EN INSTRUCTIONS FOR USE

Bromelin, liquid

CE

Electronic instructions for use see www.bag-diagnostics.com

REF 6961 10 x 10 ml

FOR IN VITRO DIAGNOSTIC USE

1. Description of product

The bromelin solution is a stable, ready-to-use reagent. The enzyme is obtained from pineapples. Its enzymatic action enhances the antigen-antibody reactions. The bromelin test can be used to detect the most frequently occurring immune antibodies, but primarily those of the Rhesus-, Kidd-, Lewis-, Vel-, P- and H-systems. The bromelin test can also be useful for detecting very weak isoagglutinins of the ABO system.

2. Principle of the test

The effect of bromelin is based both on a reduction of the erythrocyte charge, as well as an elemination of more or less long polypeptide chains that project from the erythrocyte surface. This causes the erythrocytes to come closer together and produces a stronger agglutination with IgG antibodies. The testing method indicated is based on the principle of direct hemagglutination. Once erythrocytes (patient- or test erythrocytes) and bromelin are added to the serum (patient- or test-serum), a specific antigen-antibody reaction only takes place if an antibody and the corresponding antigen are present on the erythrocytes. This reaction is visibly recognizable by the agglutination of the erythrocytes. If no antibodies are bound to the erythrocytes, then there can be no agglutination. This indicates a negative result.

3. Storage and stability

Store the reagent at 2...8°C. Do not freeze! Allow the bromelin solution to come to room temperature (18...25°C) before use and store again at 2...8°C immediately after use.
Once it 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. Do not use the bromelin solution past the expiration date indicated on the label.

4. Preparation of samples

For the determination of erythrocyte antigens, the blood samples should be collected according to the customary medical procedure. Blood-samples with and without anti-coagulants (EDTA, citrate) are suitable for testing. When determining antibodies in tube tests it must be used serum.

Do not use hemolytic samples!

Testing should take place without delay whenever possible.

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If erythrocytes are stored for too long before testing, the erythrocyte antigens may change, which can lead to false positive or false negative reactions (see 9. Important Notes/Limitations of the Method).

5. Additional materials required

Isotonic NaCl solution (isotonic saline)

Test tubes (75 x 12 mm)

Test tube rack

Single-use Pasteur pipettes

Centrifuge

Specific antisera (IgG)

Red blood cells of known phenotype

6. Test procedure

Tube test

1. Wash the erythrocytes to be examined once with isotonic saline and then make an erythrocyte suspension of 2 - 3% in isotonic saline.
2. Place 1-2 drops of the serum to be tested or the specific test serum and 1-2 drops of the erythrocyte suspension to be tested or test erythrocytes in a labeled test tube.
3. Add 1 drop bromelin solution and mix.
4. Incubate for 15 minutes at room temperature (18...25°C).
5. Centrifuge 1 minute at 400 x g (1500 rpm) or at an alternative rpm with an appropriate time adjustment.
6. Resuspend the cells by gently shaking the tube and examine macroscopically for agglutination.

The test can be stopped here or continued with a coombs test. Please follow the instructions for use for the coombs reagent.

Comments: Do not examine the test microscopically.

If it is not desired to detect cold reactive antibodies incubate the test at 37°C.

It is recommended that an auto control and a positive and a negative control with known erythrocytes should be tested in parallel with each batch of tests.

It is recommended to check the enzyme activity of every test series by testing a mix of low reacting rhesus antibodies, which reacts positive with all erythrocytes.

7. Interpretation of the results

A) Serum (antibody determination)

Agglutination with one or several erythrocyte suspensions indicates the presence of one or several antibodies in the serum tested. Antibodies can then be identified by means of an erythrocyte panel with the help of the antigram in an exclusion procedure.

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B) Erythrocytes (determination of erythrocyte antigens)
Agglutination with a defined antiserum indicates the presence of the corresponding antigen. If there is no agglutination of the erythrocytes with the antiserum, this indicates the absence of the corresponding antigen.

The limitations of the method must be considered when interpreting the results (see 9. Important Notes/Limitations of the Method).

8. Stability of reactions

All test results must be interpreted immediately once centrifugation is complete.

9. Important notes/limitations of the method

1. The proteolytic activity of the bromelin reduces the agglutination reactions of the MNSs and Duffy systems and the Xg, Pr and T antigen, so that false negative reactions may occur.
2. The reagent is suitable for in vitro diagnostic use only and may only be used by trained, qualified personnel.
3. The presence of autoagglutinins in test specimens may cause false reactions, also red cells coated with allo- or auto-antibodies (positive DAT).
4. A concentration of the enzyme too low or too high in relation to the antigen-antibody mixture will cause false results.
5. Due to the variability of antigen expression, cells may react weaker than the positive control used for the test, depending on phenotype.
6. False positive results may occur because of bacterial or chemical contamination of the reagent, the samples or the physiological NaCl solution and/or because of incorrect centrifuging.
7. False negative results or unexpected weak reactions may be caused by an insufficient cell concentration, insufficient incubation temperature or time and/or insufficient centrifugation, but also by storing the erythrocytes for too long and/or under inappropriate conditions. Reading the results of the test too late, agitating the erythrocyte sediment too strongly, and other deviations from the indicated testing procedure can also lead to weaker or false negative results.
8. In general, false negative or false positive results can result from inappropriate techniques, incorrect centrifugation or incubation, dirty tubes, incorrect pH of the isotonic NaCl solution and/or contaminated materials and samples.
9. Microbial or chemical contamination of the bromelin solution must be absolutely avoided because this shortens the shelf life of the product and can lead to false results.
10. Light cloudiness does not influence the reactivity of the product.
11. This reagent should be used as supplied without any additives (albumin, LISS) or dilution.
12. Deviation from the recommended Instructions for use may result in less than optimal product performance. User-defined deviations such as modifications of test procedures, serum dilution for use in automat or cards, freezing of serum on microtiter plates etc. may require validation by the user.
13. Whether transfusions or transplantation have taken place should always be taken into consideration when interpreting the results. Any history of transfusions and/or transplantation, as well as the patient's medication history, should be taken into consideration when interpreting results.

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10. Warnings and instructions for disposal

All materials of biological origin used for the test, especially the erythrocytes and sera to be tested, should be regarded as potentially infectious. Enzymes and preservatives can cause symptoms of poisoning if ingested. 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).

The reagent contains 0.01% neomycin sulfate as a preservative.

Disposal of all samples, unused reagent and waste should be in accordance with country, federal, state and local regulations.

A Material Safety Data Sheet (MSDS) is available to download at www.bag-diagnostics.com .

11. References

Technical manual of the American Association of Blood Banks, 18th ed., 2014

Issitt PD, Anstee DJ. Applied blood group serology. 4th ed. Durham, NC Montgomery Scientific Publication, 1998

Brecher ME. Ed. Technical manual 14th ed. Bethesda MD. Amercian Assosiation of Blood Banks, 2002

Explanation of symbols used on Labelling	
IVD	For in vitro diagnostic use
	Manufacturer
	Storage temperature / Temperature limitation
LOT	Batch code
	Use by
REF	Catalogue number
	Consult instructions for use
CONT NEOMYCINSULFAT	Contains Neomycin sulfate

Instructions for use	Version 2/2019 / Issue 2019-06

Instructions for use in other languages see:
http://www.bag-diagnostics.com
or phone +49 (0) 6404-925-125

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