

Anti-hTNF- α -hIgG4

Neutralizing human IgG4 monoclonal antibody against human TNF- α

Catalog code: htnfa-mab4, htnfa-mab4-03

<https://www.invivogen.com/anti-htnfa-higg4>

For research use only, not for diagnostic or therapeutic use

Version 23L15-MM

PRODUCT INFORMATION

Contents: Anti-hTNF- α -hIgG4 purified monoclonal antibody (mAb) is provided azide-free and lyophilized. It is available in two quantities:

htnfa-mab4: 100 μ g Anti-hTNF- α -hIgG4

htnfa-mab4-03: 3 x 100 μ g Anti-hTNF- α -hIgG4

Target: Human tumor necrosis factor- α (hTNF- α)

Isotype: Human IgG4

Source: Chinese hamster ovary (CHO) cells

Purification: Affinity chromatography

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

Tested applications: Neutralization of hTNF- α signaling

Antibody resuspension (0.1 mg/ml)

Note: Ensure you see the lyophilized pellet before resuspension.

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

Storage and stability

- Product is shipped at room temperature. Upon receipt, store at -20°C.
- Reconstituted antibody is stable for 1 month when stored at 4°C and for 6 months when stored at -20°C. Avoid repeated freeze-thaw cycles.

Quality control

- Human IgG4 isotype has been confirmed by ELISA.
- The neutralization of hTNF- α signaling pathway has been confirmed using cellular assays with the HEK-Blue™ TNF- α reporter cells.
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION

Anti-hTNF- α -hIgG4 is a neutralizing monoclonal antibody (mAb) featuring the constant region of the human IgG4 (hIgG4) isotype and the variable region of adalimumab, a fully human therapeutic mAb that targets human tumor necrosis factor- α (hTNF- α). TNF- α is a pleiotropic cytokine that notably induces NF- κ B-mediated production of pro-inflammatory cytokines^{1,2}. The variable region of adalimumab blocks the interaction of TNF- α with its receptors TFNR1 and TNFR2, thereby downregulating the inflammatory responses associated with autoimmune diseases, such as rheumatoid arthritis and Crohn's disease^{1,2}. The hIgG4 isotype displays low to no potency for Fc-mediated antibody-dependent cellular cytotoxicity (ADCC), low potency for antibody-dependent cellular phagocytosis (ADCP), and it does not mediate complement-dependent cytotoxicity (CDC). Anti-hTNF- α -hIgG4 was generated by recombinant DNA technology. It is produced in CHO cells and purified by affinity chromatography.

1. Steeland S, et al. 2018. A new venue of TNF targeting. Int. J. Mol. Sci. 19:1442. 2. Shealy D.J. & Visvanathan S. 2008. Anti-TNF antibodies: lessons from the past, roadmap for the future. Therapeutic Antibodies (book), 101-129.

APPLICATION

Anti-hTNF- α -hIgG4 is a neutralizing antibody that blocks cellular activation induced by hTNF- α . The concentration of antibody required to neutralize hTNF- α activity is dependent on the cytokine concentration, cell type and growth conditions.

Neutralization assay

The exact concentration of antibody required to neutralize recombinant hTNF- α activity is dependent on the cytokine concentration, cell type and growth conditions. Below is a protocol using recombinant hTNF- α as well as HEK-Blue™ TNF- α cells. These cells were specifically designed for the detection of bioactive human and murine TNF- α by monitoring the activation of the AP-1/NF- κ B pathway. They stably express an AP-1/NF- κ B-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene. Changes in SEAP activity in the supernatant due to inhibition of hTNF- α receptor binding can be assessed using QUANTI-Blue™ Solution, a SEAP detection reagent.

In a 96-well plate:

- Prepare a serial dilution of Anti-hTNF- α -hIgG4 or a negative control (e.g. Anti- β -Gal-hIgG4) starting 10 ng/ml to 1 μ g/ml (final conc.).
- Add 10-30 pg/ml of recombinant hTNF- α to a final volume of 40 μ l.
- Incubate for 30 minutes at 37°C, 5% CO₂.
- Prepare a suspension of HEK-Blue™ TNF- α cells in culture medium.
- Add 160 μ l (5 x 10⁴ cells/well) of the cell suspension to each well
- Incubate the plate at 37°C, 5% CO₂ for 24 hours.
- The next day: prepare QUANTI-Blue™ Solution and carry out the measurements following the instructions on the data sheet.

RELATED PRODUCTS

| Product | Description | Cat. Code |
|---------------------------------|------------------------------|-------------|
| Anti-hTNF- α -hIgG1 | Neutralizing antibody | htnfa-mab1 |
| Anti-hTNF- α -hIgA2 | Neutralizing antibody | htnfa-mab7 |
| Anti- β -Gal-hIgG4 | Isotype control | bgal-mab4 |
| HEK-Blue™ TNF- α Cells | TNF- α reporter cells | hkb-tnfdmyd |
| HEK-Dual™ TNF- α Cells | TNF- α reporter cells | hkd-tnfa |
| QUANTI-Blue™ Solution | SEAP detection reagent | rep-qbs |
| Recombinant human TNF- α | Recombinant cytokine | rcyc-htnfa |

InvivoGen offers a collection of clinically-relevant antibodies in various isotypes. Please visit www.invivogen.com/biosimilar-antibody-isotypes.

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