3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Lipopolysaccharide (LPS) Isolation Kit

Catalog Number **MAK339**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The outer membrane of Gram-negative bacteria contains lipopolysaccharide (LPS), a low molecular mass carbohydrate with a molecular mass of 10-20 kDa. It is heterogeneous and 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. Also known as endotoxin, when consumed by animals, LPS induces a strong inflammatory response and/or sepsis.

The Lipopolysaccharide (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 chloroform or phenol like traditional methods, and it will yield pure LPS in less than 2 hours that can be easily characterized and quantified.

This kit is suitable for the isolation of LPS from the outer membrane of Gram-negative bacteria.

Components

The kit is sufficient for 10 isolations.

LPS Isolation Buffer Catalog Number MAK339A	100 mL
Proteinase K (20 mg/mL) Catalog Number MAK339B	0.6 mL

Reagents and Equipment Required but Not Provided.

- Gram-negative bacteria strain
- LB medium plates
- Bacteria culture media
- PBS
- Sterile swabs
- Analytical balance
- Sonicator
- Spectrophotometer
- TruPAGE™ Precast Gel, 4-20% (Catalog Number PCG2004)
- Sample Buffer, Laemmli, 2× (Catalog Number S3401)
- TruPAGE™ Running Buffer (TEA-Tricine SDS (Catalog Number PCG3001) or Tris-MOPS SDS Express (Catalog Number PCG3003)
- SDS-PAGE apparatus
- Brilliant Blue G Solution (Catalog Number B8522)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at –20 °C, protected from light upon receiving. Before use, thaw LPS Isolation Buffer. If precipitation is observed in buffer, place bottle into 37 °C water bath for 10 minutes and gently pulse-vortex to dissolve precipitate. Centrifuge Proteinase K prior to opening.

Preparation Instructions

Sample Preparation

1. Grow an isolated culture of bacteria overnight on LB medium plate at 37 °C.
2. The next day, pre-weigh a 15 mL conical tube, then add 12 mL of cold PBS, pH 7.2.
3. With a sterile swab, sweep the bacteria growth from an LB medium plate and resuspend in cold PBS. To resuspend the bacteria, press the tip of the swab against the inside wall of the 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.
4. 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 A_{600}. Ensure that the $A_{600} \geq 0.6$.

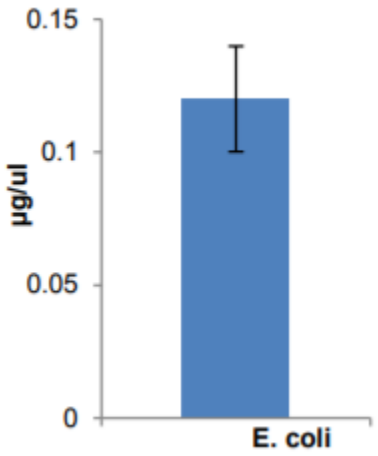
Procedure

LPS Isolation

1. Centrifuge conical tube at 2,500 × *g* for 10 minutes to pellet the bacteria.
2. Decant supernatant and repeat centrifugation.
3. Remove any remaining supernatant with pipette and discard. Complete removal of supernatant is essential to accurately determine the weight of the pellet.
4. 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 lysis buffer to add to the pellet.
   Example:
   pellet = 10 mg
   Lysis Buffer Volume to add: 100 µL
5. Sonicate the lysate 3 × 30 seconds, in a continuous pulse, with power set at 2–10 watts to break-up aggregates of bacteria. Ensure that the tube is on ice during sonication.
6. Incubate on ice for 10 minutes to complete lysis.
7. Centrifuge mixture for 10 minutes at 2,500 × *g* and 4 °C.
8. Transfer lysate to a clean 1.5 ml centrifuge tube.
9. Add Proteinase K to a final concentration of 0.1 mg/mL.
   Example:
   for 20 mg bacterial pellet
   Add 200 µL of lysis buffer and 1 µL of Proteinase K.
10. Heat lysate samples at 60 °C for 60 minutes.
11. Centrifuge heated lysates for 10 minutes at 2,500 × *g* and 4 °C.
12. Transfer supernatant to a fresh 1.5 ml tube. Quantify LPS using the Total Carbohydrate Assay Kit (MAK104). Alternatively, purity of the LPS can be evaluated by adding 2× Laemmli Sample Buffer (1:2 with sample), boiling sample for three minutes at 95 °C and then loading 20 µL of boiled sample onto a 4–20 % TruPAGE™ Precast Gel. Stain gel with Brilliant Blue G Solution.

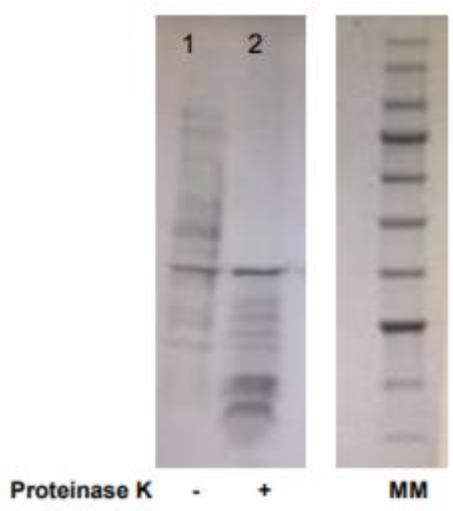
Results

Figure 1.
LPS Concentration using Carbohydrate Assay



Concentration of LPS in lysates recovered from overnight culture of *E. coli* were quantified using the Total Carbohydrate Assay Kit (MAK104). Carbohydrate standards were prepared in LPS Lysis Buffer.

Figure 2.
SDS-PAGE of LPS



LPS isolated from *E. coli* using this protocol was loaded onto a 4–20% gradient SDS gel, run for 55 minutes at 140 V, and then stained with Brilliant Blue G protein stain. LPS is a 10–20 kDa carbohydrate that is associated with low molecular mass proteins and gives a characteristic ladder banding pattern in Brilliant Blue G 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 mass marker.

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