

# LPS-EK

Standard lipopolysaccharide from *E. coli* K12 strain; TLR4 and TLR2 ligand

Catalog code: tlr1-eklps

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

For research use only

Version 23G04-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

LPS-EK 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' LPS<sup>1</sup>. LPS is the principal component of Gram-negative bacteria that activates the innate immune system. LPS recognition is predominantly mediated by Toll-like receptor 4 (TLR4), ultimately leading to the activation of NF-κB and the production of proinflammatory cytokines<sup>2</sup>.

LPS-EK is a standard lipopolysaccharide (LPS) preparation extracted by a phenol-water mixture. LPS-EK contains other bacterial components, such as lipoproteins, and therefore stimulates both TLR4 and TLR2.

1. Kuhnert P. *et al.*, 1995. Rapid and accurate identification of *Escherichia coli* K-12 strains. *Appl Environ Microbiol* 61, 4135-4139. 2. 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 and TLR2

**Solubility:** 5 mg/ml in water

**Working concentrations:**

- TLR4 activity: 1 ng-10 µg/ml
- TLR2 activity: 1 µg/ml-1 mg/ml

## METHODS

### Preparation of stock solution (5 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.

*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>.

1. Dispense 20 µl of LPS-EK 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% CO<sub>2</sub>.
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> O1111: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

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