BAG DIAGNOSTICS

EN INSTRUCTIONS FOR USE

Anti-A (ABO1)
Anti-B (ABO2)
Anti-A,B (ABO1,2)
monoclonal (IgM)

CE 0123

Clones: **Anti-A:** **F98 7C6**
Anti-B: **F84 3D6; F97 2D6**
Anti-A,B: **F98 7C6; F84 3D6; F97 2D6; F125 7B6**

Anti-A	REF	**6790**	**1 x 10 ml**	REF	**6791**	**10 x 10 ml**
Anti-B	REF	**6793**	**1 x 10 ml**	REF	**6794**	**10 x 10 ml**
Anti-A,B	REF	**6796**	**1 x 10 ml**	REF	**6797**	**10 x 10 ml**

Electronic Instructions for use see www.bag-diagnostics.com

FOR IN VITRO DIAGNOSTIC USE

1. Description of products

Anti-A, Anti-B and Anti-A,B are prepared from murine monoclonal IgM antibodies. The clone numbers are given on the labels of the test reagents. The test reagents are intended for use in a direct agglutination test in tubes and on slides and provide a specific qualitative test for the detection of the corresponding A and/or B antigens on human red blood cells. The diluent used for this low protein reagents contains NaCl, BSA, a pH buffer and EDTA. NaN_3 at a final concentration of 0.1% is added as a preservative.
Anti-A contains a blue dye, Anti-B a yellow dye and Anti-A,B contains no coloring agent.

Each lot of these Blood Grouping Reagents has been tested according to methods recommended by the US FDA. The reagents 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. When used in accordance with the recommended Instructions for Use reagents have been tested and found to specifically agglutinate human red cells if the corresponding blood group antigen is present.
The reactivity of each lot has been verified with a panel of red cells tested in accordance with the recommended Instructions for Use.

Anti-A may not react with red cells classified as A_X. Anti-A,B detects red cells classified as A_X. Part of lot release testing includes testing each lot with 2 examples of A_X red cells.
Certain subgroups of A and B may demonstrate reactions that are weaker than those obtained with A or B cells of most random donors. Depending on the subgroup involved, some may appear weak or non-reactive in direct agglutination tests by tube or slide.
The specificity of each lot has been verified by the recommended tube and slide test method with a panel of cells negative for the respective A and B antigens.
Anti-A has not demonstrated reactivity with red cells classified as B (A). Anti-B has not demonstrated reactivity with red cells that express acquired B antigen.

2. Principle of the test

The test used with this blood grouping reagents is based on the principle of direct hemagglutination. Incubation of red cells with Anti-A, Anti-B or Anti-A,B monoclonal will result in a specific antigen-antibody reaction if the corresponding antigen is present on the red cells. Visible detection of this reaction is demonstrated by agglutination of the red cells. No agglutination indicates a negative test result, and, within the accepted limitations of the test procedure, indicates the absence of the corresponding antigen from the test red cells.

3. Storage and stability

Store the test reagents at 2...8°C. Do not freeze! Allow the reagents to reach room temperature (18...25°C) before use and store again at 2...8°C immediately after use.
Once they have been opened the first time, the test reagents may be used up to the expiration date indicated on the label if the specified storage conditions are observed and no turbidity or contamination occurs. Do not use the reagents past the expiration date indicated on the label. Do not use contaminated reagents.

4. Preparation of samples

No special preparation of the patient/donor is required prior to specimen collection. Blood samples should be collected by approved aseptic medical procedure. Blood samples may be collected with or without anticoagulant if testing is performed without delay. If a delay in testing is unavoidable, red cells from clotted samples or EDTA anticoagulated samples may be tested up to 14 days from date of collection. ACD, CPD and CPDA-1 anticoagulated blood samples may be tested up to their expiration date. All red cell samples should be stored appropriately at 2...8°C. A red cell preservative solution may be used for prolonged storage of red cells.

Do not use hemolytic or contaminated samples.

Prolonged storage of red cells prior to testing may result in deterioration of red cell antigens and resultant weaker than expected test reactions.

5. Additional materials required

Isotonic saline, pH 6.5 – 7.5
Glass slides, applicator sticks
Test tubes (75 x 12 mm, glass or plastic)
Single-use Pasteur pipettes
Centrifuge (900 – 1000 rcf)
Control red cells of known ABO group

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6. Test procedure

Slide test

1. Prepare a 35 - 45% suspension of red cells to be tested in isotonic saline.
2. Place 1 drop of the monoclonal test reagent on a labeled glass slide.
3. Add 1 or 2 drops of the prepared suspension of red cells and mix thoroughly over an area of approximate 20 x 40 mm using a clean applicator stick.
4. Slowly tilt the slide back and forth for up to 2 minutes and examine for macroscopic hemagglutination. Agglutination may begin within a few seconds, but observation should not continue beyond 2 minutes.
5. At the end of 2 minutes, those tests showing no agglutination are interpreted as negative. Care should be taken not to mistake peripheral drying or fibrin strands as agglutination.

