ISO 9001/14001 Certified Company

LPS [Lipopolysaccharide] Extraction Kit

Cat. No. 17141 100 Preps

DESCRIPTION

Lipopolysaccharides(LPS) is a major constituent of the outer membrane of gram-negative bacteria. Some of its functions include a role in the resistance to phagocytosis, resistance to serum, outer membrane permeability barrier and as a receptor for adsorption of some bacteriophages. The hot phenol-water 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.

KIT CONTENTS

LPS Extraction Kit	100 Preps
Lysis Buffer	100 ml
Purification Buffer	80 ml

PREPARING SOLUTION BEFORE USE

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

CONSIDERATION BEFORE USE

- The yield of LPS extraction is proportional to increase culture volume. The yield of LPS is at its maximum when 5ml 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_{600} of 0.8-1.2.
- To get higher purity LPS from bacterial cell, treat with proteinase K to extracted LPS as following procedure.

PROTOCOL

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 ㎕ 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 ㎕ of supernatant to new 1.5 ml tube.
   Note : When pipetting the upper layer, pay attention to form any white sediments.
5. Add 800 ㎕ 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 (eg. protein, nucleic acids, lipids, etc)
6. After centrifuging the solution at 13,000rpm 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 13,000rpm 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 ㎕ 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.
   Option : To get higher purity LPS from bacterial cell, treat with proteinase K to extracted LPS. Treat 2.5㎍ proteinase K per 1㎍ LPS and incubate it at 50℃ for 30 min.
   * Usually 30㎍ LPS extracted from of *E.coli.*

Cell culture volume(OD_{600}=1.0)	2㎖ (10^9 cells)
Yield of LPS	30㎍
Amount of Proteinase K	75㎍ (2.5㎕ of 30㎎ / ml PK)