Blood Grouping Reagent

Anti-Le^a (LE1)

Seraclone® Murine Monoclonal
(LEA2)

FOR IN-VITRO DIAGNOSTIC USE
For Tube Testing
MEETS FDA POTENCY REQUIREMENTS
U.S. License Number: 1845
Rx only

PACKAGE SIZE
[REF] 808403100 [VOL] 2 mL Seraclone® Anti-Le^a (LE1)

INTENDED USE
For the determination of the Le^a (LE1) antigen of red blood cells using the tube test.

SUMMARY
Lewis antigens are not intrinsic to red blood cells but are absorbed from plasma onto the membrane. Antibodies to Le^a and Le^b are almost always IgM, do not cross the placenta and are therefore not associated with hemolytic disease of the fetus and newborn (HDFN). Anti-Le^a has been rarely implicated in hemolytic transfusion reactions (HTR).[1]

The frequencies of the common phenotypes are shown in the table.

Phenotypes in the Lewis System and their incidence %[1]

Phenotype	Whites	Blacks
Le (a+b-)	22	23
Le (a-b+)	72	55
Le (a-b-)	6	22
Le (a+b+)	Rare	Rare

Bio-Rad Seraclone® Anti-Le^a Blood Group Reagent is used to test for the presence or absence of the Le^a antigen. Bio-Rad Seraclone® Anti-Le^a is used principally in the resolution of antibody problems or in family studies.

PRINCIPLES OF THE TEST
The test principle is hemagglutination. The antibodies in Seraclone® Anti-Le^a (LE1) bind to the corresponding antigens on red blood cells and cause an antigen-antibody reaction visible as red blood cell agglutination.

REAGENT
[IVD]
OBSERVABLE INDICATIONS.
Do not use if markedly turbid.
Do not use damaged vials.

As the reactive component Anti-Le^a (LE1) contains murine monoclonal antibody of the immunoglobulin class IgM.
They are derived from hybridoma cell lines which are created by fusing mouse antibody producing B lymphocytes with mouse myeloma cells and demonstrate consistent specificity and reproducibility characteristic for monoclonal antibodies.

Antibodies are diluted in an isotonic saline solution containing bovine albumine and macromolecular potentiators.

Seraclone® Anti-Le^a (LE1) clone LEA2 (IgM)

Preservative: 0.1% Sodium azide

PRECAUTIONS
- For In-vitro diagnostic use.
- Store at 2 to 8°C.
- Do not use beyond the expiration date.
- Do not use if turbid.
- Use only Phosphate Buffered Saline (PBS) for suspension.
- Handle and dispose of reagents as potentially infectious.
- Caution: Do not pipette by mouth. The absence of murine viruses has not been determined.
- Caution: This Product Contains Natural Rubber Latex Which May Cause Allergic Reactions.
- Warning: Contains sodium azide (NaN_3), which may react with lead or copper plumbing to form explosive azides. If discarded in the sink, flush with large amounts of water to prevent the build-up of explosive metal azides.
- The bovine albumin used for the production of this reagent is sourced from donor animals of US origin that have been inspected and certified by US Veterinary Service inspectors to be disease-free.
- Consult **downloads.bio-rad.com** to download the valid version of this instruction for use.

SPECIMEN COLLECTION
Fresh samples of clotted, EDTA or citrate anticoagulated whole blood collected following general blood sampling guidelines are acceptable. The specimen should be tested as soon as possible after collection. If testing is delayed, EDTA and clotted specimens should be stored at 2 to 8°C, citrated specimens (donor segments) at 1 to 6°C.

Note: Blood specimens exhibiting gross hemolysis or contamination should not be used.
Clotted samples or those collected in EDTA may be tested within ten days from collection. Donor blood stored in citrate anticoagulant may be tested until the expiration date of the donor unit.

MATERIALS
Materials provided
- Seraclone® Anti-Le^a (LE1)

Materials required but not provided
- Pipettes
- Phospate Buffered Saline (PBS pH 7.3 ±0.2), **DO NOT** use isotonic saline
- Glass tubes 10 x 75mm or 12 x 75mm
- Serological centrifuge
- Interval timer
- Markers
- Agglutination viewer (optional).

TEST PROCEDURE
Tube test
1. Wash the red blood cells to be tested at least 2 times with PBS.
2. Prepare a 2 to 3 % suspension of the washed red blood cells to be tested in PBS pH 7.3 ±0.2.
3. Place one drop reagent into an appropriately labelled tube.
4. Add one drop (approx. 40 to 50µL) red blood cell suspension into the tube and mix.
5. Incubate for 15 minutes at room temperature (15 to 30°C).
6. Centrifuge for:
   a. 10 seconds at 800 to 1000 x g, or
   b. at a time and speed appropriate for the centrifuge calibration.
7. Gently dislodge red blood cell button and observe for macroscopic agglutination. Negative reactions may be examined with an agglutination viewer, however, microscopic examination is not recommended.
8. Record results.

STABILITY OF REACTION
Following centrifugation, all tube tests should be read immediately and results interpreted without delay. Time delays may cause a dissociation of the antigen-antibody complexes resulting to false negative or more often weak positive reactions.

