ReaCell Panel P
Panel Cells for Antibody Identification
Papainized
3 ± 0,5 % suspension

INSTRUCTIONS FOR USE

REF 43200
1011

Tests are based on the principle of haemagglutination.

Intended use:
These RBC suspensions are intended for the identification of IgG and IgM antibodies reacting in enzymatic medium, mainly the antibodies of Rh-system. Occasionally the antibodies of Kell-system also react this way. The method is suitable to identify the presence of several more cold-type IgM antibodies, too (anti-I, anti-H, anti-P1, anti-Le^a, anti-Le^b).
Do not use any additive Low Ion Strength Solution (LISS) together with papainized panel cells, because the enzyme-treated cells may cause aspecific reactions.

Composition:
The papainized panel cell kit consists of 11 vials of individual donors' blood with "0" blood group and known antigenic composition. The current antigen chart of the panel cells is always included in the packaging.
Preservative solution: 1 mmol/l chloramphenicol; 0,4 mmol/l neomycin-sulphate.
The red blood cells are prepared in 3 ± 0,5 % ready-to-use suspension and are packaged in 5 ml vials fitted with a calibrated dropper. Dropper volume is approx. 50 µl.
Do not use the reagent if the supernatant is haemolytic. Gently turn the vials upside down a few times to homogenize the suspension before use.

Storage and transporting conditions:
The reagents must be stored and transported between +2 and +8 °C. Using according to the recommended methods, their performance is guaranteed by the manufacturer from first opening until the expiry date on the label. Do not use beyond expiry date. It is advised to minimize the time outside the refrigerator. Avoid leaving the reagents at room temperature between uses.
DO NOT FREEZE!

Samples:
Serum/plasma of a blood sample not older than 48 hours stored between +2 and +8 °C can be used. (If the sample will be tested later, the serum/plasma should be stored frozen between -20 and -30 °C).

Control:
It is recommended to use **Rea IQC Total Blood Kit** (REF 44130) as internal control.

Required reagents and equipment:
Manual tube technique:
- tubes
- pipettes (30 µl, 50 µl, 100 µl)
- water bath (37°C)
- laboratory centrifuge
- buffered saline solution, pH 7,2 (REF 15015)
- ReaSol diluent (REF 11114)
- Coombs serum (AHG)
- papain

***Procedures*:**
Manual tube technique:
1. Number 12 tubes, the 12th one is for autocontrol. Write the identification number of the blood sample on each tube. **Preparation of autocontrol**: wash the RBCs of the sample to be tested with PBS (pH=7,2) twice. Prepare an appr. 3% suspension with the ReaSol diluent (e.g. add 30 µl papainized, washed RBC residue into 1 ml ReaSol diluent).

Direct method
2. Add 50-50 µl serum/plasma of the blood samples to be tested into each tube.
3. Add 50-50 µl of each panel cell suspension into the corresponding tubes.
4. Gently shake the content of the tubes and incubate them at 37 °C for 15 minutes.
5. Centrifuge the tubes for 20 seconds at 1000 g RCF, or for 60 seconds at 150 g.
6. Observe the supernatant, whether there is haemolysis. Record the result.
7. Gently shake the tubes, and observe the agglutination with a magnifier with a lamp over a white background. Record the result. Mark the strength of agglutination with crosses.
8. Evaluate the agglutination scheme with the help of the antigen chart.
9. After evaluation, if necessary, continue testing with Coombs method.

Indirect method
10. Wash the tube content 3 times with buffered saline solution.
11. Add 100-100 µl Coombs serum (AHG) into each tube.
12. Shake gently and centrifuge them for 20 seconds at 1000 g RCF or for 60 seconds at 150 g.
13. After homogenization, read the results, preferably on a white background, with a magnifier with a lamp and record the result. Mark the strength of agglutination with crosses.
14. Evaluate the agglutination scheme with the help of the antigen chart.

The papainized panel cells can be used with automated tube technique as well (e.g. ACT 24 machine, ACT robot). In this case, please follow the user's guide.
Positive, negative and washing (Coombs) control reagents must be used for every test (see OVSZ methodology letter).

