

INSTRUCTIONS FOR USE

Anti-A (ABO1)

CE 0123

Anti-B (ABO2)

Anti-A,B (ABO1,2)

monoclonal (IgM)

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

FOR IN VITRO DIAGNOSTIC USE

1. Description of products

Anti-A, Anti-B and Anti-A,B are made from monoclonal mouse IgM antibodies. The clone numbers are given on the labels of the test reagents. The test reagents aid in the detection of the corresponding antigens on red blood cells and are suitable for the direct agglutination test in tubes and on slides. The diluent used for this low protein reagents contains NaCl, BSA, a buffer and EDTA. NaN₃ 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.

The reactivity of each lot has been verified with a panel of red cells tested in accordance with the recommended Instructions for Use. Some weakened forms of antigen expression (i. e. those associated with some types of ABO subgroups) may be not detected in routine testing by Anti-A, Anti-B and Anti-A,B. The specificity of each lot is demonstrated with a panel of cells negative for the antigen in question and positive for antigens having a frequency of 1% or greater in the general North American population. When suitable test cells are available, the presence of antibodies to low frequency antigens e.g. C^w, Yt^b, M^g and Vw are excluded in routine specificity testing.

2. Principle of the test

The test used with this blood grouping reagents is based on the principle of 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.

3. Storage and stability

Store the test reagents at 2...8°C. Do not freeze! Allow the reagents to come to 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

Blood samples should be collected by approved medical procedure. Blood collected with or without anticoagulant is acceptable 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 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 of 6.5 – 7.5)

Glass slides, applicator sticks

Test tubes (75 x 12 mm)

Disposable Pasteur pipettes

Centrifuge

Control red cells of known ABO group

6. Test procedure

Slide test

1. Prepare a 35 - 45% suspension of red cells to be tested in isotonic saline.
2. Place 1 drop (40 - 50 μ l) of the monoclonal test reagent on a glass slide.
3. Add 1 drop of the prepared suspension of red cells and mix thoroughly over an approximate 20 mm circular area using a clean applicator stick.
4. Slowly rotate the slide gently 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: When Anti-A and Anti-B are used concurrently, one drop of each reagent may be applied to opposite ends of a single slide. Use for mixing a separate clean applicator stick for each reagent.

Note: 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 (40 – 50 μ l) 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 or autoagglutination, controls consisting of test red cells suspended in 6 – 8% BSA and in autologous serum or plasma should be tested in parallel.

For the determination and confirmation of the ABO blood group, reverse (serum) grouping tests with known A₁, A₂, B and O reagent red cells must be performed.

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 patient control in 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).

ABO-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. In rare cases, spontaneous and non-specific agglutinations may occur with red cells loaded with immunoglobulins in vivo. In such instances similar phenomena would most likely occur in blood grouping tests of other blood group systems as well. Therefore, a control consisting of test red cells suspended in 6 – 8% BSA and in autologous serum or plasma should be tested in parallel. If the control tests also show a positive reaction, the result of the blood group determination cannot be interpreted.
4. Suspensions of unwashed red cells in plasma or serum promote false positive reactions such as those associated with rouleaux formation or autoantibodies. The use of well-washed red cells can reduce the occurrence of such false positive reactions.
5. Red cells with weak A and/or B subgroups and red cells of newborn infants, 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. 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).
7. For determination of the ABO blood group a test for the detection of isoagglutinins must be 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 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.
8. 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 ABO-TYPE. 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.
9. Anti-A, Anti-B and Anti-A,B must not be used to test enzyme treated red cells.
10. 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.

11. If the slide test is read too late, the appearance caused by drying or fibrin strands may simulate false positive results.
12. False negative results or unexpected weak reactions may be caused by storing the red cells for too long and/or under inappropriate conditions and/or by a cell concentration that is too low.
13. False negative or false positive results can result from inappropriate techniques, incorrect centrifugation or incubation, dirty tubes, incorrect pH of the isotonic saline and/or contaminated materials and samples.
14. Hemolysis observed in ABO grouping tests should not necessarily be interpreted as a positive result. Hemolysis may be caused by bacterial contamination.
15. 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.
16. 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.
17. 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.
18. Use Anti-A, Anti-B and Anti-A,B monoclonal as supplied and as described in this Instructions for Use. Any deviation 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 albumin used in the manufacture of these products 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.

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 test reagents contain 0.1% NaN₃ as preservative. NaN₃ is harmful to health when it is inhaled or swallowed or comes in contact with the skin and mucous membranes (risk (R) and safety (S) phrases, see Explanation of symbols used in labelling). 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.

The disposal of all samples and test materials should be carried out according to legal directives.

11. Package sizes

See price list.

12. References

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






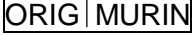



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Explanation of symbols used on Labelling	
	<i>For in vitro diagnostic use</i>
	<i>Storage temperature</i>
	<i>Batch code</i>
	<i>Use by</i>
	<i>Catalogue number</i>
	<i>Consult instructions for use</i>
	<i>Monoclonal IgM</i>
	<i>Clone</i>
	<i>Origin: mouse</i>
	<i>Contains Natriumazide</i>
	<i>Titer</i>
	<i>Harmful</i> R 22-52/53, S 02-13-22-46-56-61

- R 22 Harmful if swallowed.
- R 52/53 Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
- S 2 Keep out of the reach of children.
- S 13 Keep away from food, drink and animal feeding stuffs.
- S 22 Do not breathe dust.
- S 46 If swallowed, seek medical advice immediately and show this container or label.
- S 56 Dispose of this material and its container at hazardous or special waste collection point.
- S 61 Avoid release to the environment. Refer to special instructions, safety data sheets.

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