Instructions for use

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A_1 reagent red cells	REF **K1140**	IVD CE **0344**
B reagent red cells	REF **K1142**	IVD CE **0344**
O positive reagent red cells	REF **K1143**	IVD CE
O negative reagent red cells	REF **K1144**	IVD CE
A_2 reagent red cells	REF **K1141**	IVD CE

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For professional use only

3% Cell suspensions for the detection of alloantibodies anti-A and anti-B (A_1 and B reagent red cells) and for the use as positive or negative control (O positive, O negative and A_2 reagent red cells)

General information
The reagent red cells are suspended in a preservation medium. These reagents meet the requirements of the concerned standards and guidelines. Performance characteristics are mentioned in the release documents, which are supplied with the product upon request. The principle of the test is the agglutination technique, which is based on antigen/antibody reaction. The reagent red cells can be used in either spin tube or microplate method. These reagent red cells are also suitable for use in automated test systems and should be standardised and validated by the user. The inclusion of positive and negative controls with each series of blood group determinations is strongly recommended. As well as determining the presence or absence of the alloantibodies anti-A and anti-B in the serum or plasma of the patient, the red cells of the patient should be tested for the presence of the corresponding A and/or B antigens, using Pelikloon anti-A, anti-B and anti-A,B (IgM) monoclonal reagents (see relevant package insert).

Precautions
For in vitro diagnostic use only. Reagent red cells should be stored at 2–8°C; do not freeze. Leaking or damaged vials must not be used. Reagent red cells (unopened or opened) should not be used beyond the expiration date, which is printed on the label of the vial. Chloramphenicol 0.025%, neomycin sulfate 0.01% and gentamicin 0.005% are used as preservatives. Although all blood products are tested for infectious diseases and found negative, the reagents cannot be assumed to be free from infectious agents. Care must be taken in the use and disposal of each container and its contents. If contamination or excessive hemolysis is evident, discard. To recognise deterioration, testing of the reagent red cells as part of the laboratory quality control program using appropriate controls is recommended. Waste-disposal, after completion of the test, should be performed according to your laboratory regulations.

Specimen collection and preparation
Blood samples should be withdrawn aseptically with or without the addition of anticoagulants. If testing of the blood samples is delayed, storage should be at 2–8°C. Preparation of the specimen is described in the respective test procedures.

Test procedures
Spin tube method
Tube requirements: round bottom glass tubes; size 75 x 10/12 mm.

1. Add to a test tube:
   - 2 drops of the serum or plasma to be tested
   - 1 drop of reagent red cells 3% suspension
   and mix well.
2. Centrifuge for 20 seconds at 1000 rcf or for a time appropriate to the calibration of the centrifuge.
3. Resuspend the cells by gentle agitation and read macroscopically for agglutination.

Microplate method
Microplate requirements: polystyrene microplates with round bottom wells.

1. Add to a well:
   - 2 drops of the serum or plasma to be tested
   - 1 drop of reagent red cells 3% suspension.
2. Mix the content thoroughly for 5 seconds using a rotary shaker at 600–700 rpm.
3. Incubate for 10–15 minutes at room temperature (18–25°C) without shaking.
4. Centrifuge for 10–20 seconds at 700 rcf or for a time appropriate to the calibration of the centrifuge.
5. Reshake the microplate for 1–4 minutes on the rotary shaker at 600–700 rpm or as long as necessary to completely resuspend the cells in the wells with negative reactions.
6. Let the microplate rest for 1 minute to allow smaller agglutinates to settle.
7. The reactions can now be read either macroscopically or using an automatic reader.

Interpretation
A positive reaction (i.e. agglutination) indicates the presence of the corresponding alloantibody. A negative reaction (i.e. no visible agglutination) indicates the absence of the corresponding alloantibody. The ABO blood group is determined by the reaction pattern

obtained with the various antisera (see table overleaf). If the reaction pattern does not correspond with one of the 4 combinations below, then the reason for the discrepant results should be determined prior to assigning an ABO blood group to the patient / donor in question.

Agglutination reactions in routine ABO grouping

red cells + blood grouping reagent			serum/plasma + reagent red cells		
anti-A	anti-B	anti-A,B	A_1 cells	B cells	blood group (frequency)
0	0	0	+	+	O (46.7%)[4]
+	0	+	0	+	A (41.7%)[4]
0	+	+	+	0	B (8.6%)[4]
+	+	+	0	0	AB (3.0%)[4]

Limitations
(ABO grouping)
Unexpected positive results due to: pseudoagglutination, presence of other than anti-A and / or anti-B alloantibodies. Unexpected negative or weak results due to: the fact that serum or plasma under investigation is from a newborn, a (very) old person or from a patient with hypogammaglobulinemia, chimerism, decreased activity of the reagent red cells.

References

1. Race R.R. and Sanger R.; Blood Groups in Man, 6th ed. Oxford Blackwell Scientific Publishers 1975.
2. Issitt P.D.; Applied Blood Group Serology, 3rd ed. Montgomery Scientific Publications, Miami, Florida, USA, 1985.
3. Daniels G.; Human Blood Groups. Blackwell Science Ltd. 1995.
4. Reid M.E. and Lomas-Francis C.; The Blood Group Antigen Facts Book. Facts Book Series, 1997.
5. Mollison P.L. et al.; Blood Transfusion In Clinical Medicine, 9th ed. Blackwell, Oxford, 1993.