

# Anti-hCD14-IgG

Neutralizing IgG monoclonal antibody to human CD14

Catalog # mabg-hcd14

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

For research use only, not for diagnostic or therapeutic use

Version # 17J17-MM

## PRODUCT INFORMATION

### Content

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

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 1 year.

- 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) 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 receptor<sup>1</sup>. 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 MD2<sup>2</sup>. 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 infections<sup>3</sup>.

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.

Anti-hCD14-IgG 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 TLR2 or TLR4 ligands 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 an NF-κB-inducible SEAP reporter gene and human CD14, MD2 and TLR4).

### 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 (1-10 µg/ml final concentration) per well of a 96-well plate.

**Note:** We recommend using *Anti-β-Gal-hIgG1* (which targets *E. coli* β-galactosidase) as a negative control.

3. Add 100 µl of cell suspension (~50,000 cells) per well.
4. Incubate 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/10<sup>6</sup> cells with a goat F(ab')<sub>2</sub> anti-human kappa-PE secondary antibody for indirect immunofluorescence staining of HEK-Blue™ hTLR2 cells.

## RELATED PRODUCTS

Product	Catalog Code
Anti-β-Gal-hIgG1	bgal-mab1
HEK-Blue™ hTLR2	hkb-htr2
HEK-Blue™ hTLR4	hkb-htr4
LPS-EK Ultrapure	tlrl-pek1ps
QUANTI-Blue™	rep-qb-1

## TECHNICAL SUPPORT

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