

Anti-hTLR5-IgA

Neutralizing IgA monoclonal antibody to human TLR5

Catalog code: maba2-htlr5

<https://www.invivogen.com/anti-htlr5-iga>

For research use only, not for diagnostic or therapeutic use

Version 22K21-MM

PRODUCT INFORMATION

Contents

- 100 µg of purified anti-hTLR5-IgA antibody, provided azide-free and lyophilized

Specificity: Human TLR5 (hTLR5)

Source: Chinese hamster ovary (CHO) cells

Clone: Q2G4

Isotype: Human IgA2

Light chain type: Kappa

Clonality: Monoclonal

Purification: By affinity chromatography

Formulation: 0.2 µm filtered solution in TRIS buffer with glycine, saccharose, and stabilizing agents

Antibody resuspension

Add 1 ml of sterile water to obtain a concentration of 0.1 mg/ml.

Storage and stability

- Product is shipped at room temperature. Upon receipt, store at -20°C.
- Reconstituted antibody 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) is confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

BACKGROUND

Toll-like receptor 5 (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 IRAK1^{1,2}. TLR5 can generate a proinflammatory signal as a homodimer suggesting that it might be the only TLR participating in flagellin recognition². However, TLR5 may require the presence of a co-receptor or adaptor molecule for efficient ligand recognition and/or signaling³.

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.

DESCRIPTION

Anti-hTLR5-IgA is a chimeric monoclonal antibody specific for human Toll-like receptor 5 (TLR5). It was generated by combining the constant domains of the human IgA molecule with the murine variable regions. It was produced in CHO cells and purified by affinity chromatography. Anti-hTLR5-IgA has been selected for its ability to efficiently neutralize the biological activity of TLR5. The neutralizing activity was determined using HEK-Blue™ TLR5 cells. This antibody can also be used to detect hTLR5 using flow cytometry.

APPLICATIONS

Anti-hTLR5-IgA can be used for neutralization as described below. It can also be used for detection using flow cytometry.

Neutralization

The exact concentration of antibody required to neutralize hTLR5 activity is dependent on the cell type and growth conditions. InvivoGen has determined the neutralization dose for this antibody using **FLA-ST Ultrapure** (flagellin from *S. typhimurium*) and **HEK-Blue™ hTLR5 cells** (HEK239 cells expressing hTLR5 and an NF- κ B-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene. For more information, visit www.invivogen.com/hek-blue-tnfr5.

Procedure for neutralization using HEK-Blue™ hTLR5 cells

1. Add 100 µl of growth medium per well of a 96-well plate.
2. Add 50 µl of Anti-hTLR5-IgA or control antibody (10 ng/ml-10 µg/ml final concentration) per well.
Note: We recommend using Human IgA2 Control (which targets E. coli β -galactosidase) as a negative control antibody.
3. Add 100 µl of **HEK-Blue™ hTLR5 cell** suspension (~50,000 cells) per well.
4. Incubate for 1 hour at 37°C in a 5% CO₂ incubator.
5. Add 50 µl **FLA-ST Ultrapure** (1 ng/ml final concentration).
6. Incubate the plate at 37°C in a 5% CO₂ incubator for 18-24 h.
7. Monitor SEAP production using a SEAP detection assay such as **QUANTI-Blue™ Solution**.

RELATED PRODUCTS

Product	Description	Cat.Code
FLA-ST Ultrapure	TLR5 agonist	tlrl-epstfla
HEK-Blue™ hTLR5 Cells	hTLR5 reporter cells	hkb-htlr5
Human IgA2 Control	Isotype control	maba2-ctrl
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs

TECHNICAL SUPPORT

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