Instructions for use

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Cellbind Direct Type

REF K7012 IVD CE

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For professional use only

Micro column test for typing of blood group antigens on red cells including red cells coated in vivo with antibodies and/or complement components

General information

Cellbind Direct Type is a micro column test system in which sensitised red cells from a suspension are caught by a gel matrix in an enhancing high-density medium. Cellbind Direct Type is intended for use in typing of blood group antigens and reversed blood grouping of red cells including red cells coated in vivo with antibodies and/or complement components.
Cellbind Direct Type is suitable for use in manual as well as (semi-) automated systems. The Cellbind Direct Type assay 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 test is based on the immunofixation of sensitised red cells in a micro column containing a gel matrix. The cell suspension is added to the incubation compartment of the micro column, together with the blood grouping reagent or plasma to be tested. During the incubation phase antigen-positive red cells will bind the corresponding anti-red cell antibodies present in the reagent or plasma. Next, the cards are subjected to three phases of centrifugation. In the first phase the high-density medium will cause separation of the red cells from the reagent or plasma. In the second phase sensitised red cells will be agglutinated and caught on top of the gel matrix in the micro column, while in the third phase non-sensitised and very weakly sensitised red cells will move towards the bottom of the micro column. The inclusion of positive and negative controls with each series of blood group determinations is strongly recommended.

Precautions

For in vitro diagnostic use only. Cellbind Direct Type cards must be stored in the original polystyrene box at 2-8°C. Close the box after use. Cellbind Direct Type cards should be stored upright. If not, they should be kept in an upright position for about 15 minutes prior to use, in order to allow the gel matrix to settle again. Do not use Cellbind Direct Type cards that show signs of drying (i.e. uneven level of high-density medium in the micro columns of one card or low levels of high density medium in the columns), signs of condensation (i.e. drops in the incubation compartment or on the underside of the cover strips), damaged cover strips or have air bubbles in the high-density medium or gel matrix. Air bubbles in either high-density medium or gel matrix introduced during transport can be removed in most cases by spinning the sealed Cellbind Direct Type cards in the Cellbind Centrifuge prior to use. Cellbind Direct Type cards should not be used beyond the expiration date, which is printed on the label of the cards. After reading the results, cards can be covered and stored in an upright position at 2–8°C for up to one week. Chloramphenicol <0.1% 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. Waste-disposal, after completion of the test, should be performed according to your laboratory regulations.

Specimen collection and preparation

Specimen:

Blood samples should be withdrawn aseptically in the presence of EDTA as anticoagulant. It is strongly advised to centrifuge blood collection tubes for 5 minutes at 3000 rcf prior to collection of plasma samples. Collection of plasma samples should be performed using a pipette and not by pouring the plasma. The plasma samples must remain free of white cells, gel fragments and/or fibrin residues in order to avoid blocking of the gel matrix. For reversed typing it is advised to use fresh plasma (within 48 hours after drawing). Plasma samples that are not immediately tested may be stored for 48 hours at 2–8°C, or longer at <–18°C. It is advised to centrifuge the plasma samples after thawing for 5 minutes at 3000 rcf prior to testing in order to remove any precipitate.

Reagents:

Cellbind Direct Type	REF K7012: Box containing 48 cards with 6 micro columns each
Cellbind LISS	REF K7100: Dilution medium to prepare 0.5% red cell suspensions of patient- or donor red cells (250 ml)
	REF K7110: Dilution medium to prepare 0.5% red cell suspensions of patient- or donor red cells (100 ml)
	REF K7130: Dilution medium to prepare 0.5% red cell suspensions of patient- or donor red cells (25 ml)
Cellbind DILUENT	REF K7180: Dilution medium to prepare 0.5% red cell suspensions from 3% Sanquin reagent red cells suspensions (100 ml)
Cellbind A1 reagent red cells	REF K7240: 0.5% reagent red cell suspension for the detection of anti-A antibodies.
Cellbind B reagent red cells	REF K7242: 0.5% reagent red cell suspension for the detection of anti-B antibodies.
Cellbind O, D-positive reagent red cells	REF K7243: 0.5% reagent red cell suspension for the use as positive or negative control.

Materials:

Cellbind Centrifuge	REF K7302
Cellbind Rotor	REF K7303
Cellbind Dispenser	REF K7300
Cellbind Workstation	REF K7301

Red cell suspensions:

1. For typing a 0.5% suspension of patient or donor red cells in Cellbind LISS (REF K7100 REF K7110 or REF K7130) must be prepared.
2. For reversed typing Sanquin (0.5% or 3.0%) reagent red cell suspensions must be used. It is advised to use ready-for-use 0.5% Cellbind reagent red cell suspensions. If 3% Sanquin reagent red cells suspensions are used, a 0.5% suspension in Cellbind DILUENT (REF K7180) must be prepared according to the preparation protocol below. For use of other reagent red cells, validation by the user is mandatory.

