**Purpose:** To provide instructions for the preparation of an eluate from patient red cells that exhibit a positive direct antiglobulin test (DAT) with anti-IgG. The elution procedure dissociates the antibody bound to the red cells by the use of a low pH solution and the antibody is then recovered in a usable form by the addition of a buffering solution.

* ***Note:*** *Red blood cells that have a positive DAT due to bound complement alone will usually yield a nonreactive eluate.*

**Procedure:**

|  |  |  |
| --- | --- | --- |
|  | **Action** | **Related Documents**  |
| **1** | * Confirm sample acceptability.
* Anticoagulated samples collected in EDTA are preferred.
 | * Evaluating Patient Samples and Request Forms
 |
| **2** | **Reagent Components and Storage*** Gamma ELU-KIT II consists of 3 solutions:
	+ Concentrated Wash Solution (50 ml)
	+ Eluting Solution (11 ml)
	+ Buffering Solution (12 ml)
* Upon receipt, store at room temperature (15o to 30oC).
* Do not use if turbidity is observed in any of the solutions.
* Do not use if the Buffering Solution is not blue.
* The components of the kit may be interchanged between different lots as long as they are in date.
 | * Manufacturer Reagent Package Insert
 |
| **3** | **Preparation of Working Wash Solution*** Label a wash bottle with lot number, preparation date and expiration date of the Concentrated Wash Solution.
* Add the contents of the bottle of Concentrated Wash Solution to the wash bottle and fill to the 500 ml mark with reagent-grade water (1 volume Concentrated Wash to 9 volumes water). Mix well and store at 1o to 10oC.

***Note:*** May be used as long as it shows no obvious turbidity and is not causing hemolysis of the red blood cells. Do not use beyond expiration date of Concentrated Wash Solution. | * Manufacturer Reagent Package Insert
 |
| **4** | **Washing Phase*** Centrifuge the specimen and remove as much plasma as possible.
* To a clean 13x100 mm tube, transfer an aliquot of cells sufficient to yield 1 ml (approximately 20 large drops) of packed cells after washing is completed.
* Wash one time manually with isotonic saline in the Hettich EBA 20 centrifuge for 1 minute at 5200 rpm.
* Wash an additional 4 times manually with the Working Wash Solution. Increase the spin time to 5 minutes for the 4th wash to pack the cells.
* Reserve an aliquot of the final wash as a control.
 | * Manufacturer Reagent Package Insert
* Washing Red Cell Samples (Manual or Automated)
 |
| **5** | **Testing the Last Wash (Conventional Antiglobulin Technique Using PEG Additive)*** Test the reserved aliquot of the last wash with reagent screening cells.
* Include Aand B cells if indicated.
* Refer to reagent instructions for PEG additive.
* If antibody activity is present, discard the reserved aliquot and wash the cells an additional 2-3 times.
* Repeat testing of the last wash.
* Proceed to eluate preparation if the last wash is negative.
 | * Manufacturer Reagent Package Insert
* Antibody Screen by Polyethylene Glycol (PEG) Tube IAT Method
 |
| **6** | **Preparation of Eluate*** Transfer 1 ml (20 large drops) of the washed packed cells to a clean 12x75 mm tube.
* Add 20 drops of Eluting Solution (or an equal volume, since the volumes delivered in a drop-wise fashion may not be exact) and mix gently by inverting the tube 4 times.

***Note:*** Volume of Eluting Solution to be added should be equal to that of the packed cells in case the size of the sample is insufficient to yield 1 ml of packed cells.* Centrifuge **immediately** for 45-60 seconds at 3400 rpm.

***Note:*** Prolonged immersion of the cells in Eluting Solution causes hemolysis.* Transfer the supernatant to a clean 12x75 mm tube. Discard the cells.
* Add Buffering Solution drop by drop until a pale blue color appears and persists after mixing. This will be achieved as the volume of Buffering Solution added approaches the volume of the supernatant.

***Note:*** Addition of excessive Buffering Solution may result in dilution of antibody in the eluate.* Mix well and centrifuge for 1 minute to remove cellular debris. If debris is still visible in the eluate after centrifuging, transfer to another tube and centrifuge again.
* Transfer the eluate to a clean, properly labelled tube. It is now ready for testing.
 | * Manufacturer Reagent Package Insert
 |
| **7** | **Testing the Eluate (Conventional Antiglobulin Technique Using PEG Additive)*** Refer to reagent instructions for PEG additive.
* Due to the limited amount of eluate available, panel cells should be selected based on antibody/antibodies identified in the patient’s plasma and phenotype, if available.
* If panel cells are nonreactive and patient is non-group-O transfused with plasma products containing anti-A and/or anti-B, the eluate and the last wash should be tested against Aand B cells.
* ***Note:*** Only IgG antibodies will be recovered in the elution procedure. It is not necessary to rule out IgM antibodies during antibody identification unless otherwise instructed by the Transfusion Service Medical Director or Manager.
 | * Manufacturer Reagent Package Insert
* Guidelines for Antibody Identification
* Antibody Panel by Tube IAT Method
 |

**References:**

Package Insert Gamma ELU-KIT™ II IC3021-2

Package Insert Gamma PeG™ IC3026-3

AABB Technical Manual, Current Edition