

LPS-EK Ultrapure

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

Catalog code: tlr1-pek1ps

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

For research use only

Version 23G04-MM

PRODUCT INFORMATION

Contents

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

Storage and stability

- LPS-EK Ultrapure is shipped at room temperature. Upon receipt, store product at -20°C.
- Upon resuspension, prepare aliquots 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 endotoxin level has been assessed using a chromogenic LAL assay.
- The absence of other bacterial components (e.g. lipoproteins) has been confirmed using HEK-Blue™ TLR2 cells.

DESCRIPTION

LPS-EK Ultrapure is a preparation of a rough (r)-form lipopolysaccharide (LPS) purified from the Gram-negative *E. coli* K12. *E. coli* K12 strains are the prototypical laboratory strains and are the preferred model in biochemical genetics, molecular biology, and biotechnology. LPS isolated from *E. coli* K12 lacks the O-antigen in its structure and is therefore considered 'rough' LPS¹. LPS-EK Ultrapure is extracted by successive enzymatic hydrolysis steps and purified by the previously described phenol-TEA-DOC extraction protocol². This process removes contaminating lipoproteins, and therefore LPS-EK Ultrapure only activates TLR4.

LPS is the principal component of Gram-negative bacteria that activates the innate immune system through its recognition by Toll-like receptor 4 (TLR4). This leads to a signaling cascade that ultimately results in the activation of NF-κB and the production of proinflammatory cytokines³.

1. Kuhnert P. et al., 1995. Rapid and accurate identification of *Escherichia coli* K-12 strains. *Appl Environ Microbiol* 61, 4135-4139. **2. Hirschfeld M. et al., 2000.** Cutting edge: repurification of lipopolysaccharide eliminates signaling through both human and murine toll-like receptor 2. *J Immunol.* 165(2):618-22. **3. Kuzmich, N.N. et al., 2017.** TLR4 signaling pathway modulators as potential therapeutics in inflammation and sepsis. *Vaccines (Basel)* 5(4):34.

PRODUCT PROPERTIES

Source: *Escherichia coli* K12

Specificity: TLR4

Solubility: 1 mg/ml in water

Working concentration: 1 ng-10 µg/ml

METHODS

Preparation of stock solution (1 mg/ml)

1. Add 1 ml of endotoxin-free water (provided) and homogenize.
2. 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 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>.

1. Dispense 20 µl of LPS-EK Ultrapure at various concentrations (1 ng-10 µg/ml) per well of a 96-well plate.
2. Prepare a cell suspension ~140,000 cells per ml in HEK-Blue™ Detection.
3. Add 180 µl of the cell suspension (~25,000 cells) to each LPS-EK-containing well.
4. Incubate the plate for 6-24 h at 37°C, 5% CO₂.
5. 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
MPLA-SM*	MPLA from <i>S. minnesota</i>	tlr1-mpla2
MPLAs	Synthetic MPLA	tlr1-mpls

TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873

InvivoGen USA (International): +1 (858) 457-5873

InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Asia: +852 3622-3480

E-mail: info@invivogen.com