5. Add **800 ul** of Purification Buffer and mix well. Incubate for 10 min at -20℃. **Note :** The purpose of this step is to purify LPS from other extract of cell (e.g. Protein, nucleic acids, lipids, etc.).

6. After centrifuging the solution at 13,000 rpm for 15 min at 4℃, remove the upper layer to obtain LPS pellet.

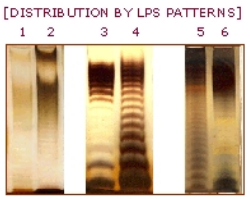
7. Add **1 ml** of 70% EtOH and washing the LPS pellet by inverting the tube 2-3 times. Centrifuge the mixtures for 3 min at 13,000 rpm at 4℃. Discard the upper layer and dry the remaining LPS pellet. **Note :** This is a washing stage to remove impurities such as salts and etc. Dry the pellet at RT.

8. Add **30 –50 ul** of 10mM Tris-HCl buffer(pH 8.0) to LPS pellet and vortex or pipetting it. And dissolve completely the LPS by boiling it for 2 min. **Note**: To get higher purity LPS from bacterial cell, treat with proteinase K to extracted LPS. Treat 2.5 ug proteinase K per 1 ug LPS and incubate it at 50℃ for 30 min.

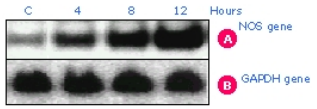
Usually 30 ug LPS extracted from of *E. coli.*

Cell culture volume(OD600=1.0)	2 ml (10^9 cells)
Yield of LPS	*30 ug*
Amount of Proteinase K	75 ug (2.5 ul of 30 mg/ml PK)

LPS Extraction from various samples & Comparison with commercial preparation



LPS was extracted from various cells using the ADI kit and LPS tested by SDS-PAGE.
Lane 1: Commercial S. enteritidis LPS
Lane 2: Extracted LPS from S. enteritidis
Lane 3: Commercial E.coli LPS
Lane 4: Extracted LPS from E.coli
Lane 5: Commercial S. enteritidis LPS
Lane 6: Extracted LPS from S. enteritidis
***Conclusion**: ADI kit extracted LPS with better yields than commercial preparation.*



Biological activity of LPS extracted by ADI's kit. LPS was extracted from Salmonella and the ability to stimulate NOS (nitric oxide synthase) was tested in RAW cell at 0 (control), 4, 8, and 12 hrs post stimulation with LPS. GAPDH (control gene) analyses by Northern blot..

Instruction Manual No. M-1000-100-LPS

Bacterial Lipopolysaccharides (LPS) Extraction Kit (up to 50 samples)

Cat. #. 1000-100-LPS

For In Vitro Research Use Only

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DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

Bacterial Lipopolysaccharides (LPS) Extraction Kit
(up to 50 samples)

Kit Components,	Cat #
Lysis Buffer, 50 ml, #1000-10-1	1 bottle
Purification Buffer, 40 ml, #1000-10-2	1 bottle
Instruction Manual	M-1000-100-LPS

Lipopolysaccharides (LPS), also known as lipoglycans, are large molecules consisting of a lipid and a polysaccharide joined by a covalent bond; they are found in the outer membrane of Gram-negative bacteria, act as endotoxins and elicit strong immune responses in animals. LPS is the major component of the Bacterial cell wall of Gram-negative bacteria, contributing greatly to the structural integrity of the bacteria, and protecting the membrane from certain kinds of chemical attack. LPS also increases the negative charge of the cell membrane and helps stabilize the overall membrane structure. It is of crucial importance to gram-negative bacteria, whose death results if it is mutated or removed. LPS is an endotoxin, and induces a strong response from normal animal immune systems. It has also been implicated in non-pathogenic aspects of bacterial ecology, including surface adhesion, bacteriophage sensitivity, and interactions with predators such as amoebae.

LPS is required for the proper conformation of Omptin activity; however, smooth LPS will sterically hinder omptins. LPS acts as the prototypical endotoxin because it binds the CD14/TLR4/MD2 receptor complex, which promotes the secretion of pro-inflammatory cytokines in many cell types, but especially in macrophages and B cells. In Immunology, the term "LPS challenge" refers to the process of exposing a subject to an LPS that may act as a toxin. LPS is also an exogenous pyrogen (external fever-inducing substance).Being of crucial importance to gram-negative bacteria, these molecules make candidate targets for new antimicrobial agents.

The hot phenolwater extraction method is usually used for extraction of LPS but takes long time and those procedure is complicated. And the method has major limitations in our hands when attempting to manipulate such small quantities of cells. LPS Extraction Kit is designed for rapid, convenient microscale extraction of LPS from bacterial cells and that is broadly applicable among different gram negative bacteria and appropriate for the small numbers of cells.

Step 1. Lysis

The bacterial cells are lysed by organic solution. Phospholipid and protein components of cell membrane disrupted and cell components are released in solution.

Step 2. LPS purification
Purification of LPS among the released cell components with high salt concentration solution.

Step 3. Washing and Elution
Salts are briefly removed by a washing step for high quality LPS.

CHARACTERISTICS
• Broadly applicable among different gram-negative bacteria
• Takes only 60minutes to extract LPS
• Gives reproducibly high yields of LPS

STORAGE
Store at 4℃ , and then stable for at least one year.

PREPARING SOLUTION BEFORE USE

• 70% EtOH, Room temperature
□ 10mM Tris-HCl buffer (pH8.0)
□ Proteinase K solution(30 mg/ml)

CONSIDERATION BEFORE USE

1. The yield of LPS extraction is proportional to increase culture volume. The yield of LPS is at its maximum when 5 ml of cultures were used. We do not recommend processing more than 5ml of bacteria culture. If excess culture volume is used, lysis will be inefficient and yield will be reduced and cellular protein contamination to LPS will be increased. Usually, the optimal culture volume is 2 ml at OD of 0.8-1.2.

2. To get higher purity LPS from bacterial cell, treat with proteinase K to 600 extracted LPS as following procedure.

LPS Extraction Procedure

1. Centrifuge at 13,000rpm at room temperature to harvest 2-5 ml of bacterial cell.
   Note : Remove all traces of supernatant. For pelleted cells, loosen the cell pellet thoroughly by repetitive tapping the tube before use. Incomplete loosening of the cell pellet may lead to inefficient lysis and reduced yields.

2. Add **1 ml** of Lysis Buffer and vortex vigorously. **Note :** To improve lysis of bacterial cell, vortex vigorously until cell clump disappeared.

3. After adding **200 ul** of chloroform, vortex vigorously for 10- 20 sec. And incubate it at room temperature for 5min. **Note :** Observe the tube before vortex. When chloroform is added, one would see a white line being formed just beneath the upper(blue layer) as the chloroform layer moves down. This region contains mixed parts of cell debris, protein, and genomic DNA and RNA. The purpose of adding the chloroform is to separate the phenol layer from aqueous layer and to eventually isolate RNA and genomic DNA/protein.

4. Centrifuge at 13,000rpm for 10 min at 4℃. Transfer **400 ul** of supernatant to new 1.5 ml tube.
   Note : When pipetting the upper layer, pay attention to form any white sediments.