

Anti-hCD14-IgA

Neutralizing IgA monoclonal antibody to human CD14

Catalog # maba-hcd14

For research use only, not for diagnostic or therapeutic use

Version # 15C23-MM

PRODUCT INFORMATION

Content

100 µg purified anti-hCD14-IgA antibody, provided azide-free and lyophilized

Clone: D3B8

Isotype: Human IgA2

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

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 at least 1 year.
- Reconstituted antibody is stable for 1 month when stored at 4 °C and for 1 year when aliquoted and stored at -20 °C. Avoid repeated freeze-thaw cycles.

Quality control

- This product has been validated for neutralization and flow cytometry.
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) is confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

BACKGROUND

CD14 is a glycosylphosphatidylinositol (GPI)-anchored membrane protein which acts as a bacterial pattern recognition receptor¹. CD14 is found on cells derived from the monocyte/macrophage lineage, as well as neutrophils and B lymphocytes. CD14 serves as a member of the heteromeric lipopolysaccharide (LPS) receptor complex that also contains TLR4 and MD2². CD14 binds LPS but is not capable of initiating a transmembrane activation signal since it does not contain a cytoplasmic domain. Upon LPS binding, CD14 physically associates with TLR4 which in turn transduces the signal. CD14 was also shown to interact with TLR2 in response to various microbial infections³.

1. Pugin J. *et al.*, 1994. CD14 is a pattern recognition receptor. *Immunity*. 1(6):509-16. 2. Da Silva Correia J. *et al.*, 2001. Lipopolysaccharide is in close proximity to each of the proteins in its membrane receptor complex. transfer from CD14 to TLR4 and MD-2. *J Biol Chem*. 276(24): 21129-35. 3. Aderem A. & Ulevitch R.J., 2000. Toll-like receptors in the induction of the innate immune response. *Nature*. 406(6797):782-7.

DESCRIPTION

Anti-hCD14-IgA is a chimeric monoclonal antibody specific for human CD14. It was generated by combining the constant domains of the human IgA molecule with murine variable regions. Anti-hCD14-IgA has been selected for its ability to efficiently neutralize the biological activity of TLR2 or TLR4.

APPLICATIONS

Anti-hCD14-IgA is a neutralizing antibody, it blocks TLR2 or TLR4 ligands induced cellular activation. It can also be used for flow cytometry.

Neutralization

The exact concentration of antibody required to neutralize human TLR2 or TLR4 activity is dependent on the TLR2 or TLR4 ligand used and their concentration, cell type and growth conditions. InvivoGen has determined the neutralization dose for this antibody using the ligand LPS-EK and HEK-Blue™ hTLR4 cells. These cells are engineered HEK293 cells stably expressing the human TLR4, MD2 and CD14 genes, as well as an NF-κB-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene.

Anti-hCD14-IgA dilution (100 ng to 10 µg/ml) and a negative control antibody (e.g. Human IgA2 Control which targets *E. coli* β-galactosidase) were incubated with HEK-Blue™ TLR4 cells for 1 hour prior to the addition of LPS-EK (1-10 ng/ml). Neutralization of TLR4-induced signaling by anti-hCD14-IgA was determined after a 24 hour incubation by assessing SEAP production using QUANTI-Blue™. QUANTI-Blue™ is a SEAP detection medium that turns blue following cytokine stimulation but remains pink if neutralization occurs. SEAP levels can be assessed by the naked eye or spectrophotometrically by reading the OD at 620-655 nm.

Flow Cytometry

This antibody was used at 200-500 ng/10⁶ cells with a goat F(ab')₂ anti-human IgA-FITC secondary antibody for indirect immunofluorescence staining of HEK-Blue hTLR2 cells.

RELATED PRODUCTS

| Product | Catalog Code |
|--------------------|--------------|
| HEK-Blue™ hTLR2 | hkb-htr2 |
| HEK-Blue™ hTLR4 | hkb-htr4 |
| Human IgA2 Control | maba2-ctrl |
| LPS-EK | tlr1-eklps |
| QUANTI-Blue™ | rep-qb-1 |

TECHNICAL SUPPORT

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