A_1B	3	3	5	4
A_2B	1	1	Rare	Rare

Principle of the test
The test principle is hemagglutination. The antibodies in Seraclone® Anti-A (ABO1), Seraclone® Anti-B (ABO2), Seraclone® Anti-A,B (ABO3) bind to the corresponding antigen on red blood cells being tested and cause an antigen-antibody reaction visible as red blood cell agglutination. The four ABO blood groups A, B, A,B and O are defined by the presence or absence of A and B antigens on red blood cells. The absence of both A and B antigens defines blood type O. The antigens A and B react with the corresponding antibody in Seraclone® Anti-A (ABO1), Seraclone® Anti-B (ABO2), and Seraclone® Anti-A,B (ABO3).

Reagent
Seraclone® Anti-A (ABO1), Seraclone® Anti-B (ABO2), and Seraclone® Anti-A,B (ABO3) contain as reactive components monoclonal antibodies of the immunoglobulin class IgM.
They are derived from hybridoma cell lines which are created by fusing mouse antibody producing B lymphocytes with mouse myeloma cells and demonstrate consistent specificity and reproducability characteristic for monoclonal antibodies. Both antibodies derived from a single clone (sister cells of one hybridoma cell) and a mixture of different antibodies derived from several clones are called monoclonal.
Antibodies are diluted in a buffered protein solution containing bovine albumine, ethylenediamine tetraacetate (EDTA), and as colorant Patent Blue (Anti-A) or Tartrazin (Anti-B).

Seraclone® Anti-A (ABO1)	clone A003 (IgM)
Seraclone® Anti-B (ABO2)	clone B005 (IgM)
Seraclone® Anti-A,B (ABO3)	clones BS 63/BS 85 (IgM/IgM)

Preservative: 0.1% sodium azide.

Precautions
- For In-vitro diagnostic use.
- Store at 2 to 8°C.
- Do not use beyond the expiration date.
- Do not use if turbid.
- Handle and dispose of reagents as potentially infectious
- Caution: Do not pipette by mouth. The absence of murine viruses has not been determined.
- Caution: This product contains Natural Rubber Latex Which May Cause Allergic Reactions.
- Warning: Contains sodium azide (NaN_3), which may react with lead or copper plumbing to form explosive azides. If discarded in the sink, flush with large amounts of water to prevent the build-up of explosive metal azides.
- The bovine albumin used for the production of this reagent is purchased from BSE-free US sources, Boval Company L.P. in Cleburne, Tx, USA and Millipore in Kankakee, IL, USA.

Specimen Collection
Fresh samples of clotted, EDTA or citrate anticoagulated whole blood collected following general blood sampling guidelines are acceptable. The specimen should be tested as soon as possible after collection. If testing is delayed, EDTA and clotted specimens should be stored at 2 to 8°C, citrated specimens (donor segments) at 1 to 6°C. Blood specimens exhibiting gross hemolysis or contamination should not be used. Clotted samples or those collected in EDTA may be tested within ten days from collection. Donor blood stored in citrate anticoagulant may be tested until the expiration date of the donor unit.

Materials
Materials provided
- Seraclone® Anti-A (ABO1), Seraclone® Anti-B (ABO2) and/or Seraclone® Anti-A,B (ABO3)

Materials required but not provided
- Pipettes (drop volume 40 to 50 µl)
- Isotonic saline solution
- Negative Control (e.g. Biotest Seraclone® Control ABO+Rh REF 805171100)
- Glass tubes 10 x 75mm or 12 x 75mm
- Serological Centrifuge
- Interval Timer
- Markers
- Optical aid (optional). The use of an optical aid for agglutination reading must be vaildated by the user.

Test Procedure
Tube test
1. Prepare a 3 to 5% suspension of red blood cells to be tested in isotonic saline.
2. Place one drop reagent into an appropriately labeled tube.
3. Add one drop of red blood cell suspension into the tube labeled for it and mix.
4. Centrifuge for 20 seconds at 800 -1000 x g.
5. Gently dislodge red blood cell button and observe for agglutination.
6. Record results

Stability of the Reaction
Following centrifugation, all tube tests should be read immediately and results interpreted without delay. Time delays may cause a dissociation of the antigen-antibody complexes resulting to false negative or more often weak positive reactions.

Quality Control
The reactivity of all blood grouping reagents should be confirmed by testing with known positive and negative red blood cells on each day of use.
To confirm the reactivity or specificity of Biotest Monoclonal ABO Blood Grouping Reagents (Anti-A, Anti-B, Anti-A,B), each should be tested with antigen-positive (preferably from heterozygous or weak antigen expression) and antigen-negative red blood cells, respectively. Each reagent is satisfactory for use if it reacts only with antigen-positive red blood cells.
Confirmation of results in forward grouping must be obtained by performing the reverse grouping test.
A negative control should be performed on samples testing positive with Anti-A, Anti-B and Anti-D. Seraclone® Control ABO+Rh may be used.

