

EN INSTRUCTIONS FOR USE

BAG-Elutions-Kit for acid elution of antibodies



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

FOR IN VITRO DIAGNOSTIC USE

REF 69501

1. Description of product


With the BAG-Elutions-Kit antibodies adsorbed onto red blood cells, either in vivo or in vitro, can be dissociated in an elution process and can be identified in a separate procedure. The recovered eluate may be used:

1. to identify a single antibody in sera containing multiple antibody specificities
2. to demonstrate the presence of a weak antigen
3. to identify the antibody responsible for a positive direct antiglobulin test in acquired hemolytic anemia or transfusion reaction
4. to identify antibodies causing hemolytic disease of the newborn

2. Principle of the test

Unadsorbed antibodies surrounding the sensitized red cells are removed by washing with a wash buffer that minimizes the loss of adsorbed antibodies from the red cells. After washing, the antibody-antigen complex is dissociated by addition of a low pH solution (elution solution). The pH of the recovered eluate is then adjusted by the addition of a neutralisation buffer. The eluate is then ready for use.

3. Contents of the kit

WASHBUF 10x	Wash buffer, 10 x concentrate, slightly yellow, containing bovine albumine and < 1% NaN ₃ 	1 x 50 ml
SOLN ELU	Elution solution, ready for use, orange-yellow, low pH glycine buffer containing a pH colour indicator and no preservatives or antimicrobial agents (Dropper and cap: orange)	1 x 13 ml
BUF	Neutralisation buffer, ready for use, light blue, Tris buffer, containing < 0,1% NaN ₃ (Dropper and cap: blue)	1 x 13 ml

4. Storage and stability

Store the wash buffer concentrate, the elution solution and the neutralisation buffer at 2...25°C. Do not freeze! Once they have been opened the first time, the reagents may be used up to the expiration date indicated on the label if the specified storage conditions are observed. Do not use the reagents if turbidity or other signs of contamination are observed and after the expiration date indicated on the label.

Store the diluted wash buffer working solution at 2...8°C in a closed labeled container. Do not freeze! The diluted wash buffer working solution may be used for up to six months if the specified storage conditions are observed and no turbidity or other signs of contamination are observed. Do not use contaminated reagents!

5. Samples

Do not use hemolytic or contaminated samples! Examine samples without delay whenever possible. A longer storage may result in lower levels of recovered antibodies and resultant weaker eluate reactivity. Additionally, the use of stored samples may result in hemoglobin stained eluates and results in difficulty adjusting the final pH of the eluate (see 12. Important Notes/Limitations of the Method).

6. Additional materials required

Isotonic NaCl solution, aqua dest., gel cards, disposable glass test tubes (75x12 mm), transfer pipettes, waterbath or incubator (37°C ± 1°C), centrifuge, reagents for testing the eluate.

7. Wash buffer dilution

Dilute the concentrated wash buffer **WASCHBUF 10x** 1:10 with aqua dest. (one volume of the concentrated wash buffer and nine volumes of aqua dest.). For the dilution measure the wash buffer concentrate always. The wash buffer working solution should be stored at 2...8°C in a closed container. The solution may be used for up to six months if no turbidity or other signs of contamination are observed during storage.

The use of **cold** wash buffer working solution may minimize antibody dissociation during the wash phase of the procedure.

8. Elution procedure

For the elution of antibodies 1 ml packed red cells are required. If smaller volumes are used, the used volume of elution solution must be adapted proportionally. Use of a packed cell volume less than 1 ml will result in a smaller final volume of eluate available for testing.

1. Perform a direct antiglobulin test on the red cell sample that has been sensitized with antibody either in vivo or in vitro. Record result.
2. Centrifuge the sensitized red cell sample in a clean, labeled test tube. Remove the excess plasma or serum and wash the red cells once with cold wash buffer working solution.
3. Transfer approx. 1.5 ml of the 1x washed packed red cells to a clean, labeled test tube. Wash the packed red cells with cold wash buffer working solution a minimum of four times to remove unbound antibodies. Reserve a small aliquot of the supernatant from the last wash to test for antibody activity (see 10. Controls). Inadequate washing could lead to serum antibody contamination.

- Transfer 1 ml of the 4x washed packed red cells to a clean, labeled test tube and add 1 ml of the elution solution **SOLN|ELU** to elute the antibodies. Mix gently. Centrifuge **immediately** for 45 - 60 seconds at 1000 rcf.

Note: Excess mixing or failure to centrifuge immediately may cause hemolysis which alters the pH of the eluate.

