FLA-BS

Flagellin from *B. subtilis*; TLR5 ligand

Catalog # tlrl-bsfla

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

For research use only

Version # 17L04-MM

PRODUCT INFORMATION

Content:

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

Storage:

- FLA-BS is shipped at room temperature and should be stored 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.

- Open vial lid carefully to avoid any loss of product.
- 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. 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-tlr5

- Dispense 20 µl of FLA-BS (10 ng -10 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% CO_2.
- 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	Catalog Code
HEK-Blue™ hTLR5 cells (human TLR5)	hkb-htlr5
HEK-Blue™ mTLR5 cells (mouse TLR5)	hkb-mtlr5
QUANTI-Blue™	rep-qb1
Other TLR5 ligands:	
FLA-BS Ultrapure (flagellin from *B. subtilis)*	tlrl-pbsfla
FLA-ST Ultrapure (flagellin from *S. typhimurium)*	tlrl-epstfla
RecFLA-ST (recombinant flagellin from *S. typhimurium*)	tlrl-flic

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 Hong Kong: +852 3622-3480
E-mail: info@invivogen.com