LPS-RS Ultrapure

Ultrapure lipopolysaccharide from *Rhodobacter sphaeroides*; TLR4 antagonist

Catalog code: tlrl-prslps

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

For research use only

Version 23G21-MM

PRODUCT INFORMATION

Contents

- 1 mg ultrapure lipopolysaccharide from *Rhodobacter sphaeroides* (LPS-RS Ultrapure)
- 1.5 ml endotoxin-free water

Storage and stability

- LPS-RS 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

- Inhibition of TLR4 activation has been confirmed using HEK-Blue™ TLR4 cells.
- The absence of TLR4 agonist activity 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

Lipopolysaccharide from the photosynthetic bacterium *Rhodobacter sphaeroides* (LPS-RS) is a potent antagonist of toxic hexa-acylated LPS in both human and murine cells[1]. It also prevents LPS-induced shock in mice[1]. LPS-RS is penta-acylated, and like other under-acylated LPS, appears to use at least two distinct mechanisms to block LPS-dependent activation of Toll-like receptor 4 (TLR4). The primary mechanism consists of direct competition between under-acylated LPS and hexa-acylated LPS for the same binding site on MD-2, while the secondary mechanism involves the ability of under-acylated LPS-MD-2 complexes to inhibit the TLR4 agonist activity of hexa-acylated LPS-MD-2 complexes[2-5].

Complete competitive inhibition of LPS activity is possible at a 100-fold excess of the antagonist. LPS-RS does not induce TLR4 signaling but is detected by the LAL assay, the standard endotoxin detection assay. LPS-RS Ultrapure is extracted by successive enzymatic hydrolysis steps and purified by the previously described phenol-TEA-DOC extraction protocol[6]. This process removes contaminating lipoproteins, and therefore LPS-RS Ultrapure does not activate TLR2 while retaining the ability to inhibit TLR4 activity.

1. Qureshi N. *et al.*, 1999. Nontoxic RsDPLA as a potent antagonist of toxic lipopolysaccharide. p. 687-98. In: Brade H., Opal S.M., Vogel S.N., and Morrison D.C., eds. Endotoxin in Health and Disease. Marcel Dekker, New York. **2. Coats SR. *et al.*, 2005.** MD-2 mediates the ability of tetra-acylated and penta-acylated lipopolysaccharides to antagonize *Escherichia coli* lipopolysaccharide at the TLR4 signaling complex. J Immunol. 175(7):4490-8. **3. Teghanemt A. *et al.*, 2005.** Molecular basis of reduced potency of underacylated endotoxins. J Immunol. 175(7):4669-76. **4. Visintin A. *et al.*, 2005.** Pharmacological inhibition of endotoxin responses is achieved by targeting the TLR4 coreceptor, MD-2. J Immunol. 175(10):6465-72. **5. Saitoh S. *et al.*, 2004.** Lipid A antagonist, lipid IVa, is distinct from lipid A in interaction with Toll-like receptor 4 (TLR4)-MD-2 and ligand-induced TLR4 oligomerization. Int Immunol. 16(7):961-9. **6. 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.

PRODUCT PROPERTIES

Species: *Rhodobacter sphaeroides*
Specificity: TLR4 antagonist
Solubility: 1 mg/ml in water

Working concentrations:

- TLR4 antagonist activity: 10 ng - 10 µg/ml

METHODS

Preparation of stock solution (1 mg/ml)

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

Inhibition of TLR4 activation

Below is a protocol using HEK-Blue™ hTLR4 cells for studying the inhibition of TLR4 by LPS-RS Ultrapure. These cells express TLR4, MD2 and CD14 genes, and an inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene placed under the control of an NF-κB-inducible promoter. Upon TLR4 activation, the cells secrete SEAP which can be readily determined using QUANTI-Blue™ Solution. For more information visit: https://www.invivogen.com/hek-blue-tlr4.

1. Prepare a HEK-Blue™ hTLR4 cell suspension at 160,000 cells/ml.
2. Add 20 µl of LPS-EB Ultrapure and 20 µl of LPS-RS Ultrapure per well of a flat bottom 96-well plate at the final concentrations indicated in the table below.

LPS-EB Ultrapure (ng/ml)	LPS-RS Ultrapure (ng/ml)					
0	0	10	30	100	300	1000
0.1	0	10	30	100	300	1000
1	0	10	30	100	300	1000
10	0	10	30	100	300	1000

- Add 160 µl of the cell suspension (~25,000 cells) to each well.
- Incubate the plate for 18-24 h at 37°C, 5% CO_2.
- Prepare QUANTI-Blue™ Solution and carry out the measurements following the instructions on the data sheet.

RELATED PRODUCTS

Product	Description	Cat. Code
HEK-Blue™ hTLR4 Cells	Human TLR4 reporter cells	hkb-htlr4
LPS-EB Ultrapure	LPS from *E. coli 0111:B4*	tlrl-3pelps
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs

TECHNICAL SUPPORT
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