

DESCRIPTION

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| Source | Chinese Hamster Ovary cell line, CHO-derived human TNF-alpha protein | |
| | Avi-tag | Human TNF alpha (Val77-Leu233) Accession # P01375.1 |
| | N-terminus | C-terminus |
| N-terminal Sequence Analysis | Gly of Avi-tag | |
| Structure / Form | Monomer, biotinylated via Avi-tag | |
| Predicted Molecular Mass | 19 kDa | |

SPECIFICATIONS

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| SDS-PAGE | 15-22 kDa, under reducing conditions |
| Activity | The biotin to protein ratio is greater than 0.7 as determined by the HABA assay. Measured by its binding ability in a functional ELISA. When Recombinant Human TNF RI/TNFRSF1A Fc Chimera (Catalog # 372-RI) is coated at 0.025 μ g/mL (100 μ L/well), Biotinylated Recombinant Human TNF- α Avi-tag binds with an ED ₅₀ of 2-12 ng/mL. 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. <i>et al.</i> (eds): IRL Press. 221. The ED ₅₀ for this effect is 20-200 μ g/mL. |
| Endotoxin Level | <0.10 EU per 1 μ g of the protein by the LAL method. |
| Purity | >95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining. |
| Formulation | Lyophilized from a 0.2 μ m filtered solution in PBS with Trehalose. See Certificate of Analysis for details. |

PREPARATION AND STORAGE

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| Reconstitution | Reconstitute at 500 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

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| <p>Binding Activity</p> <p>Biotinylated Recombinant Human TNF-alpha Avi-tag Protein Binding Activity. When Recombinant Human TNF RI/TNFRSF1A Fc Chimera (Catalog # 372-RI) is coated at 0.025 μg/mL (100 μL/well), Biotinylated Recombinant Human TNF-alpha Avi-tag binds with an ED₅₀ of 2-12 ng/mL.</p> | <p>SDS-PAGE</p> <p>Recombinant Human TNF-alpha Avi-tag Protein SDS-PAGE. 2 μg/lane of Recombinant Human TNF-alpha Avi-tag (Catalog # AV110728) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 15-22 kDa.</p> |
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BACKGROUND

Tumor necrosis factor alpha (TNF- α), 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- 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). Our Avi-tag Biotinylated human TNF- α features biotinylation at a single site contained within the Avi-tag, a unique 15 amino acid peptide. Protein orientation will be uniform when bound to streptavidin-coated surface due to the precise control of bionylation and the rest of the protein is unchanged so there is no interference in the protein bioactivity.

References:

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