Preparation of 0.5% red cell suspensions:

1. 11 µl packed patient or donor red cells + 2 ml Cellbind LISS (REF K7100, REF K7110 or REF K7130)
2. 200 µl 3% Sanquin reagent red cells suspension + 1 ml Cellbind DILUENT (REF K7180)

Operating procedure for Cellbind Centrifuge

To use the Hettich centrifuge for Cellbind cards one has to perform the following steps:

1. Insert the Cellbind Rotor according to the Hettich operating manual.
2. The rotor is recognised by the centrifuge and is automatically programmed according to the Cellbind protocol.
3. For the centrifugation step mentioned in the Cellbind test procedure below one only has to press "start" and the centrifuge will rotate in the following 3 steps:
   - 0-2 minutes 75 rcf 780 rpm
   - 2-3 minutes 200 rcf 1280 rpm
   - 3-10 minutes 1790 rcf 3840 rpm
4. After centrifugation the lid can be opened and the cards can be taken out.

Test procedure

Allow all reagents to reach room temperature (18–25°C). Do not use Cellbind Direct Type cards that show air bubbles in the gel matrix, disrupted seals or signs of drying (irregular or no liquid level above the gel matrix).

Typing of blood group antigens

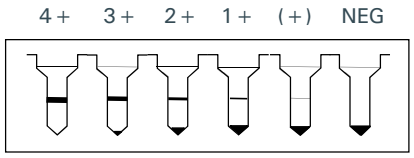
1. Remove cover strip from the required number of columns.
2. Add 40–50 µl of the 0.5% red cell suspension of patient or donor cells into the incubation compartment.
3. Add 20 µl of Sanquin blood grouping reagent into the incubation compartment.
   Note: A list of validated Sanquin blood grouping reagents is available on the website www.cellbind.nl. The use of any other typing reagent can lead to aberrant results and should therefore be validated by the user.
4. Introduce cards into the Cellbind Centrifuge (10 minutes). The centrifugation parameters have already been programmed.
5. Read the reactions.

Reversed typing

1. Remove cover strip from the required number of columns.
2. Add 40–50 µl of the 0.5% suspension of reagent red cells into the incubation compartment.
3. Add the same volume (40–50 µl) of plasma into the incubation compartment.
4. Introduce cards into the Cellbind Centrifuge (10 minutes). The centrifugation parameters have already been programmed.
5. Read the reactions.

Interpretation

In positive reactions red cells will be caught in the top layer of the gel matrix. In negative reactions only a discrete button of red cells at the bottom of the micro column will be seen. The resulting reaction patterns are shown in the figure:



The amount of red cells caught in the top layer of the gel matrix will depend upon parameters such as the antigenic density of the red cells and the titer and affinity of the antibodies. It is also determined by the duration of the initial centrifugation phases and the centrifugal force during the final phase.
Therefore, if a reaction is weaker than 4+, cells will also appear at the bottom of the micro column. The same pattern will be seen in mixed-field reactions.

Typing of blood group antigens

Positive reactions with blood grouping reagents indicate the presence of the corresponding antigens on the red cells. A positive reaction with Pelikloon control monoclonal indicates that the blood grouping results are not valid, in which case it is advised to use another technique. Negative reactions with blood grouping reagents indicate that the presence of the corresponding antigens on the red cells cannot be detected.

Reversed typing

Positive reactions with reagent red cells indicate the presence of the corresponding alloantibody. A negative reaction indicates that the presence of the corresponding alloantibody cannot be detected.

Limitations
Unexpected positive results due to: pseudoagglutination, autoagglutination, mixed field reaction, too high red cell concentrations, red cells coated in vivo with IgM or IgA antibodies, certain drugs or the presence of other than anti-A and/or anti-B alloantibodies. Unexpected negative or weak results due to: mixed field reaction, chimerism, decreased activity of reagents, insufficient interaction of the red cell suspension and the plasma or reagent in the incubation compartment and/or premature interaction between the contents of the incubation compartment and the high density medium or the fact that plasma under investigation is from a newborn, a (very) old person or from a patient with hypogammaglobulinemia. False positive or false negative results may occur through the presence of air bubbles in the gel matrix, contamination of test materials or any deviation from the recommended techniques. When strongly haemolytic samples are used, non-specific reactions may occur. If a sample contains fibrin residues, this may cause trapping of non-sensitised cells during centrifugation, resulting in a thin red line on top of the gel matrix.

References
1. Issit P.D.; Applied Blood Group Serology, 3rd ed. Montgomery Scientific Publications, Miami, Florida, USA, 1985.
2. 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.