

# MAB-hMD2

Neutralizing IgG monoclonal antibody to human MD2

Catalog # mab-hmd2

For research use only, not for diagnostic or therapeutic use

Version # 13C12-MM

## PRODUCT INFORMATION

### Content

100 µg purified MAb-hMD2 antibody, provided azide-free and lyophilized

**Clone:** 18H10

**Isotype:** Mouse IgG2b

**Formulation:** 0.2 µm filtered solution in PBS

### 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 12 months when properly stored.

- 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

MAB-hMD2 is a monoclonal antibody specific for the human MD2 accessory molecule. MAB-hMD2 has been selected for its ability to efficiently neutralize the biological activity of the human MD2.

## BACKGROUND

MD-2 (also known as lymphocyte antigen 96), a 25-kDa secreted protein, is an accessory molecule required for efficient lipopolysaccharide (LPS)-induced signaling through TLR4<sup>1</sup>. 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 process<sup>2</sup>. 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. MD-2 also increases TLR2 responsiveness to TLR2 ligands<sup>3</sup>.

1. 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.

2. Kennedy MN. *et al.*, 2004. A complex of soluble MD-2 and lipopolysaccharide serves as an activating ligand for Toll-like receptor 4. *J Biol Chem*. 279(33):34698-704.

3. Dziarski R. *et al.*, 2001. MD-2 enables Toll-like receptor 2 (TLR2)-mediated responses to lipopolysaccharide and enhances TLR2-mediated responses to Gram-positive and Gram-negative bacteria and their cell wall components. *J Immunol*. 166(3):1938-44.

## APPLICATIONS

MAB-hMD2 is a neutralizing antibody, it blocks LPS-induced cellular activation. This antibody can also be used for flow cytometry.

### Neutralization

The exact concentration of antibody required to neutralize human MD2 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 the TLR4 ligand lipopolysaccharide (LPS) from *Escherichia coli K12* (LPS-EK Ultrapure) and THP1-XBlue™-MD2-CD14 cells. These cells are derived from the THP-1 cell line, human monocytes that naturally express TLR4. THP1-XBlue™-MD2-CD14 cells stably express human MD2 and CD14 genes to increase their response to LPS. These cells also express an NF-κB-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene. 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 THP1-XBlue™-MD2-CD14 cells

- 1- Prepare a cell suspension of THP1-XBlue™-MD2-CD14 cells.
- 2- Add 100 µl of MAB-hMD2 per well of a 96-well plate (500 ng to 10 µg/ml final).
- 3- Add 100 µl of cell suspension per well (1 x 10<sup>5</sup> cells/well).
- 4- Incubate 1 hour at 37°C, 5% CO<sub>2</sub>.
- 5- Add 50 µl LPS-EK Ultrapure per well (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% CO<sub>2</sub>.
- 9- Assess SEAP levels with the naked eye or spectrophotometrically by reading the OD at 655 nm.

### Flow Cytometry

This antibody was used at 500 - 2000 ng/10<sup>6</sup> cells with a PE goat anti-mouse light chain secondary antibody for indirect immunofluorescence staining of HEK-Blue™ hTLR4 cells by flow cytometry. InvivoGen's HEK-Blue™ hTLR4 cells are engineered HEK293 cells stably expressing human TLR4, MD2 and CD14 and an NF-κB-inducible SEAP reporter gene.

## RELATED PRODUCTS

Product	Catalog Code
THP1-XBlue™-MD2-CD14 cells	thpx-mcdcdsp
HEK-Blue™ hTLR4 cells	hkb-htlr4
LPS-EK Ultrapure	tlrl-pekllps
QUANTI-Blue™	rep-qb-1

## TECHNICAL SUPPORT

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