Lipopolysaccharide Isolation Kit

Cat. No. LPS-006K **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Isolation of LPS from outer membrane of gram negative bacteria.
Applications	Isolation of LPS from outer membrane of gram negative bacteria.
Storage	Store kit at -20centigrade, protect from light. Before use, thaw LPS Isolation Buffer. If precipitation is observed in buffer, place bottle into 37centigrade water bath for 10 minutes and gently pulse-vortex to dissolve precipitate. Centrifuge Proteinase K prior to opening. Read entire protocol before performing the assay.
Kit Components	• LPS Isolation Buffer 100 ml • Proteinase K (20 mg/ml) 0.6 ml
Materials Required but Not Supplied	Gram negative bacteria strain LB media plates Bacteria culture media PBS, sterile swabs, analytical balance, sonicator Spectrophotometer SDS-PAGE Gel, running buffer, SPS-PAGE apparatus Coomassie stain
Target Species	Bacteria
Features & Benefits	• This kit does not use chlorofrom or phenol like traditional methods • Yields pure LPS in less than 2 hours that can be easily characterized and quantified

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Separation Protocol	1. Sample Preparation: Grow an isolated culture of bacteria overnight on LB media plate at 37centigrade. 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 OD600 nm. Ensure that the OD600 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 minutes 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 lysis buffer to add. Example: pellet = 10 mg; Lysis Buffer Volume to add: 100 µ 3. Sonicate the lysate 3 x 30 seconds, 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 minutes to complete lysis. 4. Centrifuge mixture 10 min, 4centigrade 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 µof lysis buffer and 1 µof Proteinase K. 6. Heat lysate samples at 60 ºC for 60 minutes. 7. Centrifuge heated lysates for 10 min, 4centigrade at 2500 x g. Transfer supernatant to a fresh 1.5 ml tube. Quantify LPS using the phenol sulfuric acid detection method for carbohydrates (K645). Alternatively, purity of the LPS can be evaluated by adding 3X SDS-PAGE Loading Buffer, boiling for three minutes at 95ºC

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	and then loading 20 µof boiled sample onto 4-20% gradient SDS-PAGE Gel. Stain gel with Coomassie Blue stain and other carbohydrate detection method system.
Sample Type	A sweep from an overnight culture of bacteria grown on an LB plate resuspended in 10 mls of PBS routinely yields an OD of 0.6-1.5. 10 mls of E. coli culture produces a pellet approximately 10-100 mgs in dry pellet weight with LPS yields 1-4% of dry weight.

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