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Lipopolysaccharide (LPS) Isolation Kit

Rev 5/19

(Catalog # K438-10; 10 isolations; Store at -20°C)

I. Introduction:

The outer membrane of gram-negative bacteria contains lipopolysaccharide (LPS), a low molecular weight carbohydrate with a molecular mass of 10-20 kDa. It is heterogeneous, and is composed of O antigen (a repeating glycan polymer), core oligosaccharide (which links the O antigen to **Lipid A** - the third component, and non-carbohydrate components such as phosphate and amino acids groups. Lipid A, has multiple fatty acids which serve to anchor LPS into the bacterial membrane allowing the O antigen and core oligosaccharide to protrude and contributes to a major part of the toxicity of gram-negative bacteria. It is also known as endotoxin. When consumed by animals, LPS induces a strong inflammatory response and/or sepsis. BioVision's LPS Isolation Kit uses bacterial membrane lysis buffer and protein digestion to yield micrograms of LPS from bacterial culture (approximately 1-4% of dry weight). This kit does not use chlorofrom or phenol like traditional methods. It will yield pure LPS in less than 2 hours that can be easily characterized and quantified!

II. Applications:

- Isolation of LPS from outer membrane of gram negative bacteria

III. Sample Types:

- A sweep from an overnight culture of bacteria grown on an LB plate resuspended in 10 ml of PBS routinely yields an OD of 0.6-1.5. 10 ml of *E. coli* culture produces a pellet approximately 10-100 mg in dry pellet weight with LPS yields 1-4% of dry weight.

IV.

Components	K438-10	Cap Code	Part Number
LPS Isolation Buffer Proteinase K (20 mg/ml)	100 ml 0.6 ml	NM Green	K438-10-1 K438-10-2

V. User Supplied Reagents and Equipment:

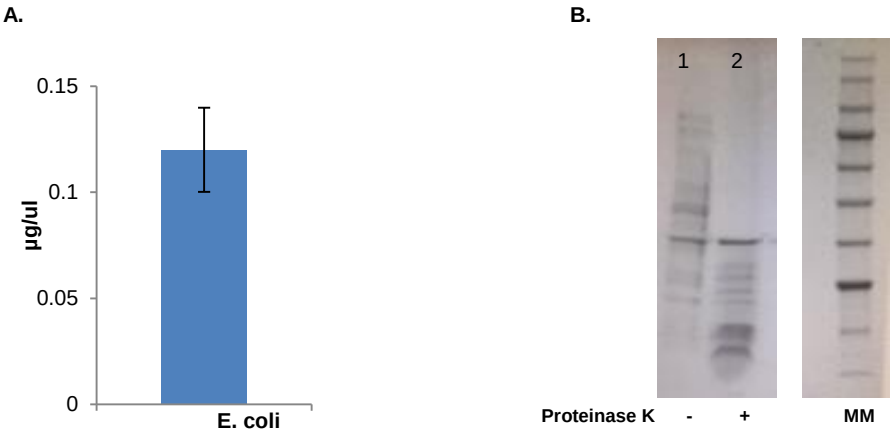
- Gram Negative Bacterial Strain
- LB Media Plates
- Bacteria culture media
- PBS
- Sterile Swabs
- Analytical Balance
- Sonicator
- Spectrophotometer
- SDS-PAGE Gel
- Running Buffer
- SDS-PAGE Apparatus
- Coomassie Stain

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protect from light. Before use, thaw LPS Isolation Buffer. If precipitation is observed in buffer, place bottle into 37°C water bath for 10 min and gently pulse-vortex to dissolve precipitate. Centrifuge Proteinase K prior to opening. Read the entire protocol before performing the assay.

VII. LPS Isolation Protocol:

1. **Sample Preparation:** Grow an isolated culture of bacteria overnight on LB media plate at 37°C. The next day, pre-weigh a 15 ml conical tube, then add 12 ml cold PBS, pH 7.2. With a sterile swab, sweep the bacteria growth from an LB media plate and resuspend (see Note) in cold PBS. Determine the concentration of bacteria in solution by evaluating the turbidity of culture with a Spectrophotometer: Remove 1 ml of bacteria suspended in PBS and add to cuvette. Place cuvette in Spectrophotometer and measure OD_{600} nm. *Ensure that the OD_{600} nm ≥0.6.*
   Note: To resuspend the bacteria, press the tip of swab against the inside wall of conical tube and rub the swab against the wall back and forth in 1 cm motions in length. This will prevent bacteria aggregates and ensure a homogeneous solution.
2. Centrifuge conical tube at 2500 x g for 10 min to pellet the bacteria. Decant supernatant and repeat centrifugation. Remove supernatant with pipette and discard. *Complete removal of supernatant is essential to accurately determine the weight of the pellet*. Reweigh conical tube and subtract weight of tube (measured previously) to determine weight of bacteria pellet. Multiply this value by 10 to determine the volume of LPS Isolation Buffer to add. Example: pellet = 10 mg; LPS Isolation Buffer Volume to add: 100 µl.
3. Sonicate the lysate 3 x 30 sec, in a continuous pulse, 2-10 watts to break-up aggregates of bacteria. *Ensure that the tube is on ice during sonication*. Incubate on ice 10 min to complete lysis.
4. Centrifuge mixture 10 min, 4°C at 2500 x g.
5. Transfer lysate to a clean 1.5 ml centrifuge tube. Then add Proteinase K to a final concentration of 0.1 mg/ml.
   Example: for every 20 mg bacterial pellet: Add 200 µl of LPS Isolation Buffer and 1 µl of Proteinase K.
6. Heat lysate samples at 60ºC for 60 min.
7. Centrifuge heated lysates for 10 min, 4°C at 2500 x g. Transfer supernatant to a fresh 1.5 ml tube. Quantify LPS using the phenol sulfuric acid detection method for carbohydrates (Cat# K645). Alternatively, purity of the LPS can be evaluated by adding 3X SDS-PAGE Loading Buffer (Cat# 2108), boiling for three min at 95ºC and then loading 20 µl of boiled sample onto 4-20% gradient SDS-PAGE Gel. Stain gel with Coomassie Blue stain (Cat# K810) and other carbohydrate detection method system.



Figures A. Concentration of LPS in lysates recovered from overnight culture of *E.coli* were quantified using the Total Carbohydrate Colorimetric Assay Kit. Carbohydrate standards were prepared in LPS Isolation Buffer. **B.** LPS isolated from *E. coli* using this protocol was loaded onto a 4-20% gradient SDS gel, run for 55 min at 140 V, and then stained with Coomassie blue protein stain. LPS is a 10-20 kDa carbohydrate that is associated with low molecular weight proteins and gives a characteristic ladder banding pattern in coomassie blue stained SDS-PAGE gels. Lane 1 represents *E. coli* lysate prior to Proteinase K digestion. Lane 2 illustrates lysate after Proteinase K digestion of proteins**. MM:** Molecular weight marker.

VII. Related Products:

3X SDS-PAGE Loading Buffer (2108-10)
Total Carbohydrate Colorimetric Assay Kit (K645)

FOR RESEARCH USE ONLY! Not to be used on humans.