Instructions for use

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PEG 4000 20%

REF K1159

IVD CE

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For professional use only

Potentiating reagent for serological tests

General information
Polyethylene glycol (PEG) 4000 is a polymer, which is used as a potentiator in serological tests. The exact way in which PEG potentiates serological reactions is not known. It is thought that PEG reduces the degree of hydration at the surface of the erythrocyte membrane. Proteins are also precipitated by PEG and both these factors may help to strengthen antigen-antibody reactions in such a way that weak antibodies are detected in this technique. The reagent has been standardised for use in serological tests according to the procedure described below. This reagent meets the requirements of the concerned standards and guidelines. Performance characteristics are mentioned in the release documents, which are supplied with the products upon request. The principle of the test is the agglutination technique, which is based on antigen/antibody reaction. The inclusion of a positive control with each series of tests is strongly recommended.

Precautions
For in vitro diagnostic use only. Reagents should be stored at 2–8°C. Leaking or damaged vials may not be used. Reagents (unopened or opened) should not be used beyond the expiration date, which is printed on the label of the vial. NaN_3 0.1% (w/v) is used as preservative. Care must be taken in the use and disposal of each container and its contents. Turbidity may indicate microbial contamination. To recognize reagent deterioration, testing of the reagent as part of the laboratory quality control program using appropriate controls is recommended. Waste-disposal, after completion of the test, should be performed according to your laboratory regulations.

Specimen collection and preparation
Blood samples should be withdrawn aseptically with or without the addition of anticoagulants. If testing of the blood samples is delayed, storage should be at 2–8°C.
Preparation of the specimen is described in the respective test procedures.

Test procedures
Indirect Antiglobulin Test with PEG 4000 20%
Tube requirements: round bottom glass tubes; size 75 x 10/12 mm.

1. Prepare a 3–5% cell suspension of red cells to be tested in isotonic saline (commercial cells should be used as supplied).
2. Add to a test tube:
   - 2 drops of patient serum
   - 1 drop of the 3–5% cell suspension
   - 4 drops of PEG 4000 20%

   and mix well.
3. Incubate in a water bath for 15–20 minutes at 37°C.
4. Resuspend the contents of the test tube completely.
5. Wash the red cells 4 times in an excess of isotonic saline. Decant the last wash completely.
6. Add 2 drops of monospecific anti-human IgG (REF K1131 or K1124) and mix well.
7. Centrifuge for 20 seconds at 1000 rcf or for a time appropriate to the calibration of the centrifuge.
8. Resuspend the cells by gentle agitation and read macroscopically for agglutination.
9. If there is no visible agglutination add 1 drop of Coombs Control Cells and repeat steps 7 and 8; the reaction should now be positive. If the test remains negative the result is invalid and the test should be repeated.

Interpretation
The presence of agglutination indicates a positive test result. The absence of agglutination indicates that a positive test result could not be detected.

Limitations
Unexpected negative or weak results due to: too vigorous shaking of the tubes during resuspension, interruptions during the test performance or ineffective washing of the red cells (causing neutralisation of the monospecific anti-human IgG by proteins (IgG) still present in the tube). In addition, the precipitate formed after PEG addition makes the washing procedure very critical. Therefore, to ensure efficient washing the tubes must be washed 4 times in an excess of isotonic saline.
The amounts of PEG, serum and cell suspension specified should be adhered to, and particular attention should be paid to the volume of the drops used. These should be of a similar volume.
PEG 4000 20% has been optimised for use by the technique recommended in this package insert.
False positive or false negative results may occur through contamination of test materials or any deviation from the recommended technique.

References

1. Race R.R. and Sanger R.; Blood Groups in Man, 6th ed. Oxford Blackwell Scientific Publishers 1975.
2. Issit P.D.; Applied Blood Group Serology, 3rd 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, 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.