Version 2a Last updated 11 July 2023

ab239718
LPS Extraction Kit

For the extraction of LPS from outer membrane of gram negative bacteria.

This product is for research use only and is not intended for diagnostic use.

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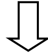
1. Overview

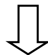
LPS Extraction Kit (ab239718) uses bacterial membrane lysis buffer and protein digestion to yield micrograms of LPS from bacterial culture (approximately 1-4% of dry weight). This kit does not use chloroform or phenol like traditional methods, and it will yield a solution of LPS and digested protein in less than 2 hours that can be easily characterized and quantified.

2. Protocol Summary

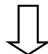
Grow an isolated culture of bacteria overnight on LB media plate at 37°C. The next day, sweep the bacteria growth from an LB media plate and resuspend in cold PBS.



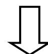
Determine the concentration of bacteria in solution by evaluating the turbidity of culture with a Spectrophotometer:



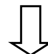
Centrifuge at 2500 x *g* for 10 min to pellet the bacteria. Decant supernatant and repeat centrifugation. Remove supernatant and discard. Determine weight of bacteria pellet. Add 10 volumes of lysis buffer.



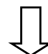
Sonicate the lysate 3 x 30 s, in a continuous pulse, 2-10 watts to break-up aggregates of bacteria. Keep on ice during sonication. Incubate on ice 10 min to complete lysis.



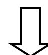
Centrifuge mixture 10 min, 4°C at 2500 x *g*.



Transfer lysate to a clean 1.5 mL centrifuge tube. Add Proteinase K to a final concentration of 0.1 mg/mL.



Heat lysate samples at 60 °C for 60 min.



Centrifuge heated lysates for 10 min, 4°C at 2500 x *g*. Transfer supernatant to a fresh 1.5 ml tube. Quantify LPS.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage conditio n
LPS Extraction Buffer	100 mL	-20°C
Proteinase K (20 mg/mL)	0.6 mL	-20°C

5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Gram negative bacteria strain.
- LB media plates.
- Bacteria culture media.
- PBS.
- Sterile swabs.
- Analytical balance.
- Sonicator.
- Spectrophotometer.
- SDS-PAGE Gel.
- Running buffer.
- SDS-PAGE apparatus.
- Coomassie stain.

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 LPS Extraction Buffer:

Before use, thaw LPS Extraction Buffer. If precipitation is observed in buffer, place bottle into 37°C water bath for 10 min and gently pulse-vortex to dissolve precipitate.

6.2 Proteinase K (20 mg/ml):

Centrifuge Proteinase K prior to opening.

7. Sample Preparation

7.1 Grow an isolated culture of bacteria overnight on LB media plate at 37°C.

7.2 The next day, pre-weigh a 15 mL conical tube, then add 12 mL cold PBS, pH 7.2.

7.3 With a sterile swab, sweep the bacteria growth from an LB media plate and resuspend (see Note) in cold PBS.

7.4 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 OD_{600} nm. Ensure that the OD_{600} nm ≥0.6.

Δ 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.
- 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 mgs in dry pellet weight with LPS yields 1-4% of dry weight.

8. Assay Procedure

8.1 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 lysis buffer to add. Example: pellet = 10 mg; Lysis Buffer Volume to add: 100 µL

8.2 Sonicate the lysate 3 x 30 s, 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.

8.3 Centrifuge mixture 10 min, 4°C at 2500 x *g*.

8.4 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 lysis buffer and 1 µL of Proteinase K.

8.5 Heat lysate samples at 60 °C for 60 min.

8.6 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. Alternatively, purity of the LPS can be evaluated by adding 3X SDS-PAGE Loading Buffer, boiling for three min at 95°C and then loading 20 µL of boiled sample onto 4-20% gradient SDS-PAGE Gel. Stain gel with Coomassie Blue stain and other carbohydrate detection method system.

9. Typical Data

Typical data provided **for demonstration purposes only**.

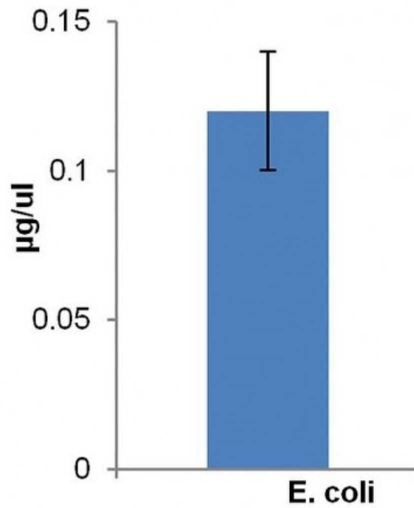


Figure 1. Concentration of LPS in lysates recovered from overnight culture of *E.coli* were quantified using a Total Carbohydrate Colorimetric Assay Kit. Carbohydrate standards were prepared in LPS Lysis Buffer.

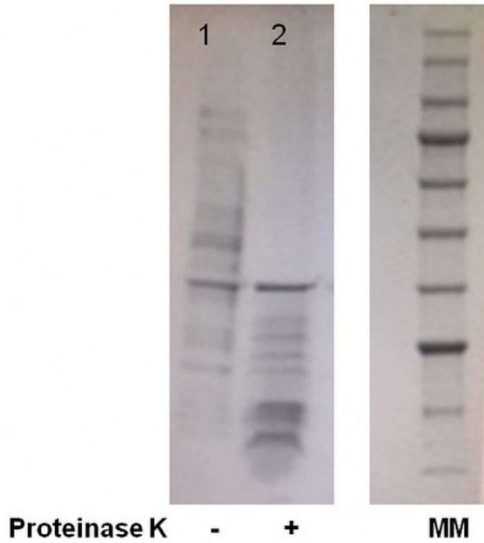


Figure 2. Digested protein extract from *E.coli* using this protocol was loaded onto a 4-20% gradient SDS gel, run for 55 min at 140 V, and then stained with Coomassie blue protein stain. The extract is associated with low molecular weight proteins and gives a characteristic ladder banding pattern in coomassie blue stained SDS-PAGE gels. LPS itself cannot be visualized with Coomassie blue staining. 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.

11. Notes

Technical Support

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