Gamma ELU-KIT™ II

For Rapid Acid Elution of Antibodies from Intact Red Blood Cells

IVD 15°C–30°C

No U. S. Standard of Potency

Do not use if markedly turbid Harmful, Preservative: 0.1% Sodium Azide

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3021-2

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INTENDED USE: Gamma ELU-KIT II is intended for use in the rapid acid elution of antibodies from intact red blood cells.

SUMMARY OF THE TEST: Elution is a procedure for the recovery of antibody bound to red blood cells. Eluates prepared from red blood cells coated in vivo or in vitro are used for any of the following purposes:

1. To identify antibodies coating the red blood cells of patients found to have a positive direct antiglobulin test.
2. To isolate specific antibodies from sera containing multiple specificities by adsorption in vitro on to selected red blood cells.
3. To determine the presence of weakly expressed antigens on red cells after prior incubation with selected antisera.
4. To prepare specific antisera free from unwanted antibodies. Eluates may also be tested for the presence of specific proteins by a variety of immunochemical or biochemical techniques.

Antibodies may be eluted from intact red blood cells or from stroma remaining after lysis of the cells. Elution of antibodies from red blood cells has been accomplished by heating [1], by treating the sensitized cells with organic solvents [2,3], or by exposing them to high or low pH [4,5,6,7].

PRINCIPLE OF THE TEST: Red blood cells coated with antibody are first thoroughly washed to remove all traces of unbound protein, using a special wash solution to maintain the association of bound antibody. The washed cells are then suspended in a glycine solution at low pH to dissociate the bound antibody. After centrifugation, the supernate containing any dissociated antibody is separated from the red cells and neutralized by adding a buffering solution. The eluate is then ready to be tested for antibody detection and/or identification.

REAGENT: Gamma ELU-KIT II consists of three solutions, in volumes sufficient for at least ten eluates using the procedure detailed in this direction insert.

- **Concentrated Wash Solution:** A concentrated, buffered solution that must be diluted 1 in 10 with laboratory reagent-grade water to prepare the Working Wash Solution. Contains 0.1% sodium azide as a preservative. The Working Wash Solution provides an isotonic buffer at an appropriate ionic strength for washing red blood cells free of unbound antibody.
- **Eluting Solution:** A low-pH glycine buffer, designed to dissociate bound antibody from washed red blood cells. This product contains no preservative.
- **Buffering Solution:** A Tris (hydroxymethyl)-aminomethane solution containing bovine albumin, which is used to neutralize the acidity of the Eluting Solution after dissociation of the bound antibody. 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. Contains 0.1% sodium azide as a preservative. A blue indicator is added to assist in adjusting the eluate to the correct pH for testing.

The component products may be interchanged between lots, providing they are in date.

PRECAUTIONS:

For in-vitro diagnostic use. Store at room temperature (15° to 30°C) when not in use. Working Wash Solution should be stored at 1° to 10°C when not in use. Effort should be made to minimize contamination during use of the product. Do not use if markedly turbid. Do not freeze. Do not use beyond expiration date. Do not use the Buffering Solution if it is not blue prior to buffering the eluate.

Key:
Underline = Addition or significant change ▲ = Deletion of text

Gamma ELU-KIT™ II

For Rapid Acid Elution of Antibodies from Intact Red Blood Cells

Concentrated Wash Solution and Buffering Solution contain 0.1% sodium azide and is classified as Harmful (Xn). R22 Harmful if swallowed.

Warning: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. If discarded into sinks, flush with a large volume of water to prevent azide build-up.

Handle and dispose of reagent as potentially infectious.

The format for the expiration date is expressed as CCYY-MM-DD (year-month-day).

SPECIMEN COLLECTION AND PREPARATION: No special preparation of the patient is required prior to specimen collection. Blood should be drawn by aseptic technique and should be tested while still fresh. If delay in testing should occur, the specimen must be stored at 1° to 10°C, preferably for not longer than 72 hours. *NOTE: The use of blood specimens greater than 72 hours old may be associated with a hemoglobin-stained eluate, and with accompanying difficulty in adjusting the final pH of the eluate for testing.*

An anticoagulated specimen is preferred from persons whose cells are coated in vivo because of the volume of cells required for the elution procedure. EDTA is the anticoagulant of choice. A defibrinated sample is also suitable.

