

**DESCRIPTION**

**Source** *E. coli*-derived  
Leu79-Leu234, with an N-terminal Met  
Accession # NP\_001075732

**N-terminal Sequence** Met - Leu79

**Analysis**

**Predicted Molecular Mass** 17.4 kDa

**SPECIFICATIONS**

**SDS-PAGE** 19 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.  
The ED<sub>50</sub> for this effect is 0.01-0.04 ng/mL.

**Endotoxin Level** <0.10 EU per 1  $\mu$ g of the protein by the LAL method.

**Purity** >95%, by SDS-PAGE under reducing conditions and visualized by silver stain.

**Formulation** Lyophilized from a 0.2  $\mu$ m filtered solution in PBS. See Certificate of Analysis for details.

**PREPARATION AND STORAGE**

**Reconstitution** Reconstitute at 100  $\mu$ g/mL in PBS.

**Shipping** The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.

**Stability & Storage** Use a manual defrost freezer and avoid repeated freeze-thaw cycles.

- 12 months from date of receipt, -20 to -70 °C as supplied.
- 1 month, 2 to 8 °C under sterile conditions after reconstitution.
- 3 months, -20 to -70 °C under sterile conditions after reconstitution.

**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). Rabbit TNF- $\alpha$  consists of a 35 amino acid (aa) cytoplasmic domain, a 21 aa transmembrane segment, and a 179 aa extracellular domain (ECD) (3). Within the ECD, rabbit TNF- $\alpha$  shares 76%-83% with bovine, canine, cotton rat, equine, feline, human, mouse, porcine, rat, and rhesus 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:**

1. Zelova, H. and J. Hosek (2013) *Inflamm. Res.* **62**:641.
2. Juhasz, K. *et al.* (2013) *Expert Rev. Clin. Immunol.* **9**:335.
3. Ito, H. *et al.* (1986) *DNA* **5**:157.
4. Tang, P. *et al.* (1996) *Biochemistry* **35**:8216.
5. Perez, C. *et al.* (1990) *Cell* **63**:251.
6. Black, R.A. *et al.* (1997) *Nature* **385**:729.
7. Moss, M.L. *et al.* (1997) *Nature* **385**:733.
8. Gearing, A.J.H. *et al.* (1994) *Nature* **370**:555.
9. Schall, T.J. *et al.* (1990) *Cell* **61**:361.
10. Loetscher, H. *et al.* (1990) *Cell* **61**:351.
11. Dembic, Z. *et al.* (1990) *Cytokine* **2**:231.
12. Smith, C.A. *et al.* (1990) *Science* **248**:1019.
13. Loetscher, H. *et al.* (1991) *J. Biol. Chem.* **266**:18324.
14. Pinckard, J.K. *et al.* (1997) *J. Biol. Chem.* **272**:10784.
15. Engelmann, H. *et al.* (1990) *J. Biol. Chem.* **265**:1531.