

LPS-PG Ultrapure

Ultrapure lipopolysaccharide from *Porphyromonas gingivalis*; TLR4 ligand

Catalog code: tlr1-pgpls

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

For research use only

Version 23G11-MM

PRODUCT INFORMATION

Contents

- 1 mg ultrapure LPS from *Porphyromonas gingivalis* (LPS-PG Ultrapure)
- 1.5 ml endotoxin-free water

Storage and stability

- LPS-PG 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 absence of bacterial contamination (e.g. lipoproteins) has been confirmed using HEK-Blue™ TLR2 cells.

DESCRIPTION

LPS-PG Ultrapure is a purified preparation of a semi-rough (sr)-form 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 R.P. 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.

PRODUCT PROPERTIES

Species: *Porphyromonas gingivalis*

Specificity: TLR4 agonist

Working concentration: 100 ng-10 µg/ml

Solubility: 1 mg/ml in water

METHODS

Preparation of stock solution (1 mg/ml)

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

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

TLR4 activation using LPS-PG Ultrapure

LPS-PG 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-tnfr4>.

- Add 20 µl of LPS-PG Ultrapure at various concentrations (100 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-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	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	tlrl-3pelps
MPLA-SM*	MPLA from <i>S. minnesota</i>	tlrl-mpla2
MPLAS	Synthetic MPLA	tlrl-mpls

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