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ELU*clear*

Acid Elution of Antibodies from Intact Red Blood Cells

Intended use:

The HBS-Elution Kit is used to elute antibodies from intact red blood cells. Unbound antibody is removed by washing the coated red blood cells with a solution which prevents the loss of adsorbed antibody from the red blood cells. After washing, the antigen-antibody complex is broken by the addition of a low pH solution. The recovered eluate is adjusted to pH 7.0 ± 0.5 by adding a buffering solution.

Summary and Explanation:

Antibodies adsorbed onto red blood cells (*in vivo* or *in vitro*) can be disassociated and recovered through elution. The eluate can then be used to:

- Identify the antibody responsible for a positive direct antiglobulin test (DAT).
- Identify a single antibody in multispecific sera by adsorption onto selected cells.
- Demonstrate the presence of a weak antigen on red blood cells after incubation with selected antisera.
- Prepare specific antibody from sera containing unwanted antibodies.

Reagents:

Eluting Solution 1 x 10mL
Buffering Solution 1 x 10mL
Concentrated Wash Solution 2 x 25mL
Wash Bottle

The Buffering Solution and Concentrated Wash Solution contain 0.1% (w/v) sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form highly explosive salts. On disposal, flush with large quantities of water.

The bovine serum albumin (BSA) contained in the Concentrated Wash Solution has been obtained from a closed herd in the female line since 1980, in which no animal has been clinically suspected of having Bovine Spongiform Encephalopathy (BSE), and which has not been fed rations containing ruminant derived protein during that period.

All components are sterile filtered to 0.22µm. All components should be clear. Turbidity may indicate bacterial contamination.

Eluting Solution and Buffer Solution are provided at optimal dilution for use.

This kit is for professional *in vitro* diagnostic use only.

Precautions

The packaging of this product contains dry natural rubber.

Storage

Reagents should be stored at 2-25° C when not in use. Prolonged storage at temperatures outside this range may result in accelerated loss of reagent activity.

Do not freeze.

Specimen Collection

Samples may be collected using an aseptic phlebotomy technique. Anticoagulated samples collected into EDTA are preferred. If testing is delayed, samples should be stored at 2-8°C. Use of cells older than 72 hours may yield less antibody and alter the pH of the final eluate.

Procedure:

Materials Provided

Hemo bioscience ELUclear kit

Materials Required But Not Provided

Test Tubes
Centrifuge (1000 rcf)
Distilled or Deionized water
Isotonic Saline
Pipettes
Timer
Anti-Human Globulin reagent containing anti-IgG
37˚C Incubator

Preparation of Working Wash Solution

To prepare Working Wash Solution using the provided wash bottle:

1. Pour contents of one bottle of Concentrated Wash Solution (25mL) into wash bottle supplied.
2. Add distilled or deionized water to fill line (250mL) and mix well.
3. The Working Wash solution is ready for use and should be stored at 2-8˚C and used for up to six months if no turbidity is observed.
4. If desired, the expiration date can be recorded on the wash bottle label in the provided space.
5. The use of cold Working Wash Solution will minimize antibody dissociation during the wash phase of the procedure.

Alternatively, the Working Wash Solution may be prepared by adding one volume of Concentrated Working Wash Solution to nine volumes of distilled or deionized water.

Recommended Technique:

1. Centrifuge the specimen and remove as much plasma as possible.
2. Wash an aliquot of the coated red blood cells once in isotonic saline, making sure to remove saline completely after wash. The aliquot should be sufficient to yield 1mL of packed cells when washing has been completed.
3. Wash the coated red blood cells an additional four times with the Working Wash Solution to remove any unbound antibody. Ensure that wash solution is removed completely after each wash. A small aliquot of the supernatant from the last wash should be reserved as a control to be tested in parallel with the eluate. (See **Quality Control**)
   Note: The use of cold (2-8°C) Working Wash Solution will minimize antibody dissociation.
4. Place 1mL (approximately 20 drops) of washed red blood cells in a clean test tube. Add 1mL (approximately 20 drops) of the eluting solution and mix gently by inverting the tube four times.
   If the volume of coated cells is less than 1mL, the eluate may be prepared by adding an equal volume of eluting solution to the coated cells.
5. Centrifuge immediately for 60 seconds at 1000 rcf.*
6. Transfer supernatant (eluate) to a clean test tube and discard cells.
7. Add Buffer Solution to eluate drop by drop, mixing well after each drop until a blue color appears and remains after mixing. The blue color indicates the pH of the eluate has been restored to within the range for testing (6.5-7.5).
8. Mix well and centrifuge to remove any precipitate or cellular debris, and then transfer the eluate to a clean test tube. For use in gel column technology, centrifuge the eluate again at a time and speed appropriate to the test method.

The eluate is now ready for antibody detection and/or identification. The eluate may be stored for up to 7 days at 2-8˚C.Centrifugation of the stored eluate prior to testing is recommended.

