Product Manual

LTA (Lipoteichoic Acid) ELISA Kit

Catalog Number

AKR-5153 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Lipoteichoic acid (LTA) is a major component of the cell wall in bacteria that are gram-positive. These bacteria contain an inner "cytoplasmic" membrane and, outside it, a thick layer of peptidoglycan (as much as 80 nanometers). The structure of LTA is different depending on the species of Gram-positive bacteria and may have long chains of ribitol or glycerol phosphate. LTA is affixed to the cell membrane via a diacylglycerol and acts as regulator of autolytic wall enzymes known as muramidases. LTA can have immunogenic properties like other antigens and are able to stimulate specific immune responses.

LTA from bacteria may bind to target mammalian cells either through membrane phospholipids, or to specific proteins such as CD14 and Toll-like receptors. Binding to TLR-2 has shown to induce expression of the transcription factor NF-κB, which results in increasing expression of both activating and inhibiting apoptotic genes. Activation of NF-κB also stimulates activation of mitogen-activated protein kinases (MAPK) as well as phosphoinositide 3-kinase (PI3K).

Cell Biolabs' LTA ELISA Kit provides a quick and convenient system to measure LTA from mammalian serum, plasma, cell, or tissue samples; it provides sufficient reagents for up to 96 tests in a 96-well plate including standard curve and unknown samples. Detection sensitivity is 15.6 ng/mL.

Related Products

1. CBA-252: MTT Cell Proliferation Assay
2. CBA-253: WST-1 Cell Proliferation Assay Reagent

Kit Components

Box 1 (shipped at room temperature)

1. Anti-LTA Antibody Coated Plate (Part No. 51531B): One 96-well strip plate (8 x 12).
2. Biotinylated Anti-LTA Antibody (1000X) (Part No. 51532C): One 10 µL vial.
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. LTA Standard (Part No. 51533C): One 50 µL vial of 50 µg/mL LTA.

Materials Not Supplied

1. PBS
2. Microcentrifuge
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store the LTA Standard and the Biotinylated Anti-LTA Antibody at -20ºC. Avoid multiple freeze/thaw cycles. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Biotinylated Anti-LTA Antibody and Streptavidin Enzyme Conjugate: Immediately before use dilute the Anti-LTA Antibody and the Streptavidin Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

Prepare a dilution series of LTA standards in the concentration range of 0 to 1000 ng/mL into Assay Diluent (Table 1).

Standard Tubes	LTA Standard (µL)	Assay Diluent (µL)	LTA (ng/mL)
1	10	490	1000
2	250 of Tube #1	250	500
3	250 of Tube #2	250	250
4	250 of Tube #3	250	125
5	250 of Tube #4	250	62.5
6	250 of Tube #5	250	31.3
7	250 of Tube #6	250	15.6
8	0	250	0

Table 1. Preparation of LTA Standards

Preparation of Samples

- Serum: Avoid hemolyzed and lipemic blood samples. Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Aliquot samples for testing and store at -20ºC or colder. Perform dilutions in Assay Diluent as necessary.

- Plasma: Avoid hemolyzed and lipemic blood samples. Collect blood with heparin or citrate and centrifuge at 2000 x g and 4ºC for 10 minutes. Remove the plasma layer and store on ice. Avoid disturbing the white buffy layer. Aliquot samples for testing and store at -20ºC or colder. Perform dilutions in Assay Diluent as necessary.
- Cells or tissues: Homogenize 50-200 mg of the cell pellet or tissue in 0.5-2 mL of ice-cold PBS using a mortar and pestle or by dounce homogenization. Incubate the homogenate at 4°C for 20 minutes. Transfer the homogenate to a centrifuge tube and centrifuge at 12000 x g for 20 minutes. Recover the supernatant and transfer to a fresh tube. Store resuspended sample at -20°C or colder until ready to test by ELISA. Perform dilutions in Assay Diluent as necessary.

Assay Protocol

1. Add 100 µL of LTA unknown sample or standard to the Anti-LTA Antibody Coated Plate. Each LTA unknown sample, standard and blank should be assayed in duplicate.
2. Incubate at room temperature for 1 hour on an orbital shaker.
3. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
4. Add 100 µL of the diluted Biotinylated Anti-LTA antibody to each well. Incubate at room temperature for 1 hour on an orbital shaker.
5. Wash the strip wells 3 times according to step 3 above.
6. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker.
7. Wash the strip wells 3 times according to step 3 above. Proceed immediately to the next step.
8. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
9. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
10. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical results with the LTA ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

