

# LPS-EK

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

Catalog # tlr1-eklps

For research use only

Version # 13106MM

## PRODUCT INFORMATION

### Content:

- 5 mg standard lipopolysaccharide from *E. coli* K12 (LPS-EK)
- 1.5 ml endotoxin-free water

### Storage:

- LPS-EK 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 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 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>. LPS with conical shape (e.g. from *E. coli*) induce cytokine production through TLR4, whereas more cylindrical LPS (e.g. from *P. gingivalis*) induce expression of a different set of cytokines through TLR2<sup>4</sup>.

High concentrations (1 µg/ml) of LPS-EK can induce TLR2 activity.

**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.

## PRODUCT PROPERTIES

**Species:** *Escherichia coli*

**Specificity:** TLR4 and TLR2 agonist

**Working concentration:** 1 ng - 10 µg/ml

**Endotoxin level:** 1 x 10<sup>6</sup> EU/mg

**Solubility:** 1 mg/ml in water

## METHODS

### Preparation of stock solution (5 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

LPS-EK can be used to activate TLR4 in HEK-Blue™ TLR4 cells, that 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-htr4](http://www.invivogen.com/hek-blue-htr4)

- Add 20 µl of LPS-EK 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-htr4
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)	tlr1-3pelps
LPS-SM Ultrapure (LPS from <i>S. minnesota</i> )	tlr1-smpls
MPLAs (synthetic MPLA)	tlr1-mpls
MPLA-SM (MPLA from <i>S. minnesota</i> )	tlr1-mpla

### TECHNICAL SUPPORT

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