*Or time and speed appropriate to the centrifuge.

Testing of the Eluate

Eluates prepared using the ELUclear kit have been evaluated and found suitable for testing by the following methods. It is the responsibility of each laboratory to evaluate the product and determine its suitability for any alternate testing method.

Conventional Antiglobulin Technique

Donor/Patient red cells should be washed three times in Normal Ionic Strength Saline (NISS) or Phosphate Buffered Saline (PBS) making a final suspension of 2-4% in the NISS or PBS.

NOTE: Reagent red blood cells may be used directly from the vial or in accordance with manufacturer's directions.

1. To a test tube add two drops of the eluate.
2. Add one drop of the red blood cell suspension.
3. Add two drops of Peg additive. **(Optional)**
   Potentiators such as low-ionic strength saline (LISS) additive or bovine serum albumin (BSA) are unnecessary; however, a polyethylene glycol (PEG) additive (i.e. HBS-PEG-XG) may be used to enhance the sensitivity of the test.
4. Mix thoroughly.
5. Incubate for 10-15 minutes at 37°C in a water bath or heat block.
6. Perform AHG test as per manufacturer's instructions.
7. Following centrifugation, all tests should be read immediately and results should be interpreted without delay. Delays may result in disassociation of antigen-antibody complexes leading to falsely negative or weakly positive reactions.

Manual Gel/Column Agglutination Technology IAGT Technique

Eluates prepared using the ELUclear kit have been evaluated and found suitable for testing in the Ortho ID-MTS™ Gel Technology System (Ortho Clinical Diagnostics, Raritan, NJ).
Use of other gel or column agglutination technology systems must be validated by the user. Follow the manual gel system manufacturer's instructions for IAGT testing.

Interpretation of Results:

Positive test
Agglutination of test red cells.

Negative test
No agglutination of test red cells.

Quality Control
When appropriate to the test method in use, all negative antiglobulin tests should be confirmed by adding IgG sensitized red blood cell.

An aliquot of the supernatant from the last wash should be tested when antibody reactivity is detected in the eluate. This is to insure that antibody present in the eluate has truly been eluted from the red blood cells and is not remaining as a result of inadequate washing.

Limitations:

1. The activity of the eluate is limited by the following:
    a. Amount of antibody bound to the red cells.
    b. Amount of dissociation of antibody during the wash procedure.
    c. Degree to which immunoglobulin is denatured by the low pH during dissociation.
    d. An antibody that does not readily dissociate in a low pH.
2 Disassociation of antibody during the wash procedure may be minimized by using cold (2-8°C) Working Wash Solution.
3. Contamination of eluate with unbound antibody due to inadequate washing of the red blood cells during the elution procedure may limit the activity of the eluate.
4. Addition of an excessive amount of Buffering Solution when adjusting pH may result in dilution of the antibody present in the eluate.
5. Failure to adjust pH to proper range may result in hemolysis of test red blood cells.
6. Red cells that have been treated with the ELUclear reagents have not been shown to be suitable for antigen typing.
7. False positive or false negative results may also occur due to: contamination of test materials, improper cell concentration, improper incubation time or temperature, improper or excessive centrifugation, improper storage of test materials or omission of reagents or deviation from the recommended techniques.
8. Red blood cells having a positive DAT due to bound complement alone will often yield an eluate showing no antibody reactivity.
9. If the test cells used in the procedure outlined in the "**Testing of the Eluate**" section of this insert are not properly washed free of human protein before incubation with the eluate, a false negative reaction may be observed. This may result in a negative test after the addition of IgG control cells. (See **Quality Control**)
10. Eluates prepared from blood samples older than 72 hours may be less potent than those prepared from fresh samples.
11. If a drug induced hemolytic anemia is suspected, the eluate should be tested against cells sensitized with the drug in question.

Specific Performance Characteristics:

The HBS-Elution Kit is used to elute antibodies from intact red blood cells. Each lot is tested to assure appropriate reactivity when used by the recommended test procedure. For technical support, contact Hemo bioscience at 1-866-332-2835.

Bibliography:

1. Rekvig OP, Hannestad K. Acid Elution of Blood Group Antibodies from Intact Erythrocytes. Vox Sang 1977;33:280-285.
2. Judd WJ. Elution of Antibody from Red Cells. In: Seminar on Antigen-Antibody Reactions Revisited. Bell CA. ED. Arlington, VA: American Association of Blood Banks 1982:175.
3. Combs MR, Telen MJ. Testing eluates in Polyethylene glycol (PEG): A sensitive technique for detecting early alloimmunization. [Abstract] Transfusion 1989; Supplement 58S.

Glossary of Symbols

Symbol	Definition
LOT	Batch code
	Manufacturer
	Temperature limitation
	Consult instructions for use.
	Use by YYYY-MM-DD
IVD	For *in vitro* diagnostic use