- Transfer the supernatant (eluate) **immediately** to a clean test tube. Discard red cells. Then add the neutralisation buffer **BUF** drop by drop (after each drop, mix well) until a distinct blue colour appears. The blue colour indicates a neutral to weak alkaline pH value (approx. pH 7.5 till 8.1).
- Centrifuge the eluate for 1 minute at 1000 rcf to remove cellular debris. Transfer the clarified eluate to a clean, labeled test tube. The eluate is now ready for testing.

Note: If not tested immediately the eluate may be stored refrigerated at 2...8°C for up to seven days and tested if no turbidity or colour deviations are observed during storage. Ensure a neutral to weak alkaline pH value for optimal reactivity of the eluate.

9. Testing the eluate

Commercial reagent red blood cells, patient or donor samples may be used as test cells. If patient or donor samples are used, wash the cells at least three times in isotonic saline prior to preparing a 3 – 5 % cell suspension. Thorough washing of the test cells is necessary since the modified antiglobulin test eliminates one wash step. If drug induced positive direct antiglobulin test is suspected, additional testing of the eluate against cells sensitized with the drug may be required to assess antibody recovery.

9.1 Testing the eluate by a modified antiglobulin test (tube test)

- Add one drop of a 3 - 5% red cell suspension to a clean labeled tube. Add 10 drops (ca. 500 µl) of isotonic saline. Centrifuge at 1000 rcf for at least 45 - 60 seconds. Completely decant or aspirate the supernatant to ensure removal of all residual saline, resulting in a "dry" red cell button.
- Add two drops (ca. 100 µl) of the eluate to the "dry" red cell button and mix well.

Note: If a weak direct antiglobulin test was demonstrated with the sensitized red cells, 3 - 4 drops (150 – 200 µl) of eluate may be used to increase the sensitivity of the test. Do not add bovine albumin or other potentiators.
- Incubate at 37°C (±1°C) for 15 minutes.
- After incubation, add 10 drops (ca. 500 µl) of the wash buffer working solution and centrifuge at 1000 rcf for at least 45 - 60 seconds. Completely decant the supernatant wash solution to ensure removal of all residual wash solution, resulting in "dry" red cell button.
- Add two drops of anti-human globulin (refer to appropriate manufacturer`s Instructions for Use for Anti-Human-Globulin) and mix gently, but thoroughly, to resuspend red cell button.
- Centrifuge for 15 seconds at 1000 rcf.
- Gently resuspend red cell button and examine for agglutination. Grade and record results.
- Negative or weak positive antiglobulin test results should be appropriately controlled by the addition of IgG sensitized reagent control cells.

9.2 Testing the eluate by gel cards

For testing the eluate by gel cards the appropriate Instructions for Use from the manufacturer of the gel cards must be observed.

10. Controls

1. Testing of the reserved wash solution (see 8. Elution Procedure, step 3) is necessary to provide verification that the antibody detected in the eluate was released from a bound state and is not residual "free" antibody remaining after inadequate washing. If a "last wash" control test is positive, the elution should be repeated using cold reagents, taking care to wash quickly and thoroughly.
2. The application of IgG sensitized reagent control cells to aid in the confirmation of the validity of negative antiglobulin test results is an essential control test for procedures that include an antiglobulin test phase (refer to the relevant manufacturer's Instructions for Use for IgG sensitized control cells).

11. Interpretation of the results

Agglutination of red cells tested against the eluate and no agglutination of the reserved „last wash“ control test indicate that serologically detectable antibodies has been recovered from the sensitized cells. Absence of agglutination indicates that no serologically detectable antibodies were recovered or perhaps that the recovered antibodies does not demonstrate blood group specificity.

If agglutination occurs with the reserved „last wash“ supernatant or if no agglutination occurs with the IgG sensitized control cells when added to negative antiglobulin test, the test results with the eluate should not be interpreted.

The limitations of the method must be considered when interpreting the results (see 12. Important Notes/Limitations of the Method).

12. Important notes/limitations of the method

The BAG-Elutions-Kit is suitable for in vitro diagnostic use only and may only be used by trained, qualified personnel.

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. Centrifuges should be calibrated individually to determine the optimal time and speed required to achieve the desired results.

The activity of the eluate is limited by the following:

1. The initial amount of antibody bound to the sensitized red cells.
2. The degree of antibody dissociation that occurs during the wash procedure. On rare occasion, the non-specific uptake of high potency antibodies has been reported to occur; this phenomenon has been reported to be associated with the combined use of low-ionic strength wash solution in the presence of strongly reactive serum antibody. In this instance, a potential false positive eluate result may occur.

