US

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: 1798

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

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

Principle 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
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 a 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 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.

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.
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 (drop volume 40 to 50 µl)
- 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
- Optical aid (optional). The use of an optical aid for agglutination reading must be vaildated by the user.

Test Procedure
Tube test
1. Wash the red blood cells to be tested at least 2 times with PBS
2. Suspend red blood cells to be tested 2 to 3 % with PBS pH 7.3 ± 0.2.
3. In an appropriately marked tube mix one drop reagent and one drop of red blood cell suspension.
4. Incubate for 15 minutes at room temperature (20 to 24°C).
5. Centrifuge for 10 seconds at 800 - 1000 x g.
6. Gently dislodge red blood cell button and observe for agglutination
7. Record results

A positive control with Le (a+b-) red blood cells and a negative control with Le (a-b+) red blood cells should performed in parallel.

Stability of the 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 Biotest 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.
It is recommended that a positive and a negative control be performed in parallel with testing.

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 Technial Manual, 15th edition).
Frequencies in the population are listed in the "Summary" section.

Limitations
- Samples with a positive direct antiglobulin test, 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. It is recommended that an appropriate control be tested in parallel.
- Samples prepared without washing will give invalid results
- Some conditions that may cause false positive results are:
  - Contamination of sample or reagents
  - Autoantibodies
  - Improper storage or preparation of red blood cells
  - Antibodies to antibiotics or other reagents
  - Cold Antibodies

Specific Performance Characteristics
Testing is performed in accordance with FDA recommended methods. The final release testing is performed according to the product specific SOPs. Each lot of Biotest Blood Group Reagent is tested in the Quality control by 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 Biotest 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 Biotest Diagnostics Corporation at 800-522-0090.

Note
Each facility should verify the optimum spin time for the specific centrifuge in use.
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
⚠	Caution, consult accompanying documents		Consult instructions for use.
	Manufacturer		Use by YYYY-MM-DD
	Contains sufficient quantity for <n> tests.	REF	Catalog number
	Temperature limitation	VOL	Volume

Bibliography
1. Mark E. Brecher, MD et al. Technical Manual 15th Edition, Bethesda, MA: AABB, 2005.

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