PROCEDURE:

Materials Provided: Gamma ELU-KIT II

Additional Materials Required: Test tubes (12x75 mm for elution, 10x75 mm or 12x75 mm for eluate testing), pipettes, laboratory reagent-grade water, 37°C waterbath or incubator, isotonic saline or phosphate-buffered (approximately 15 mM) isotonic saline pH 6.5-7.5, timer and centrifuge. Anti-Human Globulin containing anti-IgG, IgG-sensitized red blood cells, and an optical aid such as a hand lens or concave mirror for eluate testing. Use of Gamma PeG™ is optional.

TEST METHODS:

Preparing Eluates

Applies to red blood cells that have been coated with antibody in vivo, or to selected red blood cells that have been coated in vitro by prior incubation with an antiserum. *NOTE: The purpose of the special Working Wash Solution recommended is to reduce to a minimum the amount of antibody that dissociates from the red blood cells during the washing phase. It has been reported, however, that the use of this low-ionic strength solution may cause non-specific uptake of antibody to antigen-negative cells during the washing phase, especially when the antibody is particularly strong [8].* As an option, saline may be used to wash the red blood cells before preparing the eluate. This should avoid the uptake of antibody to antigen-negative cells, but may result in the dissociation of low-affinity antibody during the wash procedure, leading to an eluate containing no activity, or to less antibody than expected. Step 1 of the following procedure is not required if the decision is made not to wash with the Working Wash Solution.

1. Prepare Working Wash Solution by adding one volume of Concentrated Wash Solution to nine volumes of laboratory reagent-grade water. Mix thoroughly before use. *NOTE: Dilution of the Concentrated Wash Solution reduces the concentration of sodium azide to a level at which it is no longer effective as a preservative. If stored at 1° to 10°C, the Working Wash Solution may be used for as long as it shows no obvious turbidity and is not causing hemolysis of red blood cells.*
2. Centrifuge the specimen and remove as much serum or plasma as possible.
3. Wash an aliquot of the coated red blood cells one time with saline. The volume of the aliquot should be sufficient to yield 1 mL (approximately 20 large drops) of packed cells when washing has been completed.

4. Use the Working Wash Solution to wash the cells an additional four times to remove all unbound antibody. Alternatively, four further washes with saline can be substituted in case non-specific binding of antibody to antigen-negative red blood cells is suspected. Adequate washing of up to 1 mL of packed red blood cells can be achieved in a 12x75 mm test tube if these directions are followed. Reserve a small aliquot of the supernate from the final wash to serve as a control.
5. Place 1 mL (20 large drops) of washed red blood cells in a clean 12x75 mm test tube, add 20 drops (approximately 1 mL) of Eluting Solution and mix GENTLY by inverting the tube four times. If the size of the test sample is insufficient to yield as much as 1 mL of packed cells, the eluate may be prepared from a lesser volume of cells, but will yield a correspondingly lesser volume of eluate to test. In such a case, the volume of Eluting Solution added would not be 20 drops, but would be a volume EQUAL to the volume of packed cells in the tube.
6. Centrifuge immediately for 45 to 60 seconds at 3,400 rpm (rcf 900 to 1,000). Prolonged immersion of the cells in Eluting Solution causes hemolysis. The consequent release of hemoglobin into the eluate alters the pH and may affect the volume of Buffering Solution required to adjust the pH of the eluate to neutral.
7. Transfer the supernatant eluate into a clean test tube. The deposited red blood cells should be discarded, as they are no longer suitable for antigen typing procedures.
8. To the separated acid eluate, add sufficient Buffering Solution to restore the pH of the eluate to within the required range for testing (6.4 to 7.6). The presence of a blue indicator in the Buffering Solution provides a means to determine that the eluate has been adjusted appropriately. Do not use the Buffering Solution if it is not blue prior to buffering the eluate. This can be observed when the Buffering Solution is aspirated with a pipette. When Buffering Solution is first added to a freshly prepared eluate, it will be noted to turn yellow on contact with the acid solution. As the volume of Buffering Solution approaches the original volume of the eluate, however, the color changes to a pale blue. This persists upon mixing if the pH of the eluate has been adjusted to within the desired range. The volume of Buffering Solution required for this purpose may vary with different eluates, depending on a number of factors, the most prominent being the extent to which hemolysis occurs during the eluting step. Besides undue prolongation of the eluting step, already mentioned, the degree of hemolysis may be influenced by the age and osmotic fragility of the red blood cells from which the eluate is being prepared. It should also be noted that volumes might not be exact when solutions are being delivered in dropwise fashion. The presence of an indicator in the Buffering Solution facilitates the adjustment of the eluate to the required pH range for testing. If the color of the eluate remains yellow after adding 20 drops (approximately 1 mL) of Buffering Solution, continue to add Buffering Solution, one drop at a time, until a pale blue color persists upon thorough mixing.
9. Mix well and centrifuge to remove any precipitate or cellular debris, then transfer the eluate to a clean, properly labeled test tube.

