

# Instructions for use



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**Cellbind Screen**

**REF K7000**

**IVD CE**

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*For professional use only*

Micro column test for the detection or identification of red cell antibodies as well as for blood grouping

## General information

The Cellbind Screen assay is a micro column test system in which sensitised red cells from a suspension are caught by a gel matrix containing anti-IgG, anti-IgM, and anti-C3d in an enhancing high-density medium. Each screen card consists of six micro columns containing the gel in the high-density medium. Cellbind Screen is intended for use in the detection or identification of red cell antibodies, as well as for blood grouping, crossmatching and the modified direct antiglobulin test (DAT, for the detection of *in vivo* coating of red cells with antibodies and complement components). Cellbind Screen is suitable for use in manual as well as (semi-) automated systems. The Cellbind Screen 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 plasma, serum or blood grouping reagent to be tested. During the incubation phase antigen-positive red cells will bind the corresponding anti-red cell antibodies present in the plasma, serum or reagent. 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 plasma, serum or reagent. 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 Screen cards must be stored in the original polystyrene box at 2-8°C. Close the box after use. Cellbind Screen 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 Screen 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 Screen cards in the Cellbind Centrifuge prior to use. Cellbind Screen 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 with or without the addition of anticoagulants. It is strongly advised to centrifuge blood collection tubes at 3000 rcf prior to collection of serum samples (for 10 minutes) or plasma samples (for 5 minutes) in order to prevent false positive reactions. Collection of serum or plasma samples should be performed using a pipette and not by pouring the plasma or serum. The plasma or serum samples must remain free of white cells, gel fragments and/or fibrin residues in order to avoid blocking of the gel matrix. For the detection or identification of red cell antibodies it is advised to use fresh plasma or serum (within 48 hours after drawing). Serum or 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 serum or plasma samples after thawing for 5 minutes at 3000 rcf prior to testing in order to remove any precipitate. For the modified direct antiglobulin test fresh blood should be used (within 48 hours after drawing), preferably drawn into EDTA, so as to prevent *in vitro* coating of red cells with complement components. Plasma is not suitable for the detection of complement-binding antibodies, since anticoagulants will inhibit complement activation.

### Reagents:

Cellbind Screen	<b>REF</b> K7000	: Box containing 48 cards with 6 micro columns each.
Cellbind LISS	<b>REF</b> K7100	: Dilution medium to prepare 0.5% red cell suspensions of patient- or donor red cells (250 ml).
	<b>REF</b> K7110	: Dilution medium to prepare 0.5% red cell suspensions of patient- or donor red cells (100 ml).
	<b>REF</b> K7130	: Dilution medium to prepare 0.5% red cell suspensions of patient- or donor red cells (25 ml).
Cellbind DILUENT	<b>REF</b> K7180	: Dilution medium to prepare 0.5% red cell suspensions from 3% Sanquin panels or Sanquin reagent red cells suspensions (100 ml).
Cellbind P2	<b>REF</b> K7200	: (2 x 10 ml) 0.5% reagent red cell suspensions for the detection of red cell antibodies.
Cellbind P3	<b>REF</b> K7210	: (3 x 10 ml) 0.5% reagent red cell suspensions for the detection of red cell antibodies.

Cellbind P3-P (papain)	<b>REF</b> K7211	: (3 x 10 ml) papain treated 0.5% reagent red cell suspensions for the detection of red cell antibodies.
Cellbind ID16	<b>REF</b> K7230	: (16 x 3 ml) 0.5% reagent red cell suspensions for the identification of red cell antibodies.
Cellbind ID16-P (papain)	<b>REF</b> K7231	: (16 x 3 ml) papain treated 0.5% reagent red cell suspensions for the identification of red cell antibodies.
Cellbind A <sub>1</sub> reagent red cells	<b>REF</b> K7240	: 0.5% reagent red cell suspension for the detection of anti-A antibodies.
Cellbind A <sub>2</sub> reagent red cells	<b>REF</b> K7241	: 0.5% reagent red cell suspension for the use as positive or negative control.
Cellbind B reagent red cells	<b>REF</b> K7242	: 0.5% reagent red cell suspension for the detection of anti-B antibodies.
Cellbind O, D-positive reagent red cells	<b>REF</b> K7243	: 0.5% reagent red cell suspension for the use as positive or negative control.