Evaluation of results:
Positive result:
- if agglutination is visible from + to ++++
- if haemolysis is observed

Negative result:
- if the reaction mixture remains a homogeneous suspension

The test results should be compared with the antigen chart. The antigen chart is unique and specific for each batch produced. It is included in the packaging. Autocontrol test should be performed strictly under the same conditions (medium, method). Positive result of a control should be carefully cleared up (certain diseases or medication may be the cause). Occasionally other test cells may be needed for antibody identification. The results of antibody identification can be supported by determining the RBC antigens of the subject.

Cause of possible errors:
Reasons of false negative results:
- the test sample and/or the reagents were not stored correctly and they lost their reactivity
- the incubation time and/or temperature was not correct
- the RBCs were not centrifuged properly
- shaking too hard can extinguish weak reactions
- the AHG reagent is neutralized (i.e.: inadequate cell washing)

Reasons of false positive results:
- bacterial contamination or other impurity of test cells
- inadequate centrifugation and homogenization
- in rare cases the tested serum or plasma may contain antibodies which are against some of the used diluent's components
- in LISS, the enzyme-treated cells give aspecific reactions, thus do not add any reaction accelerator to papainized cells!

Limitations of the method:
- The reactivity of preserved test cells may decrease during shelf life, depending on the individual characteristics of each donor, which can neither be controlled nor predicted by the manufacturer.
- In case of positive autocontrol result, the tested serum/plasma may contain autoantibodies, which requires further investigation.
- Negative test results do not exclude the presence of very rare antibodies.
- In case of the presence of very frequent antibodies or multiple antibodies, corresponding rare cells or other capable methods for differentiating the antibodies may be needed.
- Enzyme treatment destroys M, N, S, s, Fy^a, Fy^b antigens, so the corresponding antibodies will not react with enzyme-treated RBCs.

Precautions:
All reagents of human origin and all substrates that have come into contact with the samples must be handled as potentially infectious materials.
Red Blood Cell concentrates, and the plasma used in Rea IQC Total Blood Kit has been tested by the Hungarian Blood Transfusion Service under the operative decree 3/2005. (II. 10.) EüM regarding blood products for transfusion. All human blood products used in production were found non-reactive for Lues, HIV1-2, HbsAg and HCV by procedures recommended by the European Council, however, none of the methods currently known can absolutely guarantee that the products do not contain any transmissible pathogen.
It is recommended to wear gloves and safety goggles.
Special protective measures, conditions for waste disposal and disinfection should be implemented following local regulations.

Packaging:

REF 43200 11 x 5 ml

Bibliography:
1.) Transzfúziós szabályzat – Az OVSZ módszertani levele 2. kiadás, OVSZ, Bp. 2008. (Transfusion Guideline – 2nd Edition of Methodology Letters HNBTS, Hungary)
2.) AABB Technical Manual 18th Edition Bethesda, Maryland USA, 2015
3.) Guidelines of Transfusion Services in the United Kingdom 7th Edition 2005
4.) Decree 2/2005 (II. 10.) of EüM regulation of quality and safety for collecting, testing, processing, storing and distribution of human blood and blood components, and their individual technical requirements (localization of Directive 2002/98/EC and Directive 2004/33/EC)

Incident reporting:
For end-user/third party in the European Union and in countries with identical regulatory regime (Regulation 2017/746/EU on In vitro Diagnostic Medical Devices); if during the use of this device or as a result of this use, a serious incident occurs, please report it to REANGENS Ltd. to info@reagenskft.hu email address and to your (local) national Competent Authority.

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Hungary

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Version: 12v

ReaCell Panel P
Panel Cells for Antibody Identification
Papainized
0,8 ± 0,1 % suspension
for micro technique

INSTRUCTIONS FOR USE

REF 43800

Tests are based on the principle of haemagglutination.

Intended use:
These RBC suspensions are intended for the identification of IgG and IgM antibodies reacting in enzymatic medium, mainly the antibodies of Rh-system. Occasionally the antibodies of Kell-system also react this way. The method is suitable to identify the presence of several more cold-type IgM antibodies, too (anti-I, anti-H, anti-P1, anti-Le^a, anti-Le^b).
Do not use any additive Low Ion Strength Solution (LISS) together with papainized panel cells, because the enzyme-treated cells may cause aspecific reactions.