Note: Use for mixing a separate clean applicator stick for each reagent and each red cell suspension.

Do not place slides on heated surfaces.

Attention: Slide test procedures may not be sufficiently sensitive for reliable detection of weakened antigen expression.

Tube test

1. Wash the red cells to be tested in isotonic saline and prepare a 2 - 4% suspension in isotonic saline.
2. Dispense 1 drop of monoclonal test reagent and 1 drop of the red cell suspension in a labeled test tube and mix thoroughly.
3. Centrifuge 15 seconds at 900 – 1000 rcf or at an alternative rcf with an appropriate time adjustment.
4. Resuspend the cells by gently shaking the tube and examine macroscopically for agglutination.

Note: Do not examine the test microscopically.
Some weak reactions may be enhanced by incubating the test for up to 15 minutes at room temperature (18...25°C) followed by centrifugation as in steps 3 – 4 above.

Controls

Red cells that are positive with regard to the respective antigen and red cells that are negative with regard to the respective antigen must also be tested as controls. Positive control cells should be selected to represent weak expression of the specific antigen.

The determination of the antigens should be carried out with at least 2 different test reagents. When using two monoclonal test reagents, two different clones should be used. Anti-A,B should be used as a confirmatory control for tests with Anti-A and Anti-B.

For excluding spontaneous red cell aggregation, controls consisting of 6 – 8% BSA or autologous serum or plasma may be tested in parallel.

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For the determination and confirmation of the ABO blood group, reverse (serum) grouping tests with known A_1, A_2, B and O reagent red cells must be performed (see statutory and local provisions).

It is strongly recommended that the reactivity and specificity of the reagents be confirmed in regular intervals of use by control tests with known antigen positive and negative red cells (see statutory and local provisions).

7. Interpretation of the results

Within the accepted limitations of the test procedure, agglutination of test red cells with Anti-A or Anti-B indicates the presence of the corresponding antigen. Similarly, agglutination of test red cells with Anti-A,B indicates the presence of A and/or B antigen.

Within the accepted limitations of the test procedure, no agglutination of test red cells with Anti-A or Anti-B indicates the absence of the corresponding antigen. Similarly, no agglutination of test red cells with Anti-A,B indicates the absence of A and B antigen.

No valid conclusion concerning the test result can be achieved, if there is no agglutination with the known positive red cells, or if agglutination occurs with the known negative red cells or if the control with 6- 8% BSA or autologous serum/plasma shows agglutination.

If discrepant results occur with two different test reagents when determining the antigen or between antigen determination and isoagglutinin detection, the blood group determination must be repeated with another test method and/or an additional test reagent.

The limitations of the method must be considered when interpreting the results (see 9. Important Notes/Limitations of the Method).

AB0-blood group typing with test reagents			Blood Group	Determination of isoagglutinins with test erythrocytes			
Anti-A	Anti-B	Anti-AB		A1	A2	B	0
+	-	+	**A**	-	-	+	-
-	+	+	**B**	+	+	-	-
-	-	-	**0**	+	+	+	-
+	+	+	**AB**	-	-	-	-

8. Stability of reactions

All test results must be interpreted immediately once the test is performed.

9. Important notes/limitations of the method

1. The test reagents are suitable for in vitro diagnostic use only and may only be used by trained, qualified personnel.
2. Hemolytic samples should not be used.
3. On rare occasion, red cells coated in vivo with immunoglobulin may agglutinate spontaneously and non-specifically in some reagent media. This phenomenon is usually associated with reagents formulated with high protein and macromolecular additives. Anti-A, Anti-B and Anti-A,B are formulated in a low protein medium which does not promote spontaneous agglutination. However, very rarely, examples of red cells heavily coated with immunoglobulin may agglutinate non-specifically in low protein media. In

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such instances a similar occurance would most likely be observed in other blood grouping test as well. If the test cells are reactive with Anti-A, Anti-B and Anti-D an additional control may be desirable. A control test consisting of either 6 - 8% BSA or autologous patient serum/plasma may be suitable. If the control test yields a positive reaction, a valid interpretation of the blood grouping results cannot be made.

