

FLA-PA Ultrapure

Purified flagellin from *P. aeruginosa*; TLR5 ligand

Catalog # tlr1-pafla

<http://www.invivogen.com/fla-pa>

For research use only

Version # 17L04-MM

PRODUCT INFORMATION

Content:

- 50 µg FLA-PA Ultrapure (purified flagellin from *P. aeruginosa*)
- 1.5 ml endotoxin-free water

Storage:

- FLA-PA is shipped at room temperature and should be stored at -20 °C.
- Upon resuspension, prepare aliquots of FLA-PA Ultrapure and store at -20 °C. Resuspended product is stable for 6 months at -20 °C when properly stored. Avoid repeated freeze-thaw cycles.

Quality Control:

- Purity greater than 90% as determined by SDS-PAGE
- Endotoxin levels: <0.05 EU/µg
- Biological activity has been confirmed using HEK-Blue™ hTLR5 cells.

DESCRIPTION

FLA-PA Ultrapure, a ~52 kDa protein, is a high purity grade of flagellin isolated from *Pseudomonas aeruginosa*, a virulent Gram negative bacterial pathogen. This bacterium is implicated in respiratory tract infections, particularly in cystic fibrosis patients. Flagellin from *P. aeruginosa* is extracted by acid hydrolysis and is purified by ultrafiltration and chromatography.

Flagellin is a proinflammatory molecule recognized by distinct types of pattern recognition receptors (PRRs); the surface localized Toll-like receptor (TLR5)¹ and the cytosolic NOD-like receptors (NLRs), NLRC4 and NAIP5². Extracellular flagellin is detected by TLR5 resulting in MyD88-mediated NF-κB activation, cytokine and nitric oxide production depending on the nature of the TLR5 signaling complex³. Intracellular flagellin is detected by NLRC4 (also known as IPAF) and NAIP5. Recognition by NLRC4 and NAIP5, leads to inflammasome assembly, triggering caspase-1 activation of IL-1β and IL-18.

1. Hayashi F. *et al.*, 2001. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature* 410(6832):1099-103. 2. Zhao *et al.*, 2011. The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus. *Nature*. 2011 Sep 14;477(7366):596-600. 3. Mizel SB. *et al.*, 2003. Induction of macrophage nitric oxide production by Gram-negative flagellin involves signaling via heteromeric Toll-like receptor 5/Toll-like receptor 4 complexes. *J Immunol.* 170(12):6217-23.

METHODS

Preparation of stock solution (500 µg/ml)

- Stimulation of TLR5 can be achieved with FLA-PA Ultrapure at a concentration of 1 ng - 1 µg/ml.
- Open vial lid carefully to avoid any loss of product.
 - Add 100 µl of the endotoxin-free water provided and mix by pipetting. Do not vortex.

TLR5 stimulation using FLA-PA Ultrapure

FLA-PA Ultrapure can be used to stimulate TLR5 in HEK-Blue™ TLR5 cells. HEK-Blue™ TLR5 cells stably overexpress the TLR5 gene and an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP).

For more information, visit: www.invivogen.com/hek-blue-trl5

- Dispense 20 µl of FLA-PA Ultrapure (1 ng - 1 µg/ml final concentration) per well of a 96-well plate.
- Prepare a cell suspension of HEK-Blue™ TLR5 cells according to the data sheet.
- Add 180 µl of HEK-Blue™ TLR5 cell suspension per well.
- Incubate the plate for 6-24 h at 37 °C, 5% CO₂.
- Collect 20 µl of supernatant and add to a well of a 96-well plate containing 180 µl of QUANTI-Blue™.
- Incubate the plate at 37 °C for 1-3 h.
- Determine SEAP levels using a spectrophotometer at 620-655 nm.

RELATED PRODUCTS

Product	Cat. Code
HEK-Blue™ hTLR5 cells (human TLR5)	hkb-htrl5
HEK-Blue™ mTLR5 cells (mouse TLR5)	hkb-mtrl5
QUANTI-Blue™	rep-qb1
Other TLR5 ligands:	
FLA-BS Ultrapure (flagellin from <i>B. subtilis</i>)	tlr1-pbsfla
FLA-ST Ultrapure (flagellin from <i>S. typhimurium</i>)	tlr1-epstfla
RecFLA-ST (recombinant flagellin from <i>S. typhimurium</i>)	tlr1-flc

TECHNICAL SUPPORT

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