3. Cells stored longer than 72 hours may result in lower levels of recovered antibody and resultant weaker eluate reactivity. Additionally, the use of stored samples may result in hemoglobin stained eluates and results in difficulty adjusting the final pH of the eluate.
4. The degree to which immunoglobulin is denatured by the low pH during dissociation (this is expected to be minimal if the procedure is carried out as recommended).
5. False positive results may occur from contamination of the eluate with unbound antibody due to inadequate washing of the red blood cells before starting the elution procedure.
6. False negative tests may occur if the test red cell suspensions are not washed sufficiently before incubation with the eluate, or if the test system becomes contaminated in any way with human protein other than antibody recovered during the elution phase.
7. After addition of neutralisation buffer different blue colour variations (pigeon blue to blue-lilac) have been observed, which do not affect the test results. In the case of a large colour deviation the pH value must be checked via indicator strips. The pH value range must be neutral to weak alkaline (approx. pH 7.5 till 8.1).
8. Failure to adjust pH to proper range may result in hemolysis of the test cells. Additionally, the activity of recovered antibody may be adversely affected by pH variation above or below the optimal range.
9. Excess dilution of the eluate from incorrect volume addition of the elution solution or by addition of excessive amounts of neutralisation buffer during adjustment of the pH range of the eluate could result in weakened or false negative results.
10. Elution procedures performed on red cells that are sensitized only with complement are unlikely to yield reactive eluates.
11. Red blood cells used for elution studies cannot be used for phenotyping.
12. Other tests variables such as the use of too large volumes of packed red cells, improper technique, inappropriate centrifugation or incubation, improperly cleaned glassware and/or contaminated materials and samples may cause false negative or false positive results.
13. Turbidity may indicate bacterial contamination or reagent deterioration. Microbiological contamination of the reagents must be avoided as this may reduce the life of the product and cause erroneous results. Do not use contaminated reagents!

13. Performance characteristics

For the performance evaluation clinical samples from blood donor services (sensitized with antibodies in-vivo) and samples, which were sensitized in vitro with different human antibodies, were tested (EDTA- and citrate blood). The antibody elution was performed with the BAG-Elutions-Kit and with two established C€-marked products for antibody elution. The eluates obtained were tested in an antibody screening and identification test with test cell panels.

In all cases the antibodies could be eluated with the BAG-Elutions-Kit. The eluated antibodies could be detected and identified with test cell panels in tube test and gel cards. There was an agreement of 100% with the results of the established C€-marked products for antibody elution.

14. 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).

Any bovine albumin used in the manufacture of this product is sourced from donor animals of United States origin that have been inspected and certified by US veterinary service inspectors to be disease-free. This ruminant-based product is deemed to have low TSE (Transmissible Spongiform Encephalopathy) risk.

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 neutralisation buffer and the wash buffer concentrate contain NaN_3 as preservative. Do not swallow and avoid contact with the skin and mucous membranes (H- and P-phrases, see Explanation of symbols used in labelling). Copper and lead, which are used in some piping systems, can form explosive salts with sodium azide. Therefore disposal of azide-containing material should be followed by rinsing with copious water.

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

Material Safety Data Sheets (MSDS) are available to download at www.bag-diagnostics.com .






15. References

Technical manual of the American Association of Blood Banks, 17th ed., 2011

Rekvig OP, Hannestad K. Acid Elution of Blood Group Antibodies From Intact Erythrocytes. Vox Sang 1977: 33:280

Judd WJ. Elution of Antibodies From Red Cells. In: Seminar On Antigen-Antibody Reactions Revisited. Bell CA, ed. Arlington, VA: American Association of Blood Banks 1982: 175

Leger RM, Arndt PA, Ciesielski DJ, Garratty G. False-positive eluate reactivity due to the low-ionic wash solution used with commercial acid-elution kits. Transfusion 1998: 38:565-572

Explanation of symbols used on Labelling	
Ab ACID ELUTION	Intended purpose: Acid elution of red blood cell antibodies
CONT	Contents of the kit
BUF	Neutralisation buffer
SOLN ELU	Elution solution
WASHBUF 10x	Wash buffer, 10 x concentrate
IVD	For in vitro diagnostic use
	Storage temperature / Temperature limitation
LOT	Batch code
	Use by
REF	Catalogue number
	Manufacturer
	Consult instructions for use
CONT NaN ₃	Contains Sodium Azide
	<p>Warning</p> <p>H302 Harmful if swallowed.</p> <p>H412 Harmful to aquatic life with long lasting effects.</p> <p>P264: Wash hands thoroughly after handling.</p> <p>P270: Do not eat, drink or smoke when using the product.</p> <p>P273: Avoid release to the environment</p> <p>P301+P312 IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell.</p> <p>P330: Rinse mouth.</p> <p>P501: Dispose of contents/container to the hazardous waste with special marking.</p>

Instructions for use	Version: 5/2019 / Issue: 2019-06
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Instructions for use in other languages see:

<http://www.bag-diagnostics.com>

or phone: +49 (0)6404-925-125