

LPS-EB Ultrapure

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

Catalog # tlr1-3pelps

For research use only

Version # 13106-MM

PRODUCT INFORMATION

Content:

- 5 x 10⁶ EU of ultra pure LPS from *E. coli* 0111:B4 (LPS-EB Ultrapure)
- 1.5 ml endotoxin-free water

Storage:

- LPS-EB Ultrapure is shipped at room temperature and should be stored at -20°C. Lyophilized product is stable 1 year when properly stored.
- Upon resuspension, prepare aliquots of LPS-EB Ultrapure and store at 4°C for short term storage or -20°C for long term storage. Resuspended product is stable 1 month at 4°C and 6 months at -20°C. Avoid repeated freeze-thaw cycles.

DESCRIPTION

Lipopolysaccharide (LPS), the major structural component of the outer wall of Gram-negative bacteria, is a potent activator of the immune system. Large quantities of LPS induce the overproduction of cytokines causing septic shock while suboptimal doses of LPS induce tolerance to subsequent exposure to LPS¹. LPS recognition is predominantly mediated by TLR4². 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 MD2³. 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 TLRs⁴.

Most LPS preparations on the market are contaminated by other bacterial components, such as lipoproteins, thus activating TLR2 signaling as well as TLR4 signaling. Ultra-Pure LPS-EB was extracted by successive enzymatic hydrolysis steps and purified by the phenol-TEA-DOC extraction protocol. The Ultra-Pure LPS-EB preparation provided by InvivoGen only activates the TLR4 pathway.

1. **Fujihara M. et al., 2003.** Molecular mechanisms of macrophage activation and deactivation by lipopolysaccharide: roles of the receptor complex. *Pharmacol Ther.* 100(2):171-94. 2. **Poltorak A. et al., 1998.** Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science*, 282(5396): 2085-8. 3. **Re F. & Strominger JL., 2003.** Separate Functional Domains of Human MD-2 Mediate Toll-Like Receptor 4-Binding and Lipopolysaccharide Responsiveness. 4. **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. 5. **Schindler U. & Baichwal VR., 1994.** Three NF-κB 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 a stock solution (5 x 10⁶ EU/ml)

- Add 1 ml of endotoxin-free water (provided).
 - Vortex until complete solubilization.
 - Store at 4°C for short term storage or -20°C for long term storage.
- Note:** 5 x 10⁶ EU/ml corresponds to 5 mg/ml.

TLR4 stimulation

Stimulation of TLR4 with LPS-EB Ultrapure can be achieved with concentrations ranging from 10¹ to 10⁴ EU/ml.

- Transfect your cell line with a pNiFty plasmid, an NF-κB reporter plasmid, i.e. a plasmid carrying a reporter gene such as SEAP or luciferase, under the control of an NF-κB-inducible ELAM-1 (E-selectin) promoter⁵.

If your cell line does not naturally express TLR4, MD2 and CD14, cotransfect with a TLR4 expression plasmid such as pUNO1-TLR4 and an MD2/CD14 expression plasmid such as pDUO2-hMD2/CD14.

Note: Alternatively, evaluate TLR4 activation using reporter cells, such as InvivoGen's HEK-Blue™ hTLR4 cells which express the human TLR4, MD2, CD14 and SEAP reporter genes. NF-κB production in these cells can be easily quantified using a SEAP detection medium, such as QUANTI-Blue™ or HEK-Blue™ Detection.

- Twenty-four to forty-eight hours after transfection, stimulate cells with 10¹ to 10⁴ EU/ml LPS-EB Ultrapure for 6 to 24 hours.
- Determine LPS stimulation on TLR4 by assessing reporter gene expression using the appropriate detection system.

RELATED PRODUCTS

Product	Catalog Code
HEK-Blue™ Detection	hb-det1
QUANTI-Blue™	rep-qb1
HEK-Blue™ hTLR4 cells	hkb-htr4
293/hTLR4-MD2-CD14 cells	293-htr4md2cd14
pNiFty2-Luc (Zeo ^R)	pnifty2-luc
pNiFty2-SEAP (Zeo ^R)	pnifty2-seap
pUNO1-hTLR4 (human gene)	puno1-htr4a
pDUO2-hMD2/CD14	pduo2-hmd2cd14

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