Composition:
The papainized panel cell kit consists of 11 vials of individual donors' blood with "0" blood group and known antigenic composition. The current antigen chart of the panel cells is always included in the packaging.
Preservative solution: 1 mmol/l chloramphenicol; 0,4 mmol/l neomycin-sulphate.
The red blood cells are prepared in 0,8 ± 0,1 % ready-to-use suspension and are packaged in 5 ml vials fitted with a calibrated dropper. Dropper volume is approx. 50 µl.
Do not use the reagent if the supernatant is haemolytic. Gently turn the vials upside down a few times to homogenize the suspension before use.

Storage and transporting conditions:
The reagents must be stored and transported between +2 and +8 °C. Using according to the recommended methods, their performance is guaranteed by the manufacturer from first opening until the expiry date on the label. Do not use beyond expiry date. It is advised to minimize the time outside the refrigerator. Avoid leaving the reagents at room temperature between uses. DO NOT FREEZE!

Samples:
Serum/plasma of a blood sample not older than 48 hours stored between +2 and +8 °C can be used. (If the sample will be tested later, the serum/plasma should be stored frozen between -20 and -30 °C).

Control:
It is recommended to use **Rea IQC Total Blood Kit** (REF 44130) as internal control.

Required reagents and equipment:
Microplate method:
- microplates
- pipettes (10 µl, 50 µl)
- buffered saline solution, pH 7,2 (REF 15015)
- ReaSol diluent (REF 11114)
- papain
- incubator (37 °C)
- microplate centrifuge
- microplate shaker
- mirror reader / automate reader

Gel column method:
- Coombs and neutral cards
- pipettes (10 µl, 25 µl, 50 µl)
- buffered saline solution, pH 7,2 (REF 15015)
- ReaSol diluent
- papain
- incubator (37 °C)
- card centrifuge

Procedures:
Microplate method:
Before use, let the reagents, blood samples and devices reach room temperature.
1. Write the identification number of the blood sample on the microplates.
2. Add 50-50 µl serum/plasma of the blood sample to be tested into 12 wells.
3. Add 50-50 µl of each panel cell suspension into the corresponding wells.
4. The 12th well is for autocontrol. **Preparation of autocontrol**: wash the RBCs of the sample to be tested with PBS (pH=7,2) twice. Add 100 µl 1% papain solution to 100 µl red blood cell residue, and incubate it for 5 minutes at 37 °C. Wash it with PBS twice, and prepare an appr. 1 % suspension with the ReaSol diluent (e.g. add 10 µl papainized, washed RBC residue into 1 ml ReaSol diluent).
5. Shake the content of the microplate at maximum grade for 1-2 minutes.
6. Incubate the microplate for 15 minutes at 37 °C.
7. Centrifuge at 1000 RPM for 1 minute.
8. Shake the plates at maximum grade for a few seconds, then at low grade for 2-3 minutes.
9. Read the results on mirror reader, record them, and mark the strength of agglutination with crosses on the antigen chart. The tests can be evaluated on automated reader, too.
10. Evaluate the agglutination scheme with the help of the antigen chart.

Gel column method:
Before use, let the reagents, blood samples and devices reach room temperature. (The instructions for use of different manufacturer's gel cards may differ, so always make sure to read the manual provided by the manufacturer carefully.)
Direct method
1. Neutral cards should be used. Mark the cards with the identification number of the patient and the panel cell's number.
2. Remove the film and add 50-50 µl of each panel cell suspension into the corresponding column of the gel card.
3. The 12th column of the gel card is for autocontrol. Preparation: see above.
4. Add 25-25 µl of the serum/plasma to be tested into each gel column.
5. Incubate the card for 15 minutes at 37 °C.
6. Centrifuge for 10 minutes.
7. Read and record the reactions on the antigen chart accordingly. Mark the strength of agglutination with crosses.
8. Evaluate the agglutination scheme with the help of the antigen chart.

Note: In case of 0,8 % papainized panel cells, the antiglobulin test can be carried out on gel column using Coombs cards.

Evaluation of results:
Positive result:
- if agglutination or haemolysis is observed on the microplate
- if any reaction is observed in the gel columns

Negative result:
- if the reaction mixture remains a homogeneous suspension
- if the cells are at the bottom of the gel column

The test results should be compared with the antigen chart. The antigen chart is unique and specific for each batch produced. It is included in the packaging. Autocontrol test should be performed strictly under the same conditions (medium, method). Positive result of a control should be carefully cleared up (certain diseases or medication may be the cause). Occasionally other test cells may be needed for antibody identification. The results of antibody identification can be supported by determining the RBC antigens of the subject.

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