

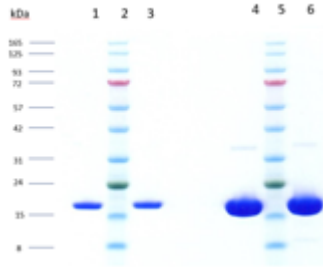
DESCRIPTION

Human tumor necrosis factor alpha (TNF- α), also known as cachectin and TNFSF2, is the prototypic ligand of the TNF superfamily. TNF- α is produced by a wide variety of immune, epithelial, endothelial, and tumor cells^{1,2}. It is a primary mediator of numerous immunologic functions, including hemorrhagic tumor necrosis/cytotoxicity, inflammation and regulation of antiviral and immune proliferative and activation responses. As a central player in the cytokine network, TNF- α has been implicated in a variety of disease states, including cachexia, endotoxic (septic) shock, acute respiratory distress syndrome and a number of necrotic, proliferative and autoimmune diseases. Human TNF- α encoded by a 7124 gene, is a type II integral membrane protein consisting of a 35 amino acid (aa) cytoplasmic domain, a 21 aa transmembrane segment, and a 177 aa extracellular domain (ECD). 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 assembled intracellularly to form a noncovalently linked homotrimer which is expressed on the cell surface^{3,4}. Shedding of membrane bound TNF- α by TACE/ADAM17 releases the bioactive cytokine, a 60 kDa soluble trimer of the TNF- α extracellular domain⁵⁻⁷. TNF- α binds the ubiquitous 55-60 kDa TNFR I^{8,9} and the hematopoietic cell-restricted 80 kDa TNFR II, both of which are also expressed as homotrimers^{1,2,10}. The TNF- α transmembrane protein is proteolytically cleaved to yield a soluble protein, which subsequently forms a non-covalently linked homotrimer in solution¹¹. The individual subunits of this homotrimer have a relative molecular mass each of 20 KDa. TNF- α binds two receptors TNFR I and TNFR II inducing NF- κ B and MAPK signaling pathways. Recombinant human TNF- α is intended for use in cell culture applications.

- Homotrimer conformation as determined by SEC chromatography.
- TNF- α ELISA for TNF- α folding validation.
- Biological activity is tested using L929 cells for cytotoxicity assay.
- Endotoxin levels <0.05 EU/ μ g as determined using a kinetic chromogenic LAL assay.

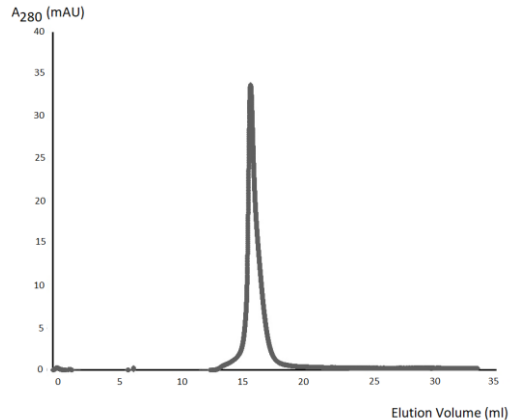
DATA

SDS PAGE



Recombinant Human TNF- α (active) (Catalog # AT-0001H/CF) was resolved by SDS-PAGE with Coomassie Blue staining, under reducing (R) conditions, showing a band at 20.6 kDa. (Lane 1, 3 loading 0.5 μ g/ μ l without or with DTT after SEC analysis; Lane 2, 5 Broad range protein markers; Lane 4, 6 loading 5 μ g/ μ l without or with DTT before SEC analysis)

SEC chromatography



Over 98% of Recombinant Human TNF- α (Catalog # AT-0001H/CF) presents as active trimers with a molecular mass of 60 kDa, as determined by SEC chromatography.

SPECIFICATION

Recombinant human TNF- α is produced in Chinese hamster ovary (CHO) cells transfected with the ECD sequence of the human TNF- α gene with an 8-His tag at C-terminals.

Source: Mammalian; Chinese hamster ovary (CHO) cells

Species: Human; Gene ID: 7124; UniProt ID: P01375

Molecular weight: 20.6 kDa including tags

Tags: 8-His tag at C-terminals

Concentration: determined by A_{280} with the Extinction coefficient of 1.044 l/g \cdot cm⁻¹ to produce 1 mg/vial, 200 μ g/vial product form.

Amino acids: encoding the extracellular (57-233) domain of human TNF with an 8-His tag at C-terminals.

