LPS-RS

Lipopolysaccharide from *Rhodobacter sphaeroides*- TLR4 antagonist

Catalog # tlrl-rslps

For research use only

Version # 13I06-MM

PRODUCT INFORMATION

Content:

- 5 mg *Rhodobacter sphaeroides* LPS (LPS-RS)
- 1.5 ml endotoxin-free water

Storage:

- LPS-RS is shipped at room temperature and should be stored at -20˚C. Lyophilized product is stable 1 year at -20°C when properly stored.
- Upon resuspension, prepare aliquots of LPS-RS and store at 4°C for short term storage or -20˚C for long term storage. Resuspended product is stable 1 month at 4°C and 6 months at -20°C. Avoid repeated freeze-thaw cycles.

APPLICATION

TLR4-specific inhibition in TLR2 non-responsive cells.

DESCRIPTION

Bacterial lipopolysaccharide (LPS) is the major structural component of the outer wall of all Gram-negative bacteria and a potent activator of the immune system. LPS is recognized by Toll-like receptor 4 (TLR4) which interacts with three different extracellular proteins: LPS binding protein (LBP), CD14 and, myeloid differentiation protein 2 (MD-2), to induce a signaling cascade leading to the activation of NF-κB and the production of proinflammatory cytokines.

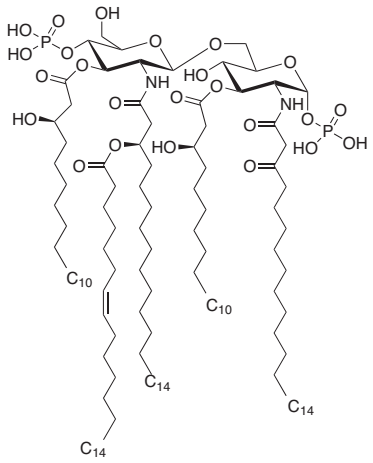
LPS consists of a polysaccharide region that is anchored in the outer bacterial membrane by a specific carbohydrate lipid moiety termed lipid A. Lipid A, also known as endotoxin, is responsible for the immunostimulatory activity of LPS. Lipid A is a glucosamine disaccharide linked to hydroxy fatty acids that are further substituted by nonhydroxylated fatty acids. The number of fatty acids is a major determinant of the immunogenicity of endotoxin. The most active form of lipid A contains six fatty acyl groups and is found in pathogenic bacteria such as *Escherichia coli* and *Salmonella* species. Underacylated lipid A structures, containing four or five fatty acids, induce markedly less host defense responses and can inhibit in a dose-dependent manner the strong endotoxic response triggered by hexa-acylated LPS.

LPS from the photosynthetic bacterium *Rhodobacter sphaeroides* (LPS-RS) is a potent antagonist of toxic LPS in both human and murine cells and also prevents LPS-induced shock in mice[1].

LPS-RS is penta-acylated that appears to utilize at least two distinct mechanisms to block LPS-dependent activation of TLR4. The main 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 hexa-acylated endotoxin:MD-2 complexes function at TLR4[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 preparations (cat. code tlrl-rslps) contain lipopeptide contaminants, and therefore stimulate Toll-like receptor 2 (TLR2).

1. Qureshi, N., B. W. Jarvis, K. Takayama. 1999. Nontoxic RsDPLA as a potent antagonist of toxic lipopolysaccharide. H. Brade, and S. M. Opal, and S. N. Vogel, and D. C. Morrison, eds. Endotoxin in Health and Disease 687. 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.



Lipid A from *R. sphaeroides*

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METHODS

Preparation of a stock solution and dilutions

- Add 1 ml endotoxin-free water (provided) to the 5 mg of LPS-RS and homogenize to obtain a stock solution at 5 mg/ml.
- Prepare serial dilutions using endotoxin-free water, typically from 10 ng to 10 µg/ml.

Inhibition of TLR4 activation

InvivoGen has developed a simple and convenient cell-based method to study TLR4 activation. This method relies on the use of HEK-Blue™-hTLR4 cells that coexpress TLR4, MD2 and CD14 genes, and an optimized 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™, a detection medium that turns blue in the presence of SEAP.

Note: *Be aware that if your cells express TLR2, inhibition of TLR4 with LPS-RS will not be detectable, as LPS-RS activates TLR2.*

The protocol described below is performed using HEK-Blue™-hTLR4 Cells QUANTI-Blue™, the ultrapure LPS from *E. coli 0111:B4* (LPS-EB ultrapure) and the antagonist LPS from *R. sphaeroides* (LPS-RS). Alternatively, the ultrapure LPS from *E. coli K12* (LPS-EK ultrapure) can be used to activate TLR4.

Day 1:

- Prepare a HEK-Blue™-hTLR4 cell suspension at 160,000 cells/ml in DMEM supplemented with 10% heat-inactivated FBS.

Note: Some fetal bovine serum (FBS) may contain alkaline phosphatases that can interfere with SEAP quantification. We recommend to use heat-inactivated FBS to inactivate these enzymes which are thermosensitive. Heat-inactivated FBS is available commercially or can be prepared by incubating 30 min at 56°C.

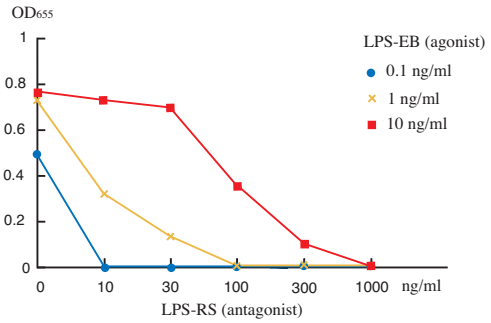
- Add 20 µl of LPS-EB and 20 µl of LPS-RS per well of a flat-bottom 96-well plate at the final concentrations indicated in the table below:

LPS-EB Conc. (ng/ml)	LPS-RS Conc. (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 HEK-Blue™-hTLR4 cell suspension (~25,000 cells) per well.
- Incubate the plate at 37°C in a 5% CO_2 incubator for 18-24 h.

Day 2:

- Prepare QUANTI-Blue™ following the instructions on the pouch.
- Add 180 µl of resuspended QUANTI-Blue™ per well of a flat-bottom 96-well plate.
- Add 20 µl of induced HEK-Blue™-hTLR4 Cells supernatant.
- Incubate the plate at 37°C incubator for 1 to 3 h.
- Determine SEAP levels using a spectrophotometer at 620-655 nm.



Antagonistic effect of LPS-RS on LPS-stimulated TLR4: HEK-Blue™-4 cells were incubated with increasing concentrations of LPS-EB ultrapure (*E. coli* 0111:B4) and LPS-RS. After 24h incubation, TLR4 inhibition was assessed by measuring the levels of SEAP secreted in the supernatant by using QUANTI-Blue™, which turns blue in the presence of SEAP.

RELATED PRODUCTS

Product	Catalog Code
HEK-Blue™-hTLR4 Cells	hkb-htlr4
LPS-EB Ultrapure	tlrl-3pelps
LPS-EK Ultrapure	tlrl-peklps
QUANTI-Blue™	rep-qb1

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