LPS-EB Ultrapure

Purified lipopolysaccharide from *E. coli* 0111:B4 strain; TLR4 ligand

Catalog code: tlrl-3pelps

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

For research use only

Version 23F20-MM

PRODUCT INFORMATION

Contents

- 5 x 10^6 EU of ultrapure LPS from *E. coli* 0111:B4 (LPS-EB Ultrapure)
- 1.5 ml endotoxin-free water

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* 0111:B4. It is extracted by successive enzymatic hydrolysis steps and purified by the previously described phenol-TEA-DOC extraction protocol[1]. 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* 0111:B4, a pathogenic serotype of *E. coli* known to cause significant gastric disease[2, 3]. 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[4].

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: 10^1 - 10^4 EU/ml
Solubility: 5 x 10^6 EU/ml in water

METHODS

Preparation of stock solution (5 x 10^6 EU/ml)

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

Note: 5 x 10^6 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 10^1 - 10^4 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_2.
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 *S. minnesota*	tlrl-smlps
MPLA-SM*	MPLA from *S. minnesota*	tlrl-mpla2
MPLAs	Synthetic MPLA	tlrl-mpls

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