

LPS-EK

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

Catalog code: tlr1-eklps

<https://www.invivogen.com/lps-ek>

For research use only

Version 21B01-MM

PRODUCT INFORMATION

Contents

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

Storage and stability

- LPS-EK is shipped at room temperature. Upon receipt, store product at -20°C.
- Upon resuspension, prepare aliquots of LPS-EK and store at 4°C for 1 month or -20°C for 6 months at -20°C. Avoid repeated freeze-thaw cycles.

Quality control

- Activation of TLR4 has been confirmed using HEK-Blue™ TLR4 cells.
- The presence of other bacterial components (e.g. lipoproteins) has been assessed using HEK-Blue™ TLR2 cells.

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. 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 TLR2⁴.

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

Source: *Escherichia coli* K12

Specificity: TLR4 and TLR2 agonist

Working concentration: 1 ng-10 µg/ml

Solubility: 5 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.

Note: LPS-EK stock solution may appear cloudy.

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 [HEK-Blue™ Detection](#), a cell culture medium that allows the detection of SEAP as the reporter protein is secreted by the cells.

For more information, visit: <https://www.invivogen.com/hek-blue-tlr4>.

- Dispense 20 µl of LPS-EK at various concentrations (1 ng-10 µg/ml) per well of a 96-well plate.
- Prepare a cell suspension ~140,000 cells per ml in [HEK-Blue™ Detection](#).
- Add 180 µl of the cell suspension (~25,000 cells) to each LPS-EK-containing well.
- Incubate the plate for 6-24 h at 37°C, 5% CO₂.
- Determine SEAP levels using a spectrophotometer at 620-655 nm.

RELATED PRODUCTS

Product	Description	Cat.Code
CRX-527	Synthetic lipid A analog	tlr1-crx527
HEK-Blue™ Detection	SEAP Detection reagent	hb-det2
HEK-Blue™ hTLR4 Cells	Human TLR4 reporter cells	hkb-htlr4
HEK-Blue™ mTLR4 Cells	Mouse TLR4 reporter cells	hkb-mtlr4
LPS-EB Ultrapure	LPS from <i>E. coli</i> O111:B4	tlr1-3pelps
LPS-SM Ultrapure	LPS from <i>S. minnesota</i>	tlr1-smlps
MPLAs	Synthetic MPLA	tlr1-mpsls
MPLA-SM	MPLA from <i>S. minnesota</i>	tlr1-mpla

TECHNICAL SUPPORT

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