

# LPS-EB Ultrapure

Purified lipopolysaccharide from *E. coli* O111:B4 strain; TLR4 ligand

Catalog code: tlrl-3pelps; tlrl-3pelps-4

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

For research use only

Version 25B04-MM

## PRODUCT INFORMATION

### Contents

Ultrapure lipopolysaccharide from *E. coli* O111:B4 (LPS-EB Ultrapure) is available in two quantities:

- **tlrl-3pelps:** 5 x 10<sup>6</sup> EU
- **tlrl-3pelps-4:** 20 x 10<sup>6</sup> EU (four vials of 5 x 10<sup>6</sup> EU)
- endotoxin-free water; 1.5 ml with tlrl-3pelps and 4 x 1.5 ml with tlrl-3pelps-4

### Storage and stability

- LPS-EB Ultrapure is shipped at room temperature. Upon receipt, store at -20°C.
- Resuspended LPS-EB Ultrapure may be stored for 1 month at 4°C or for 6 months when aliquoted and stored 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-EB Ultrapure is an ultrapure preparation of lipopolysaccharide (LPS) from the Gram-negative bacteria *E. coli* O111:B4. It is extracted by successive enzymatic hydrolysis steps and purified by the previously described phenol-TEA-DOC extraction protocol<sup>1</sup>. This process removes contaminating lipoproteins, and therefore LPS-EB Ultrapure only activates TLR4.

LPS-EB Ultrapure is a preparation of smooth (s)-form LPS purified from *E. coli* O111:B4, a pathogenic serotype of *E. coli* known to cause significant gastric disease<sup>2,3</sup>. 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 cytokines<sup>4</sup>.

1. 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.
2. Coleman, W.G., Jr. *et al.*, 1977. Genetic analysis of *Escherichia coli* O111:B4, a strain of medical and biochemical interest. *J Bacteriol* 130:656-60.
3. Viljanen, M.K. *et al.*, 1990. Outbreak of diarrhea due to *Escherichia coli* O111:B4 in schoolchildren and adults: association of Vi antigen-like reactivity. *Lancet* 336:831-4.
4. Kuzmich, N.N. *et al.*, 2017. TLR4 signaling pathway modulators as potential therapeutics in inflammation and sepsis. *Vaccines (Basel)* 5(4):34.

## PRODUCT PROPERTIES

**Species:** *Escherichia coli*

**Specificity:** TLR4

**Working concentration:** 10<sup>1</sup> - 10<sup>4</sup> EU/ml

**Solubility:** 5 x 10<sup>6</sup> EU/ml in water

## METHODS

### Preparation of stock solution (5 x 10<sup>6</sup> EU/ml)

1. Add 1 ml of endotoxin-free water (provided).
2. Vortex until completely dissolved.

**Note:** 5 x 10<sup>6</sup> EU/ml corresponds to 5 mg/ml.

### TLR4 activation using LPS-EB Ultrapure

LPS-EB 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-htlr4>.

1. Add 20 µl of LPS-EB Ultrapure at 10<sup>1</sup> - 10<sup>4</sup> EU/ml in a 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-EB-Ultrapure-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
HEK-Blue™ Detection	SEAP Detection reagent	hb-det2
HEK-Blue™ hTLR4 Cells	Human TLR4 reporter cells	hkb-htlr4
HEK-Blue™ mTLR4 Cells	Murine TLR4 reporter cells	hkb-mtlr4
LPS-SM Ultrapure	LPS from <i>S. minnesota</i>	tlrl-smlps
MPLA-SM	MPLA from <i>S. minnesota</i>	tlrl-mpla
MPLAs	Synthetic MPLA	tlrl-mpis

## 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](mailto:info@invivogen.com)