

Anti-hTLR5-IgA2

Detection and neutralizing IgA2 monoclonal antibody to human TLR5

Catalog code: htlr5-maba7-2

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

For research use only

Version 24F24-NJ

PRODUCT INFORMATION

Contents

- 2 x 100 µg of purified anti-hTLR5-IgA2 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 (0.1 mg/ml)

Add 1 ml of sterile water per 100 µg vial.

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 using cellular assays.
- 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 IRAK^{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.

TECHNICAL SUPPORT

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DESCRIPTION

Anti-hTLR5-IgA2 is a chimeric monoclonal antibody specific for human Toll-like receptor 5 (TLR5). It was generated by combining the constant domains of the human IgA2 molecule with the murine variable regions. Anti-hTLR5-IgA2 has been selected for its ability to efficiently neutralize the biological activity of TLR5. It can also be used to detect hTLR5 using flow cytometry. This antibody was produced in Chinese hamster ovary (CHO) cells and purified by affinity chromatography.

APPLICATIONS

Anti-hTLR5-IgA2 is a detection and neutralizing antibody. It can be used to block hTLR5-induced cellular activation *in vitro*, as described below.

NEUTRALIZATION PROTOCOL

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-htlr5.

In a 96-well plate:

1. Add 50 µl of Anti-hTLR5-IgA2 (10 ng/ml - 10 µg/ml final concentration) per well.
*Note: We recommend using Anti- β -Gal-hlgA2 (which targets *E. coli* β -galactosidase) as a negative control antibody.*
2. Add 100 µl of HEK-Blue™ hTLR5 cell suspension (~50,000 cells) per well.
3. Incubate for 1 hour at 37°C in a 5% CO₂ incubator.
4. Add 50 µl human TLR5 agonist, such as FLA-ST Ultrapure (1 ng/ml final concentration).
5. Incubate the plate at 37°C in a 5% CO₂ incubator for 18-24 h.
6. Monitor SEAP production using QUANTI-Blue™ Solution following the instructions on the data sheet.

RELATED PRODUCTS

Product	Description	Cat.Code
FLA-ST Ultrapure	TLR5 agonist	tlr-epstfla
HEK-Blue™ hTLR5 Cells	hTLR5 reporter cells	hkb-htlr5
Anti- β -Gal-hlgA2	Isotype control	bgal-mab7
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs