

**DESCRIPTION**

<b>Source</b>	<i>E. coli</i> -derived 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-Val <sub>77</sub> -Arg-Ser-Ser-Ser-Arg-Thr-Pro-Ser Val <sub>77</sub> -Arg-Ser-Ser-Ser-Arg-Thr-Pro-Ser-Asp
<b>Predicted Molecular Mass</b>	17 kDa

**SPECIFICATIONS**

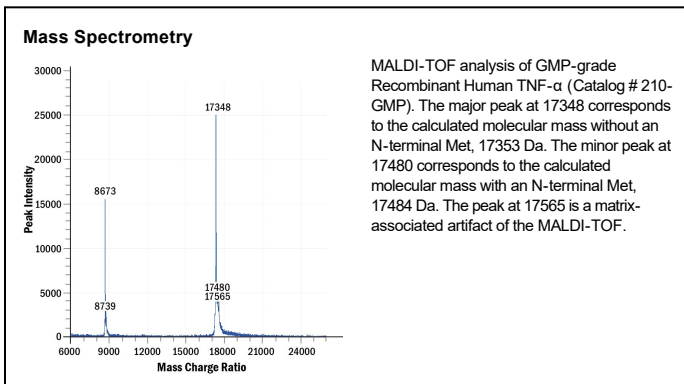
<b>SDS-PAGE</b>	17 kDa, reducing conditions
<b>Activity</b>	Measured in a cytotoxicity assay using L-929 mouse fibroblast cells in the presence of the metabolic inhibitor actinomycin D. Matthews, N. and M.L. Neale (1987) in <i>Lymphokines and Interferons, A Practical Approach</i> . Clemens, M.J. <i>et 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 x 10 <sup>4</sup> 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 100-200 $\mu$ g/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</math> -20 °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</math> -20 °C under sterile conditions after reconstitution.</li> </ul>

**DATA**

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

R&D Systems' quality focus includes:

- Manufactured and tested under an ISO 9001:2015 and ISO 13485:2016 certified quality system
- Documented processes and QA control of documentation and process changes
- Personnel training programs
- Raw material testing and vendor qualification/monitoring
- Fully validated equipment, processes and test methods
- Equipment calibration schedules using a computerized calibration program
- Facility maintenance, safety programs and pest control
- Material review process for variances
- Monitoring of stability over product shelf-life

R&D Systems strives to provide our customers with the analytical characteristics of each product so that customers may determine whether our products are appropriate for their research. The Certificate of Analysis provided contains the following lot specific information:

- 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

Additional testing and documentation requested by the customer can be arranged at an additional cost. Testing may include, but is not limited to, USP <61> bioburden testing, positive identity testing, testing for adventitious agents and testing for residual host cell content.

Production records and facilities are available for examination by appropriate personnel on-site at R&D Systems in Minneapolis, Minnesota USA.

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### Production

- All molecular biology procedures use animal-free media and dedicated labware.
- Dedicated fermentors are utilized in committed animal-free areas.

### Purification

- Protein purification columns are animal-free.
- Bulk proteins are filtered using animal-free filters.
- Purified proteins are stored in animal-free containers in a dedicated cold storage room.

### Quality Assurance

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- No impairment of biological activity.
- High quality product obtained under stringent conditions.
- For *ex vivo* research or bioproduction, [additional documentation](#) can be provided.

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