LPS-B5

Standard preparation of lipopolysaccharide from *E. coli* 055:B5; TLR4 & TLR2 ligand

Catalog code: tlrl-b5lps

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

For research use only

Version 21B01-MM

PRODUCT INFORMATION

Contents

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

DESCRIPTION

LPS-B5 is a standard preparation of a smooth (S)-form lipopolysaccharide (LPS) from the Gram-negative bacteria *E. coli* 055:B5. LPS-B5 is a 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. LPS recognition is predominantly mediated by TLR4[1]. LPS is composed of three domains; an O-antigenic polysaccharide, a core oligosaccharide, and an amphipathic domain known as lipid A. The presence or absence of O-antigenic polysaccharide chains (O-chains) determines whether the LPS is considered smooth or rough. Full-length O-chains render the LPS smooth, whereas the absence or reduction of O-chains make the LPS rough. The core domain contains an oligosaccharide component that attaches directly to lipid A. The lipid A domain is responsible for the endotoxic activity of LPS.

LPS-B5 is highly pyrogenic[2] and a potent activator of TLR4 with the subsequent induction of NF-κB and the production of pro-inflammatory cytokines[3,4]. LPS-B5 is extracted by successive enzymatic hydrolysis steps and purified by the phenol-TEA-DOC extraction protocol as described previously[5]. LPS-B5 contains other bacterial components, such as lipoproteins, and therefore stimulates both TLR4 and TLR2.

1. Poltorak A. *et al.*, 1998. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science, 282: 2085-8. **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. Gutschow M. *et al.*, 2013.** Single-cell and population NF-κB dynamic responses depend on lipopolysaccharide preparation. PLoS One. 8:e53222. **4. Hsu R. *et al.*, 2011.** LPS-induced TLR4 signaling in human colorectal cancer cells increases beta1 integrin-mediated cell adhesion and liver metastasis. Cancer Res. 1:1989-98. **5. 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.

METHODS

Preparation of stock solution (5 mg/ml)

- Add 1 ml of endotoxin-free water (provided) and homogenize.
- Prepare aliquots of stock solution and store at 4°C. Further dilutions can be prepared using water.

Note: LPS-B5 stock solution may appear cloudy.

Working concentrations:

- TLR4 activity: 100 pg-1 µg/ml
- TLR2 activity: 100 ng/ml-1 µg/ml

TLR4 activation using LPS-B5

LPS-B5 can be used to activate TLR4 in cells expressing this receptor such as HEK-Blue™ TLR4 cells. These cells 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.

- Dispense 20 µl of LPS-B5 at various concentrations (100 pg-1 µg/ml) per well of a 96-well plate.
- Prepare a cell suspension ~140,000 cells per ml in HEK-Blue™ Detection.
- Add 180 µl of the cell suspension (~25,000 cells) to each LPS-B5-containing well.
- Incubate the plate for 6-24 h at 37°C, 5% CO_2.
- Determine SEAP levels using a spectrophotometer at 620-655 nm.

RELATED PRODUCTS

Product	Description	Cat. Code
HEK-Blue™ Detection	SEAP detection medium	hb-det2
HEK-Blue™ hTLR2 Cells	Human TLR2 reporter cells	hkb-htlr2
HEK-Blue™ hTLR4 Cells	Human TLR4 reporter cells	hkb-htlr4
LPS-B5 Ultrapure	LPS from *E. coli 055:B5*	tlrl-pb5lps
LPS-EB Ultrapure	LPS from *E. coli 0111:B4*	tlrl-3pelps
LPS-SM Ultrapure	LPS from *S. minnesota*	tlrl-smlps
MPLA-SM	MPLA from *S. minnesota*	tlrl-mpla
MPLAs	Synthetic MPLA	tlrl-mpls

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