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.

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. 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).


Lipid A from *R. sphaeroides*
**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:

<table>
<thead>
<tr>
<th>LPS-EB Conc. (ng/ml)</th>
<th>LPS-RS Conc. (ng/ml)</th>
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<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

- Add 160 µl of HEK-Blue™-hTLR4 cell suspension (~25,000 cells) per well.
- Incubate the plate at 37°C in a 5% CO2 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.

**RELATED PRODUCTS**

<table>
<thead>
<tr>
<th>Product</th>
<th>Catalog Code</th>
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</thead>
<tbody>
<tr>
<td>HEK-Blue™-hTLR4 Cells</td>
<td>hkb-htrl4</td>
</tr>
<tr>
<td>LPS-EB Ultrapure</td>
<td>trl-3pelps</td>
</tr>
<tr>
<td>LPS-EK Ultrapure</td>
<td>trl-peklps</td>
</tr>
<tr>
<td>QUANTI-Blue™</td>
<td>rep-qb1</td>
</tr>
</tbody>
</table>

**Antagonistic effect of LPS-RS on LPS-stimulated TLR4:**

HEK-Blue™-hTLR4 cells were incubated with increasing concentrations of LPS-EB ultrapure (*E. coli* 0111:B4) and LPS-RS. After 24 h 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.