ReaCell I, II, III **INSTRUCTIONS FOR USE**
Screening Cells for Antibody Detection
3 ± 0,5 % suspension

IVD CE 1011

REF 41100, 41120

Tests are based on the principle of haemagglutination.

Intended use:
These RBC suspensions are intended for the detection of IgG (Rh-, Kell-, Duffy-, Kidd-, etc.) and IgM (mainly anti-M, anti-N) irregular RBC antibodies with direct agglutination and IAT methods. During direct agglutination, IgM antibodies of the serum/plasma react with the corresponding RBC antigens and perform agglutination.
The sensibility of the method can be increased and the reaction time can be shortened by adding Low Ionic Strength Solution (LISS) at the same time. The additive LISS has to be used in compliance with the manufacturer's instructions for use.

Composition:
The screening cell kit consists of 3 vials of individual donors' blood with "0" blood group and known antigenic composition. The current antigen chart of the screening 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 and 10 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)
- buffered saline solution, pH=7,2 (REF 15015)
- ReaSol diluent (REF 11114)
- LISS (AGGI-LISS, REF 13110)
- Coombs serum (AHG)
- laboratory cell washer/centrifuge

Procedure::
Manual tube technique:
1. Mark 4 tubes with number 1 to 4 for each blood sample: I, II, III and one 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 washed RBC residue into 1 ml ReaSol diluent).

Direct method
2. Add 100-100 µl serum/plasma of the blood samples to be tested into each tube.
3. Add 50-50 µl of each screening cell suspension into the corresponding tubes.
4. Gently shake the content of the tubes and incubate them at 37 °C for 60 minutes or add 100 µl AGGI-LISS to reduce the incubation time to 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, over a white background, and observe the agglutination with a magnifier with a lamp. Record the result. Mark the strength of agglutination with crosses.
8. Evaluate the agglutination scheme with the help of the antigen chart.

Indirect Method
9. Wash the tubes three times with buffered saline solution.
10. Add 100-100 µl Coombs serum (AHG) into each tube.
11. Shake gently and centrifuge for 20 seconds at 1000 g RCF, or for 60 seconds at 150 g.
12. 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.
13. Evaluate the agglutination scheme with the help of the antigen chart.

The screening 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.
Any LISS can be used, but the instructions for use (provided by the manufacturer) must be followed strictly and steps must be made accordingly.
Positive, negative and washing (Coombs) control reagents must be used for every test (see OVSZ methodology letter).

Evaluation of results:
If agglutination or haemolysis is visible in any of the tubes during the screening test procedure (step 7, 8), or even when reading the agglutination (step 12), the antibody-screening is positive, continue testing to identify the antibody with a kit of panel cells.
If there is no way to identify the antibodies, blood sample should be forwarded to a special laboratory, where identification can be performed.

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

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.

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 41100	3 x 5 ml
REF 41120	3 x 10 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 REAGENS Ltd. to info@reagenskft.hu email address and to your (local) national Competent Authority.

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ReaCell I, II, III **INSTRUCTIONS FOR USE**
Screening Cells for Antibody Detection
0,8 ± 0,1 % suspension
for micro technique

IVD CE 1011

REF 41150, 41180

Tests are based on the principle of haemagglutination.

Intended use:
These RBC suspensions are intended for the detection of IgG (Rh-, Kell-, Duffy-, Kidd-, etc.) and IgM (mainly anti-M, anti-N) irregular RBC antibodies with direct agglutination and indirect antiglobulin techniques on gel cards.

Composition:
The screening cell kit consists of 3 vials of individual donors' blood with "0" blood group and known antigenic composition. The current antigen chart of the screening 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 and 10 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:
Gel column method:
- LISS/Coombs cards or Neutral cards
- card centrifuge
- incubator (37 °C)
- pipettes (10µl, 25 µl, 50 µl)
- ReaSol diluent (REF 11114), or any other solutions recommended by the card manufacturer
- buffered saline solution, pH=7,2 (REF 15015)

Procedure:
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 screening cell's number.
2. Remove the film and add 50-50 µl of each screening cell suspension into the corresponding column of the gel card.
3. Add 25-25 µl of the serum/plasma to be tested into each gel column.
4. Incubate the card for 15 minutes at 37 °C.
5. Centrifuge it for 10 minutes.
6. Read and record the reactions on the antigen chart accordingly. Mark the strength of agglutination with crosses.
7. Evaluate the agglutination scheme with the help of the antigen chart.

Indirect method
1. LISS/Coombs cards should be used. Mark the cards with the identification number of the patient and the screening cell's number.
2. Remove the film and add 50-50 µl of each screening cell suspension into the corresponding column of the gel card. Always add the RBCs FIRST to the gel chamber.
3. The 4th column of the gel card is for autocontrol. **Preparation of autocontrol**: wash the sample with PBS (pH=7,2) twice. Prepare an appr. 1% suspension with the ReaSol diluent (e.g. add 10 µl washed RBC residue into 1 ml ReaSol diluent).
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 them 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.

Positive, negative and washing (Coombs) control reagents must be used for every test (see OVSZ methodology letter).

Evaluation of results:
If agglutination or haemolysis is visible during the screening in any of the columns of a gel card, the antibody-screening is positive, continue testing to identify the antibody with a kit of panel cells. If there is no way to identify the antibodies, blood sample should be forwarded to a special laboratory, where identification can be performed.

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
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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.
- Do not use LISS with gel column method!

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 41150	3 x 5 ml
REF 41180	3 x 10 ml

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