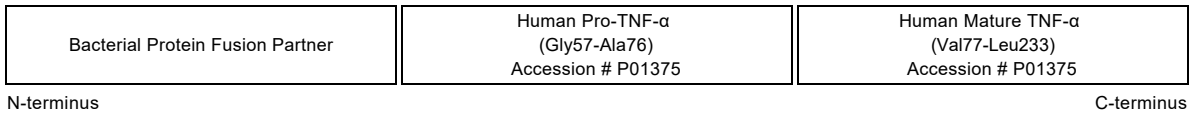
Recombinant Human Pro TNF-α Fusion Protein

Catalog Number: 1012-PS

DESCRIPTION

Source

E. coli-derived human TNF-alpha protein



N-terminal Sequence Analysis	Bacterial Protein Fusion Partner
Predicted Molecular Mass	45 kDa

SPECIFICATIONS

SDS-PAGE	42 kDa, reducing conditions
Activity	Measured by its ability to be used as a protein substrate for TACE/ADAM17. Under the described conditions TACE/ADAM17 will cleave pro-TNF-α to produce mature TNF-α.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>90%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Lyophilized from a 0.2 µm filtered solution in Urea, NaCl, NaH_2PO_4 and DTT. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 25 mM Tris, 200 mM NaCl and 0.02%(w/v) Brij-35, pH 8.0
- Recombinant Human Pro TNF-α Fusion Protein (rhPro-TNF-α) (Catalog # 1012-PS)
- Recombinant Human TACE/ADAM17 (rhTACE) (Catalog # 930-ADB)
- Positive Control: Recombinant Human TNF-α (rhTNF-α) (Catalog # 210-TA)
- Goat Anti-Human TNF-α Biotinylated Antigen Affinity-purified Polyclonal Antibody (Catalog # BAF210)
- SDS-PAGE followed by Western Blotting

Assay

1. Prepare the following vials for a final volume of 20 µL.
   a. 10 µL of rhPro-TNF-α at 0.2 mg/mL + 10 µL of rhTACE at 0.2 mg/mL
   b. 10 µL of rhPro-TNF-α at 0.2 mg/mL + 10 µL of Assay Buffer (control-with incubation)
   c. 10 µL of rhPro-TNF-α at 0.2 mg/mL + 10 µL of Assay Buffer (control-without incubation)
2. Incubate vials at 37 °C overnight (except for the non-incubated control, store at ≤-20 °C).
3. Stop the reactions and controls by adding the reducing gel loading buffer for SDS-PAGE to all vials for a final concentration of 50 ng rhPro-TNF-α/15 µL. Heat the samples at 95 °C for 3-5 minutes.
4. Prepare a sample of rhTNF-α (Catalog # 210-TA) at 5 ng/15 µL in reducing gel loading buffer as a control on the gel. Heat the sample at 95 °C for 3-5 minutes.
5. Load the samples on a 15% gel.
   a. 50 ng/lane (15 µL) of rhPro-TNF-α of the incubated reactions (including the control with incubation).
   b. 50 ng/lane (15 µL) of rhPro-TNF-α of the control (without incubation).
   c. 5 ng/lane (15 µL) of rhTNF-α (Catalog # 210-TA) control.
6. Follow SDS-PAGE/Western blotting procedures.
   a. Use the Biotinylated Anti-human TNF-α/TNFSF1A antibody at 0.1 µg/mL. (Catalog # AF-210-NA) antibody may be used in place of (Catalog # BAF210).
7. Visually determine processing of rhPro-TNF-α to the mature form by rhTACE by comparing the incubated reactions to the rhTNF-α control.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile, deionized water.
Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 6 months from date of receipt, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after reconstitution.

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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 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-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 TNF-α-converting-enzyme (TACE or 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). TACE/ADAM17 cleaves the 26 kDa form at the Ala76-Val77 bond to produce the 17 kDa form (6, 16). ADAM10 processes the 26 kDa form at the same site as TACE (17). ADAM9 cleaves the 26 kDa form at alternative sites, Ala74-Glu75 and Ser79-Ser80 (18). The use of the recombinant Pro-TNF-α fusion protein as a protein substrate for Pro-TNF-α processing proteases has been tested with recombinant TACE/ADAM17 (R&D Systems, Catalog # 930-ADB), ADAM10 (Catalog # 936-AD and 946-AD), or ADAM9 (Catalog # 949-AD). The disappearance of the fusion protein (45 kDa) and the appearance of the mature TNF-α (17 kDa) were followed by Western blot analysis using an anti-human TNF-α polyclonal antibody (Catalog # AF210 or BAF210).

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