

FLA-ST

Flagellin from *S. typhimurium*; TLR5 ligand

Catalog # tlr1-stfla

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

For research use only

Version # 17L04-MM

PRODUCT INFORMATION

Content:

- 100 µg FLA-ST (standard flagellin from *S. typhimurium*)
- 1.5 ml endotoxin-free water

Storage:

- FLA-ST is shipped at room temperature and should be stored at -20°C for 1 year.
- Upon resuspension, prepare aliquots of FLA-ST and store -20°C. Product is stable for 6 months at -20°C when properly stored. Avoid repeated freeze-thaw cycles.

Quality control:

- Purity: ≈ 10% (SDS-PAGE)
- Endotoxin levels: 1-10 EU/µg
- Biological activity has been confirmed using HEK-Blue™ hTLR5 cells.

DESCRIPTION

FLA-ST is isolated from the Gram-negative bacteria *S. typhimurium*. 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-ST 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-ST

FLA-ST 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-tnfr5

- Dispense 20 µl of FLA-ST (10 ng to 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% 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	hkb-htlr5
HEK-Blue™ mTLR5 Cells	hkb-mtlr5
QUANTI-Blue™	rep-qb1
Other TLR5 ligands:	
FLA-BS Ultrapure (flagellin from <i>B. subtilis</i>)	tlr1-pbsfla
FLA-PA Ultrapure (flagellin from <i>P. aeruginosa</i>)	tlr1-pafila
FLA-ST Ultrapure (flagellin from <i>S. typhimurium</i>)	tlr1-epstfla
RecFLA-ST (recombinant flagellin from <i>S. typhimurium</i>)	tlr1-flic

TECHNICAL SUPPORT

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