QUALITY CONTROL
The reactivity of all blood typing reagents should be confirmed by testing with known positive and negative red blood cells on each day of use.
To confirm the reactivity or specificity of Bio-Rad Monoclonal Anti-Le^a Blood Grouping Reagent, it should be tested with antigen-positive and antigen-negative red blood cells, respectively. The reagent is satisfactory for use if it reacts only with antigen-positive red blood cells.

INTERPRETATION OF RESULTS
Agglutination of the red blood cells is a positive result and indicates the presence of the corresponding antigen. No agglutination is a negative result and indicates the absence of the corresponding antigen.
An agglutination viewer may facilitate the reading of tube tests (as recommended by the AABB Technical Manual)[1].
Frequencies in the population are listed in the "Summary" section of this package insert.

LIMITATIONS
- The Lewis antigen status varies greatly between the red cells of one individual and another. As a consequence, positive reactions obtained with specimens of different patients may vary in strength. In properly calibrated test systems, Seraclone™ Anti-Le^a (LE1) should produce positive reactions of 2+ to 4+ with group B or O cells. Reactions weaker than 1+ with group B or O test or control red cells should be investigated, as they may be an indication that the environmental temperature, centrifugation speed or time, or volume of reagent or cell suspension used are not optimum or that the reagent is deteriorating.
- Red cell Lewis antigens are known to deteriorate with age. Stronger test reactions will be obtained with red cells from fresh specimens than with older samples.
- The red cells of most newborns will type as Le$^{(a-b-)}$ with murine monoclonal, animal, or human Anti-Lewis reagents. However, some infants' specimens may be encountered that will produce weakly positive reactions in direct agglutination tests with murine monoclonal Anti-Le$^{(a)}$, but not with human or animal reagents of the same specificity.
- The Lewis phenotypes of children under six years of age cannot be accurately determined.
- The Le$^{(a)}$ and Le$^{(b)}$ antigens may be reduced during pregnancy.
- Samples with cold agglutinins or rouleaux formation may show false positive results in testing with monoclonal antibodies. Results on these samples must be interpreted with caution. False positive results or reaction suspected to be due to cold agglutinins should be resolved according to in-house procedures.
- Samples prepared without washing will give invalid results.
- Grossly icteric blood samples, blood samples with abnormally high concentrations of protein or blood samples from patients who have received plasma expanders of high molecular weight may give false positive results.
- Some conditions that may cause false positive results are:

M Bio-Rad Medical Diagnostics GmbH
Industriestr. 1, D-63303 Dreieich, Germany

- Contamination of sample or reagents
- Autoantibodies
- Improper storage or preparation of red blood cells
- Cross reactions with patient's medication (e.g. antibiotics)
- Mixed field reaction due to patients pre-transfusion history

- The formulation (enhancing diluent in combination with antibody concentration) of this Blood Grouping Reagent limits the test procedure to:
  - Tests must not be interpreted microscopically
  - Prolonged incubation time may increase rouleaux formation which might be interpreted as false positive results
  - Unspecific +/- results may be interpreted sometimes, but can clearly be differentiated from true positive results
- Lewis substance present in human serum or plasma can neutralize the Lewis antibodies, thus red blood cells must be washed and resuspended in Phosphat Buffered Saline (PBS) for testing.
- It is recommended to use the Seraclone Anti-Le^{a} in parallel with an Anti-Le^{b} Blood Grouping Reagent.

SPECIFIC PERFORMANCE CHARACTERISTICS

Testing is performed in accordance with FDA recommended methods.
The final release testing is performed according to the product specific SOPs. As part of the release process each lot of Bio-Rad Blood Group Reagent is tested according to the package insert method against a panel of antigen positive red blood cells to insure suitable reactivity. The products meet FDA potency requirements. The specificity testing for the presence of contaminating antibodies is performed according to the product specific SOPs.
For the product performance it is necessary to adhere to the recommended method in the instructions for use.

The performance of the Bio-Rad Anti-Le^{a} was confirmed against a FDA approved reference reagent in a Multi Center Field Trial.

For Technical Support or further product information, contact Bio-Rad Laboratories, Inc., at 800-224-6723.

NOTE

Manual techniques are to be performed according to the manufacturer's instructions. Each deviation from these instructions is the sole responsibility of the user.
Used tests must be discarded as hazardous material. Manage waste according to local, state and national regulations.

GLOSSARY OF SYMBOLS

Symbol	Definition	Symbol	Definition
[LOT]	Batch Code	[IVD]	*In vitro* diagnostic medical device
!	Consult the instructions for use for important cautionary information such as warnings and precautions	I	Consult instructions for use
M	Manufacturer	e	Use by YYYY-MM-DD
s	Contains sufficient quantity for <n> tests	[REF]	Catalog number
t	Temperature limitation	[VOL]	Volume

BIBLIOGRAPHY

1. John D. Roback, MD et al. Technical Manual 17th Edition, Bethesda, MA: AABB, 2011.

Key: Underline = Addition of changes ◄ = Deletion of text