

**ACID ELUTION KIT  
DIRECTIONS FOR USE**

**Red Cell Elute: For Acid Elution Of Antibodies From Intact Red Cells.**

**SUMMARY**

Allo/ Auto antibodies adsorbed onto red cells, either *in vivo* or *in vitro*, can be dissociated and recovered through elution. The eluate can then be used to identify a single antibody in multispecific sera, demonstrate the presence of a weak antigen, identify the antibody responsible for a positive direct antiglobulin test in acquired haemolytic anaemia or transfusion reaction, identify the antibodies causing haemolytic disease of the newborn or prepare specific antibody from sera containing unwanted antibodies.

**PRINCIPLE**

Unadsorbed antibodies in the sample are removed by washing with the working wash solution. 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 base buffered solution (See **Limitations**). To ensure that the eluate only contains red-cell-bound antibody, the supernatant from last wash of red cells to be eluted, must be tested in parallel with eluate.

**KIT DESCRIPTION**

Lorne Red Cell Elute is an acid elution kit. The kit consists of Concentrated Wash Solution, which is used to minimise antibody dissociation during washing, Acid Eluting Solution, which is a low pH glycine buffer containing a colouring agent (pH indicator) and a Base Buffering Solution, Tris (hydroxymethyl aminomethane) solution containing bovine albumin. All the solutions are supplied at optimal dilution for use with all recommended techniques stated below without the need for further dilution or addition with the exception of the Concentrated Wash Solution. For lot reference number and expiry date see **Vial Label**.

**STORAGE**

Do not freeze. Reagent vials should be stored at room temperature on receipt. Prolonged storage at temperatures outside this range may result in accelerated loss of reagent activity. This reagent has undergone transportation stability studies at 37°C and -25°C as described in document BS EN ISO 23640:2015.

**SAMPLE COLLECTION AND PREPARATION**

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

**PRECAUTIONS**

1. The reagent is intended for *in vitro* diagnostic use only.
2. If a vial is cracked or leaking, discard the contents immediately.
3. Do not use reagent past the expiration date (see **Vial Label**).
4. Do not use reagent if a precipitate is present.
5. Protective clothing should be worn when handling the reagent, such as disposable gloves and a laboratory coat.
6. The reagents have been filtered through a 0.2 µm capsule to reduce the bio-burden. Once a vial has been opened the contents should remain viable up until the expiry date as long as there is no marked turbidity, which can indicate reagent deterioration or contamination.
7. Wash solution contains 0.1% sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive metal azides. On disposal flush away with large volumes of water.
8. Care must be taken in the use and disposal of each vial and its contents.

**DISPOSAL OF REAGENT AND DEALING WITH SPILLAGES**

For information on disposal of kit reagents and decontamination of a spillage site see **Material Safety Data Sheets**, available on request.

**CONTROLS AND ADVICE**

1. Testing of reserved solution from last wash will confirm that the antibody detected in the eluate was released from a bound state and not from free antibody remaining after inadequate washing. If control tubes are positive the elution should be repeated taking care to wash thoroughly.
2. The antiglobulin tube technique can only be considered valid if all negative tests react positively with IgG sensitised red cells (Coomb's control cell).
3. In **Recommended Techniques** one volume is approximately 50µl when using the vial dropper provided.
4. Use of the kit and the interpretation of results must be carried out by properly trained and qualified personnel in accordance with the requirements of the country where the kit is in use. The user must determine the suitability of the kit for use in other techniques.

**KIT COMPONENTS SUPPLIED**

- Acid Eluting Solution 1 x 10 ml (Solution I).
- Base Buffering Solution 1 x 10 ml (Solution II).
- Concentrated Wash Solution 2 x 25 ml.
- Wash Bottle.

**REAGENTS AND MATERIALS REQUIRED**

- Anti-human globulin i.e. Lorne AHG Elite (Cat # 435010 or 415010).
- Distilled or Deionised water.
- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- IgG sensitised red cells i.e. Lorne Coombs Control Cells (Cat # 970010).
- PBS solution (pH 6.8-7.2) or Isotonic saline solution (pH 6.5-7.5).
- Test tube centrifuge.
- Volumetric pipettes.
- Water bath or dry heat incubator equilibrated to 37°C ± 2°C.
- Bio-Rad LISS/Coombs or Coombs Anti-IgG ID Gel Cards.
- Bio-Rad ID-Centrifuge.
- Bio-Rad ID-Diluent 2.
- Bio-Rad ID-Incubator equilibrated to 37°C ± 2°C.
- Ortho BioVue AHG polyspecific or AHG Anti-IgG System Cassettes.
- Ortho BioVue System Centrifuge.
- Ortho BioVue System Heat Block equilibrated to 37°C ± 2°C.
- Ortho 0.8% Red Cell Diluent.

