ALBA Elution Kit

For the acid elution of antibodies from intact red blood cells

℞ REF **Z313U** IVD

INTENDED USE

The ALBA Elution Kit is intended for use in the acid elution of antibodies from intact red blood cells to allow further detection and/or identification.

It is intended for professional use within a blood donor or clinical laboratory environment to perform acid elution of antibodies relating to transfusion in the general population.

This pre-analytical procedure is performed manually.

The ALBA Elution Kit is an accessory for sample preparation only and does not return diagnostic results for an analyte.

SUMMARY AND EXPLANATION

Antibodies bound to red blood cells in vivo or in vitro can be disassociated and recovered through acid elution. Eluted antibodies can then be further detected/identified by serological methods.

PRINCIPLE OF THE PROCEDURE

Unbound antibodies are removed from the sample by thorough washing with the Working Wash Solution. After washing, the antigen-antibody complex is dissociated by the addition of a low pH Elution Solution. The recovered eluate is then returned to a suitable pH for serological testing by the addition of a Buffering Solution.

REAGENT

The ALBA Elution Kit consists of:

Vial Identifier	Name	Description
1	Concentrated Wash Solution	A glycine buffered solution containing bovine serum albumin used to minimize antibody dissociation during washing. 1 x 30 mL vial. Preservative: 0.85% sodium azide.
2	Elution Solution	A low pH glycine buffer containing a pH indicator. 1 x 10 mL vial.
3	Buffering Solution	A Tris (hydroxymethyl aminomethane) solution containing bovine serum albumin. 1 x 11 mL vial. Preservative: <0.1% sodium azide.

The kit components may be interchanged between lots, providing they are in date.

Bovine serum albumin has been sourced from animals declared free from Bovine Spongiform Encephalopathies (BSE) disease and deemed to have low Transmissible Spongiform Encephalopathies (TSE) risk.

WARNINGS AND PRECAUTIONS

For in vitro diagnostic use only.

Use of recommended equipment/material and strict adherence to the procedures are essential.

Do not use if product integrity is compromised.

Wear appropriate personal protective equipment and follow local laboratory safety procedures.

Do not use beyond the expiration date (year, month, day: YYYY-MM-DD).

Do not use if turbid.

This product has components (dropper bulbs) containing dry natural rubber.

Refer to the Safety Data Sheet for full details of safety information.

STORAGE, HANDLING AND DISPOSAL

The ALBA Elution Kit 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.

Crystallization of the Concentrated Wash Solution may occur at lower temperatures. Should this occur, incubation at 37°C will re-dissolve crystals.

Refer to the Reagent Preparation section for recommended storage of prepared Working Wash Solution.

Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive compounds. If discarded into a sink, flush with a large volume of water to prevent azide build-up.

Follow local disposal regulations based on your location along with recommendations and content in the Safety Data Sheet and/or refer to the chemical information provided in the Reagent section to determine the safe disposal of this product.

SPECIMEN COLLECTION AND PREPARATION

Specimens should be collected by a standard collection technique. The specimen should be processed as soon as possible after collection. If processing is delayed, the specimen should be stored at refrigerated temperatures. Use of red blood cells older than 72 hours may result in lower yields of recovered antibodies. Anticoagulated samples collected into EDTA are preferred.

REAGENT PREPARATION

Prepare the Working Wash Solution by adding one part Concentrated Wash Solution 1 to nine parts distilled or deionized water. The solution is stable for up to six months when stored at 2-8 °C or up to 10 days when stored at 20-25 °C. The use of cold Working Wash Solution may minimize antibody dissociation during washing.

If preparing the full 30 mL volume of provided Concentrated Wash Solution, combine with 270 mL of water.

The other components of the kit are supplied ready for use.

PROCEDURE

This reagent has been standardized for use only by the procedure described below

Material Provided

- ALBA Elution Kit

Materials Required but Not Provided

- Distilled or deionized water
- Isotonic saline
- Pipettes
- Centrifuge
- 12 x 75 mm test tubes

Elution Procedure

When using supplemental testing equipment (i.e. centrifuge), follow the procedures that are contained in the operator's manual provided by the device manufacturer.

