FLA-BS

Flagellin from *B. subtilis*; TLR5 ligand

Catalog code: tlrl-bsfla

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

For research use only

Version 24B29-MM

PRODUCT INFORMATION

Contents

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

Storage and stability

- FLA-BS is shipped at room temperature. Upon receipt, store at -20 °C.
- Upon resuspension, prepare aliquots of FLA-BS 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

- Endotoxin levels: <0.05 EU/µg
- Biological activity has been confirmed using HEK-Blue™ hTLR5 cells.

DESCRIPTION

FLA-BS, a ~32 kDa protein, is isolated from the Gram-positive bacteria *Bacillus subtilis*. 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 NAIP5[2]. 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 complex[3]. 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 at a concentration of 10 ng -10 µg/ml.

1. Open vial lid carefully to avoid any loss of product.
2. Add 200 µl of the endotoxin-free water provided and mix by pipetting. Do **not** vortex.

TLR5 stimulation using FLA-BS

FLA-BS 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 (10 ng to 10 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-containing well.
4. Incubate the plate at 37°C in a CO_2 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 *S. typhimurium*	tlrl-epstfla
FLA-PA Ultrapure	Flagellin from *P. aeruginosa*	tlrl-pafla

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Asia: +852 3622-3480
E-mail: info@invivogen.com