LORNE LABORATORIES LTD.

GREAT BRITAIN

MONOCLONAL BLOOD GROUPING REAGENTS

DIRECTIONS FOR USE

Anti-Le^b Monoclonal: For Tube Technique.

SUMMARY

The Lewis system antigens are not an integral part of the red cell membrane and are produced by tissue cells and found primarily in plasma and watery secretions. Red cells acquire Lewis antigens by adsorption from surrounding plasma. The amount of Lewis antigen expressed on a cell can vary with the cell's ABO phenotype. Anti-Le^a and Anti-Le^b have not been associated with Haemolytic Disease of the Newborn.

Anti-Le^a	Anti-Le^b	Phenotype	Caucasians[1]	Afro-Americans[1]
+	0	**Le(a+b-)**	22%	23%
0	+	**Le(a-b+)**	72%	55%
0	0	**Le(a-b-)**	6%	22%
+	+	**Le(a+b+)**	Rare	Rare

INTENDED PURPOSE

This reagent is a blood grouping reagent intended to be used to qualitatively determine the presence or absence of Le^b antigens (LE2) on the red cells of blood donors or patients requiring a blood transfusion when tested in accordance with the recommended techniques stated in this IFU.

Principle

The reagent contains antibodies to the Le^b antigen on human red cells and will cause direct agglutination (clumping) of red cells, that carry the Le^b antigen, after centrifugation. No agglutination (no clumping) generally indicates the absence of the Le^b antigen (see **Limitations**).

REAGENTS

Lorne Monoclonal Anti-Le^b blood grouping reagent contains mouse monoclonal IgM antibodies, diluted in a phosphate buffer containing sodium chloride, EDTA, bovine albumin and macromolecular potentiators (10.0 g%). Anti-Le^b is manufactured with Clone LEB2. The reagent does not contain or consist of CMR substances, or endocrine disrupting substances or that could result in sensitisation or an allergic reaction by the user. The reagent is supplied at optimal dilution for use with all the recommended techniques stated below without the need for further dilution or addition. For lot reference number and expiry date see **Vial Label**.

STORAGE

Reagent vials should be stored at 2 - 8ºC on receipt. Prolonged storage at temperatures outside this range may result in accelerated loss of reagent reactivity. This reagent has undergone transportation stability studies at 37ºC and –25ºC as described in document BS EN ISO 23640:2015.

SAMPLE COLLECTION AND PREPARATION

Blood samples can be collected into EDTA, citrate, CPDA anticoagulants or as a clotted sample. The samples should be tested as soon as possible following collection. If a delay in testing should occur, store the samples at 2-8°C. Samples displaying gross haemolysis or microbial contamination should not be used for testing. Blood samples showing evidence of lysis may give unreliable results. It is essential (see "Limitations" section) to wash all blood samples with PBS or Isotonic saline before being tested.

PRECAUTIONS

1. The reagent is intended for *in vitro* diagnostic use only.
2. If a reagent vial is cracked or leaking, discard the contents immediately.
3. Do not use the reagents past the expiration date (see **Vial Label**).
4. Do not use the reagents if a precipitate is present.
5. Protective clothing should be worn when handling the reagents, such as disposable gloves and a laboratory coat.
6. The reagent has been filtered through a 0.2 µm capsule to reduce the bio-burden, but is not supplied sterile. 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. The reagent 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. No known tests can guarantee that products derived from human or animal sources are free from infectious agents. 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 the reagent and decontamination of a spillage site see **Material Safety Data Sheets**, available on request.

CONTROLS AND ADVICE

1. It is recommended a positive and negative control be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected results.
2. When typing red cells from a patient who is diagnosed with a disease that causes the red cells to become coated with antibody or other proteins (such as HDN, AIHA), it is important to test the patient's red cells using Lorne's reagent negative control (Monoclonal Rh Control (catalogue 640010).
3. Before use, let the reagent warm up to room temperature. As soon as the reagent has been used, put the reagent back in storage at 2-8ºC.
4. In the **Tube Technique** one volume is approximately 50µl when using the vial dropper provided.
5. The use of the reagent 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 reagent is in use.
6. The user must the determine suitability of the reagent for use in other techniques.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED

- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- PBS solution (pH 6.8–7.2) or Isotonic saline solution (pH 6.5–7.5).
- Positive and negative control red cells:
  Le(b+)(positive control) and Le(b–) (negative control).
- Test tube centrifuge.
- Volumetric pipettes.

