

FLA-BS Ultrapure

Purified Flagellin from *B. subtilis*; TLR5 ligand

Catalog code: tlr1-pbsfla

<https://www.invivogen.com/fla-bs>

For research use only

Version 24B29-MM

PRODUCT INFORMATION

Contents

- 50 µg FLA-BS Ultrapure (purified flagellin from *Bacillus subtilis*)
- 1.5 ml endotoxin-free water

Storage and stability

- FLA-BS Ultrapure is shipped at room temperature. Upon receipt, store at -20°C.

- Upon resuspension, prepare aliquots of FLA-BS 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: >95% (SDS-PAGE)
- Endotoxin levels: <0.05 EU/µg
- Biological activity has been confirmed using HEK-Blue™ hTLR5 cells.

DESCRIPTION

FLA-BS Ultrapure, a ~32 kDa protein, is a high purity grade of flagellin isolated from the Gram-positive bacteria *Bacillus subtilis*. FLA-BS Ultrapure is extracted by violent agitation and purified by different successive separation techniques. Flagellin, the principal component of the flagella present on many Gram-negative and Gram-positive bacteria, 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-BS Ultrapure at a concentration of 1 ng -1 µg/ml.

1. Open vial lid carefully to avoid any loss of product.
2. Add 100 µl of the endotoxin-free water (provided) and mix by pipetting. Do **not** vortex.

TLR5 stimulation using FLA-BS Ultrapure

FLA-BS Ultrapure can be used to stimulate TLR5 in HEK-Blue™ TLR5 cells. These cells stably overexpress the TLR5 gene and an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP). Levels of SEAP can be easily determined using a SEAP detection medium, such as HEK-Blue™ Detection.

For more information, visit: www.invivogen.com/hek-blue-tlr5.

1. Dispense 20 µl of FLA-BS Ultrapure (1 ng to 1 µg/ml final concentration) per well of a 96-well plate.
2. Prepare a suspension of HEK-Blue™ TLR5 cells in HEK-Blue™ Detection medium according to the data sheet.
3. Immediately add 180 µl of the cell suspension to each FLA-BS Ultrapure-containing well.
4. Incubate the plate at 37°C in a CO₂ incubator for 16-24 hours.
5. Determine SEAP levels using a spectrophotometer at 620-655 nm.

RELATED PRODUCTS

Product	Description	Cat. Code
HEK-Blue™ hTLR5 Cells	Human TLR5 reporter cells	hkb-htlr5
HEK-Blue™ mTLR5 Cells	Murine TLR5 reporter cells	hkb-mtlr5
HEK-Blue™ Detection	SEAP detection medium	hb-det2
FLA-ST Ultrapure	Flagellin from <i>S. typhimurium</i>	tlr1-epstfla
FLA-PA Ultrapure	Flagellin from <i>P. aeruginosa</i>	tlr1-pafla

TECHNICAL SUPPORT

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