

## TNF alpha Protein, Human, Recombinant (aa 57-233, His)

### General Information

Synonyms:	TNFA;TNF- $\alpha$ ;Cachectin;TNF-Alpha;TNF-a;TNF $\alpha$ ;Tumor Necrosis Factor;TNFSF2;TNF;Tumor Necrosis Factor Ligand Superfamily Member 2
Protein Construction:	Gly57-Leu233
Species:	Human
Expression Host:	E. coli
Accession:	P01375
Molecular Weight:	18 KDa (reducing condition)
AA Sequence:	Gly57-Leu233

### QC Testing

Biological Activity:	1. Loaded Human TNF alpha-His on HIS1K Biosensor, can bind Human TNF RII-mFc with an affinity constant of 1.83 nM as determined in BLI assay. (Regularly tested) 2. Measured in a cytotoxicity assay using L-929 mouse fibroblast cells in the presence of the metabolic inhibitor actinomycin D. The ED50 for this effect is 30-150 pg/ml. (QC verified)
Purity:	Greater than 95% as determined by reducing SDS-PAGE. (QC verified)
Endotoxin:	< 0.1 ng/ $\mu$ g (1 EU/ $\mu$ g) as determined by LAL test.
Formulation:	Lyophilized from a solution filtered through a 0.22 $\mu$ m filter, containing 20 mM PB, 100 mM NaCl, pH 8.0.

### Preparation and Storage

#### Reconstitution:

Reconstitute the lyophilized protein in distilled water. The product concentration should not be less than 100  $\mu$ g/ml. Before opening, centrifuge the tube to collect powder at the bottom. After adding the reconstitution buffer, avoid vortexing or pipetting for mixing.

#### Stability & Storage:

Lyophilized powders can be stably stored for over 12 months, while liquid products can be stored for 6-12 months at -80°C. For reconstituted protein solutions, the solution can be stored at -20°C to -80°C for at least 3 months. Please avoid multiple freeze-thaw cycles and store products in aliquots.

Actual storage temperature shall be subject to the COA.

#### Shipping:

In general, lyophilized powders are shipped with blue ice, while solutions are shipped with dry ice.

### Protein Background

Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ) is secreted by macrophages, monocytes, neutrophils, T-cells, and NK-cells following stimulation by bacterial LPS. Cells expressing CD4 secrete TNF- $\alpha$  while cells that express CD8 secrete little

or no TNF- $\alpha$ . Synthesis of TNF- $\alpha$  can be induced by many different stimuli including interferons, IL2, and GM-CSF. The clinical use of the potent anti-tumor activity of TNF- $\alpha$  has been limited by the proinflammatory side effects such as fever, dose-limiting hypotension, hepatotoxicity, intravascular thrombosis, and hemorrhage. Designing clinically applicable TNF- $\alpha$  mutants with low systemic toxicity has been of intense pharmacological interest. Human TNF- $\alpha$  that binds to murine TNF-R55 but not murine TNF-R7, exhibits retained anti-tumor activity and reduced systemic toxicity in mice compared with murine TNF- $\alpha$ , which binds to both murine TNF receptors. Based on these results, many TNF- $\alpha$  mutants that selectively bind to TNF-R55 have been designed. These mutants displayed cytotoxic activities on tumor cell lines in vitro and have exhibited lower systemic toxicity in vivo. Recombinant Human TNF- $\alpha$  High Active Mutant differs from the wild-type by amino acid substitution of amino acids 1-7 with Arg8, Lys9, Arg10 and Phe157. This mutant form has been shown to have increased activity with less inflammatory side effects in vivo.

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