

# LPS-EK Ultrapure

Ultrapure lipopolysaccharide from *E. coli* K12 strain - TLR4 ligand

Catalog # tlrl-pekllps

For research use only

Version # 14D09-MM

## PRODUCT INFORMATION

### Content:

- 1 mg ultrapure lipopolysaccharide from *E. coli* K12 (LPS-EK Ultrapure)
- 1.5 ml endotoxin-free water

### Storage:

- LPS-EK Ultrapure is shipped at room temperature and should be stored at -20°C. Lyophilized product is stable 1 year at -20°C when properly stored.
- Upon resuspension, prepare aliquots of LPS-EK 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.

### Quality control

- The presence of other bacterial components (e.g. peptidoglycans and lipoproteins) is controlled using HEK-Blue™ TLR2 cells.
- The endotoxin level is controlled using a chromogenic LAL assay.

## 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 LPS<sup>1</sup>. LPS recognition is predominantly mediated by TLR4<sup>2</sup>. 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<sup>3</sup>.

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<sup>4</sup>.

Most LPS preparations on the market are contaminated by other bacterial components, such as lipoproteins, thus activating TLR2 signaling as well as TLR4 signaling. The ultrapure preparation that InvivoGen provides 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 J., 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.

## METHODS

### Preparation of stock solution (1 mg/ml)

- Add 1 ml of endotoxin-free water (provided) and homogenize.
- Prepare aliquots of stock solution and store at -20°C. Further dilutions can be prepared using water.

### TLR4 activation using LPS-EK Ultrapure

LPS-EK Ultrapure can be used to activate TLR4 in cells expressing this receptor such as HEK-Blue™ TLR4 cells. These cells were designed to study TLR4 stimulation by monitoring NF-κB activation.

Stimulation of HEK-Blue™ TLR4 cells with a TLR4 agonist activates NF-κB which induces the production of SEAP (secreted embryonic alkaline phosphatase). Levels of SEAP can be easily determined using a SEAP detection medium, such as QUANTI-Blue™.

For more information visit: [www.invivogen.com/hek-blue-htlr4](http://www.invivogen.com/hek-blue-htlr4)

- Add 20 µl of LPS-EK Ultrapure at 1 ng - 10 µg/ml in a well of a 96-well plate.
- Add 180 µl of HEK-Blue™ TLR4 cell suspension per well.
- Incubate the plate for 16 - 24 h at 37°C, 5% CO<sub>2</sub>.
- Collect 20 µl of supernatant and add to a well of a 96-well plate containing 180 µl of QUANTI-Blue™.
- Incubate the plate at 37°C for 1 - 3 h.
- Determine SEAP levels using a spectrophotometer at 620 - 655 nm.

## RELATED PRODUCTS

Product	Catalog Code
HEK-Blue™ hTLR4 Cells (human TLR4)	hkb-htlr4
HEK-Blue™ mTLR4 Cells (mouse TLR4)	hkb-mtlr4
QUANTI-Blue™	rep-qb1
<b>Other TLR4 agonists</b>	
LPS-EB Ultrapure (LPS from <i>E. coli</i> 0111:B4)	tlrl-3pelps
LPS-SM Ultrapure (LPS from <i>S. minnesota</i> )	tlrl-smllps
MPLAs (synthetic MPLA)	tlrl-mpls
MPLA-SM (MPLA from <i>S. minnesota</i> )	tlrl-mpla

## TECHNICAL SUPPORT

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