

INSTRUCTIONS FOR USE

Chloroquin-Diphosphat



for removing antibodies from the surface of sensitized red blood cells

FOR IN VITRO DIAGNOSTIC USE

1. Description of product

Chloroquin-Diphosphat is used to remove IgG antibodies from the surface of sensitized red blood cells, e. g. if red blood cells with an positive direct antiglobulin test cannot be used directly for blood group typing, because the cells has been sensitized with IgG in vivo. Under controlled conditions, Chloroquin-Diphosphat dissociates IgG from red blood cells without or only a little damage to the red blood cell antigens and so permits a complete phenotyping of the red blood cells after treatment with Chloroquin Diphosphat. After treatment with Chloroquin-Diphosphat an identification of the removed antibodies is not possible.

2. Principle of the test

Red blood cells sensitized with IgG antibodies are incubated with Chloroquin-Diphosphat at room temperature. Chloroquin-Diphosphat dissociates IgG from the red blood cells. The detached antibodies are removed by washing with Alsever solution. The red blood cells are now ready for use for antigen determination.

3. Storage and stability

Store Chloroquin-Diphosphat at 2...8°C. Do not freeze! Once Chloroquin-Diphosphat has been opened the first time, the reagent may be used up to the expiration date indicated on the label if the specified storage conditions are observed and no turbidity or other signs of contamination are noticed. Do not use the reagent after the expiration date indicated on the label. Do not use contaminated reagents!

4. Samples

Blood samples should be collected by approved medical procedure. Do not use hemolytic or contaminated samples! Examine samples without delay whenever possible. Cells stored longer than 5 days may render more difficulties in removing antibodies respectively may result in strong hemolysis and damage to or loss of red blood cell antigens if cells are incubated with Chloroquin-Diphosphat (see 7. Important Notes/Limitations of the Method).

5. Additional materials required

Alsever solution
Anti-Human-Globulin (Anti-IgG)
Red blood cells of known phenotype
IgG sensitized reagent control cells
Disposable test tubes (75 x 12 mm)
Disposable Pasteur Pipettes
Centrifuge
Reagents for determination of red blood cell antigens

6. Performance

Perform a direct antiglobulin test with Anti-Human-Globulin (Anti-IgG) of the red cell sample that has been sensitized with antibody in vivo. Record result.

6.1 Removing IgG antibodies with Chloroquin-Diphosphat

1. Wash the sensitized red cell sample three times with Alsever solution in a clean, labeled test tube (in each case centrifuge 5 minutes at 400 x g (1500 rpm)). Discard supernatant.
2. Pipete 10 drops of the washed test red cell button into a labelled test tube.
3. Add 40 drops Chloroquin-Diphosphat and mix thoroughly.

4. Incubate 30 minutes at room temperature.
5. Wash the red cells three times with Alsever solution (in each case centrifuge 5 minutes at 400 x g (1500 rpm)). Discard supernatant.

Note: A mild hemolysis can be neglected.

6.2 Check of antibody removing in a direct antiglobulin test

After the last washing step perform a direct antiglobulin test with Anti-Human-Globulin (Anti-IgG) with a part of the red cells (see Directions for Use for Anti-Human-Globulin in use).

The direct antiglobulin test should be negative. If the initial amount of antibody bound to the sensitized red cells was strong, a positive result can occur. In this case the treatment with Chloroquin-Diphosphat can be repeated (according to steps 2 - 5). Check again the antibody removing in a direct antiglobulin test.

If the direct antiglobulin test result is still positive, the antibodies could not or only incomplete removed. In this case a determination of red blood cell antigens is not possible respectively a false phenotyping can occur.

A third treatment with Chloroquin-Diphosphat is not recommended, since strong hemolysis and damage to or loss of red blood cell antigens can occur.

6.3 Determination of red blood cell antigens

If the antibodies are removed from the surface of the red blood cells, the antigens can be determined with monoclonal and/or human antibodies. The phenotyping of the red blood cells should be done according to manufacturer's Directions for Use for test reagents in use.

6.4 Controls

It is recommended as control to use red blood cells positive for the antigen for which the test sample is to be phenotyped. Treat these cells with Chloroquin-Diphosphat in the same manner as the test sample and determine the red cell antigens.

If the control red cells does not react as expected, the test results of the phenotyping with the Chloroquin-Diphosphat treated test sample should not be interpreted (see 7. Important Notes/Limitations of the Method).

A negative antiglobulin test results should be appropriately controlled by the addition of IgG sensitized reagent control cells (see Directions for Use for IgG sensitized control cells in use). If no agglutination occurs with the IgG sensitized control cells when added to negative antiglobulin test, the test results should not be interpreted.

