

MAb mTLR4/MD2

Purified monoclonal antibody to mouse TLR4/MD2

Catalog # mab-mtlr4md2

For research use only, not for diagnostic or therapeutic use

Version # 14B27-MM

PRODUCT INFORMATION

Content

100 µg purified monoclonal anti-mTLR4/MD2 antibody (MAb-mTLR4/MD2), provided lyophilized

Clone: MTS510

Isotype: Rat IgG2a

Formulation: PBS pH 7.4

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 MAb-mTLR4/MD2 at -20°C. Product is stable for 1 year.

- Upon resuspension, prepare aliquots of MAb-mTLR4/MD2 and store at -20°C. Resuspended product is stable 1 year when properly stored. Avoid repeated freeze-thaw cycles.

Description

MAb mTLR4/MD2 (MTS510) monoclonal antibody reacts preferentially, especially in flow cytometry, with mouse Toll-like receptor 4 (TLR4, CD284) that is associated with MD2. MAb mTLR4/MD2 is a blocking antibody that is useful to study the role of TLR4 as a receptor for LPS induced cytokine production by TLR4 expressing cells. MAb mTLR4/MD2 was shown to coprecipitate MD2 (30 kDa) with TLR4 (100 kDa).

BACKGROUND

Toll-like Receptor 4 (TLR4; also known as CD284) plays a crucial role in initiating immune responses. TLR4 was identified as the receptor that responds to lipopolysaccharide (LPS; also known as endotoxin), a component of the outer membrane of Gram-negative bacteria^{1, 2}. However, TLR4 alone is not sufficient to confer LPS responsiveness. TLR4 requires MD-2 (also known as lymphocyte antigen 96), a secreted molecule, to functionally interact with LPS³. MD-2 binds to both LPS and the extracellular domain of TLR4. Following synthesis, MD-2 is either secreted directly into the medium as a soluble, active protein, or binds directly to TLR4 in the endoplasmic reticulum before migrating to the cell surface. The secreted form of MD-2 forms a stable complex with LPS, in a CD14-dependent process⁴. The MD-2/LPS complex activates TLR4 in the absence of CD14 or free LPS, indicating that the activating ligand of TLR4 is the MD-2/LPS complex. Additional proteins, called CD14 and LPS-binding protein (LBP), are also involved in LPS binding⁵. Recognition of LPS by TLR4 induces the production of pro-inflammatory cytokines via MyD88-dependent or TRIF-dependent pathways.

1. **Poltorak A. et al., 1998.** Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science*, 282:2085-8. 2. **Chow JC. et al., 1999.** Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction. *J Biol Chem*. 274(16):10689-92. 3. **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. 4. **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. 5. **Kawai T. & Akira S., 2010.** The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat Immunol*. 11(5):373-84.

APPLICATIONS

MAb mTLR4/MD2 (MTS510) can be used for flow cytometry (intracellular staining), immunoprecipitation and immunohistochemical staining of frozen tissue sections. The antibody recognizes the mouse TLR4/MD2 complex and is not useful for detection of TLR4 alone. Furthermore, MAb TLR4 can be used as an antagonist of TLR4, it blocks LPS-induced cellular activation.

Neutralization

The exact concentration of antibody required to neutralize mouse TLR4/MD2 activity is dependent on the TLR4 agonist used and its concentration, cell type and growth conditions.

In vivoGen has determined the neutralization dose for this antibody using the TLR4 ligand lipopolysaccharide (LPS) from *Escherichia coli 0111:B4* (LPS-EB Ultrapure) and HEK-Blue™ mTLR4 cells. HEK-Blue™ mTLR4 cells were obtained by co-transfection of the murine TLR4, MD-2 and CD14 co-receptor genes, and an inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene into HEK293 cells. SEAP expression can be easily detected and quantified using QUANTI-Blue™, a SEAP detection medium that turns blue following TLR stimulation but remains pink if neutralization occurs.

Procedure for neutralization using HEK-Blue™ mTLR4 cells

- 1- Prepare a cell suspension of HEK-Blue™ mTLR4 cells.
- 2- Add 20 µl of MAb-mTLR4/MD2 per well of a 96-well plate (1-10 µg/ml final).
- 3- Add 160 µl of cell suspension per well (5 x 10⁴ cells/well).
- 4- Incubate 1 hour at 37°C, 5% CO₂.
- 5- Add 20 µl LPS-EB Ultrapure per well (0.5-1 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, 5% CO₂.
- 9- Assess SEAP levels with the naked eye or spectrophotometrically by reading the OD at 655 nm.

RELATED PRODUCTS

Product	Catalog Code
pUNO-mTLR4 (mouse TLR4 gene)	puno-mtlr4
pUNO-mMD2 (mouse MD2 gene)	puno-mmd2
HEK-Blue™ mTLR4 cells	hkb-mtlr4
QUANTI-Blue™	rep-qb-1
LPS-EB ultrapure (<i>E. coli 0111:B4</i>)	tlr1-3pelps
293/mTLR4-MD2-CD14 cells	293-mtlr4md2cd14

TECHNICAL SUPPORT

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