

LPS-PG Ultrapure

Ultrapure lipopolysaccharide from *Porphyromonas gingivalis* - TLR4 ligand

Catalog # tlr1-pgpls

For research use only

Version # 14F18-MM

PRODUCT INFORMATION

Content

- 1 mg of an ultra pure preparation of lipopolysaccharide from *Porphyromonas gingivalis* (LPS-PG Ultrapure)
- 1.5 ml endotoxin-free water

Storage

- LPS-PG Ultrapure is provided lyophilized and shipped at room temperature. Store at -20°C.
- Upon resuspension, prepare aliquots of LPS-PG Ultrapure and store at 4°C for 1 month or -20°C for 6 months. Avoid repeated freeze-thaw cycles.

Quality control

- The TLR4 activity is controlled using HEK-Blue™ TLR4 cells.
- The presence of other bacterial components (e.g. lipoproteins) is controlled using HEK-Blue™ TLR2 cells.

DESCRIPTION

LPS-PG Ultrapure is a highly purified preparation of lipopolysaccharide (LPS) from the Gram-negative bacteria *Porphyromonas gingivalis*. LPS-PG is an important virulence factor in the mechanisms of periodontal disease. LPS is the principal component of Gram negative bacteria that activates the innate immune system. LPS recognition is predominantly mediated by TLR4¹. The TLR4 response to LPS-PG is dependent on the presence of key accessory molecules, CD14 and MD2².

LPS-PG presents a unique and heterogenous chemical structure, which differs from traditionally recognized enteric bacterium-derived LPS. The fact that LPS-PG exhibits activity in C3H/HeJ mice, which are deficient for TLR4, led to a common belief that this LPS is a TLR2 ligand^{3, 4}. However, structural and functional studies of LPS-PG have revealed that it activates cells through TLR4. The TLR2 activity of LPS-PG is ascribed to a contaminant lipoprotein⁵. LPS-PG Ultrapure underwent enzymatic treatment to remove lipoproteins and hence only activates TLR4.

1. **Poltorak A. et al., 1998.** Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science*, 282:2085-8. 2. **Darveau RP. et al., 2004.** *Porphyromonas gingivalis* lipopolysaccharide contains multiple lipid A species that functionally interact with both toll-like receptors 2 and 4. *Infect Immun.* 72(9):5041-51. 3. **Kirikae T. et al., 1999.** Lipopolysaccharides (LPS) of oral black-pigmented bacteria induce tumor necrosis factor production by LPS-refractory C3H/HeJ macrophages in a way different from that of *Salmonella* LPS. *Infect Immun.* 67(4):1736-42. 4. **Hirschfeld M. et al., 2001.** Signaling by toll-like receptor 2 and 4 agonists results in differential gene expression in murine macrophages. *Infect Immun.* 69(3):1477-82. 5. **Ogawa T. et al., 2007.** Chemical structure and immunobiological activity of *Porphyromonas gingivalis* lipid A. *Front Biosci.* 12:3795-812.

METHODS

Preparation of stock solution (1 mg/ml)

- Add 1 ml of endotoxin-free water (provided) and homogenize.
- Prepare aliquots of stock solution and store at 4°C or at -20°C. Further dilutions can be prepared using water.

Working concentration 100 ng - 10 µg/ml

TLR4 activation using LPS-PG Ultrapure

LPS-PG Ultrapure can be used to activate TLR4 in cells expressing this receptor such as HEK-Blue™ TLR4 cells. These cells 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: www.invivogen.com/hek-blue-tlr4

- Dispense 20 µl of LPS-PG Ultrapure at different concentrations per well of a 96-well plate.
- Prepare a cell suspension ~140,000 cells per ml in HEK-Blue™ Detection medium and immediately add 180 µl of the cell suspension (~25,000 cells) to each LPS-PG 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	Catalog Code
HEK-Blue™ hTLR4 Cells (human TLR4)	hkb-htlr4
HEK-Blue™ Detection	hb-det2
Other TLR4 agonists	
LPS-B5 Ultrapure (LPS from <i>E. coli</i> 055:B5)	tlr1-pb5lps
LPS-EB Ultrapure (LPS from <i>E. coli</i> 0111:B4)	tlr1-3pelps
MPLAs (synthetic monophosphoryl lipid A)	tlr1-mpls

TECHNICAL SUPPORT

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