hTLR5-Fc

Soluble ectodomain of human TLR5 fused to an IgG1 Fc domain

Catalog code: fc-htlr5-2

https://www.invivogen.com/htlr5-fc

For research use only

Version 24E27-MM

PRODUCT INFORMATION

Contents

- 2 x 50 µg lyophilized hTLR5-Fc

Formulation

0.2 µm filtered solution in phosphate buffer with glycine, saccharose and stabilizing agents

Storage and stability

- Product is shipped at room temperature. Upon receipt, store at -20 °C.
- Reconstituted hTLR5-Fc is stable for 1 month when stored at 4 °C and for 1 year when stored at -20 °C. Avoid repeated freeze-thaw cycles.

Quality control

- This product has been validated for neutralization.
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION

The soluble TLR5 receptor, hTLR5-Fc, was generated by fusing the N-terminal extracellular domain of human TLR5 (aa 21-639) to the N-terminus of a human IgG1 Fc domain with a 2 amino acid linker. The hTLR5-hFc fusion has an apparent molecular weight of 110 kDa on SDS-PAGE. Fc-hTLR5 is expressed in CHO cells and purified by protein G affinity chromatography.

BACKGROUND

Toll-like receptor 5 (TLR5) is a Type 1 transmembrane receptor comprising an N-terminal extracellular leucine rich repeat domain and a C-terminal intracellular TIR signaling domain. TLR5 recognizes flagellin from both Gram-positive and Gram-negative bacteria. Activation of the receptor stimulates the production of proinflammatory cytokines, such as TNF-α, through signaling via the adaptor protein MyD88 and the serine kinase IRAK[1, 2]. TLR5 can generate a proinflammatory signal as a homodimer suggesting that it might be the only TLR participating in flagellin recognition[2]. However, TLR5 may require the presence of a co-receptor or adaptor molecule for efficient ligand recognition and/or signaling[3].

1. Gewirtz AT. *et al.*, 2001. Cutting edge: bacterial flagellin activates basolaterally expressed TLR5 to induce epithelial proinflammatory gene expression. J Immunol, 167(4):1882-5. **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. Tallant T. *et al.*, 2004.** Flagellin acting via TLR5 is the major activator of key signaling pathways leading to NF-kappa B and proinflammatory gene program activation in intestinal epithelial cells. BMC Microbiol. 4(1):33.

METHOD

Preparation of stock solution (100 µg/ml)

1. Add 500 µl of sterile water to the 50 µg of hTLR5-Fc.
2. Mix by pipetting. Do **not** vortex.

APPLICATIONS

hTLR5-Fc can be used for receptor binding assays and to neutralize human TLR5-induced cellular activation. The optimal working concentration of hTLR5-Fc must be determined empirically for a given set of experimental conditions.

Receptor binding assays

Typically, 1 ng-1 µg/ml hTLR5-Fc is incubated with 0.1-5 µg of an immobilized TLR5 ligand. The receptor-ligand binding is detected using a labeled secondary anti-IgG antibody.

Neutralization studies

We recommend to incubate various concentrations of hTLR5-Fc (typically 10 ng-1 µg/ml) with human TLR5-expressing cells, prior to the addition of a TLR5 ligand (typically 10 ng-5 µg/ml). The neutralizing activity of hTLR5-Fc is determined by assessing flagellin-induced cell activation.

RELATED PRODUCTS

Product	Catalog Code
TLR5 ligands	
FLA-BS Ultrapure (flagellin from *B. subtilis*)	tlrl-pbsfla
FLA-PA Ultrapure (flagellin from *P. aeruginosa*)	tlrl-pafla
FLA-ST Ultrapure (flagellin from *S. typhimurium*)	tlrl-epstfla
Anti-Flagellin antibodies	
Anti-Flagellin FliC	mabg-flic-2
TLR5 expressing cells	
HEK-Blue™ hTLR5 cells (SEAP reporter cells)	hkb-htlr5
TLR5 plasmid	
pUNO1-hTLR5	puno1-htlr5

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