

RecFLA-ST

Recombinant flagellin from *Salmonella typhimurium*; TLR5 ligand

Catalog code: tlr1-flic-10, tlr1-flic-50

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

For research use only

Version 18D16-MM

PRODUCT INFORMATION

Contents

- Recombinant flagellin from *S. typhimurium* (RecFLA-ST) is provided lyophilized and is available in two quantities:
 - 10 µg: tlr1-flic-10
 - 50 µg: tlr1-flic-50
- Endotoxin-free water; 1.5 ml is provided with tlr1-flic-10 and 10 ml is provided with tlr1-flic-50

Storage and stability

- RecFLA-ST is shipped at room temperature. Upon receipt, store at -20°C.
- Upon resuspension, prepare aliquots and store at -20°C. Resuspended product is stable 6 months at -20°C when properly stored. Avoid repeated freeze-thaw cycles.

Quality Control

- Biological activity has been verified using HEK-Blue™ hTLR5 cells.
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using cellular assays.

BACKGROUND

Flagellin is the major component of the bacterial flagellar filament, which confers motility on a wide range of bacterial species. This proinflammatory molecule is present in both Gram-negative and Gram-positive bacteria. It is recognized by distinct types of pattern recognition receptors (PRRs). Extracellular flagellin is recognized by surface localized Toll-like receptor 5 (TLR5), which promotes the activation of NF-κB and the subsequent production of cytokines^{1, 2}. Flagellin is also recognized by the cytosolic NOD-like receptors (NLRs) NAIP5/NAIP6 sensors of the NLRC4 inflammasome^{3, 4}. Flagellin monomers are translocated into the host cell cytosol by a mechanism that requires bacterial secretion systems. This triggers the formation of a NAIP-NLRC4 inflammasome leading to caspase-1-mediated secretion of IL-1β and IL-18^{3, 4}.

1. Song WS. *et al.*, 2017. A conserved TLR5 binding and activation hot spot on flagellin. *Sci Rep.* 7:40878. 2. Hayashi F. *et al.*, 2001. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature.* 410(6832):1099-103. 3. Duncan JA. & Canna SW., 2018. The NLRC4 Inflammasome. *Immunol Rev.* 281(1):115-123. 4. Zhao Y. *et al.*, 2011. The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus. *Nature.* 477(7366):596-600.

DESCRIPTION

RecFLA-ST is a recombinant flagellin protein encoded by the fliC gene from the Gram-negative bacteria *Salmonella typhimurium*. This protein is produced in CHO cells and purified by affinity chromatography. RecFLA-ST is intended for use in cell culture applications.

METHODS

Preparation of stock solution (500 µg/ml)

- Open vial lid carefully to avoid any loss of product.
- Resuspend RecFLA-ST with the endotoxin-free water (provided).
 - Add 20 µl of endotoxin-free water to the 10 µg vial.

Note: Alternatively, 10 µg of RecFLA-ST can be resuspended in 40 µl of endotoxin-free water to provide a stock solution at 250 µg/ml.

- Add 100 µl of endotoxin-free water to the 50 µg vial.
- Mix by pipetting. Do not vortex.

Working concentration: 10-100 ng/ml

TLR5 activation using RecFLA-ST

Below is a protocol for TLR5 activation 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 RecFLA-ST (10-100 ng/ml final concentration) per well of a 96-well plate.
- Distribute 180 µl of HEK-Blue™ TLR5 cell suspension (1.4 x 10⁵ cells) 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™ Solution.
- Incubate the plate at 37°C for 1-3 h.
- 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-htr5
HEK-Blue™ mTLR5 cells	Mouse TLR5 reporter cells	hkb-mtr5
HEK-Dual™ hTLR5 cells	Human TLR5 dual reporter cells	hkd-htr5ni
QUANTI-Blue™ Solution	SEAP detection medium	rep-qbs
Other TLR5 ligands:		
FLA-BS Ultrapure	Flagellin from <i>B. subtilis</i>	tlr1-pbsfla
FLA-PA Ultrapure	Flagellin from <i>P. aeruginosa</i>	tlr1-pafla
FLA-ST Ultrapure	Flagellin from <i>S. typhimurium</i>	tlr1-epstfla
Flagellin FliC VaccciGrade™	Preclinical grade RecFLA-ST	vac-fla

TECHNICAL SUPPORT

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