

INSTRUCTIONS FOR USE

Elution System



for acid elution of antibodies

FOR IN VITRO DIAGNOSTIC USE

1. Description of product

With the Elution-System antibodies adsorbed onto red blood cells, either in vivo or in vitro, can be dissociated and recovered through the process of elution. 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,
5. to prepare specific antibody from sera containing unwanted antibodies.

2. Principle of the test

Unadsorbed antibody surrounding the sensitized red cells is removed by washing with a wash solution that minimizes the loss of adsorbed antibody 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 base buffer solution (buffer solution). The eluate is then ready for use.

3. Contents of the kit

WASHBUF 10x	Wash buffer, 10 x concentrate, contains 1% NaN ₃	1 x 50 ml
ELU	Elution solution, ready for use, low pH glycine buffer containing a coloring agent (pH indicator) and no preservatives or antimicrobial agents	1 x 13 ml
BUF	Buffer solution, ready for use, Tris (hydroxymethylaminomethan) solution, containing bovine albumin and 0,1% NaN ₃	1 x 13 ml

4. Storage and stability

Store the wash buffer concentrate, the elution solution and the buffer solution at 15...30°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 and no turbidity or other signs of contamination are observed. Do not use the reagents past the expiration date indicated on the label.

Store with distilled water diluted wash buffer working solution at 2...8°C in a closed labelled container. Do not freeze! The 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. 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 to the required range for testing (see 12. Important Notes/Limitations of the Method).

6. Additional materials required

Isotonic NaCl solution

Aqua dest.

Disposable glass test tubes (75 x 12 mm)

Transfer pipettes

Waterbath or incubator (37°C ± 1°C)

Centrifuge

Reagents for testing of the eluate

7. Wash buffer dilution

Dilute the concentrated wash buffer by adding one volume of the concentrated wash buffer to nine volumes of Aqua dest. This wash buffer working solution should be stored at 2...8°C in a closed container. This solution may be used for up to six months if no turbidity or other signs of contamination are observed during storage.

Use of a "cold" wash buffer working solution may further minimize antibody dissociation during the wash phase of the procedure.

8. Elution procedure

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 isotonic saline.
This procedure is based on a recommended sample volume of 1 ml of packed red cells. However, smaller volumes may be used if the proportionate ratio of packed cells to elution solution is maintained. Use of a packed cell volume less than 1 ml will result in a smaller final volume of eluate available for testing.
3. Transfer 1 ml of packed red cells to a clean, labeled test tube. Wash the packed red cells with the 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. Inadequate washing could lead to serum antibody contamination.
4. Transfer the washed red cells to a clean, labeled test tube. To 1 ml of washed, packed red cells, add 20 drops (~ 1ml) of the elution solution to elute the antibody. Mix gently. Centrifuge immediately for 45 - 60 seconds at 900 - 1000 rcf.

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

5. Transfer the supernatant (eluate) to a clean test tube. Discard red cells. Add 5 drops of the buffer solution to buffer the eluate. Mix well. Continue adding buffer solution dropwise, mixing well after each drop until a distinct blue color appears. The blue color indicates a pH range of ~ 6.5 - 7.5.
6. Centrifuge the eluate to remove cellular debris. Transfer the clarified eluate to a clean, labeled test tube. The eluate is 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 is observed during storage. Optimum reactivity with eluates which are within the pH range of 6.5 - 7.5 should be obtained.

9. Testing the eluate by the modified antiglobulin test

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 the cell suspension. Thorough washing of the test cells is necessary since the modified antiglobulin test method eliminates the wash phase of the antiglobulin test.

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.

1. Add one drop of a 3 - 5% cell suspension to a clean labeled tube. Add 5 - 10 drops of isotonic saline. Centrifuge at 900 - 1000 rcf for at least 30 seconds. Completely decant the supernatant saline to ensure removal of all residual saline, resulting in a "dry" red cell button.
2. Add two drops 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 of eluate may be used to increase the sensitivity of the test. Do not add bovine albumin or other potentiators.

3. Incubate at 37°C ($\pm 1^\circ\text{C}$) for 15 minutes.
4. After incubation, add 5 - 10 drops of the wash buffer working solution and centrifuge at 900 - 1000 rcf for at least 30 seconds. Completely decant the supernatant wash solution to ensure removal of all residual wash solution, resulting in "dry" red cell button.
5. Add two drops of anti-human globulin (refer to appropriate manufacturer's Directions for Use for Anti-Human-Globulin). Gently, but thoroughly, mix contents to resuspend red cell button.
6. Centrifuge for 15 seconds at 900 - 1000 rcf.
7. Gently resuspend red cell button and examine for agglutination. Grade and record results.

Note: The use of a microscope or an optical aid is recommended only for confirmation of negative or weak hemagglutination reactions.

8. Negative or weak positive antiglobulin test results should be appropriately controlled by the addition of IgG sensitized reagent control cells.

10. Controls

1. Testing of the reserved wash solution ("Last Wash" – 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 wash buffer working solution, 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 Directions 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 antibody has been recovered from the sensitized cells. Absence of agglutination indicates that no serologically detectable antibody was recovered or perhaps that the recovered antibody 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 reagent 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 or test applications. 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 the 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 to the required range for testing.

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. 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.
8. Excess dilution of the eluate from incorrect volume addition of the Elution System components or by addition of excessive amounts of reagents during adjustment of the pH range of the eluate could result in weakened or false negative results.
9. Elution procedures performed on red cells that are sensitized only with complement are unlikely to yield reactive eluates.
10. Red blood cells used for elution studies cannot be used for phenotyping.
11. Other test variables such as improper technique, inappropriate centrifugation or incubation, improperly cleaned glassware and/or contaminated materials and samples may cause false negative or false positive results.
12. 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. 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 buffer solution and the wash buffer concentrate contain NaN_3 as preservative. NaN_3 is harmful to health in a concentration of 0.1% and toxic in a concentration of 1%. Do not inhale or swallow or come in contact with the skin and mucous membranes (risk (R) and safety (S) 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.

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














14. References

Technical manual of the American Association of Blood Banks, 15th ed., 2005

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	
	Contents of the kit
	Buffer solution
	Elution solution
	Wash buffer, 10 x concentrate
	For in vitro diagnostic use
	Storage temperature
	Batch code
	Use by
	Catalogue number
	Consult instructions for use
	Contains Sodium Azide
	Toxic R 25-52/53, S 45
	Harmful R 22-52/53, S 02-13-22-46-56-61

- R 22 Harmful if swallowed.
R 25 Toxic if swallowed
R 52/53 Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
S 2 Keep out of the reach of children.
S 13 Keep away from food, drink and animal feeding stuffs.
S 22 Do not breathe dust.
S 45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
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.
S 61 Avoid release to the environment. Refer to special instructions, safety data sheets.

Instructions for use	Issue: August 2007
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