Pelikloon anti-A (IgM) monoclonal  REF  K1188  €0344
Pelikloon anti-B (IgM) monoclonal  REF  K1189  €0344
Pelikloon anti-A,B (IgM) monoclonal  REF  K1190  €0344

blood grouping reagents for the detection of the A and/or B antigen on human red cells

General information
Pelikloon anti-A, anti-B, and anti-A,B (IgM) monoclonal blood grouping reagents (clone numbers are mentioned on the corresponding certificate of analysis/release document) are prepared from culture supernatants from stable hybridoma cell lines as first described by Köhler and Milstein (Nature 1975). These monoclonal reagents contain murine 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 product upon request. The principle of the test is the agglutination technique, which is based on antigen/antibody reaction. The reagents can be used in either spin tube, microplate or slide method. These reagents 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 ABO blood group of the red cells, the serum of the patient should be tested for the presence of the corresponding anti-A and/or anti-B alloantibodies, using A1 and B reagent red cells (see relevant package insert).

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. NaN₃ 0.1% (w/v) is used as preservative. The anti-A and anti-B reagents have been coloured for easy recognition. 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Blood samples should be withdrawn aseptically with or without the addition of anticoagulants.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.
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.

Slide method

1. Place 1 drop of Pelikloon reagent on a clean glass slide.
2. Add 1 drop of a 35–45% cell suspension of the red blood cells to be tested in isotonic saline or in their own plasma or serum.
3. Mix well using a clean applicator stick over a circular area approximately 20 mm in diameter.
4. Move the slide slowly and observe for agglutination of the red blood cells for a period not exceeding 2 minutes.
5. Read macroscopically for agglutination.

Interpretation

A positive reaction (i.e. agglutination) indicates the presence of the corresponding antigen. A negative reaction (i.e. no visible agglutination) indicates the absence of the corresponding antigen. 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

<table>
<thead>
<tr>
<th>red cells + blood grouping reagent</th>
<th>serum/plasma + reagent red cells</th>
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</thead>
<tbody>
<tr>
<td>anti-A</td>
<td>anti-B</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>+</td>
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<tr>
<td>+</td>
<td>+</td>
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</tbody>
</table>

Limitations

Unexpected positive results due to: polyagglutination, 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.

References