1. Wash an aliquot of red blood cells one time in isotonic saline. The aliquot should be sufficient to yield 1 mL of packed cells. Remove as much saline as possible after washing.
2. Wash the red blood cells four times with Working Wash Solution to remove any unbound antibody. Remove as much supernatant as possible after each wash. Reserve a small aliquot of the supernatant from the last wash as a control to be tested in parallel with the eluate.

   NOTE: Use of Working Wash Solution is recommended to minimize antibody dissociation during washing; however, isotonic saline may be used instead, if desired.
3. Transfer 1 mL (approximately 20 drops) of the washed red blood cells to a clean tube.
4. Add 1 mL (approximately 20 drops) of Elution Solution 2 to the tube and mix gently by inverting the tube four times.

   NOTE: If an alternative volume of packed red blood cells is being used to prepare the eluate, the volume of Elution Solution used should equal the volume of washed, packed red blood cells.
5. Centrifuge immediately for 60 seconds at 1000 rcf (approximately 3400 rpm)*.
6. Transfer eluate to a clean tube and discard the red blood cells.

   NOTE: If excessive cellular debris is present, eluate may be centrifuged again and transferred to a second clean tube prior to adding the Buffering Solution.
7. Add Buffering Solution 3 to the 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 suitable for testing (approximately 7.0 ± 0.5).
8. Centrifuge the eluate for 2 minutes at 1000 rcf (approximately 3400 rpm)* to remove any residual cellular debris.
9. Transfer the centrifuged eluate to a clean tube.
10. If the eluate is to be tested by column agglutination techniques, it should be centrifuged again at a time and speed appropriate to the test method and transferred to a clean tube.
11. The eluate is now ready for testing

The eluate may be tested using a conventional indirect antiglobulin test, with or without enhancement media, or column agglutination techniques, following your laboratory's standard operating procedures or the manufacturer's instructions for use. Laboratories are responsible for validating the performance of their selected test methods to confirm suitability for the intended application.

When tested by column agglutination technique, if fibrin or other particulate matter is observed at the top of the gel after the Indirect Antiglobulin Test is performed, the eluate can be recentrifuged for 2 minutes at 1000 rcf (approximately 3400 rpm)* and retested.

The eluate can be stored at 2-8 °C and tested up to seven days after preparation. Stored eluates should be centrifuged for 2 minutes at 1000 rcf (approximately 3400 rpm)* prior to testing.

* Or a time and speed appropriate for the centrifuge used.

QUALITY CONTROL

An aliquot of the supernatant from the last wash must be tested as a control. A negative control result indicates that any antibody detected in the eluate was released from a bound state and not from unbound antibody remaining after inadequate washing.

If the control test result is positive, the elution should be repeated taking care to wash the red blood cells thoroughly.

LIMITATIONS

This kit is designed for use with a minimum of 1 mL of packed red cells for elution.

The antibody yield obtained from the elution process is dependent on the following variables:

1. The amount of antibody bound to the red blood cells.
2. The degree of dissociation of bound antibody during the washing process.
3. The degree of recombination of antibody that occurs before the eluate is separated from the red blood cells.
4. The degree of denaturation of the immunoglobulin by the low pH Elution Solution during dissociation.

The degree of hemolysis present in the eluate is impacted by extended exposure of the red blood cells to the Elution Solution, as well as sample-specific characteristics, such as the age and osmotic fragility of the red blood cells used to prepare the eluate.

Failure to adjust the pH of the eluate to the proper range may result in hemolysis of test red blood cells or inhibition of antibody activity in subsequent testing.

Grossly hemolyzed samples must not be used for elution procedures. If the eluate becomes grossly hemolyzed during the process, the procedure must be discontinued. Hemolysis may interfere with the color change produced by the buffering solution, which is used to confirm that the eluate has reached a suitable pH for testing. In such cases, interpretation of the pH indicator may be unreliable.

Cord blood and/or neonatal samples were not included in performance studies to assess the elution kit's performance on red blood cells from this patient population. Kit performance has therefore not been assessed for this patient population.

