Instructions for use

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Pelikloon anti-C (IgM) monoclonal	REF K1195 K1202	IVD CE 0344
Pelikloon anti-c (IgM) monoclonal	REF K1196 K1203	IVD CE 0344
Pelikloon anti-E (IgM) monoclonal	REF K1191 K1204	IVD CE 0344
Pelikloon anti-e (IgM) monoclonal	REF K1197 K1205	IVD CE 0344
002_v04 07/2019 (en)		*For professional use only*

Blood grouping reagents for the detection of the Rhesus antigens C, c, E or e on human red cells

General information
Pelikloon anti-C, c, E, and both e (IgM) monoclonal blood grouping reagents (clone number is mentioned on the corresponding certificate of analysis/release document and product label) are prepared from culture supernatant from stable hybridoma cell lines as first described by Köhler and Milstein (Nature 1975). Two different lines of Pelikloon reagents (varying in clone composition) are available and, if desired, can be used for confirmation of test results obtained with either line of reagents. These monoclonal reagents contain human IgM antibodies and have been specially selected and developed to provide a reliable alternative to polyclonal reagents. These reagents meet the requirements of the concerned standards and guidelines. Performance characteristics are mentioned in the release documents, which are supplied with the products upon request. The principle of the test is the agglutination technique, which is based on antigen/antibody reaction. These reagents can be used in either spin tube or microplate method. 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 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. 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Blood samples should be withdrawn aseptically with or without the addition of anticoagulants.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 or in their own plasma or serum.
2. Add to a test tube:
   - 1 drop of Pelikloon reagent
   - 1 drop of the 3–5% cell suspension

   and mix 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.
5. In case of negative or doubtful test results incubate for 15–20 minutes at room temperature (18–25°C) and repeat step 3 and 4.

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 or in their own plasma or serum.
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 corresponding antigen. In case of other discrepancies between the first and the second line of reagents, please contact your Sanquin distributor.
A negative reaction (i.e. no visible agglutination) indicates the absence of the corresponding antigen.

Occurrence	Caucasians	Negroids
C antigen	68%	27%
c antigen	80%	96%
E antigen	29%	22%
e antigen	98%	98%

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
Antigen variant cells may produce unexpected positive or negative reactions with samples previously typed with blood grouping reagents of polyclonal or other cell line-derived monoclonal sources. 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 optimized 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. et al.; The Blood Group Antigen FactsBook. FactsBook Series, 3rd ed. 2012.
5. Mollison P.L. et al.; Blood Transfusion In Clinical Medicine, 9th ed. Blackwell, Oxford, 1993.

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.