Human TNFα ELISA Kit

Catalogue Number: SEL103

ORDERING INFORMATION

Components
Capture Antibody 20 ug
Detection Antibody 5 ug
Standard protein 100 ng

Formulation: Lyophilized powder

Storage: -20℃

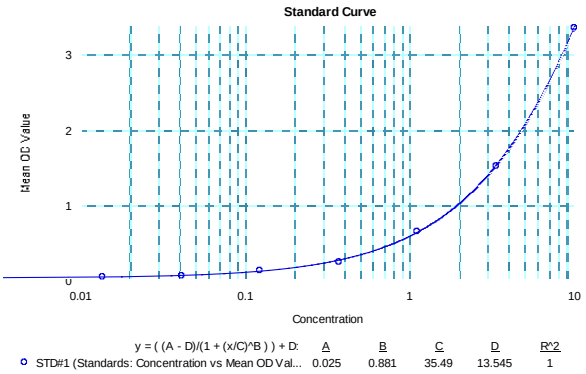
Reconstitution or dilution: PBS

Specificity: human TNFα

Recommended Usage:
Capture antibody: 1 ug/ml in PBS
Detection antibody: 0.25 ug/ml in PBS
Standard protein: 3-fold

General protocol of ELISA:
Coat 100 ul of the capture antibody on a 96-well immunoplate overnight at 4˚C or for 2 hours at room temperature. Block the plate with 200 ul of 3% albumin containing PBS for 2 hours at room temperature. Wash the plate 3 times with 0.1% tween-20 containing PBS. Add samples to detect IL-32 or standard protein overnight at 4˚C or for 2 hours at room temperature. Wash the plate 3 times with 0.1% tween-20 containing PBS. Add 100 ul of the detection antibody for 2 hours at room temperature. Wash the plate 3 times with 0.1% tween-20 containing PBS. Add 100 ul of HRP-conjugated streptavidin for 30 min at room temperature. Wash the plate 3 times with 0.1% tween-20 containing PBS. Add 100 ul of tetramethyl benzidine (TMB) solution until the standard wells turn to blue. This may not take more than 20 minutes. Add another 100 ul of 1N H_2SO_4 and read the optical density at 450 nm wave.

Standard Curve



hTNFa (ng/ml)	OD Values
10	3.361
3.333	1.517
1.111	0.649
0.370	0.241
0.123	0.131
0.041	0.056
0.013	0.041

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).

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82-2-457-0868