RECOMMENDED TECHNIQUE

A. Tube Technique

1. Prepare a 2-5% suspension of washed red cells in PBS or Isotonic saline.
2. Place in a labelled test tube: 1 volume of Lorne Anti-Leb reagent and 1 volume of red cell suspension.
3. Mix thoroughly and incubate at room temperature for 15 minutes.
4. Centrifuge all tubes for 20 seconds at 1000 rcf or for a suitable alternative time and force.
5. Gently resuspend red cell button and read macroscopically for agglutination

INTERPRETATION OF TEST RESULTS

1. **Positive:** Agglutination of the red cells constitutes a positive test result and within accepted limitations of test procedure, indicates the presence of the Lewis b antigen on the red cells.
2. **Negative:** No agglutination of the red cells constitutes a negative result and within accepted limitations of the test procedure, indicates absence of the Lewis b antigen on the red cells.
3. **Control:** Test results of cells that are agglutinated using the reagent negative control shall be excluded, as the agglutination is most probably caused by the effect of the macromolecular potentiators in the reagent on sensitised cells.

STABILITY OF THE REACTIONS

1. Tests should be read immediately after centrifugation. Delays may result in dissociation of antigen-antibody complexes leading to false negative, or weak positive reactions.
2. Caution should be exercised in the interpretation of results of tests performed at temperatures other than those recommended.

LIMITATIONS

1. Lorne Anti-Leb reagent must only be used with washed red cells suspended in PBS or Isotonic saline because Lewis antigens are present in plasma. Cells suspended in plasma/serum **cannot** be used since the soluble antigen present may neutralise the test reagent, giving false negative results.
2. Weaker reactions may occur when Anti-Le^b is tested against A_1 or A_1B Le(b+) red cells because amount of Lewis antigen expressed on red cell can vary with cell's ABO phenotype.
3. Red cells of most new-borns will type Le(a-b-) with monoclonal or human anti-Lewis reagents.
4. The Lewis phenotypes of children under six years of age cannot be accurately determined. Red cell Lewis antigens are weaker during pregnancy and some women with red cells of the Le(a-b+) phenotype may type as Le(a-b-) whilst pregnant.
5. Stored blood may give weaker reactions than fresh blood
6. False positive or false negative results may also occur due to:
   - Contamination of test materials
   - Improper storage, cell concentration, incubation time or temperature
   - Improper or excessive centrifugation
   - Deviation from the recommended techniques

SPECIFIC PERFORMANCE CHARACTERISTICS

1. Prior to release, each lot of reagent was tested using the recommended test methods listed in this IFU. The tests complied with the test requirements as stated in the current version/issue of the "Guidelines for the Blood Transfusion Services in the United Kingdom".
2. Specificity of source Monoclonal antibodies is demonstrated using a panel of antigen-negative cells.
3. The Quality Control of the reagents was performed using red cells with phenotypes that were verified by a UK blood transfusion centre and had been washed with PBS or Isotonic saline prior to use.

DISCLAIMER

1. The user is responsible for the performance of the reagent by any method other than those mentioned in the **Recommended Technique**.
2. Any deviations from the **Recommended Technique** should be validated prior to use[5].

BIBLIOGRAPHY

1. Marion E.Reid & Christine Lomas-Francis, Blood Group Antigens & Antibodies, SBB Books, New York 2007; Page 189.
2. Issitt PD. Applied Blood Group Serology, 3rd Edition. Montgomery Scientific, Miami 1985; Chapter 7.
3. AABB Technical Manual, 16th edition, AABB 2008.
4. Guidelines for the Blood Transfusion Service in the United Kingdom, 6th Edition 2002. The Stationary Office.
5. 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.

AVAILABLE REAGENT SIZES

	Vial Size	Catalogue Number	Tests per vial
Anti-Le^b Monoclonal	2 ml	631002	40
	1000 ml	631000*	20,000

***This size is For Further Manufacturing Use (FFMU) only is therefore not CE marked.**

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