Instructions for use

Sanquin Reagents B.V.
Plesmanlaan 125
1066 CX Amsterdam
The Netherlands

Phone: +31 20 5123599
Fax: +31 20 5123570
Reagents@sanquin.nl
www.sanquin.org/reagents

Cellbind Direct

REF K7011

IVD CE

061_v02 01/2017 (en)

For professional use only

Micro column test for the detection of in vivo coating of red cells with antibodies and/or complement components

General information
The Cellbind Direct assay is a micro column test system in which suspended red cells, which are sensitized in vivo with antibodies and/or complement components, are caught by a gel matrix in an enhancing high-density medium. A Cellbind Direct card consists of two micro columns containing anti-IgG, two micro columns containing monoclonal anti-C3d and two micro columns as a control (Ctrl.). Cellbind Direct is suitable for use in manual as well as (semi-) automated systems. The Cellbind Direct 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 sensitized red cells in a micro column containing a gel matrix. The cell suspension is added to the upper compartment of the micro column. The cards are subjected to centrifugation. In the initial phases of centrifugation the antibody and/or complement coated red cells will be agglutinated and caught on top of the gel matrix in the micro column. In the final phase non-coated or very weakly coated red cells will move towards the bottom of the micro column.

Precautions
For in vitro diagnostic use only. Cellbind Direct cards must be stored in the original polystyrene box at 2-8°C. Close the box after use. Cellbind Direct 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 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 cards in the Cellbind Centrifuge prior to use. Cellbind Direct 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:
For the Cellbind Direct assay it is advised to use fresh blood (within 48 hours after drawing). The blood should be preferably drawn into EDTA, so as to prevent *in vitro* coating of red cells with complement components. It is strongly advised to centrifuge blood collection tubes for 5 minutes at 3000 rcf prior to collection of samples.

Reagents:

Cellbind Direct	REF K7011	: Box containing 48 cards with 2x (IgG, C3d, Ctrl.) tests 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).

Materials:

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

Red cell suspensions:
For the Cellbind Direct test a 0.5% suspension of patient red cells in Cellbind LISS must be prepared.

Preparation of 0.5% red cell suspensions:
11 µl packed red cells + 2 ml Cellbind LISS or
200 µl 3% patient red cell suspension + 1 ml Cellbind LISS

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.For the centrifugation step mentioned in the Cellbind test procedure below one only has to press on "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
3. After centrifugation the lid can be opened and the cards can be taken out.

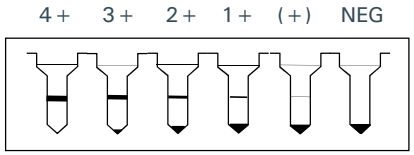
Test procedure

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

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

Interpretation

In positive reactions red cells will be caught in the top layer of the gel matrix. Positive reactions indicate the in vivo coating of red cells with antibodies and/or complement components.
In negative reactions only a discrete button of red cells at the bottom of the micro column will be seen. Test results are not reliable if the Ctrl. column does not show a negative reaction.
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 level of sensitization of the red cells, the affinity of the antibodies for the red cell antigens, or the amount of coated complement components on the red cells. 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.

Limitations

Unexpected positive results due to: pseudoagglutination, autoagglutination, mixed field reaction, certain drugs or too high red cell concentrations. Unexpected negative or weak results due to: mixed field reaction or decreased activity of reagents. False positive or false negative results may occur through 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.

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.