LPS-B5 Ultrapure

Ultrapure preparation of lipopolysaccharide from *E. coli* 055:B5; TLR4 ligand

Catalog code: tlrl-pb5lps

https://www.invivogen.com/lps-b5

For research use only

Version 23F20-MM

PRODUCT INFORMATION

Contents

- 5 mg LPS-B5 Ultrapure (lipopolysaccharide from *E. coli* serotype 055:K59(B5)H$^-$)
Source strain: ATCC 12014; CDC 5624-50 [NCTC 9701]
- 1.5 ml endotoxin-free water

Storage and stability

- LPS-B5 Ultrapure is shipped at room temperature. Upon receipt, store product at -20 °C.
- Resuspended LPS-B5 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 absence of other bacterial components (e.g. lipoproteins) has been confirmed using HEK-Blue™ TLR2 cells.

DESCRIPTION

LPS-B5 Ultrapure is an ultrapure preparation of lipopolysaccharide (LPS) from the Gram-negative bacteria *E. coli* 055:B5. 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-B5 Ultrapure only activates TLR4.

LPS-B5 is a preparation of smooth (s)-form LPS from *E. coli* 055:B5. It is the prototypical endotoxin and is often used as an endotoxin standard in Limulus amebocyte lysate (LAL) assays. 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. Dogan M. et al., 2000.** Effects of different serotypes of Escherichia coli lipopolysaccharides on body temperature in rats. Life Sci. 67(19):2319-29.. **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

Species: *Escherichia coli*
Specificity: TLR4
Working concentration: 100 pg-1 µg/ml
Solubility: 5 mg/ml in water

METHODS

Preparation of stock solution (5 mg/ml)

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

TLR4 activation using LPS-B5 Ultrapure

LPS-B5 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-B5 Ultrapure at 100 pg-1 µg/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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