4. Suspensions of unwashed red cells promote false positive reactions such as those associated with rouleaux formation or autoantibodies. The routine use of well-washed, saline suspended red cells for tube tests may reduce the risk of such false positive reactions.
5. Red cells with weak A and/or B subgroups and red cells of newborn infants or cord cells, which have no full expression of A and B antigens, may demonstrate only weak or no reactions. In particular slide test procedures may not be sufficiently sensitive for reliable detection of weakened antigen expression.
6. Anti-A may not react with red cells classified as A_X. Anti-A,B detects red cells classified as A_X.
7. If A or B variants are suspected or the blood group determination shows doubtful or discrepant results further determinations with molecular genetic tests are recommended, e.g. with BAGene kits (BAG-SSP kits for the determination of ABO attributes on a molecular genetic basis). With this method detection of weak antigens is possible. It is important to detect such weak expressions of antigens in donor blood units so that such blood is not transfused to group O recipients.
8. Some diseases can cause false positive or false negative results (e.g. weakened A antigen by acute leukemia, acquired B antigen by older patients with blood group A and colon carcinoma). Anti-A has not demonstrated reactivity with red cells classified as B (A). Anti-B has not demonstrated reactivity with red cells that express acquired B antigen.
9. For determination of the ABO blood group a test for the detection of isoagglutinins must carried out always too. Serological anomalies can occur that may result in unexpected reactions or discrepancies between antigen and isoagglutinin determination. In particular sera from newborn infants or cord cells 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. Discrepancies must be resolved prior to assigning a blood group.
10. Anti-A, Anti-B and Anti-A,B must not be used to test enzyme treated red cells.
11. Reading the results of the tube test too late, agitating the red cell sediment too strongly, and other deviations from the indicated testing procedure can lead to weaker or false negative results.
12. If the slide test is read too late, the appearance caused by drying or fibrin strands may simulate false positive results.
13. False negative results or unexpected weak reactions may be caused by storing the red cells for too long and/or under inappropriate storage conditions and/or by a cell concentration that is too low.
14. False negative or false positive results also may be caused by inappropriate techniques, incorrect centrifugation or incubation, dirty tubes or slides, incorrect pH of the isotonic saline and/or contaminated materials and samples.
15. Hemolysis observed in ABO grouping tests should not necessarily be interpreted as a positive result. Hemolysis may be caused by bacterial contamination. The validity of the test results, and the correct interpretation thereof, is dependent on the demonstration of the expected control results obtained with the positive and negative control cells. If a

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patient control is run simultaneously with the test and shows agglutination, no valid conclusion concerning the test result can be reached.

16. Marked turbidity may indicate microbial contamination. A microbial contamination of the test reagents must be absolutely avoided because this shortens the shelf life of the product and can lead to false results. Do not use contaminated reagents.
17. Whether transfusions or transplantation have taken place should always be taken into consideration when interpreting the results. Any history of transfusions and/or transplantation, as well as the patient's medication history, should be taken into consideration when interpreting results.
18. 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 centrifugation 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.
19. Use Anti-A, Anti-B and Anti-A,B monoclonal as supplied and as described in this Instructions for Use. Deviation from the recommended Instructions for Use may result in less than optimal product performance. Any deviation (e.g. dilutions, other test methods) must be validated by the user.

10. Warnings and instructions for disposal

All materials of biological origin used for the test should be regarded as potentially infectious. The absence of murine virus has not been determined. Any bovine source materials, used in the manufacture of these products, are sourced from donor animals that have been inspected and certified by veterinary service inspectors to be disease-free. This ruminant-based product is deemed to have low TSE (Transmissible Spongioform Encephalopathy) risk.

Appropriate safety precautions are recommended when handling biological materials (do not pipette using the mouth; wear protective gloves when performing the test; disinfect hands after testing).

Biological materials must be deactivated before disposal (e.g., by autoclaving). Single-use materials must be autoclaved or incinerated after use. Spills of potentially infectious material should be removed without delay with an absorbent paper towel and the contaminated area disinfected with an appropriate disinfectant or 70% ethanol. Materials used for the removal of spills must be deactivated before disposal (e.g., by autoclaving).

The dropper bulbs of these products contain natural rubber latex, which is known to cause allergic reactions in some individuals.

The reagent contains 0.1% NaN_3 as preservative. Sodium azide is toxic. Do not ingest, avoid contact with the skin and mucous membranes. The copper and lead used in some plumbing systems can react with azides to form explosive salts. Therefore, when disposing of azide-containing materials, they should be flushed away with a large volume of water.

Disposal of all samples, unused reagents and waste should be in accordance with country, federal, state and local regulations.

A Material Safety Data Sheets (MSDS) is available to download at www.bag-diagnostics.com.

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Explanation of symbols used on Labelling	
IVD	For in vitro diagnostic use
	Manufacturer
	Storage temperature / Temperature limitation
LOT	Batch code
	Use by
REF	Catalogue number
	Consult instructions for use
MONOCL | IGM	Monoclonal IgM
CLONE	Clone
ORIG | MURIN	Origin: mouse
CONT | NaN_3	Contains Natriumazide
TIT	Titer
	Warning H302 Harmful if swallowed P301 + P312 IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell P264 Wash hands thoroughly after handling P270 Do not eat, drink or smoke when using this product P281 Use personal protective equipment as required

Instructions for use	Version: 5/2019 / Issue: 2019-06

Instructions for use in other languages see:
http://www.bag-diagnostics.com
or phone +49 (0) 6404-925-125

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