Adding an excessive amount of Buffering Solution when adjusting the pH may result in dilution of the antibody present in the eluate.

Other factors that can lead to false results, include using unequal amounts of packed red blood cells and Elution Solution, improper technique, improper centrifugation parameters, use of blood samples over 72 hours or contaminated equipment, materials, or samples.

Red blood cells used in the elution procedure must not be used for antigen typing.

SPECIFIC PERFORMANCE CHARACTERISTICS

The ALBA Elution Kit is released following satisfactory quality control testing performed by the manufacturer.

Comparator Study

Elution procedures were performed on in vivo and in vitro IgG sensitized red blood cell (RBC) samples using the ALBA Elution Kit and an established comparator kit. The eluates obtained were tested in manual tube serology, with and without PEG potentiation, and column agglutination techniques, including ORTHO ID-MTS™, and results compared for equivalence.

Sample Type	Number Tested	Concordance (%) of ALBA Elution Kit with comparator kit
In vitro IgG sensitized RBC	45	100
In vivo IgG sensitized RBC	16	100

100% equivalence in results was demonstrated between eluates produced by the ALBA Elution Kit and those from the comparator kit.

Precision Study

Repeatability: 10 elution procedures were performed on one red blood cell sample sensitized in vitro with an IgG class human polyclonal blood group antibody using the ALBA Elution Kit. The elutions were performed on the same day by the same operator.

Reproducibility: One elution procedure was performed by two operators on one red blood cell sample sensitized in vitro with IgG class human polyclonal blood group antibody using two lots of ALBA Elution Kit twice per day for five non-consecutive days.

The eluates obtained were tested in antibody screening tests by manual tube serology and column agglutination techniques (ORTHO ID-MTS™) and results compared for equivalence between test occasions to demonstrate precision of kit performance.

Test Method	Repeatability	Reproducibility
Manual Tube	100%	100%
ORTHO ID-MTS™	80%*	100%

*Two false positive results were returned on ORTHO ID-MTS™ which were not reproduced on repeat of the study. The reason for these results was not determined, however use of in vitro sensitized red blood cell samples was thought to be a contributing factor. All other results returned the expected reactions.

BIBLIOGRAPHY

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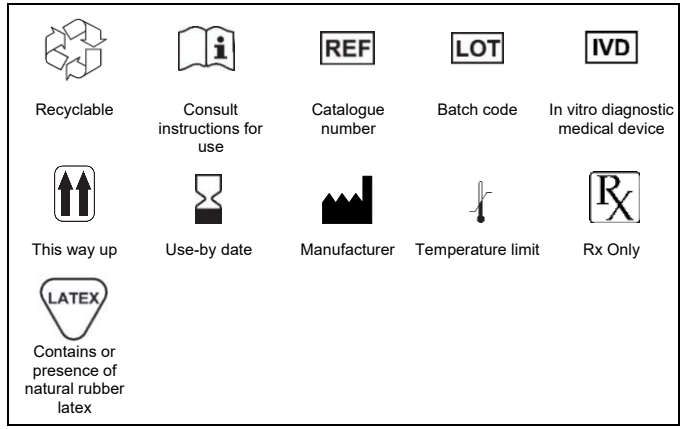
Judd WJ, Johnson ST, Storry J. Judd's Methods in Immunohematology. 4th ed. Bethesda, MD: AABB Press 2022: Chapter 4-E, 130-134.

SYMBOLS



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REVISION HISTORY

Version	Section	Change
7	Limitations	Addition of the following limitations: This kit is designed for use with a minimum of 1 mL of packed red cells for elution. Grossly hemolyzed samples must not be used for elution procedures. If the eluate becomes grossly hemolyzed during the process, the procedure must be discontinued. Hemolysis may interfere with the color change produced by the buffering solution, which is used to confirm that the eluate has reached a

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		suitable pH for testing. In such cases, interpretation of the pH indicator may be unreliable. Cord blood and/or neonatal samples were not included in performance studies to assess the elution kit's performance on red blood cells from this patient population. Kit performance has therefore not been assessed for this patient population.