**PREPARATION OF WORKING WASH SOLUTION**

1. Pour contents of 1 bottle of Concentrated Wash Solution into wash bottle supplied.
2. Add enough good quality distilled or deionised water to fill wash bottle up to fill line (250 ml) and mix well.
3. The Working Wash Solution is ready for use and may be stored at 2-8°C for up to six months and used if no turbidity is observed.
4. The use of cold Working Wash Solution will minimise antibody dissociation during the wash phase of the procedure.

**TESTING OF RED CELL SAMPLE PRIOR TO ELUTION**

1. Perform a direct antiglobulin test on the red cell specimen to be tested and record results.
2. If specimen gives a positive direct antiglobulin test then there is bound antibody, either due to *in vivo* or *in vitro* sensitisation, present on red cells.
3. To determine whether sensitisation is due to immunoglobulin or complement, test the specimen against anti-IgG and anti-C3d.
4. If the specimen gives a positive result with anti-IgG then the positive direct antiglobulin test is due to immunoglobulin and an elution can be carried to remove and identify antibody present.
5. If the specimen gives a positive result with anti-C3d then the positive direct antiglobulin test is due to complement and an elution should not be carried out because it will fail to demonstrate antibody activity.
6. The stronger the direct antiglobulin test result the more antibody that will be readily eluted from surface of the red cells.

**RECOMMENDED ELUTION TECHNIQUE**

1. Take a 2 ml sample of the red cell specimen to be tested and wash the red cells once in isotonic saline and take care to completely decant saline after washing.
2. Wash the red cells four times with the Working Wash Solution to remove any unbound antibody.
3. Reserve a small aliquot of the supernatant from the last wash, which will be used later to test for antibody activity.
4. Make sure to completely decant the wash solution after each wash.
5. Place in a labelled test tube: 1 ml of the washed sensitized packed red cells and add 1 ml of Solution I to elute the antibody.
6. Mix thoroughly and centrifuge the tube for 60 seconds at 1000 rcf or for a suitable alternative time and force.
7. Transfer supernatant (eluate) to a clean tube and discard cells.
8. To the eluate add Solution II drop by drop, mixing well after each drop until blue colour appears. The blue colour indicates a pH range of 6.5-7.5 and means the eluate has been buffered to the correct pH.
9. If precipitates appear, centrifuge for 60 seconds at 1000 rcf or for a suitable alternative time and force.
10. For testing of the eluate see below. The eluate can be stored for up to 7 days at 2-8°C.

## SELECTION OF RED CELLS FOR ELUATE / LAST WASH SUPERNATANT

1. The choice of red cells used for testing against the eluate is dependent on the individual Laboratory.
2. Commercial 3-5% or 0.8% reagent red cells may be used.
3. Patient and donor red cell samples can be used so long as the cells are washed at least three times in isotonic saline and then suspended to 3% or 0.8% before use.
4. If a drug induced haemolytic anaemia is suspected then eluate should be tested against cells sensitised with drug in question.

## TESTING OF THE ELUATE/ LAST WASH SUPERNATANT

### A. Tube Testing Technique

1. Place in a labelled test tube: 2 volumes\* eluate or last wash supernatant and 1 volume red cells.
2. Mix well and incubate at 37°C for 15 minutes.
3. After incubation add 10 drops of the Working Wash Solution.
4. Mix thoroughly and centrifuge all tubes for 30 seconds at 1000 rcf or for a suitable alternative time and force.
5. Decant solution and then add two drops of anti-human globulin.
6. Mix thoroughly and centrifuge for 20 seconds at 900-1000 rcf.
7. Resuspend the cells and read for agglutination. Record results.
8. Confirm validity of all negative reactions by using IgG sensitised red cells (see **Controls and Advice**).