The eluate is now ready for testing to detect or identify antibodies. If delay in testing should occur, the eluate should be stored at 1° to 10°C and may be tested up to seven days after preparation, providing no turbidity develops. Turbidity may indicate bacterial contamination, which may cause false results. Longer storage is not precluded if the eluate is sterilized by membrane filtration and stored thereafter at 1° to 10°C, and if subsequent testing is controlled with red blood cells established as giving positive and negative reactions with the freshly prepared eluate. The blue color of the indicator may be expected to fade during storage.

Testing Eluates

Eluates may be tested by conventional antibody detection methods, by an alternative procedure the user can validate by producing data to demonstrate consistently reliable results, or by the following procedure using the modified indirect antiglobulin technique. Potentiators such as bovine albumin, or a low-ionic-strength additive, are ordinarily unnecessary, as the eluate is already in a low-ionic substrate. If a polyethylene glycol additive reagent (e.g. Gamma PeG™) is used to enhance the sensitivity of the test, the modified antiglobulin test must not be used. In this case, the cells should be washed at least three times with saline after incubation with the eluate, as it is necessary to wash away the additive before adding Anti-Human Globulin.

NOTE: The modified antiglobulin technique omits repeated washing of the test red blood cells after incubation with the eluate, but depends on adequate removal of human protein from the cell suspensions before incubation. Commercial Reagent Red Blood Cells may be sufficiently washed during manufacture to be used directly from the vial, but red blood cells from patient or donor samples must be thoroughly washed with at least two changes of saline, taking care to decant the saline completely between washes and to resuspend the red blood cells thoroughly when adding saline for the next wash. After the second wash, the saline is decanted completely and sufficient saline is added to make a 3-4% suspension of the red blood cells.

1. Place 1 drop of the washed red blood cell suspension in a properly labeled test tube. Add a small volume of saline (5-10 drops). Centrifuge for 30 seconds at 3,400 rpm (rcf 900 to 1,000), decant the saline and blot the tubes dry.
2. Add 2 drops of eluate to the dry red blood cell button in each tube.
3. Mix the contents of all tubes thoroughly and incubate for 10 minutes at 37°±1°C. Incubation may be extended up to 30 minutes. Incubating for the upper end of the time range may enhance reactivity.
4. After appropriate incubation, add 5-10 drops of Working Wash Solution to the tube and mix. Adding 10 drops of Working Wash Solution may enhance the efficiency of the wash process.
5. Centrifuge for 30 seconds at 3,400rpm (rcf 900 to 1,000).
6. Decant the Working Wash Solution completely and blot the tubes dry.
7. Add 1 or 2 drops of Gamma-clone® Anti-Human Globulin to each dry button of red blood cells or follow the directions of the Anti-Human Globulin manufacturer. Adding 2 drops of AHG may enhance reactivity.
8. Mix well and centrifuge tubes for:
   (a) 1 minute at 1,000 rpm (rcf 100 to 125), or
   (b) 15 seconds at 3,400 rpm (rcf 900 to 1,000) or
   (c) a time appropriate to the calibration of the centrifuge.
9. Resuspend the cells by gentle shaking and examine for agglutination. Negative reactions may be examined with an optical aid. Record results.

Stability of Reaction: The washing phases of the antiglobulin test must be carried out without interruption, and final test results must be interpreted immediately upon completion of the test.