Interpretation of results
Agglutination of the test red blood cells is a positive result and indicates the presence of the corresponding antigen. No agglutination is a negative result and indicates the absence of the corresponding antigen.
Frequencies in the population are listed in the "Summary" section.
An agglutination viewer may facilitate the reading of tube tests (as recommended by the AABB Technial Manual, 15th edition).

Reaction patterns, red blood cell antigens and isoagglutinins

The interpretation of results in testing infant blood samples may be difficult due to the fact that infant serum does not necessarily contain the natural occurring ABO antibodies for antigens absent from the red blood cells.
In all other cases, any discrepancy between forward and reverse grouping has to be resolved before the ABO blood group is recorded. The reagents do not react with cryptoantigens (T-, Tn-, Tk activated cells). Anti-B reacts correctly negative with acquired B characteristics.

Reagent with Patient red blood cells Phenotype			Reagent Red Blood Cells with Patient serum/plasma			Blood Group
Anti-A	Anti-B	Anti-AB	A_1	A_2 *	B	
+	0	+	0	0	+	A
0	+	+	+	+	0	B
0	0	0	+	+	+	O
+	+	+	0	0	0	AB

+ = agglutination 0 = no agglutination
*Testing with A_2 cells is not required

Reactions of Anti-A (ABO1), Anti-B (ABO2) and Anti-A,B (ABO3) with ABO variants

Cells	Seraclone® Anti-A (ABO1)	Anti-B (ABO2)	Anti-AB (ABO3)
A_2	++++	0	++++
A_2B	++++	++++	++++
A_3	+++(+)	0	++(+)
A_3B	+++(+)	++++	++++
A_x	++(+)	0	+(+)
A_xB	++(+)	++++	++++
B weak	0	++(+)	++(+)
A_1B weak	++++	++(+)	++++

Limitations
- Samples with a positive direct antiglobulin test, cold agglutinins, or rouleaux formation may show false positive results in testing with monoclonal antibodies. Results on these samples must be interpreted with caution. False positive results or reaction suspected to be due to cold agglutinins should be resolved according to in-house procedures. It is recommended that an appropriate control be tested in parallel.
- Incubation for 20 minutes may be performed to enhance weak reactions.
- Recurrent alternating storage of Anti-A,B (ABO3) from 2 to 8°C to room temperature may result in protein precipitation which does not affect the efficacy of the reagent.
- Some conditions that may cause false positive results are:
  - Contamination of sample or reagents
  - Autoantibodies
  - Improper storage or preparation of red blood cells
  - Antibodies to antibiotics or other reagents
  - Cold Antibodies

Specific Performance Characteristics
Testing is performed in accordance with FDA recommended methods.
The final release testing is performed according to the product specific SOPs. Each lot of Biotest blood group reagent is tested in the Quality control by package insert method against a panel of antigen positive red blood cells (heterozygous antigen expression and if possible weakened antigen expression) to insure suitable reactivity. The products meet FDA potency requirements. The specificity testing for the presence of contaminating antibodies is performed according to the product specific SOPs.
For the product performance it is necessary to adhere to the recommended method in the instructions for use.

The performance of the Biotest Anti-A, Anti-B and Anti-A,B was confirmed against FDA approved reference reagents in a Multi Center Field Trial.

For Technical Support or further product information, contact Biotest Diagnostics Corporation at 800-522-0090.

Note
Each facility should verify the optimum spin time for the specific centrifuge in use.
Manual techniques are to be performed according to the manufacturer's instructions. Each deviation from these instructions is the sole responsibility of the user.
Used tests must be discarded as hazardous material. Manage waste according to local, state and national regulations.

Glossary of Symbols

Symbol	Definition	Symbol	Definition
LOT	Batch Code	IVD	*In vitro* diagnostic medical device
⚠	Caution, consult accompanying documents		Consult instructions for use.
	Manufacturer		Use by YYYY-MM-DD
	Contains sufficient quantity for <n> tests.	REF	Catalog number
	Temperature limitation	VOL	Volume

Bibliography

1. Mark E. Brecher, MD et al. Technical Manual 15th Edition, Bethesda, MA: AABB, 2005.
2. Marion E. Reid, Christine Lomas-Francis, The Blood Group Antigen FactsBook, New York, NY: Academic Press, 2004.

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Biotest Medical Diagnostics GmbH, Industriestr. 1,
D-63303 Dreieich, Germany, www.biotest.de, mail@biotest.de
Tel.: +49-6103-801-0, Fax: +49-6103-801-140