# Recombinant Human TNF-α Hyperactive Mutant

Catalog Number: 8599-TA/CF

## DESCRIPTION

| | |
|---|---|
| **Source** | *E. coli*-derived human TNF-alpha protein<br>Lys87-Leu233 (Leu233Phe), with an N-terminal Met-Arg-Lys-Arg<br>Accession # P01375 |
| **N-terminal Sequence Analysis** | Met |
| **Structure / Form** | Non-covalent trimer |
| **Predicted Molecular Mass** | 17 kDa |

## SPECIFICATIONS

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|---|---|
| **SDS-PAGE** | 16 kDa, reducing conditions |
| **Activity** | 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 Lymphokines and Interferons, A Practical Approach. Clemens, M.J. *et al.* (eds): IRL Press. 221.<br>The $ED_{50}$ for this effect is 2.5-12.5 pg/mL. |
| **Endotoxin Level** | <0.10 EU per 1 µg of the protein by the LAL method. |
| **Purity** | >95%, by SDS-PAGE with silver staining. |
| **Formulation** | Lyophilized from a 0.2 µm filtered solution in PBS. See Certificate of Analysis for details. |

## PREPARATION AND STORAGE

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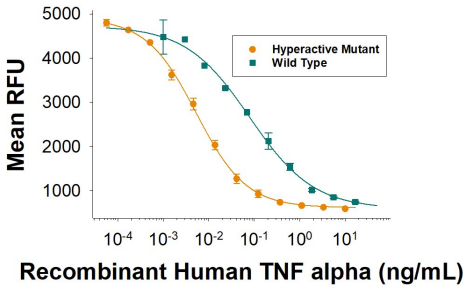
|---|---|
| **Reconstitution** | Reconstitute at 100 µg/mL in PBS. |
| **Shipping** | The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below. |
| **Stability & Storage** | **Use a manual defrost freezer and avoid repeated freeze-thaw cycles.**<br>• 12 months from date of receipt, -20 to -70 °C as supplied.<br>• 1 month, 2 to 8 °C under sterile conditions after reconstitution.<br>• 3 months, -20 to -70 °C under sterile conditions after reconstitution. |

## DATA

**Bioactivity**




Recombinant Human TNF-alpha Hyperactive Mutant (Catalog # 8599-TA/CF) induces cytotoxicity in the L-929 mouse fibroblast cell line in the presence of the metabolic inhibitor actinomycin D. The $ED_{50}$ for this effect is 2.5-12.5 pg/mL. This is an approximate 10-fold activity increase over the wild-type TNF-alpha (Catalog # 210-TA).



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## BACKGROUND

Tumor necrosis factor alpha (TNF-α), also known as cachectin and TNFSF1A, 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-α consisits 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-α shares 97% aa sequence identity with rhesus and 71%-92% with bovine, canine, cotton rat, equine, feline, mouse, porcine, and rat TNF-α. TNF-α is produced by a wide variety of immune, epithelial, endothelial, and tumor cells (1, 2). TNF-α is assembled intracellularly to form a noncovalently linked homotrimer which is expressed on the cell surface (4). Cell surface TNF-α 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-α by TACE/ADAM17 releases the bioactive cytokine, a 55 kDa soluble trimer of the TNF-α extracellular domain (6-8). TNF-α 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-α 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-α (15). This recombinant protein contains an N-terminal insertion and a Leu233Phe substitution which have been shown to increase the bioactivity of TNF-α *in vitro* and *in vivo* (16).

**References:**

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