# Epiclone™ Anti-Le$^a$ and Anti-Le$^b$

**Murine Monoclonal IgM Phenotyping Reagents**

**FOR TUBE METHOD**

WARNING:
Contains
Sodium Azide 0.1%,
Harmful if swallowed.

IVD

Caution:
Handle as if capable
of transmitting
infection

immulab

**METHOD SUMMARY**

| | Tube | |
|---|---|---|
| **Reagent** | Anti-Le$^a$ | Anti-Le$^b$ |
| **Validated Methods** | Yes | Yes |
| **Reagent Volume** | 2 | 1 |
| **Cell Volume** | 1 | 1 |
| **Cell Concentration** | 3-5% | 2-3% |
| **Incubation Time** | 15 mins | 15 mins |
| **Temperature** | Room Temp | Room Temp |
| **Spin (Speed/Time)** | Low for 30 secs | High for 10 secs |

**REAGENT DESCRIPTION**

Epiclone™ Anti-Le$^a$ and Epiclone™ Anti-Le$^b$ monoclonal phenotyping reagents are prepared from murine monoclonal IgM antibodies. When used by the recommended methods these reagents will cause agglutination of the red cells carrying the specific Le$^a$ and/or Le$^b$ antigens. Epiclone™ Anti-Le$^a$ and Anti-Le$^b$ phenotyping reagents have been tested against red cell samples that are positive for the appropriate antigen to ensure adequate potency, and against a panel of cells that are negative for the appropriate antigen to ensure specificity. The reagents contain Bovine Albumin, macromolecular potentiators and Sodium Azide as a preservative. Each reagent has been optimised for use without further dilutions or additions. The clones used to produce these reagents are: Epiclone™ Anti-Le$^a$ is 3C9 and Epiclone™ Anti-Le$^b$ is LEB1.

**STORAGE CONDITIONS**

Store at 2° to 8°C (Refrigerate. Do Not Freeze).

**PRINCIPLE OF THE TEST**

The agglutination of red cells by a specific reagent indicates the presence of the corresponding antigen on those cells, whilst a negative reaction signifies the absence of the corresponding antigen. Red cells expressing the Le$^a$ and Le$^b$ antigens will agglutinate in the presence of the corresponding specific antibody in Epiclone™ Anti-Le$^a$ and/or Epiclone™ Anti-Le$^b$.

**BACKGROUND**

**Blood Group System**

The Lewis blood group system was first reported by Mourant who described the antibody anti-Le$^a$ in 1946. The antithetical antibody, anti-Le$^b$ was reported by Andresen in 1948.

Most examples of the antibodies are non-immune and of minor clinical significance, although anti-Le$^a$ has been reported as the cause of haemolytic transfusion reactions. Neither antibody causes Haemolytic Disease of the Foetus and Newborn (HDFN).

**Antigen Characteristics**

The Lewis system antigens are not intrinsic red cell membrane antigens, but are located on glycosphingolipids that are readily adsorbed onto the red cells from plasma. The production of Lewis antigens is dependent on the genes of the Secretor and Lewis systems.

Lewis antigens can be found in soluble form in saliva and all body fluids, except CSF (Cerebrospinal Fluid). They are also expressed on lymphocytes, monocytes, platelets and tissues of the pancreas, stomach mucosa, small and large intestines, skeletal muscle, renal cortex and adrenal glands. Lewis antigens are not fully developed on cord cells. Lewis antigen expression may be lost in some disease states such as infectious mononucleosis and hepatic cirrhosis, and is often greatly reduced during pregnancy. Women with a transient Le(a-b-) phenotype during pregnancy may produce Lewis antibodies.

Four Lewis phenotypes occur; Le(a+b-), Le(a-b+), Le(a+b+) and Le(a-b-). The majority of anti-Le$^a$ and anti-Le$^b$ antibodies are formed by Le(a-b-) individuals. They are usually IgM, often bind complement, react best within the 4° to 25°C range and are commonly enhanced by enzyme treatment of the red cells.

**Gene Frequency**

The expected results and frequencies of the phenotypes in the Australian blood donor population are given below:

| Reactions obtained with: | | Phenotype | Frequency |
|---|---|---|---|
| Anti-Le$^a$ | Anti-Le$^b$ | | |
| + | 0 | Le(a+b-) | 0.2170 |
| 0 | + | Le(a-b+) | 0.7210 |
| + | + | Le(a+b+) | <0.0001 |
| 0 | 0 | Le(a-b-) | 0.0620 |

The phenotype Le(a+b+), whilst absent or rare in most other populations, is frequent in Australian Aboriginals (10%), Polynesians (10-40%), and Asians (3-27%). The Le(a-b-) phenotype is almost 30% in Negroids and may reach 40% in some African populations.

