Instructions for use

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Bromelin (Iyophilized) REF K1121 IVD CE

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For professional use only

Enzyme solution (0.5%) for use in blood group serology

General information
Bromelin is a buffered extract prepared from the root of the pineapple plant which is suitable for use in serological methods. Bromelin is a proteolytic enzyme solution which can hydrolyse peptide bonds on proteins present on the red cell membrane. This results in a reduction of the surface charge of the membrane. This membrane modification is useful in serological testing since it leads to an enhancement of some antigen-antibody interactions and the elimination of others. In general, the reactions of Rhesus, ABO, Lewis and P system antibodies are enhanced by enzyme modification of red cells. By denaturing Fy^a, Fy^b, M, N, S, s and Xg^a antigens, enzyme modification can diminish or eliminate the reactions of the corresponding antibodies. 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.

Precautions
For in vitro diagnostic use only. Bromelin (Iyophilized) REF K1121 should be stored at 2–8°C and reconstituted in 5 ml distilled water before use. The reconstituted bromelin should be used within 24 hours. For a longer storage up to the expiration date the reconstituted bromelin should be stored at <-18°C. Repeated freezing and thawing should be avoided. 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. The bromelin does not contain preservatives. Care must be taken in the use and disposal of each container and its contents. To recognise 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Blood samples should be withdrawn aseptically with or without the addition of anticoagulants.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 procedure
Enzyme treatment of a 3% cell suspension:

1. Wash the red cells to be enzyme-treated once in an excess of isotonic saline and prepare a 3% cell suspension in isotonic saline.
2. Add to a glass test tube:
   - 1 volume of bromelin (Sanquin)
   - 9 volumes of a 3% cell suspension

   and mix well.
3. Incubate in a water bath for 15–20 minutes at 37°C.
4. Wash the enzyme-treated cells 3 times in an excess of isotonic saline.
5. After the last washing prepare a 3% cell suspension in isotonic saline.

Test for antibody detection:
Tube requirements: round bottom glass tubes; size 75 x 10/12 mm.

6. Add to a test tube:
   - 2 drops of patient serum (diluted 1:2 in isotonic saline)
   - 1 drop of enzyme-treated cell suspension

   and mix well.
7. Incubate in a water bath for 15–20 minutes at 37°C.
8. Centrifuge for 20 seconds at 1000 rcf or for a time appropriate to the calibration of the centrifuge.
9. Resuspend the cells by gentle agitation and read macroscopically for agglutination.
   Note: An autocontrol (patient serum + patient enzyme-treated cells) should be included as a negative control in antibody identification procedures

Interpretation
A positive reaction (i.e. agglutination) indicates the presence of an antibody (or antibodies) in the patient serum. A negative reaction (i.e. no visible agglutination) indicates the serum does not contain antibodies against the antigens present on the enzyme-treated cells.

Limitations
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.