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Instruction for use

Use by professionals only
Tests/ml: 25: with drop size 40µl when using separate volumetric pipettes

CE 0483

Revision: **29/07-2019**

Product-Name:	Product-Code:	Product-Name:	Product-Code:
Anti-A A-11H5	A-mono-11H5	**Anti-B B-6F9**	B-mono-6F9
Anti-A BIRMA-1	A-mono-BIRMA	**Anti-B LB-2**	B-mono-LB2
Anti-AB A-5E10-B-2D7	AB-mono-5E10	(Mouse IgM)	

Reagent for specific detection of the corresponding antigen. Bloodgroup testreagent for microplate- , tube-, slide- and plate tests.
All described test methods are only valid for manual applications as recommended in this instruction. The user must determine their suitability for use in other techniques (automates, semi-automates, gel-cards, others) according to recognized techniques and hints of the machine- or cards manufacturer in individual responsibility.
Only for in-vitro diagnostic laboratory use. Store at + 2 - 8 °C when not in use.

Product description:	Anti-A, Anti-B and Anti-AB are monoclonal *Mouse IgM* reagents which detect the corresponding red cell antigens in a direct agglutination reaction. Lack of agglutination indicates the absence of the corresponding antigen. The respective monoclonal antibodies derived from cultured hybridoma cell lines (*murine*). The antibodies (IgM) are diluted in a buffered isotonic saline solution containing bovine albumin (without stabilizers), EDTA and ingredients to facilitate the resuspension of red cell buttons following centrifugation. Sodium azide at a final concentration of < 0,1% w/w is added as a preservative.
Clone:	**Anti-A: A-11H5 and BIRMA-1, Anti-B: B-6F9 and LB-2, Anti-AB: A-5E10-B-2D7** Reagents Anti-A and Anti-B are coloured (blue respectively yellow) in order to avoid confusion of the reagents and to allow better control of the preparations.
Note/Caution:	Sodium azide can cause high explosive metal azide combinations with lead and cooper. When pouring rinse with a lot of water. All blood products should be treated as potentially infectious. No known regime of testing can completely guarantee that any product derived from human blood is incapable of transmitting infectious agents. Care should be exercised in the use and the disposal of the container and its contents.
Shelf life	Test-reagents can be used until the end of the product shelf life indicated on the label if stored correctly between 2 – 8° C when not in use. After the first opening the reagents must be stored properly closed at 2 – 8° C. Bacterial contamination are to be avoided. Clearly clouded reagents should not be used any longer.
Test methods:	Samples may be drawn aseptic into the common anticoagulants (EDTA, ACD,). Testing should be performed as soon as possible after blood withdrawal to minimise the chance that falsely positive or falsely negative reactions will be encountered due to contamination or improper storage of a specimen. Samples that cannot be tested immediately should be stored at +2 – 8 °C..
Additional materials required:	Isotonic saline, transfer pipettes, glass slides, applicator slicks, slides or plates, test tubes and test tube racks, validated serological centrifuge, cell panel, timer. Microplate tests: microplates, microplate shaker (optional), validated serological centrifuge, when used with reading machine or on automates it is the users responsibility to validate any accessory device for the intended use, NaCl solution, timer, transfer pipette, if necessary Bovine Albumin.
Microplate test:	MTP from different suppliers show different characteristics which might have non specific reaction of the red blood cells as a consequence. It is recommended to pre-treat new MTP before its first use in order to minimize the fastening of the red cells. Recommended are MTP with U-profile out of plastic. 1. Add 1 drop (30-50µl) of Bovine Albumin 22% to each well. 2. Through careful movements or on a shaker mix well so that all wells uniformly are coated. 3. Incubate 10-15 min. at RT (18-25°C). 4. Pour off Bovine Albumin and give the contents of the wells in suitable waste containers 5. Rinse MTP at least 10 x with tap water. 6. Rinse MTP subsequently 2 x with distilled water. 7. MTP tip over and dap away in order to remove surplus water. 8. Dry MTP before use at the air. Alternative methods possible as far as validated by the users. 1. Prepare a 2-4 % suspension of test red cells in isotonic solution. (Recommendation 2% suspension) 2. Add one drop of the respective reagent (30-50µl) to the appropriate test wells of a U well microplate. 3. Add an equal volume of the cell suspension to the appropriate test wells. 4. Mix the contents of each well using manual means or a microplate shaker. (30 sec.) 5. No incubation time necessary except for titrations or to the strengthening of weak Phenotypes. 6. Centrifuge the microplates at 1.500 UpM until 60 sec. or other appropriate time and UpM. 7. Re-suspend the red cells using the microplate shaker. (as in no. 4.) 8. Read tests macroscopically or with an automated plate reader. The use of an automated plate reader must be validated by the customer. The use of additional visual remedies as mirror or magnifier can ease the reading.
Tube test:	In order to improve the test-results it is recommended to wash the cells before re-suspension at least one time in 0,9% isotonic saline. 1. Prepare a 2 – 3 % suspension of the washed red cells in 0,9% isotonic saline, plasma or serum. 2. Add 1 drop of the respective test serum Anti-A or Anti-B or Anti-AB and one drop of red cells to the appropriately labelled tubes and mix. 3. Centrifuge for 1 Min. at 400g (1.500 UpM) or with alternative UpM at appropriate time. 4. Control supernatant on absence of Hemolysis which can have its origin on bacteriological contamination. Gently agitate each tube to re-suspend the cell buttons and examine for agglutination just after centrifugation. 5. Record results and reactivity strengths.