#### Materials:

Cellbind Centrifuge	<b>REF</b> K7302
Cellbind Rotor	<b>REF</b> K7303
Cellbind Incubator	<b>REF</b> K7304
Cellbind Dispenser	<b>REF</b> K7300
Cellbind Workstation	<b>REF</b> K7301

#### Red cell suspensions:

- For typing, crossmatching, the modified direct antiglobulin test and the autocontrol a 0.5% suspension of patient or donor red cells in Cellbind LISS (**REF** K7100 **REF** K7110 or **REF** K7130) must be prepared.
- For antibody detection or identification Sanquin (0.5% or 3.0%) panels or reagent red cell suspensions must be used. It is advised to use ready-for-use 0.5% Cellbind panels or Cellbind reagent red cell suspensions. If 3% Sanquin panels or 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 panels or reagent red cells, validation by the user is mandatory.  
Note: this protocol cannot be applied to cells that have been treated with enzymes (**REF** K1384 and **REF** K1393). If there is a need for testing with enzyme treated cells, Cellbind P3-P (**REF** K7211) or Cellbind ID16-P (**REF** K7231) must be used.

#### Preparation of 0.5% red cell suspensions:

- 11 µl packed patient- or donor red cells + 2 ml Cellbind LISS (**REF** K7100, **REF** K7110 or **REF** K7130)
- 200 µl 3% Sanquin panel or 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:

- Insert the Cellbind Rotor according to the Hettich operating manual.
- 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 procedures 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
- After centrifugation the lid can be opened and the cards can be taken out.

#### Test procedures

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

#### Antibody detection or identification

- Remove cover strip from the required number of columns.
- Add 40–50 µl of the 0.5% red cell suspension of test cells into the incubation compartment.
- Add the same volume (40–50 µl) of plasma or serum into the incubation compartment.
- Incubate for 15 minutes at 37°C in the Cellbind Incubator.
- Introduce cards into the Cellbind Centrifuge (10 minutes). The centrifugation parameters have already been programmed.
- Read the reactions.

#### Typing of blood group antigens

- Remove cover strip from the required number of columns.
- Add 40–50 µl of the 0.5% red cell suspension of patient or donor cells into the incubation compartment.
- 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](http://www.cellbind.nl). For some of these reagents an additional incubation step is required, these reagents are indicated in this list. The use of any other typing reagent can lead to aberrant results and should therefore be validated by the user.
- Introduce cards into the Cellbind Centrifuge (10 minutes). The centrifugation parameters have already been programmed.
- Read the reactions.

#### Reversed typing

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

#### Modified Direct Antiglobulin Test (DAT)

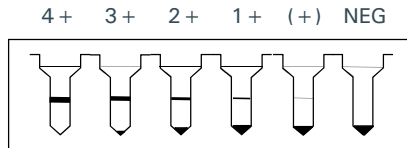
1. Remove cover strip from the required number of columns.
2. Add one drop (40–50 µl) of the 0.5% red cell suspension of patient cells into the incubation compartment.
3. Introduce cards into the Cellbind Centrifuge (10 minutes). The centrifugation parameters have already been programmed.
4. Read the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

#### Crossmatch

1. Remove cover strip from the required number of columns.
2. Add 40–50 µl of the 0.5% red cell suspension of donor red cells into the incubation compartment.
3. Add the same volume (40–50 µl) of patient plasma or serum into the incubation compartment.
4. Incubate for 15 minutes at 37°C in the Cellbind Incubator.
5. Introduce cards into the Cellbind Centrifuge (10 minutes). The centrifugation parameters have already been programmed.
6. 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 antigenic density of the red cells, and the titer and affinity of the antibody. It is also determined by the duration of the second centrifugation phase and the centrifugal force during the third 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.

#### Antibody detection or identification

Positive reactions indicate the presence of red cell antibodies in the plasma or serum. Negative reactions indicate the absence of red cell antibodies. A positive autocontrol may indicate the presence of autoantibodies.

#### Typing of blood group antigens

Positive reactions with blood grouping reagents indicate the presence of the corresponding antigens on the red cells. 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.

#### Modified Direct Antiglobulin Test (DAT)

Positive reactions indicate the *in vivo* coating of red cells with antibodies and/or complement components.

#### Crossmatch

Positive reactions indicate incompatibility of the donor blood with the recipient. Negative reactions indicate compatibility of the donor blood with the recipient.

#### Limitations

Unexpected positive results due to: pseudoagglutination, autoagglutination, mixed field reaction, certain drugs, too high red cell concentrations or red cells sensitised *in vivo* with antibodies and/or complement components. Unexpected negative or weak results due to: weak antigens, weak antibodies, low titers of antibodies, mixed field reaction, decreased activity of reagents, insufficient interaction of the red cell suspension and the plasma, serum or reagent in the incubation compartment and/or premature interaction between the contents of the incubation compartment and the high density medium. 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. 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. Race R.R. and Sanger R.; Blood Groups in Man, 6<sup>th</sup> ed. Oxford Blackwell Scientific Publishers 1975.
2. Issit P.D.; Applied Blood Group Serology, 3<sup>rd</sup> ed. Montgomery Scientific Publications, Miami, Florida, USA, 1985.
3. Daniels G.; Human Blood Groups. Blackwell Science Ltd. 1995.
4. Mollison P.L. et al.; Blood Transfusion In Clinical Medicine, 9<sup>th</sup> 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.*