

#### **Synonym**

DIF,TNF-alpha,TNFA,TNFSF2,cachexin,cachectin,TNFα

#### Source

Human TNF-alpha, premium grade(TNA-H4211) is expressed from human 293 cells (HEK293). It contains AA Val 77 - Leu 233 (Accession # P01375-1). Predicted N-terminus: Val 77

It is produced under our rigorous quality control system that incorporates a comprehensive set of tests including sterility and endotoxin tests. Product performance is carefully validated and tested for compatibility for cell culture use or any other applications in the early preclinical stage.

GMP-TNAH23 is the GMP version of this TNA-H4211. These two proteins display indistinguishable performance profiles, thereby ensuring a seamless transition for end users from early preclinical stag to later clinical phases.

#### **Molecular Characterization**

# TNF-alpha(Val 77 - Leu 233) P01375-1

This protein carries no "tag".

The protein has a calculated MW of 17.4 kDa. The protein migrates as 17 kDa±3 kDa when calibrated against <u>Star Ribbon Pre-stained Protein Marker</u> under reducing (R) condition (SDS-PAGE) due to glycosylation.

#### Endotoxin

Less than 0.01 EU per µg by the LAL method / rFC method.

#### **Host Cell Protein**

<0.5 ng/µg of protein tested by ELISA.

## **Host Cell DNA**

<0.02 ng/μg of protein tested by qPCR.

#### **Sterility**

Negative

## Mycoplasma

Negative

#### **Purity**

>95% as determined by SDS-PAGE.

>95% as determined by SEC-MALS.

# **Formulation**

Lyophilized from  $0.22~\mu m$  filtered solution in PBS, pH7.4 with trehalose as protectant.

Contact us for customized product form or formulation.

#### Reconstitution

Please see Certificate of Analysis for specific instructions.

For best performance, we strongly recommend you to follow the reconstitution protocol provided in the CoA.

#### **Storage**

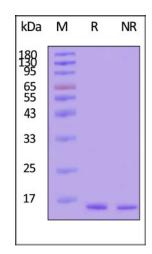
For long term storage, the product should be stored at lyophilized state at -20°C or lower.

Please avoid repeated freeze-thaw cycles.

This product is stable after storage at:

- -20°C to -70°C for 24 months in lyophilized state;
- -70°C for 3 months under sterile conditions after reconstitution.

#### **SDS-PAGE**



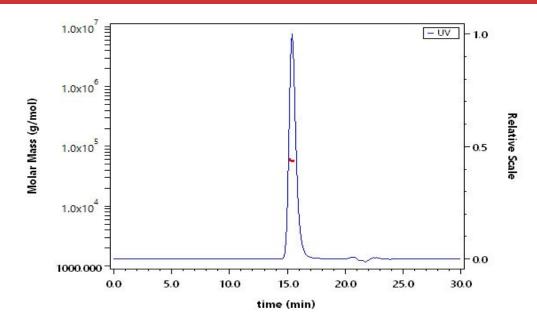
Human TNF-alpha, premium grade on SDS-PAGE under reducing (R) and non-reducing (NR) conditions. The gel was stained with Coomassie Blue. The

# SEC-MALS





purity of the protein is greater than 95% (With <u>Star Ribbon Pre-stained Protein</u> <u>Marker</u>).

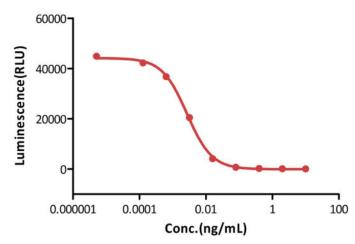


The purity of Human TNF-alpha, premium grade (Cat. No. TNA-H4211) is more than 95% and the molecular weight of this protein is around 52-62 kDa verified by SEC-MALS.

Report

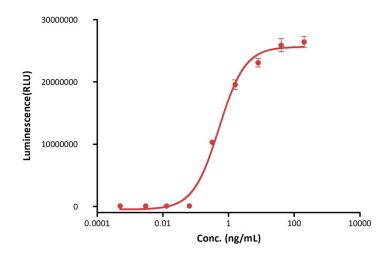
# **Bioactivity-CELL BASE**

# Human TNF-alpha, premium grade induced cytotoxicity in WEHI-13VAR cells

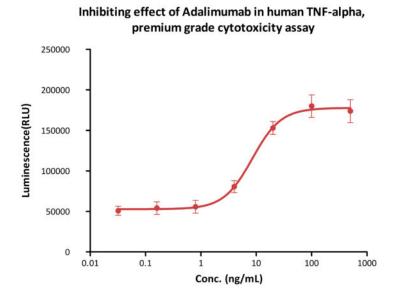


Human TNF-alpha, premium grade (Cat. No. TNA-H4211) induces cytotoxicity effect on the WEHI-13VAR cells in the presence of the metabolic inhibitor actinomycin D. The specific activity of Human TNF-alpha, premium grade is >2.00×10^7 IU/mg, which is calibrated against human TNF-alpha WHO International Standard (NIBSC code: 12/154) (QC tested).