**SPECIMEN COLLECTION AND PREPARATION**

Blood samples should be withdrawn aseptically with or without the addition of anticoagulants. Tests should be performed as soon as possible after collection of the sample. If testing the blood samples is delayed, samples should be stored between 2° to 8°C. Samples collected into EDTA or Heparin may be tested up to 7 days from the date of withdrawal provided storage has been at 2° to 8°C. Clotted samples may be tested up to 14 days from the date of withdrawal provided storage has been at 2° to 8°C.

Samples collected in Citrate may be tested up to 35 days from the date of withdrawal provided storage has been at 2° to 8°C. Cells may also be stored in Celpresol™ for up to 42 days.

Red cells should be washed three times in buffered isotonic saline, pH 7.0 to 7.2 or Celpresol™ prior to use.

**RECOMMENDED METHODS**

**Tube Method - Epiclone™ Anti-Le$^a$**

1. Prepare a 3-5% suspension of test red cells in buffered isotonic saline or in Celpresol™.
2. Add 2 drops of Epiclone™ Anti-Le$^a$ phenotyping reagent to an appropriately labelled, clean small glass test tube (10x75mm or 12x75mm).
3. Add 1 drop of the suspension of test red cells.
4. Mix well and incubate at room temperature for 15 minutes.
5. Centrifuge at low speed (500rcf) for 30 seconds*.
6. Gently agitate the tube to dislodge the red cells and examine for agglutination. Record results.

Note: * Or centrifuge at a speed and time appropriate for the centrifuge in use.

**Tube Method - Epiclone™ Anti-Le$^b$**

1. Prepare a 2-3% suspension of test red cells in buffered isotonic saline or Celpresol™.
2. Add 1 drop of Epiclone™ Anti-Le$^b$ phenotyping reagent to an appropriately labelled, clean small glass test tube (10x75mm or 12x75mm).
3. Add 1 drop of the suspension of test red cells.
4. Mix well and incubate at room temperature for 15 minutes.
5. Centrifuge at high speed (1000rcf) for 10 seconds*.
6. Gently agitate the tube to dislodge the red cells and examine for agglutination. Record results.

Note: * Or centrifuge at a speed and time appropriate for the centrifuge in use.

**CONTROLS**
The use of controls is essential in the performance of all blood grouping tests. Control samples should be tested in parallel with the test sample.

Positive Control – red cells known to be positive for the antigen as appropriate for the phenotyping reagent in use. Where possible, heterozygous cells should be used.

Negative Control – red cells known to lack the antigen as appropriate for the phenotyping reagent in use.

**INTERPRETATION OF RESULTS**
Results should be interpreted immediately upon completion of the test procedure. Agglutination of the test red cells constitutes a positive result and indicates the presence of the appropriate Lewis antigen. No agglutination of the test red cells indicates the absence of the relevant Lewis antigen.

Most murine monoclonal IgM Lewis antibodies are unable to directly agglutinate antigen-positive cells in saline, presumably due to low antigen numbers on the red cells. For this reason potentiators are commonly used to enable these antibodies to be used in saline environments as direct agglutinators. As such, the use of Positive and Negative Controls are important when assessing patient Lewis results. Persistent trace or gritty reactions should be interpreted as negative.

**LIMITATIONS OF PROCEDURE**
False results may occur due to:
1. Incorrect technique.
2. Presence of gross rouleaux.
3. Use of aged blood samples, reagents or supplementary materials.
4. Contaminated blood samples, reagents or supplementary materials.
5. Other deviation from the recommended test methods.
6. Incorrect concentrations of red cells or expired reagents.
7. Incorrect reading of results.
8. The reagents must only be used with washed red cells suspended in isotonic saline (buffered to pH 7.0 to 7.2) or Celpresol™. Traces of the patient's plasma may contain sufficient Lewis substance to neutralise the reagents and cause a false negative result.
9. Weaker reactions may be obtained if red cells are suspended in low ionic strength solutions.
10. Red cells treated with proteolytic enzymes may give false positive reactions.
11. Lewis antigens are not fully developed on the red cells of infants and valid results cannot be guaranteed on samples from children under two years of age.

**PRECAUTIONS**
1. For *in vitro* diagnostic use only.
2. The material from which this product was derived is from non-human sources, there is no risk of HIV or HBsAg infection. However good laboratory practice requires safe handling procedures are used.
3. Contains Sodium Azide 0.1% as a preservative. Products containing Sodium Azide can react with acids or oxidisers. Harmful if swallowed. May be harmful if inhaled. May cause irritation to skin and eyes. No chronic health effects known.
4. This product should be clear; turbidity may indicate bacterial contamination. The reagent should not be used if a precipitate or particles are present.
5. The bovine material used is from an approved source free from Bovine Spongiform Encephalopathy (BSE).

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|  | Consult instructions for use | IVD | *In vitro* diagnostic medical device | REF | Catalogue number |  | Temperature limitation |  | Manufacturer |

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|---|---|
|  | WARNING: Health Hazard |


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