QUALITY CONTROL:

1. All negative antiglobulin tests should be confirmed by adding IgG-sensitized red blood cells, such as Checkcell®, and then repeating centrifugation and reading. A positive test result at this point confirms that active antiglobulin (anti-IgG) was added to the test system and was present when the original antiglobulin test was interpreted as negative.
2. A sample from the final wash should be tested for antibody activity in parallel with the eluate. The purpose of this control is to assure that antibody present in the eluate has been derived from a bound state on the original cells, and is not merely remaining as a result of inadequate washing. If antibody activity is present in the last wash, the elution procedure must be repeated after more thorough washing of the cells. The possibility should be considered, however, that the presence of antibody activity in the wash solution could be due to the dissociation of antibody from the cells during washing. Whenever there is reason to suspect this possibility, dissociation of antibody can be minimized by using Working Wash Solution at 1° to 10°C to wash the cells prior to the elution procedure.

INTERPRETATION OF TEST RESULTS: An agglutination reaction, occurring when the eluate is tested against red blood cells of appropriate phenotypes, indicates that an antibody has been recovered from the original cells, subject to satisfactory control tests. In suspected drug-related positive direct antiglobulin tests, the eluate should be tested against red blood cells coated with the appropriate drug (e.g. penicillin) before concluding that no antibody has been eluted from the cells.
The absence of agglutination when the eluate is tested against appropriate red blood cells indicates that no antibody has been recovered from the cells.

LIMITATIONS: The yield of antibody obtained upon elution from coated red blood cells is dependent on the following variable factors:

1. The amount of antibody bound to the cells. Where elution is being attempted from red blood cells that have been incubated with a serum or plasma sample in vitro, this is related to the sample to cell ratio in the incubating mixture, the mean binding constant of the particular antibody, the number, type and accessibility of the antigen receptor sites, and also to whether or not the reaction has been allowed to reach equilibrium.
2. The degree of dissociation of antibody that occurs during the washing procedures. This may be minimized by washing in 1° to 10°C Working Wash Solution. In most cases, satisfactory eluates can be made after washing the cells with Wash Solution at room temperature.
3. The degree of recombination of antibody that occurs before the eluate is separated from the cells. This is minimal with the acid elution procedure, as separation of the eluate from the cells occurs at a pH that is unfavorable for antibody association.
4. The degree to which immunoglobulin is denatured by the low pH during dissociation. This is minimal if the procedure is carried out as recommended.

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Other factors to be considered are:

5. Red blood cells having a positive direct antiglobulin test attributable to bound complement alone will normally yield an eluate showing no antibody reactivity.
6. Incorrect restoration of pH in the eluate may cause hemolysis of red blood cells, or may inhibit antibody activity in subsequent testing.
7. Cells from blood samples stored for longer than 72 hours may yield less potent eluates than those from freshly drawn samples. An alternative elution procedure may be preferable for the preparation of eluates from stored cells.
8. A false-negative test may occur if the modified antiglobulin test procedure is used and the test red cell suspension is not washed sufficiently free of human protein before incubation with the eluate, or if the test system becomes contaminated in any way with human protein other than antibody dissociated during the elution phase. A negative test after the addition of IgG-sensitized red blood cells (Step 1 of Quality Control) should alert the investigator to this source of error.
9. Use of the low-ionic strength wash solution provided has been reported [8] to give rise to the possibility of non-specific uptake of particularly strong antibody to antigen-negative red blood cells, leading to an eluate that is, in effect, falsely positive.
10. Failure to develop a pale blue color when Buffering Solution is added to a freshly prepared eluate may indicate degradation of the Buffering Solution and the eluate should then be discarded. In this case confirm that the Buffering Solution is blue prior to use and the eluate should then be repeated.

SPECIFIC PERFORMANCE CHARACTERISTICS: The solutions comprising Gamma ELU-KIT II are tested and found to give satisfactory results when used by the procedure detailed in this direction insert to prepare eluates from red blood cells coated with a wide range of IgG antibodies. The performance of this product is dependent on adhering to the recommended methods found in this insert. For additional information or for technical support, contact Immucor Technical Service at 800-492-BLUD (2583) or 770-441-2051.

BIBLIOGRAPHY:

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Disregard section on testing eluates using the modified antiglobulin technique ⌈ ⌋. HMC TSL will test eluates using the conventional antiglobulin technique with PEG additive.

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