# Anti-hTNF-α-hlgG3

## Neutralizing human IgG3 monoclonal antibody against human TNF- $\alpha$

Catalog # htnfa-mab3

## For research use only, not for diagnostic or therapeutic use

Version # 11J21-MM

## **PRODUCT INFORMATION**

Content: 100  $\mu g$  purified anti-hTNF- $\alpha$ -hIgG3 antibody, provided azide-free and lyophilized.

## Isotype: Human IgG3

Formulation: 0.2  $\mu$ m filtered solution in 68 mM phosphate buffer with 91 mM glycine, 5% w/v saccharose 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. Lyophilized product is stable for 1 year 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**

Anti-hTNF- $\alpha$ -hIgG3 is a neutralizing monoclonal antibody featuring the constant region of the human IgG3 isotype and the variable region of adalimumab. Adalimumab is a fully human monoclonal antibody against the pro-inflammatory cytokine human tumor necrosis factor alpha (hTNF- $\alpha$ ). Adalimumab binds to TNF- $\alpha$  and blocks its interaction with TNF receptors thereby downregulating the inflammatory reactions associated with autoimmune diseases, such as rheumatoid arthritis and Crohn's disease.

Human IgG3 is the third most common antibody present in serum. Human IgG3 is very susceptible to cleavage by proteolytic enzymes, due an extended hinge region. Human IgG3 displays strong antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC).

Anti-hTNF- $\alpha$ -hIgG3 was generated by recombinant DNA technology. It has been produced in CHO cells and purified by affinity chromatography with protein G / agarose. The neutralizing activity of this IgG3 antibody was determined using HEK-Blue<sup>™</sup> TNF- $\alpha$ /IL-1 $\beta$  Cells.

#### **Antibody Isotype Collection**

For your neutralization experiments, InvivoGen proposes an anti-hTNF- $\alpha$  isotype collection. This collection consists of monoclonal antibodies comprising the variable region of adalimumab, and the constant region of the most common human and murine isotypes; eight in humans (IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgE) and three in mice (IgG1, IgG2a, IgA). The isotypes differ in their functional locations and effector functions, such as CDC and ADCC, as presented in the table above.

## **ANTIBODY ISOTYPES**

| Name | Types | Description   |  |
|------|-------|---|--|
| IgG  | 4     | Major Ig in serum, placental transfer<br>CDC (hIgG3>hIgG1>hIgG2>hIgG4; mIgG2a>mIgG1)<br>ADCC (hIgG1≥hIgG3>hIgG2≥IgG4; mIgG2a>mIgG1) |  |
| IgM  | 1     | Third most common serum Ig, first Ig to be made<br>Good CDC, some ADCC  |  |
| IgA  | 2     | Major class in secretions, second most common serum Ig<br>monomer in serum, dimer in secretions. No CDC, some ADCC                  |  |
| IgE  | 1     | Least common serum Ig, involved in allergic reaction<br>Strong binding to Fc receptors on basophils, no CDC                         |  |

## APPLICATION

Anti-hTNF- $\alpha$ -hIgG3 is a neutralizing antibody that blocks cellular activation induced by human TNF- $\alpha$  (hTNF- $\alpha$ ). The concentration of antibody required to neutralize hTNF- $\alpha$  activity is dependent on the cytokine concentration, cell type and growth conditions.

#### **Neutralization**

InvivoGen has determined the neutralization dose for this antibody using recombinant hTNF- $\alpha$  and HEK-Blue<sup>tot</sup> TNF- $\alpha$ /IL-1 $\beta$  Cells. These cells are HEK293 cells stably expressing an NF- $\kappa$ B-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene.

Recombinant hTNF- $\alpha$  was incubated with anti-hTNF- $\alpha$ -hIgG3 for 30 min prior to the addition of the HEK-Blue<sup>TM</sup> TNF- $\alpha$ /IL-1 $\beta$  Cells. Neutralization of TNF- $\alpha$ -induced signaling by anti-hTNF- $\alpha$ -hIgG3 was determined after a 24 hour incubation by assessing SEAP production using QUANTI-Blue<sup>TM</sup>. QUANTI-Blue<sup>TM</sup> 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.

## **RELATED PRODUCTS**

| Product  | <b>Catalog Code</b>   |
|--|---|
| HEK-Blue <sup>™</sup> TNFα/IL-1β Cells<br>QUANTI-Blue <sup>™</sup><br>Anti-hTNF-α-hIgG1<br>Anti-hTNF-α-hIgG2<br>Anti-hTNF-α-hIgG4<br>Anti-hTNF-α-hIgA1<br>Anti-hTNF-α-hIgA2<br>Anti-hTNF-α-hIgA2<br>Anti-hTNF-α-hIgE<br>Anti-hTNF-α-mIgG1<br>Anti-hTNF-α-mIgG1 | hkb-tnfil<br>rep-qbl<br>htnfa-mabl<br>htnfa-mab2<br>htnfa-mab4<br>htnfa-mab5<br>htnfa-mab6<br>htnfa-mab7<br>htnfa-mab7<br>htnfa-mab9<br>htnfa-mab10 |
| Anti-hTNF-α-mIgA   | htnfa-mab11   |

An anti-CD20 (rituximab) isotype collection is also available, for more information visit www.invivogen.com/antibody-isotypes

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