

LPS-EB

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

Catalog code: tlr1-eb1ps

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

For research use only

Version 21B01-MM

PRODUCT INFORMATION

Contents

- 5 mg LPS-EB (lipopolysaccharide from *E. coli* 0111:B4)
- 1.5 ml endotoxin-free water

Storage and stability

- LPS-EB is shipped at room temperature. Upon receipt, store product at -20°C.
- Resuspended LPS-EB 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 been assessed using HEK-Blue™ TLR2 cells.

DESCRIPTION

LPS-EB is a standard preparation of lipopolysaccharide (LPS) from the Gram negative bacteria *E. coli* 0111:B4. LPS is the principal component of Gram negative bacteria that activates the innate immune system. LPS recognition is predominantly mediated by TLR4¹. This recognition involves the binding of LPS with lipopolysaccharide-binding protein (LBP) and subsequently with CD14 which physically associates with a complex including TLR4 and MD2². Formation of the TLR4-centered LPS receptor complex induces the production of proinflammatory cytokines through the MyD88 pathway.

LPS signaling also involves a MyD88-independent cascade that mediates the expression of IFN-inducible genes. Furthermore, the shape of lipid A, the component responsible for the immunostimulatory activity of LPS, has been shown to direct the interaction of LPS with TLRs. LPS with conical shape (e.g. from *E. coli*) induce cytokine production through TLR4, whereas more cylindrical LPS (e.g. from *P. gingivalis*) induce expression of a different set of cytokines through TLR2³.

High concentrations (1 µg/ml) of LPS-EB can induce TLR2 activity.

1. **Poltorak A., 1998.** Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in TLR4 gene. *Science*, 282(5396): 2085-8. 2. **Shimazu R., 1999.** MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. *J Exp Med*, 189(11):1777-82. 3. **Netea MG., 2002.** Does the shape of lipid A determine the interaction of LPS with Toll-like receptors? *Trends Immunol*, 23(3):135-9.

PRODUCT PROPERTIES

Species: *Escherichia coli*

Specificity: TLR4 and TLR2 agonist

Working concentration: 10 ng-10 µg/ml

Solubility: 5 mg/ml in water

METHODS

Preparation of stock solution (5 mg/ml)

- Add 1 ml of endotoxin-free water (provided) and homogenize.

Notes:

- This product can be reconstituted by injecting water through the rubber cap using a needle and syringe.
- LPS-EB stock solution may appear cloudy.

TLR4 activation using LPS-EB

LPS-EB 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-htr4>.

- Add 20 µl of LPS-EB at 10 ng-10 µg/ml in a 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-EB-containing well.
- Incubate the plate for 6-24 h at 37°C, 5% CO₂.
- 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-htr4
HEK-Blue™ mTLR4 Cells	Murine TLR4 reporter cells	hkb-mtr4
LPS-EB Ultrapure	LPS from <i>E. coli</i> 0111:B4	tlr1-3pelps
LPS-SM Ultrapure	LPS from <i>S. minnesota</i>	tlr1-smlps
MPLA-SM	MPLA from <i>S. minnesota</i>	tlr1-mpla
MPLAs	Synthetic MPLA	tlr1-mpls

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 Hong Kong : +852 3622-3480

E-mail: info@invivogen.com