Amino Acid Sequence:

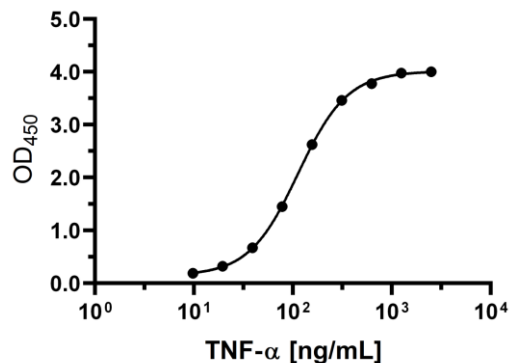
GPQREEFPRDLISPLAQAVRSSSRTPSDKPVAVHVVANPQAE
GQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKG
QGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEA
KPWYEPYIYLGGVFQLEKGDRLSAEINRPDYLDFAESGQVYFGII
ALHHHHHHHHH

Formulation: Carrier free recombinant human TNF- α was lyophilized from a 0.22 mm filtered solution in sterile ammonium bicarbonate buffer solution (pH 7.4) (Catalog # AT-0001H/CF; carrier free product formulation) or Recombinant human TNF- α was lyophilized from a 0.22 mm filtered solution in sterile ammonium bicarbonate buffer solution (pH 7.4) and 5% trehalose containing (Catalog # AT-0001H; Stabilier product formulation).

Quality control:

- Purity greater than 98% as determined by SDS-PAGE.

TNF- α ELISA

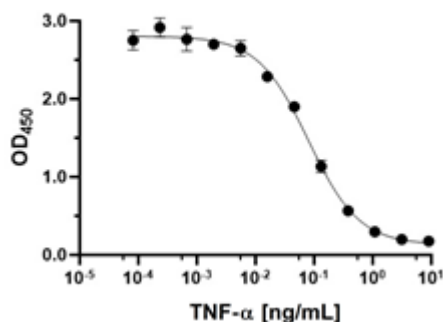


Recombinant Human TNF- α (Catalog # AT-0001H/CF) is detected with ELISA analysis with mouse anti-His capture antibody, human anti-TNF- α primary antibody and rabbit anti-human IgG-HRP secondary antibody.

Recombinant Human TNF- α

Catalog Number: AT-0001H-1000/CF

Cytotoxicity analysis



Recombinant Human TNF- α (Catalog # AT-0001H/CF) induces cytotoxicity in the L-929 mouse fibroblast cell line in the presence of the metabolic inhibitor actinomycin D. The ED₅₀ of cytotoxicity effect is 66 pg/mL.

This protocol uses Cell Proliferation Assay to determine bioactivity of TNF- α .

1. Maintain and culture L929 cells to confluence in RPMI 1640 complete medium (medium contain 10% heat inactivated FBS).
2. Trypsinization L929 cell and plating cell number 2x 10⁴/well in 96-well microplate.
3. Incubate for 16–20 hours (37°C in a 5% CO₂).
4. Prepare serial dilutions of TNF- α (0.01 pg/mL to 9 ng/mL) in fresh RPMI complete medium containing 1 μ g/mL actinomycin D.
5. Remove original medium and add 100 μ L/well of these medium with TNF- α dilutions.
6. Incubate the plate for 24 hours at 37°C in a 5% CO₂, humidified atmosphere.
7. Remove the medium and add 100 μ L/well of fresh medium with cell proliferation reagent WST-1 (Roche), each well contain 90 μ L medium and 10 μ L WST-1.
8. Incubate the plate for 4 hour at 37°C in a 5% CO₂, humidified atmosphere.
9. Shake thoroughly for 1 min on a shaker.
10. Record the absorbance at 450nm using an ELISA plate reader.

PREPARATION AND STORAGE

- Recombinant human TNF- α in lyophilized carrier free powder form is shipped at room temperature.
- It should be stored at -20 °C; stored at -80°C upon receiving for long term storage.
- Preparation of stock solution (100 μ g/ml):
 1. Add 10 ml endotoxin-free water to 1000 μ g of recombinant human TNF- α .
 2. Mix by pipetting. Do not vortex.
 3. Prepare aliquots of recombinant human TNF- α . Avoid freeze-thaw cycles.
 4. Further dilutions can be prepared in the appropriate
- Note: Avoid repeated freeze-thaw cycles.

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RELATED PRODUCTS

Product	Catalog Code
Anti-hTNF- α -hlgG1	AT-hTNF α -Ab001
Human TNF- α ELISA Set	AT-hTNF α -ELISA001
Recombinant Human TNF- α	AT-0001H-200/CF
Recombinant Human TNF- α	AT-0001H-50
Recombinant Human TNF- α	AT-0001H-200
Recombinant Human TNF- α	AT-0001H-1000

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