## ActiveMax® Human TNF-alpha Protein, Tag Free, low endotoxin (active trimer) (MALS verified)

Catalog # AMS.TNA-H4211



## **Synonym**

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

#### **Source**

ActiveMax® Human TNF-alpha, Tag Free, low endotoxin (active trimer) (MALS verified) (AMS.TNA-H4211) is expressed from human 293 cells (HEK293). It contains AA Val 77 - Leu 233 (Accession # P01375-1). Predicted N-terminus: Val 77

#### **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 and 18 kDa under reducing (R) condition (SDS-PAGE).

#### Endotoxin

Less than  $0.1\ EU$  per  $\mu g$  by the LAL method.

## **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. Normally trehalose is added as protectant before lyophilization.

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**

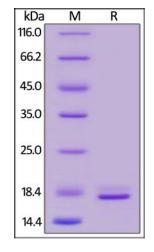
For long term storage, the product should be stored at lyophilized state at -20°C or lower.

Please avoid repeated freeze-thaw cycles.

This product is stable after storage at:

- -20°C to -70°C for 12 months in lyophilized state;
- -70°C for 6 months under sterile conditions after reconstitution.

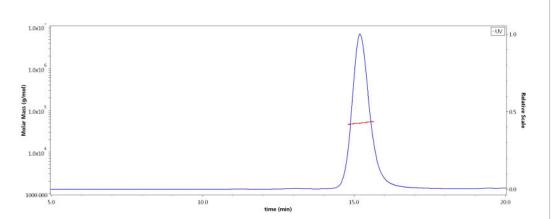
## **SDS-PAGE**



ActiveMax® Human TNF-alpha, Tag Free, low endotoxin (active trimer) (MALS verified) on SDS-PAGE under reducing (R) condition. The gel was stained overnight with Coomassie Blue. The purity of the protein is greater than 95%.

# **Bioactivity-ELISA**

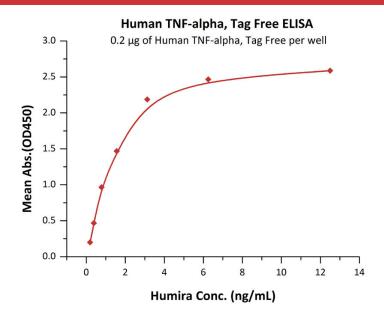
## **SEC-MALS**



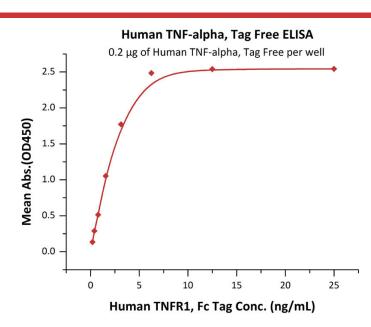
The purity of ActiveMax® Human TNF-alpha, Tag Free, low endotoxin (active trimer) (MALS verified) (Cat. No. AMS.TNA-H4211) was more than 95% in HP-SEC, and around 47-60 kDa verified by SEC-MALS.

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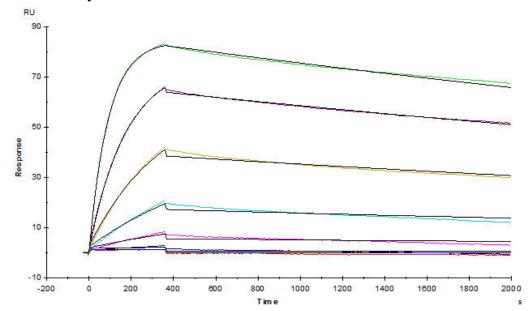


Immobilized ActiveMax® Human TNF-alpha, Tag Free, low endotoxin (active trimer) (MALS verified) at 2  $\mu$ g/mL (100  $\mu$ L/well) can bind Humira with a linear range of 0.2-2 ng/mL (QC tested).