#### Human TNF-alpha Protein, premium grade stimulates human NF-KB (Luc) HEK293 Reporter Cells



The NF-kB (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of Human TNF-alpha, premium grade (Cat. No. TNA-H4211). The EC50 value

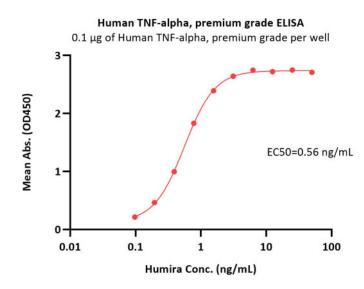


Neutralization assay shows that the cytotoxicity effect of human TNF-alpha, premium grade (Cat. No. TNA-H4211) was inhibited by increasing concentration of Adalimumab. The concentration of TNF-alpha used is 20 pg/mL. The IC50 is 8.48 ng/mL (Routinely tested).

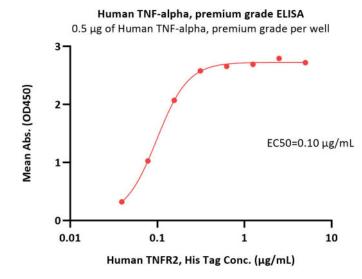


of this effect is 0.5143 ng/mL (Routinely tested).

### **Bioactivity-ELISA**

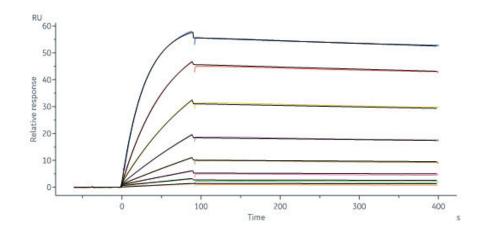


Immobilized Human TNF-alpha, premium grade (Cat. No. TNA-H4211) at 1  $\mu$ g/mL (100  $\mu$ L/well) can bind Humira with a linear range of 0.1-2 ng/mL (QC tested).

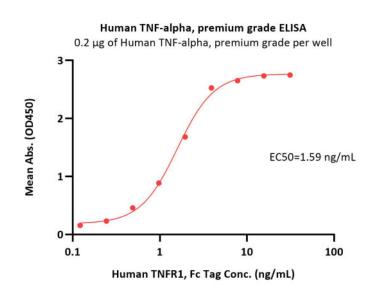


Immobilized Human TNF-alpha, premium grade (Cat. No. TNA-H4211) at 5  $\mu$ g/mL (100  $\mu$ L/well) can bind Human TNFR2, His Tag (Cat. No. TN2-H5227) with a linear range of 0.039-0.313  $\mu$ g/mL (Routinely tested).

# **Bioactivity-SPR**



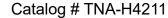
Monoclonal Anti-TNF-alpha Antibody, Human IgG1 (13B8) (Cat. No. TNA-AM493) captured on Protein A Chip can bind Human TNF-alpha, premium



Immobilized Human TNF-alpha, premium grade (Cat. No. TNA-H4211) at 2  $\mu$ g/mL (100  $\mu$ L/well) can bind Human TNFR1, Fc Tag (Cat. No. TN1-H5251) with a linear range of 0.1-4 ng/mL (Routinely tested).



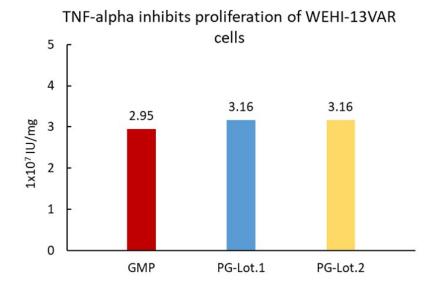
# Human TNF-alpha Protein, premium grade





grade (Cat. No. TNA-H4211) with an affinity constant of 0.267 nM as determined in a SPR assay (Biacore 8K) (Routinely tested).

# **Bioactivity-Stability**



The Cell based assay shows batch-to-batch consistency between Acro's GMP and PG TNF-alpha.

# Background

Tumor necrosis factor alpha (TNF $\alpha$ ) is a cytokine produced primarily by monocytes and macrophages. It is found in synovial cells and macrophages in the tissues. The primary role of TNF $\alpha$  is in the regulation of immune cells. TNF $\alpha$  is able to induce apoptotic cell death, to induce inflammation, and to inhibit tumorigenesis and viral replication. Dysregulation of TNF $\alpha$  production has been implicated in a variety of human diseases, including major depression, Alzheimer's disease and cancer. Recombinant TNF $\alpha$  is used as an immunostimulant under the INN tasonermin. TNF $\alpha$  can be produced ectopically in the setting of malignancy and parallels parathyroid hormone both in causing secondary hypercalcemia and in the cancers with which excessive production is associated.

