E. coli LPS

Purified lipopolysaccharide from *E. coli* 0111:B4 strain- TLR4 ligand

Catalog # tlrl-eblps

For research use only

Version # 05A18-SV

PRODUCT INFORMATION

Content:

- 5 mg purified *E. coli* 0111:B4 LPS

Storage:

- *E. coli* 0111:B4 LPS is shipped at room temperature and should be stored at 4˚C.
- Resuspended LPS may be stored at 4˚C for 1 month.

DESCRIPTION

Lipopolysaccharide (LPS) is the principal component of Gram negative bacteria that activates the innate immune system. LPS recognition is predominantly mediated by TLR4[1]. This recognition involves the binding of LPS with lipopolysaccharide-binding protein (LBP) and subsequently with CD14 which physically associates with a complex including TLR4 and MD2[2]. Formation of the TLR4-centered LPS receptor complex induces the production of proinflammatory cytokines through the MyD88 pathway. LPS signaling also involves a MyD88-independent cascade that mediates the expression of IFN-inducible genes. Furthermore, the shape of lipid A, the component responsible for the immunostimulatory activity of LPS, has been shown to direct the interaction of LPS with TLRs. LPS with conical shape (eg from *E. coli*) induce cytokine production through TLR4 whereas more cylindrical LPS (eg from *P. gingivalis*) induce expression of a different set of cytokines through TLR2[3].

References

1. Poltorak A. *et al.*, 1998. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science, 282(5396): 2085-8.
2. Shimazu R. *et al.*, 1999. MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. J Exp Med, 189(11):1777-82.
3. Netea MG. *et al.*, 2002. Does the shape of lipid A determine the interaction of LPS with Toll-like receptors? Trends Immunol, 23(3):135-9.
4. Schindler U. & Baichwal VR., 1994. Three NF-kB binding sites in the human E-selectin gene required for maximal tumor necrosis factor alpha-induced expression. Mol Cell Biol, 14(9):5820-5831.

METHODS

Preparation of sterile stock solution (5 mg/ml)

- In a sterile 2 ml vial, add 200 µl of ethanol to sterilize the product and vortex for 30 seconds.
- Add 800 µl of sterile water and homogenize.

Preparation of sterile working solution

Stimulation of TLR4 with *E. coli* LPS can be achieved with concentrations ranging from 10 ng to 10 µg/ml.

- Prepare a 1000X or 100X working solution by mixing one volume of LPS stock solution and the appropriate volume of sterile water (see table below).

Working concentration	10 ng/ml	100 ng/ml	1 µg/ml	10 µg/ml
Working solution	10 µg/ml	10 µg/ml	1 mg/ml	1 mg/ml
Dilution	1000X	100X	1000X	100X
LPS solution (5 mg/ml)	10 µl	10 µl	100 µl	100 µl
Sterile H_2O	5 ml	5 ml	400 µl	400 µl

LPS stimulation

- Transfect your cell line with an NF-κB reporter plasmid, i.e. a plasmid carrying a reporter gene such as GFP, SEAP or luciferase, under the control of the NF-κB-inducible ELAM-1(E-selectin) promoter[4].

If your cell line does not naturally express TLR4, cotransfect with a TLR4 expression plasmid, such as pUNO-TLR4 or pDUO-MD2/TLR4 plasmids.

- Twenty-four to forty-eight hours after transfection, stimulate cells with 10 ng to 10 µg/ml *E. coli* 011:B4 LPS for 6 hours.
- Determine LPS stimulation on TLR4(MD2) by assessing reporter gene expression using the appropriate detection system.

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