

Anti-hCD14-IgG

Neutralizing IgG monoclonal antibody to human CD14

Catalog code: mabg-hcd14-2

<https://www.invivogen.com/anti-hcd14-igg>

For research use only, not for diagnostic or therapeutic use

Version 24A02-MM

PRODUCT INFORMATION

Contents: 2 x 100 µg purified anti-hCD14-IgG provided azide-free and lyophilized

Target: Human CD14 (hCD14)

Species reactivity: Reacts with hCD14

Clonality: Monoclonal antibody

Clone: D3B8

Isotype: Human IgG1

Source: CHO cells

Formulation: 0.2 µm filtered solution in sodium phosphate buffer with glycine, saccharose, and stabilizing agents

Purity: Purified by affinity chromatography with protein G

Antibody resuspension (0.1 mg/ml)

Add 1 ml of sterile water to 100 µg and gently pipette until completely resuspended.

Storage and stability

- Product is shipped at room temperature. Store lyophilized antibody 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 and flow cytometry.

- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been 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-IgG is a chimeric monoclonal antibody specific for human CD14. It has been selected for its ability to efficiently neutralize the biological activity of TLR2 or TLR4. This antibody was generated by recombinant DNA technology. It has been produced in CHO cells and purified by affinity chromatography.

APPLICATIONS

Anti-hCD14-IgG is a neutralizing antibody, it blocks CD14-dependent TLR2-induced cellular activation and CD14-dependent TLR4-induced cellular activation. It can also be used for flow cytometry.

Neutralization

The exact concentration of antibody required is dependent on the cell type and growth conditions. InvivoGen has determined the neutralization dose for this antibody using LPS-EK Ultrapure and HEK-Blue™ hTLR4 cells (HEK293 cells expressing hCD14, human MD2 and human TLR4 and an NF-κB-inducible SEAP reporter gene).

Procedure for neutralization using HEK-Blue™ TLR4 cells

1. Prepare a cell suspension at ~500,000 cells/ml.
2. Add 100 µl of Anti-hCD14-IgG or control antibody (1-5 µg/ml final concentration) per well of a 96-well plate.
Note: We recommend using Anti-β-Gal-hlgG1 (which targets E. coli β-galactosidase) as a negative control.
3. Add 100 µl of cell suspension (~50,000 cells) per well.
4. Incubate for 1 hour at 37°C.
5. Add 50 µl of LPS-EK Ultrapure (1 ng/ml final concentration).
6. Incubate overnight at 37°C.
7. Add 20 µl of 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 optical density (OD) at 655 nm.

Flow Cytometry

This antibody was used at 500-2000 ng/10⁶ cells with a goat F(ab')₂ anti-human kappa-PE secondary antibody for indirect immunofluorescence staining of HEK-Blue™ hTLR2 cells (HEK293 cells expressing hCD14, hTLR2, and an NF-κB-inducible SEAP reporter gene).

RELATED PRODUCTS

Product	Description	Cat.Code
Anti-β-Gal-hlgG1	Isotype control	bgal-mab1
HEK-Blue™ hTLR2	TLR2 reporter cells	hkb-htlr2
HEK-Blue™ hTLR4	TLR4 reporter cells	hkb-htlr4
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs
LPS-EK Ultrapure	TLR4 agonist	tlrl-peklps

TECHNICAL SUPPORT

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