

LPS-SM Ultrapure

Ultrapure lipopolysaccharide from *Salmonella minnesota* R595; TLR4 ligand

Catalog code: tlr1-smlps

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

For research use only

Version 23G11-MM

PRODUCT INFORMATION

Contents

- 5 mg ultrapure LPS from *S. minnesota* R595 (LPS-SM Ultrapure)
- 1.5 ml endotoxin-free water

Storage and stability

- LPS-SM Ultrapure is shipped at room temperature. Upon receipt, store product at -20°C.
- Upon resuspension, prepare aliquots and store at -20°C. Resuspended product is stable for 6 months when properly stored. 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 the HEK-Blue™ LPS Detection Kit 2.
- The absence of bacterial contamination (e.g. lipoproteins) has been confirmed using HEK-Blue™ TLR2 cells.

DESCRIPTION

LPS-SM Ultrapure is a preparation of a rough (r)-form lipopolysaccharide (LPS) purified from *Salmonella minnesota* R595, a mutant strain that produces LPS lacking O-antigens and a truncated core region¹. It is extracted by successive enzymatic hydrolysis steps and purified by the phenol-TEA-DOC extraction protocol². This process removes contaminating lipoproteins, and therefore LPS-SM Ultrapure only activates TLR4.

LPS activates the innate immune system through its recognition by Toll-like receptor 4 (TLR4). At the cell surface, activation of TLR4 initiates the MyD88-dependent pathway, ultimately leading to the activation of NF-κB³. Also, the TLR4 complex can be endocytosed in a CD14-mediated fashion. This results in TRIF-dependent production of type I interferons (IFNs)³. Unlike wild-type smooth LPS, rough LPS can interact with TLR4 in a CD14-independent manner⁴.

1. Paracini N. *et al.*, 2022. Lipopolysaccharides at solid and liquid interfaces: Models for biophysical studies of the Gram-negative bacterial outer membrane. *Adv Colloid Interface Sci.* 01:102603. 2. 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. 3. Kuzmich N.N. *et al.*, 2017. TLR4 signaling pathway modulators as potential therapeutics in inflammation and sepsis. *Vaccines (Basel)* 5(4):34. 4. Huber M. *et al.*, 2006. R-form LPS, the master key to the activation of TLR4/MD-2-positive cells. *Eur J Immunol.* 36(3):701-11.

PRODUCT PROPERTIES

Species: *Salmonella enterica* serovar minnesota mutant R595

Specificity: TLR4 agonist

Working concentration: 10 ng-10 µg/ml

Endotoxin level: 1 x 10³ EU/mg

Solubility: 5 mg/ml in water

METHODS

Preparation of stock solution (5 mg/ml)

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

Note: LPS-SM Ultrapure stock solution may appear cloudy.

TLR4 activation using LPS-SM Ultrapure

LPS-SM 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-tlr4>.

- Add 20 µl of LPS-SM Ultrapure at various concentrations (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-SM Ultrapure-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-htlr4
HEK-Blue™ mTLR4 Cells	Murine TLR4 reporter cells	hkb-mtlr4
LPS-EB Ultrapure	LPS from <i>E. coli</i> O111:B4	tlr1-3pelps
MPLA-SM*	MPLA from <i>S. minnesota</i>	tlr1-mpla2
MPLAs	Synthetic MPLA	tlr1-mpls

TECHNICAL SUPPORT

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