

## Biotinylated Recombinant Human TNF-α Avi-tag

Catalog Number: AVI10728

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	e Gly of Avi-tag		
Structure / Form	Monomer, biotinylated via Avi-tag		
Predicted Molecular Mass	19 kDa		
SPECIFICATIONS			
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.		

	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 <sub>50</sub> for this effect is 20-200 pg/mL.	
Endotoxin Level	<0.10 EU per 1 µg 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 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details.	

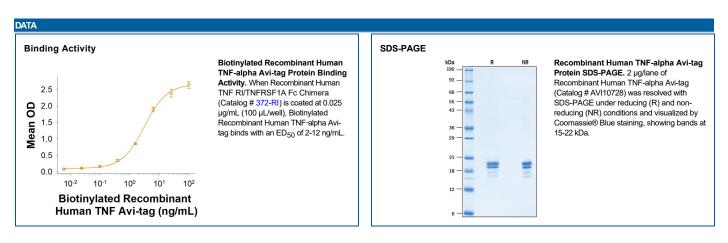
When Recombinant Human TNF RI/TNFRSF1A Fc Chimera (Catalog # 372-RI) is coated at 0.025 µg/mL (100 µL/well),

Measured in a cytotoxicity assay using L-929 mouse fibroblast cells in the presence of the metabolic inhibitor actinomycin D. Matthews, N.

Measured by its binding ability in a functional ELISA.

Biotinylated Recombinant Human TNF-α Avi-tag binds with an ED<sub>50</sub> of 2-12 ng/mL.

PREPARATION AND STORAGE		
Reconstitution	Reconstitute at 500 μg/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.	



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## 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). Human TNF- $\alpha$  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- $\alpha$  shares 97% aa sequence identity with rhesus and 71%-92% with bovine, canine, cotton rat, equine, feline, mouse, porcine, and rat 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). Our Avi-tag Biotinylated human TNF- $\alpha$  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 streptavidincoated 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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