Anti-hTLR4-IgG

Neutralizing IgG monoclonal antibody to human TLR4

Catalog # mabg-htlr4

For research use only, not for diagnostic or therapeutic use

Version # 12C02-MM

PRODUCT INFORMATION

Content

100 µg purified anti-hTLR4-IgG antibody, provided azide-free and lyophilized

Clone: W7C11

Isotype: Mouse IgG1

Formulation: 0.2 µm filtered solution in PBS with 5% saccharose

Antibody resuspension

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

Storage

- Product is shipped at room temperature. Store lyophilized antibody at -20˚C. Product is stable for 6 months.
- Reconstituted antibody is stable 1 month when stored at 4°C and 6 months when aliquoted and stored at -20˚C. Avoid repeated freeze-thaw cycles.

Description

Anti-hTLR4-IgG is a monoclonal antibody specific for human Toll-like receptor 4 (hTLR4). Anti-hTLR4-IgG has been selected for its ability to efficiently neutralize the biological activity of hTLR4. The neutralizing activity of this IgG antibody was determined using InvivoGen's HEK-Blue™ hTLR4 cells, which express the human TLR4 and the SEAP reporter genes.

BACKGROUND

Toll-Like receptors (TLRs) play a critical role in early innate immunity to invading pathogens by sensing microorganisms. These evolutionary conserved receptors recognize highly conserved structural motifs only expressed by microbial pathogens, called pathogen-associated microbial patterns (PAMPs). Stimulation of TLRs by PAMPs initiates a signaling cascade leading to the secretion of proinflammatory cytokines following NF-κB activation. To date ten human and twelve murine TLRs have been characterized, TLR1 to TLR10 in humans, and TLR1 to TLR9, TLR11, TLR12 and TLR13 in mice, the homolog of TLR10 being a pseudogene.

TLR4, the first human TLR identified, is the receptor for Gram-negative lipopolysaccharide (LPS). The TLR4 gene was shown to be mutated in C3H/HeJ and C57BL/10ScCr mice, both of which are low responders to LPS[1]. However, TLR4 alone is not sufficient to confer LPS responsiveness. TLR4 requires MD-2, a secreted molecule, to functionally interact with LPS[2]. Furthermore, a third protein, called CD14, was shown to participate in LPS signaling, leading to NF-κB translocation. This signaling is mediated through several adaptor proteins: MyD88 TIRAP/Mal[3] , TRIF/TICAM1 and TRAM/TICAM2[4].

APPLICATIONS

Anti-hTLR4-IgG is a neutralizing antibody, it blocks LPS-induced cellular activation.

Neutralization

The exact concentration of antibody required to neutralize human TLR4 activity is dependent on the TLR4 agonist used and its concentration, cell type and growth conditions. InvivoGen has determined the neutralization dose for this antibody using ultra-pure LPS from *Escherichia coli 0111:B4* (LPS-EB ultra-pure) and HEK-Blue™ hTLR4 Cells. These cells are engineered HEK293 cells stably expressing human TLR4, MD2 and CD14 and an NF-κB-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene.

Procedure for neutralization using HEK-Blue™ hTLR4 cells

1- Prepare a cell suspension at 250,000 cells/ml.
2- Add 100 µl of cell suspension per well of a 96-well plate.
3- Add 100 µl of anti-hTLR4-IgG dilution (500 ng to 10 µg/ml final).
4- Incubate 1 hour at 37°C.
5- Add 50 µl LPS-EB ultra-pure (2 ng/ml final).
6- Incubate overnight at 37°C
7- Add 20 µl supernatant to 180 µl QUANTI-Blue™ in a 96-well plate.
8- Incubate 1-3 hours at 37°C
9- Assess SEAP levels with the naked eye or spectrophotometrically by reading the OD at 655 nm.

References

1. Poltorak A. *et al.*, 1998. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science, 282(5396):2085-8. **2. Shimazu R. *et al.*, 1999.** MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. J Exp Med, 189(11):1777-82.**3. Horng T. GM. Barton, and R. Medzhitov, 2001.** TIRAP: an adapter molecule in the Toll signaling pathway. Nat Immunol, 2(9):835-41. **4. Fitzgerald KA. *et al.*, 2003.** LPS-TLR4 Signaling to IRF-3/7 and NF-{kappa}B Involves the Toll Adapters TRAM and TRIF. J Exp Med. 198(7):1043-1055.

RELATED PRODUCTS

Product	Catalog Code
HEK-Blue™ hTLR4 cells	hkb-htlr4
LPS-EB ultra-pure	tlrl-3pelps
QUANTI-Blue™	rep-qb-1

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