\* If a weak direct antiglobulin test (2+ or less) was observed on the sensitised cells, 3-4 drops of eluate may be used to increase the sensitivity of the test.

### B. Bio-Rad-ID Micro Typing Technique

1. Prepare a 0.8% suspension of washed red cells in Bio-Rad ID-Diluent 2.
2. Remove aluminium foil from as many Bio-Rad LISS/Coombs or Coombs Anti-IgG gel card microtubes as needed.
3. Place in appropriate microtube: 50µl of red cell suspension and 25µl of eluate.
4. Place in another microtube: 50µl of red cell suspension and 25µl of last wash supernatant.
5. Incubate the LISS/Coombs ID-Card(s) for 15 minutes at 37°C.
6. Centrifuge the LISS/Coombs ID-Card(s) in the Bio-Rad ID-Card centrifuge.
7. Read macroscopically for agglutination.

### C. Ortho BioVue Typing Technique

1. Prepare a 0.8% suspension of washed red cells in 0.8% Ortho Red Cell Diluent.
2. Remove aluminium foil from as many Ortho AHG polyspecific or AHG Anti-IgG cassette reaction chambers as needed.
3. Place in appropriate reaction chamber: 50µl of test red cell suspension and 40µl of eluate.
4. Place in another reaction chamber: 50µl of red cell suspension and 40µl of last wash supernatant.
5. Incubate the cassette(s) for 15 minutes at 37°C.
6. Centrifuge cassette(s) the cassette in an Ortho BioVue System Centrifuge.
7. Read macroscopically for agglutination.

## INTERPRETATION OF RESULTS

1. **Positive:** Agglutination of red cells constitutes a positive test result and within the accepted limitations of the test procedure, indicates that one or more antibodies have been recovered from the sensitised red cells.
2. **Negative:** No agglutination of red cells constitutes a negative result and within the accepted limitations of the test procedure, indicates that no antibody has been recovered from the sensitised red cells.
3. Results of last wash supernatant must be negative. The test must be repeated if positive results are obtained.

## LIMITATIONS

1. The activity of the eluate is limited by the amount of antibody bound to the red cells, the amount of dissociation of antibody during the wash procedure and the degree to which immunoglobulin is denatured by the low pH during dissociation.
2. Contamination of eluate with unbound antibody due to inadequate washing of the red cells during the elution procedure may limit the activity of the eluate.
3. Failure to adjust pH to proper range may result in haemolysis.
4. Excess dilution of the eluate by adding excessive amounts of "Base Buffering Solution" when adjusting the pH of the eluate may cause weaker or false negative results.
5. Red cells used for elution studies should not be used for phenotyping.
6. 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
  - Deviation from the recommended techniques

## SPECIFIC PERFORMANCE CHARACTERISTICS

1. The kit has been characterised by all the procedures mentioned in the **Recommended Techniques**.
2. Prior to release, each lot of Lorne Red Cell-Elute has been tested by **Recommended Techniques** and been shown to elute a wide range of IgG antibodies from sensitised red cells.
3. The kit complies with the recommendations contained in the latest issue of the Guidelines for the UK Blood Transfusion Services.

## DISCLAIMER

1. The user is responsible for the performance of the kit by any method other than those mentioned in the **Recommended Technique**.
2. Any deviations from the **Recommended Technique** should be validated prior to use.

## BIBLIOGRAPHY

1. British Committee for Standards in Haematology, Blood Transfusion Task Force. Recommendations for evaluation, validation and implementation of new techniques for blood grouping, antibody screening and cross matching. *Transfusion Medicine*, 1995, **5**, 145-150.
2. Guidelines for the Blood Transfusion Service in the United Kingdom. H.M.S.O. Current Edition.
3. 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.
4. Rekvig OP, Hannestad K. Acid Elution of Blood Group Antibodies from Intact Erythrocytes. *Vox Sang* 1977: 33:280.
5. Widmann FK. Ed. Arlington. VA: American Association of Blood Banks 1985:235.

## AVAILABLE REAGENT SIZES







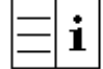
	Pack size	Catalogue Number
Red Cell Elute	10 tests/kit	930110

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## TABLE OF SYMBOLS

	Batch Number		<i>In-vitro</i> Diagnostic
	Catalogue Reference		Store At
	Expiry Date		Manufacturer
	Read Pack Insert		