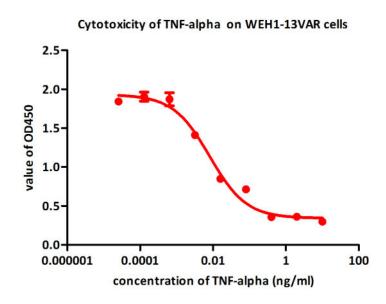
Immobilized ActiveMax® Human TNF-alpha, Tag Free, low endotoxin (active trimer) (MALS verified) at 2  $\mu$ g/mL (100  $\mu$ L/well) canbind Human TNFR1, Fc Tag with a linear range of 0.2-3 ng/mL (Routinely tested).

## **Bioactivity-SPR**

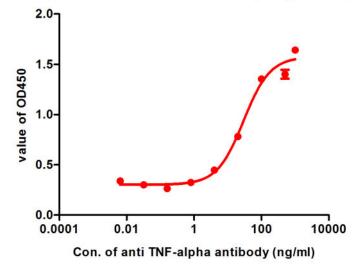


Humira (Adalimumab) captured on CM5 chip via anti-human IgG Fcantibodies surface, can bind ActiveMax® Human TNF-alpha, Tag Free, lowendotoxin (active trimer) (MALS verified) (Cat. No. AMS.TNA-H4211) with an affinity constant of 0.255 nM as determined in a SPR assay (Biacore T200) (Routinely tested).

# **Bioactivity-Cell based assay**



## Inhibiting effect of Adalimumab in TNF-alpha cytotoxicity assay



# ActiveMax® Human TNF-alpha Protein, Tag Free, low endotoxin (active trimer) (MALS verified)

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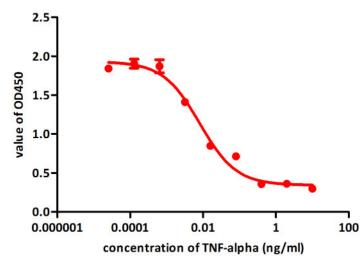
ActiveMax® Human TNF-alpha, Tag Free, low endotoxin (active trimer) (MALS verified) (Cat. No. AMS.TNA-H4211) induces cytotoxicity effect on theWEH1-13VAR cells in the presence of the metabolic inhibitor actinomycin D.

The EC50 for this effect is 0.007-0.014 ng/mL (Routinely tested).

Neutralization assay shows that the cytotoxicity effect of ActiveMax® Human TNF-alpha, Tag Free, low endotoxin (active trimer) (MALS verified) (Cat. No. AMS.TNA-H4211) was inhibited by increasing concentration of Adalimumab. The Concentration of TNF-alpha used is 1 ng/mL. The IC50 is 29 ng/mL (Routinely tested).

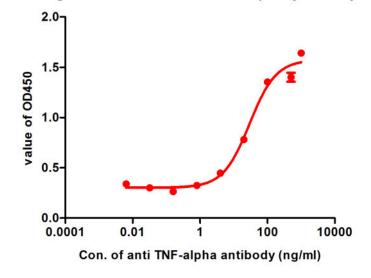
#### **Bioactivity-Cell based assay**

Cytotoxicity of TNF-alpha on WEH1-13VAR cells



ActiveMax® Human TNF-alpha, Tag Free, low endotoxin (active trimer) (MALS verified) (Cat. No. AMS.TNA-H4211) induces cytotoxicity effect on theWEH1-13VAR cells in the presence of the metabolic inhibitor actinomycin D.The EC50 for this effect is 0.007-0.014 ng/mL (Routinely tested).

#### Inhibiting effect of Adalimumab in TNF-alpha cytotoxicity assay



Neutralization assay shows that the cytotoxicity effect of ActiveMax® Human TNF-alpha, Tag Free, low endotoxin (active trimer) (MALS verified) (Cat. No. AMS.TNA-H4211) was inhibited by increasing concentration of Adalimumab. The Concentration of TNF-alpha used is 1 ng/mL. The IC50 is 29 ng/mL (Routinely tested).

## 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.

## References

- (1) <u>Dowlati Y, Herrmann N, et al., 2010, Biol Psychiatry 67 (5): 446–457.</u>
- (2) <u>Swardfager W, Lanctot K, et al., 2010, Biol Psychiatry 68 (10): 930–941.</u>
- (3) Locksley RM, Killeen N et al., 2001, Cell 104 (4): 487–501.