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Slide-test/plate-test:	1. Slide-tests are performed with whole blood, plate-tests with washed Erythrocytes or whole blood. 2. Place one drop of the reagent on a clean glass-, plastic slide or plate. 3. Add one drop of whole blood (respectively 35-45% suspension of red cells) to the slides or one drop of whole blood in 0,9% saline solution (respectively 10% red-cell suspension) to the plate using a transfer pipette or applicator stick. 4. Mix blood and reagent. This is achieved by slow rotation over a period up to 2 minutes (slides) and on plates after an incubation time of 5 – 10 minutes. Incubation time for whole blood testing is limited to 5 min. maximum. 5. Observe macroscopically for agglutination and record results. Care should be taken not to mistake peripheral drying as agglutination. Do not place slides on or before a heated illuminated surface.
Interpretation of test results:	There must be agreement between the results of the antigen determination (cell grouping)and the determination of the alloantibodies (serum grouping) of a blood specimen. 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 cells, and passive anti-a and/or anti-B antibodies from the mother´s circulation may yield conflicting results when tests are performed on cord blood specimens. Cord blood specimens may also give weaker-than-normal reactions in the cell grouping, as the ABH antigens are imperfectly developed at birth.
Advice to users:	- Control: On each test, positive and negative control red cells have to be tested in parallel, - On plates, blood samples may occasionally react by rouleaux formation, which can be mistaken for a weak agglutination and may incorrectly be read as a positive result. This phenomenon has non-immunological causes. Rouleaux formation also occurs in heparin blood, blood from patients treated with plasma expanders (e.g. dextran) as well as in blood from patients with plasmacytoma (high protein content, changes in protein composition), oncological disease (abnormal blood count) or coagulation dysfunctions. For testing these patients, use the tube test, which usually avoids this phenomenon. - The reagent agglutinates weakly expressed antigens with normal or weaker agglutination strength (A3, Bweak) or weaker to negative reaction (Ax). To determine weakly expressed antigens the tube test should be used because of its higher sensitivity, if necessary with incubation for 30 minutes. - Unusually weak reactions until non reactivity may occur from A- and B subgroups. The red cells of people with some disease states may give falsely positive or falsely negative reactions with Anti-A or Anti–B. - Cord cells contaminated with Wharton´s jelly may give falsely positive reactions. - Do not use monoclonal reagents with mouse antibodies in indirect Anti-Human-Globulin tests with AHG-reagent. - Certain subgroups of A and B may produce reactions that are weaker or even no reactions than those obtained with A or B cells of most random donors. The presence of such antibodies cannot be predicted. When sufficiently strong they can cause the non-specific agglutination of reagent A1 and B cells in serum (reverse) grouping tests. They can also produce non-specific agglutination in cell (forward) tests with anti-A, and –B and –AB when unwashed, plasma- or cells respectively in serum suspended cells or plasma samples are determined. Consequently for the determination of blood groups always ABO and Isoagglutinin determinations are mandatory. - 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.
Performance data:	The reagent fulfils the common technical specifications' requirements according to Annex II, List A der Directive 98/79/EC for in vitro diagnostics. It has the same or a better performance characteristics as comparable reagents in use. It was tested on more than 1000 samples with sensitivity and specificity of 100%.
Limitations:	Strengths of the test results are depending from the age of the blood. Falsely positive or falsely negative test results can occur from insufficient cell-concentration, inadequate incubation time or temperature, improper centrifugation, improper storage of materials or non- consideration of the instructions for the different test methods. They can occur as well from bacterial or chemical contamination of the anti-serum, cells or the saline solution. When less sensitive test methods than the tube test are used negative results can occur with Anti-AB and weak Ax cells. Than it is recommended to repeat the test with the tube method. *Clone BIRMA-1 has not been tested by the company with regard to recognizing Ax-cells.* The use of the anti-sera in machines or on gel cards may require dilutions. The use of such manipulated sera asks for re-validation under the responsibility of the user. This is valid for all manipulations as for example the cold freezing of the sera on microplates.
References:	1. Kohler C. & Milstein C. (1975), Continuous cultures of fused cells secreting antibody of predefined specificity. Nature, 256, 495-497. 2. Lee H.H., Rouger P., Germain C., Muller A. & Salmon C. (1983). The production and standardisation of monoclonal antibodies as AB blood group typing reagents. Symposium of International Association of Biological Standardisation on monoclonal antibodies. 3. Human Blood Groups, by Geoff Daniels, 1st Ed., Blackwell Science, Oxford 1995. 4. HMSO, Guidelines for Blood Transfusion Services., 2nd Ed., 1994.