7. Important notes/Limitations of the method

1. Chloroquin-Diphosphat is suitable for in vitro diagnostic use only and may only be used by trained, qualified personnel.
2. Chloroquin-Diphosphat does not dissociate complement components from red blood cells. If the red blood cells are coated with IgG antibodies and complement components in vivo, tests should be done after Chloroquin-Diphosphat treatment using Anti-IgG specific Anti-Human-Globulin only.
3. After treatment with Chloroquin-Diphosphat an identification of the removed antibodies is not possible.
4. The centrifugal force applied should be the minimum required to produce a clear supernatant and a clearly delineated red cell button that can be easily resuspended. No single centrifugation speed or time can be recommended for all types of available centrifuges or test applications. Centrifuges should be calibrated individually to determine the optimal time and speed required to achieve the desired results.
5. Cells stored longer than 5 days may render more difficulties in removing antibodies respectively may result in strong hemolysis and damage to or loss of red blood cell antigens through treatment with Chloroquin-Diphosphate.
6. If the procedure is carried out as recommended, Chloroquin-Diphosphat dissociates IgG from red blood cells without or only a little damage to the red blood cell antigens. Test variables such as prolonged incubation time, too high incubation temperature or more than two treatments with Chloroquin-Diphosphat, using of hemolytic or contaminated red blood cells may result in strong hemolysis and damage to or loss of red blood cell antigens, an accurate phenotyping is not possible.
7. Do not use hemolytic or contaminated samples!

8. Inadequate washing of the red blood cells may result in false or unsatisfied test results.
9. Other test variables such as improper technique, inappropriate centrifugation or incubation, improperly cleaned glassware and/or contaminated materials and samples may cause false results.
10. If the direct antiglobulin test result is still positive after the second treatment with Chloroquin-Diphosphat, the antibodies could not or only incomplete removed. In this case a determination of red blood cell antigens is not possible respectively a false phenotyping can occur.
11. Chloroquin-Diphosphat does not contain preservatives. Turbidity may indicate bacterial contamination or reagent deterioration. Microbiological contamination of Chloroquin-Diphosphat must be avoid as this may reduce the shelf life of the product and cause false results. Do not use contaminated reagents!

8. Performance characteristics

100 red blood cell suspensions were treated with Chloroquin-Diphosphat. 10 samples were coated with antibodies in vivo and 90 samples were coated with several antibodies in vitro (Anti-D, -C, -c, -C^w, -E, -e, -K, -k, -Jk^a, -Jk^b, -Fy^a, -Fy^b, -S, -s). Chloroquin-Diphosphat removed the antibodies from all 100 samples completely. In 6 cases it was necessary to repeat the treatment with Chloroquin-Diphosphat. Extensive phenotyping of the samples showed that antigens of the ABO, Rh, Kell, Duffy, Kidd and MNSs systems could be determined and were not destroyed after treatment with Chloroquin-Diphosphat.

9. Warnings and instructions for disposal

All materials of biological origin used for the test, especially the specimens to be tested, should be regarded as potentially infectious. Therefore, appropriate safety precautions are recommended when handling biological materials (do not pipette using the mouth; wear protective gloves when performing the test; disinfect hands after testing).

Biological materials must be deactivated before disposal (e.g., by autoclaving). Single-use materials must be autoclaved or incinerated after use.

Spills of potentially infectious material should be removed without delay with an absorbent paper towel and the contaminated area disinfected with an appropriate disinfectant or 70% ethanol. Materials used for the removal of spills must be deactivated before disposal (e.g., by autoclaving).







Chloroquin-Diphosphat is harmful to health. Do not inhale or swallow or come in contact with the skin, mucous membranes and eyes (risk (R) and safety (S) phrases, see Explanation of symbols used in labelling). Dispose Chloroquin-Diphosphat at special waste collection point.

The disposal of all samples and test materials should be carried out according to legal directives.

10. References

Edwards, JM, Moulds, JJ, and Judd, WJ: Chloroquine dissociation of antigen-antibody complexes: A new technique for typing red blood cells with a positive direct antiglobulin test. Transfusion 22:59, 1982

Modern Blood Banking and Transfusion Practices, Denise M. Harmening, F. A. Davis Company Philadelphia, 3. Auflage, 1994

Explanation of symbols used on Labelling	
	For in vitro diagnostic use
	Storage temperature
	Batch code
	Use by
REF	Catalogue number
	Consult instructions for use
	Harmful R 22, S 02-13-24/25 46-56

- R 22 Harmful if swallowed.
S 2 Keep out of the reach of children.
S 13 Keep away from food, drink and animal feeding stuffs.
S 24/25 Avoid contact with skin and eyes.
S 46 If swallowed, seek medical advice immediately and show this container or label.
S 56 Dispose of this material and its container at hazardous or special waste collection point.

Instructions for use	Issue: August 2007
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