

DESCRIPTION

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

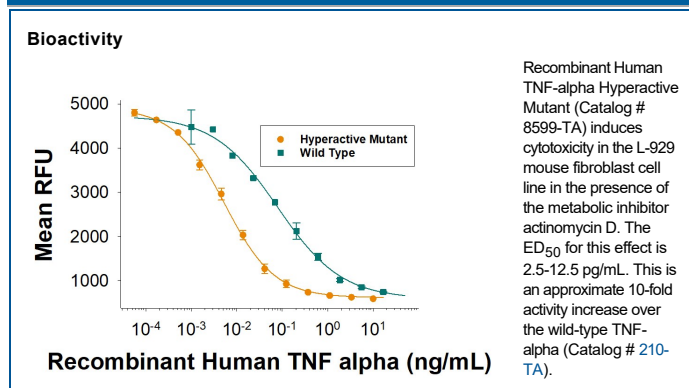
SPECIFICATIONS

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 <i>Lymphokines and Interferons, A Practical Approach</i> . Clemens, M.J. <i>et al.</i> (eds): IRL Press. 221. The ED ₅₀ for this effect is 2.5-12.5 pg/mL.
Endotoxin Level	<0.10 EU per 1 μ g of the protein by the LAL method.
Purity	>95%, by SDS-PAGE with silver staining.
Formulation	Lyophilized from a 0.2 μ m filtered solution in PBS with BSA as a carrier protein. See Certificate of Analysis for details.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 100 μ 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. <ul style="list-style-type: none"> • 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.

DATA



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 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- 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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