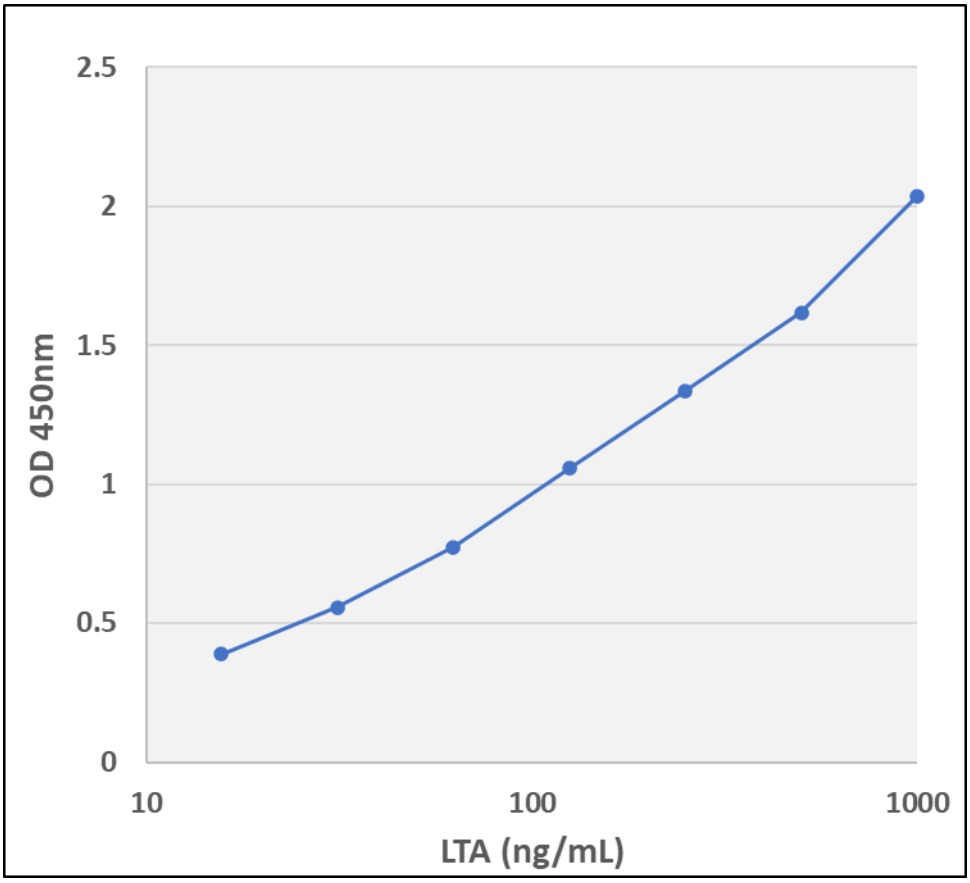

Figure 1: LTA ELISA Kit Standard Curve.

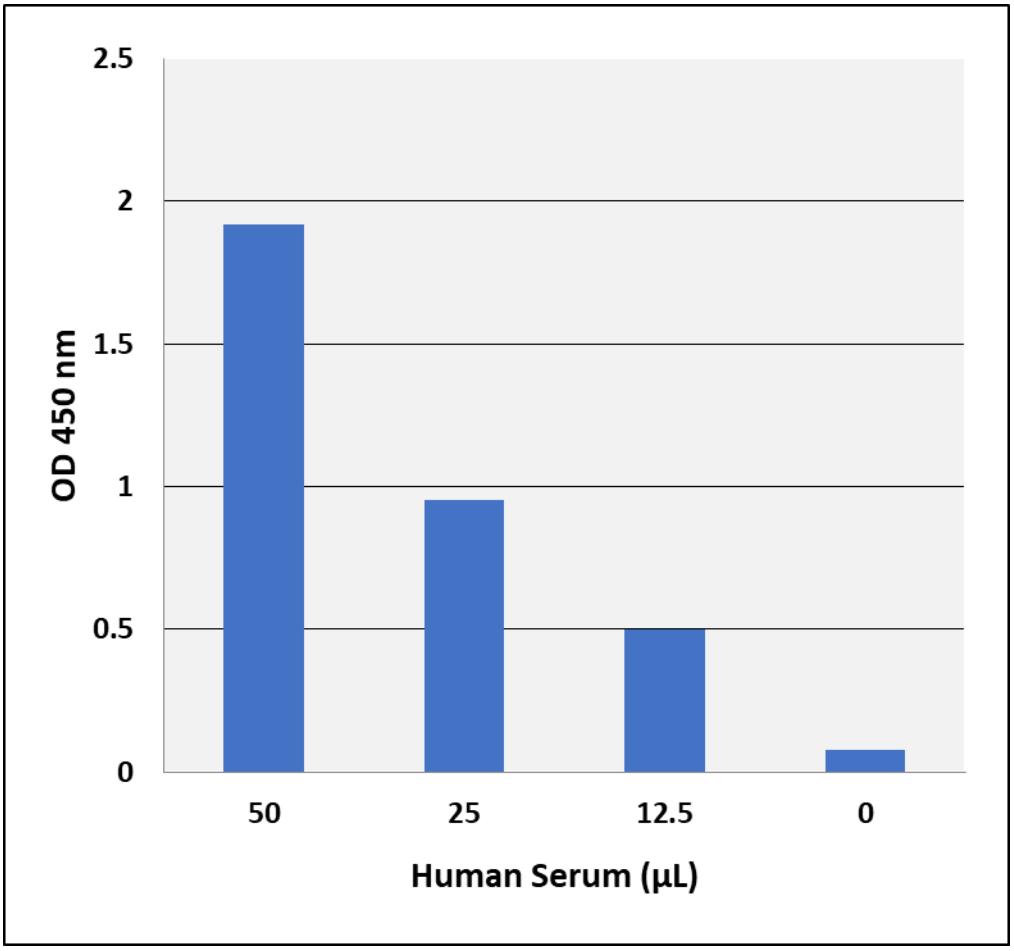

Figure 2: Detection of LTA in serum. Human serum samples were tested using the LTA ELISA Kit.

References

1. Ginsburg I (2002) *Lancet Infect. Dis*. **2**:171-179.
2. Richter SG, Elli D, Kim HK, Hendrickx APA, Sorg JA, Olaf Schneewind O, and Missiakas D (2013) *PNAS* **110**: 3531-3536
3. Percy MG Gründling A (2014) *Ann. Rev. Microbiol*. **68**: 81-100.

Warranty

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