

**DESCRIPTION**

<b>Source</b>	<i>E. coli</i> -derived human TNF-alpha protein Val77-Leu233, with and without an N-terminal Met Accession # P01375 Produced using non-animal reagents in an animal-free laboratory. Manufactured and tested under cGMP guidelines.
<b>N-terminal Sequence Analysis</b>	Met-Val77-Arg-Ser-Ser-Ser-Arg-Thr-Pro-Ser Val77-Arg-Ser-Ser-Ser-Arg-Thr-Pro-Ser-Asp
<b>Predicted Molecular Mass</b>	17 kDa

**SPECIFICATIONS**

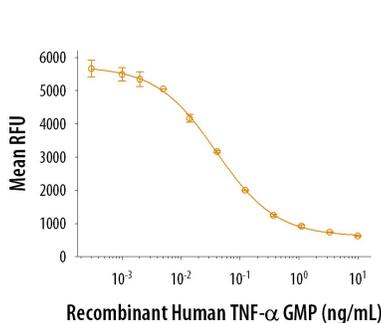
<b>SDS-PAGE</b>	17 kDa, reducing conditions
<b>Activity</b>	Measured in a cytotoxicity assay using L-929 mouse fibroblast cells in the presence of the metabolic inhibitor actinomycin D. Matthews, N. and M.L. Neale (1987) in <i>Lymphokines and Interferons, A Practical Approach</i> . Clemens, M.J. <i>et al.</i> (eds); IRL Press. 221. The ED <sub>50</sub> for this effect is 25-100 pg/mL.  The specific activity of recombinant human TNF- $\alpha$ GMP is approximately $7.6 \times 10^4$ IU/ $\mu$ g, which is calibrated against human TNF- $\alpha$ WHO International Standard (NIBSC code: 88/786).
<b>Endotoxin Level</b>	<0.10 EU per 1 $\mu$ g of the protein by the LAL method.
<b>Purity</b>	>97%, by SDS-PAGE with silver staining.
<b>Host Cell Protein</b>	< 0.5 ng per $\mu$ g of protein when tested by ELISA.
<b>Mycoplasma</b>	Negative when tested in a ribosomal RNA hybridization assay.
<b>Formulation</b>	Lyophilized from a 0.2 $\mu$ m filtered solution in PBS. See Certificate of Analysis for details.

**PREPARATION AND STORAGE**

<b>Reconstitution</b>	Reconstitute at 0.1-1 mg/mL in PBS.
<b>Shipping</b>	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.
<b>Stability &amp; Storage</b>	<b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b> <ul style="list-style-type: none"> <li>• A minimum of 6 months when stored at <math>\leq -20</math> °C as supplied. Refer to lot specific COA for the Use by Date.</li> <li>• 1 month, 2 to 8 °C under sterile conditions after reconstitution.</li> <li>• 3 months, <math>\leq -20</math> °C under sterile conditions after reconstitution.</li> </ul>

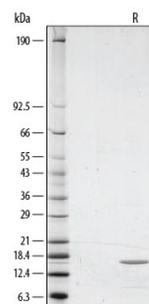
**DATA**

**Bioactivity**



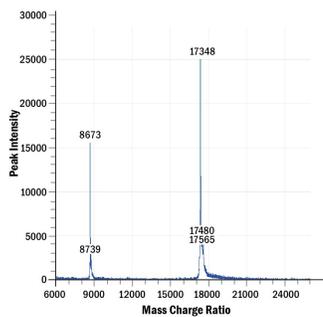
GMP-grade Recombinant Human TNF- $\alpha$  (Catalog # 210-GMP) induces cytotoxicity in the L-929 mouse fibroblast cell line in the presence of the metabolic inhibitor actinomycin D. The ED<sub>50</sub> is 25-100 pg/mL.

**SDS-PAGE**



1  $\mu$ g/lane of GMP-grade Recombinant Human TNF- $\alpha$  (Catalog # 210-GMP) was resolved by SDS-PAGE with silver staining, under reducing (R) conditions, showing a single band at 17 kDa.

**Mass Spectrometry**



MALDI-TOF analysis of GMP-grade Recombinant Human TNF- $\alpha$  (Catalog # 210-GMP). The major peak at 17348 corresponds to the calculated molecular mass without an N-terminal Met, 17353 Da. The minor peak at 17480 corresponds to the calculated molecular mass with an N-terminal Met, 17484 Da. The peak at 17565 is a matrix-associated artifact of the MALDI-TOF.

**BACKGROUND**

Tumor necrosis factor alpha (TNF- $\alpha$ ), also known as cachectin and TNFSF2, is the prototypic ligand of the TNF superfamily. It is a pleiotropic molecule that plays a central role in inflammation, immune system development, apoptosis, and lipid metabolism (1, 2). Human TNF- $\alpha$  consists of a 35 amino acid (aa) cytoplasmic domain, a 21 aa transmembrane segment, and a 177 aa extracellular domain (ECD) (3). Within the ECD, human TNF- $\alpha$  shares 97% aa sequence identity with rhesus and 71%-92% with bovine, canine, cotton rat, equine, feline, mouse, porcine, and rat TNF- $\alpha$ . TNF- $\alpha$  is produced by a wide variety of immune, epithelial, endothelial, and tumor cells (1, 2). TNF- $\alpha$  is assembled intracellularly to form a noncovalently linked homotrimer which is expressed on the cell surface (4). Cell surface TNF- $\alpha$  can induce the lysis of neighboring tumor cells and virus infected cells, and it can generate its own downstream cell signaling following ligation by soluble TNFR I (2, 5). Shedding of membrane bound TNF- $\alpha$  by TACE/ADAM17 releases the bioactive cytokine, a 55 kDa soluble trimer of the TNF- $\alpha$  extracellular domain (6-8). TNF- $\alpha$  binds the ubiquitous 55-60 kDa TNF RI (9, 10) and the hematopoietic cell-restricted 80 kDa TNF RII (11, 12), both of which are also expressed as homotrimers (1, 2, 13). Both type I and type II receptors bind TNF- $\alpha$  with comparable affinity (14), although only TNF RI contains a cytoplasmic death domain which triggers the activation of apoptosis. Soluble forms of both types of receptors are released and can neutralize the biological activity of TNF- $\alpha$  (15).

**References:**

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## MANUFACTURING SPECIFICATIONS

### GMP Proteins

R&D Systems, a Bio-Techne Brand's GMP proteins are produced according to relevant sections of the following documents: WHO TRS, No. 822, 1992 Annex 1, Good Manufacturing Practices for Biological Products; USP Chapter 1043, Ancillary Materials for Cell, Gene and Tissue-Engineered Products and USP Chapter 92, Growth Factors and Cytokines Used in Cell Therapy Manufacturing.

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- Monitoring of stability over product shelf-life

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- N-terminal amino acid analysis, SDS-PAGE analysis, and endotoxin level (as determined by LAL assay) performed on each bulk QC lot, not on individual bottlings of each QC lot
- Post-bottling lot-specific bioassay results (compliance with an established range) and results of microbial bioburden testing (using broth culture, Sabourand's dextrose and blood agar plates with results reported at 3 days and at 7 days)
- Host Cell Protein testing performed by ELISA
- Mycoplasma testing by ribosomal RNA hybridization assay

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- Purified proteins are stored in animal-free containers in a dedicated cold storage room.

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