Instructions for use

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Pelikloon anti-D enhanced (IgM) monoclonal

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For professional use only

Blood grouping reagent for the detection of the D antigen on human red cells

General information
Pelikloon anti-D enhanced (IgM) monoclonal blood grouping reagent (clone numbers are mentioned on the corresponding certificate of analysis/release document and product label) are prepared from culture supernatants from stable hybridoma cell lines as first described by Köhler and Milstein (Nature 1975). This monoclonal reagent contains human IgM antibodies and has been specially selected and developed to provide a reliable alternative to polyclonal reagents. This reagent meets 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 can be used in either spin tube or microplate method and will detect all of the D antigens and most D variant category VI and weak D antigens. The inclusion of positive and negative controls with each series of blood group determinations is strongly recommended.

Precautions
For in vitro diagnostic use only. Reagents should be stored at 2-8 °C. Leaking or damaged vials may not be used. Reagents (unopened or opened) should not be used beyond the expiration date, which is printed on the label of the vial. NaN_3 0,1% (w/v) is used as preservative. The reagent cannot be assumed to be free from infectious agents. Care must be taken in the use and disposal of each container and its contents. Turbidity may indicate microbial contamination. To recognise reagent deterioration, testing of the reagent 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Blood samples should be withdrawn aseptically with or without the addition of anticoagulants.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. Prepare a 3-5% cell suspension of red cells to be tested in isotonic saline .
2. Add to a test tube:
   - 1 drop of Pelikloon reagent
   - 1 drop of the 3-5% cell suspension

   and mix the contents well.
3. Centrifuge for 20 seconds at 1000 rcf or for a time appropriate to the calibration of the centrifuge.
4. Resuspend the cells by gentle agitation and read macroscopically for agglutination.

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

1. Prepare a 2-3% cell suspension of red cells to be tested in isotonic saline.
2. Add to a well:
   - 1 drop of Pelikloon reagent
   - 1 drop of the 2-3% cell suspension.
3. Mix the content thoroughly for 5 seconds using a rotary shaker at 600-700 rpm.
4. Incubate for 10-15 minutes at room temperature (18-25 °C) without shaking.
5. Centrifuge for 10-20 seconds at 700 rcf or for a time appropriate to the calibration of the centrifuge.
6. 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.
7. Let the microplate rest for 1 minute to allow smaller agglutinates to settle.
8. 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 Rh D antigen. A positive reaction, while weak or negative by other techniques, indicates the presence of a weak or partial D antigen and further investigation to elucidate the Rh D status is recommended. A negative reaction (i.e. no visible agglutination) indicates the absence of the Rh D antigen.

Occurrence	Caucasians	Negroids
D antigen	85%	92%

Limitations
Unexpected positive results due to: pseudoagglutination, autoagglutination, mixed field reaction, the presence of Whartons jelly together with umbilical cord cells.
Unexpected negative or weak results due to: weak antigens, mixed field reaction, decreased activity of the reagent.
False positive or false negative results may occur through contamination of test materials or any deviation from the recommended technique.
Red cells that have a positive direct antiglobulin test (DAT) may produce false positive test result. Use of Pelikloon control monoclonal is recommended for detection of such invalid test results.
Pelikloon monoclonal blood grouping reagents have been optimised for use by the technique(s) recommended in this package insert. Unless otherwise stated their suitability for use by other techniques must be determined by the user.

References

1. Race R.R. and Sanger R.; Blood Groups in Man, 6th ed. Oxford Blackwell Scientific Publishers 1975.
2. Issit 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.
6. Engelfriet C.P. et al.; Immunohaematology. Sanquin Blood Supply Foundation, 2003.

Sanquin products are guaranteed to perform as described in the original manufacturer's instructions for use. Strict adherence to the procedures, test layouts and recommended reagents and equipment is essential. Sanquin declines all responsibility arising from any deviation thereof.