For research purpose only. Not for use in diagnostic procedures for clinical purposes. For IN VITRO USE ONLY.

LPS Extraction Kit

Cat. No. **17141** 100 rxn

DESCRIPTION

Lipopolysaccharides (LPS) is a major constituent of the outer membrane of gram-negative bacteria. Some of LPS is a disease-causing endotoxin; it is also important in classification of serum type. Commonly using method is the complicated hot phenol-water extraction that takes long period of time.
LPS Extraction Kit is designed for rapid and convenient extraction of LPS. It even enables LPS extraction from small volume of bacterial cells.

STORAGE

Store all components at 4℃.

CHARACTERISTICS

- Takes only 60minutes to extract LPS
- Gives reproducibly high yields of LPS

KIT CONTENTS

• Lysis buffer	100ml
• Purification buffer	80ml

CONSIDERATION BEFORE USE

The yield of LPS extraction is proportional to volume of culture. The yield of LPS is at its maximum when 5ml of culture is used. The optimal culture volume is 5ml at OD_{600} of 0.8-1.2.

PROTOCOL

1. Centrifuge for approximately 30 sec. at 13,000rpm at RT to harvest 5ml of bacterial cell.
   Note : Remove all traces of supernatant.

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

3. After adding 200ul of chloroform, vortex vigorously for 10-20sec., and incubate it at RT for 5min.
   Note : Observe the tube before vortexing. With addition of chloroform, white line formation just beneath the upper blue layer should be observed as the chloroform layer moves down. This region is composed of mixture of cell debris, protein, and genomic DNA and RNA. The purpose of chloroform addition is to separate the phenol layer from aqueous layer to eventually isolate RNA and genomic DNA/protein.

4. Centrifuge at 13,000rpm for 10min at 4℃. Transfer 400ul of supernatant to a new 1.5ml tube.
   Note : Following centrifugation two layers are formed and white precipitate that contains protein and gDNA is observed between two layers. Therefore, when pipetting the supernatant, one should be careful to avoid the white precipitation .

5. Add 800ul of purification buffer and mix well. Incubate for 10min at -20 ℃.
   Note : The purpose of this step is to purify LPS from other components of cell extract (eg. protein, nucleic acids, lipids, etc).

6. Centrifuge at 13,000 rpm for 15min at 4 ℃.

7. After washing the LPS pellet with 1ml of 70% EtOH, dry it complytely.
   Note : This is a washing stage to remove impurities such as salts. When drying the LPS, carefully remove any moisture on the wall of tube with 3MM paper then dry the pellet at RT.

8. Add 70 ul of 10mM Tris-HCl buffer(pH 8.0) to the LPS pellet and sonicate it.
   Note : LPS also can be dissolved by boiling it for 1min.