**Product Datasheet**

Recombinant human TNF alpha protein (Active) ab259410

**Description**

- **Product name**: Recombinant human TNF alpha protein (Active)
- **Biological activity**: Fully biologically active when compared to standard. The ED₅₀ as determined by the dose-dependent Killing/apoptosis of L-929 cells is 0.71ng/mL corresponding to a Specific Activity of 1.41 x 10⁶ IU/mg.
- **Purity**: >= 95 % SDS-PAGE, >= 95 % HPLC.
- **Endotoxin level**: < 0.005 Eu/µg
- **Expression system**: HEK 293 cells
- **Accession**: P01375
- **Protein length**: Full length protein
- **Animal free**: Yes
- **Carrier free**: Yes
- **Nature**: Recombinant
- **Species**: Human
- **Sequence**: VRSSRTPSDKPVAHV/VANPQAEGQLQWLNRANALLA NGVELRDNQLVV
  PSEGFLYIYSQVLFGKQGCPSTHVLLHTTISR/AVSYQTKVNL
  LLSA/KSP
  CQRETPEGAEAKPWyEPYLGGVFQLEKGDRLSAEINRPDYLDFAESGQV YFGI/AL
- **Predicted molecular weight**: 17 kDa
- **Amino acids**: 77 to 233
- **Additional sequence information**: Full length mature chain soluble form. N-terminal glycine.

**Specifications**

Our Abpromise guarantee covers the use of **ab259410** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

**Applications**

- Sandwich ELISA
### Form
- Lyophilized

### Additional notes
- This protein is filter sterilised prior to aliquoting and lyophilisation. All aliquoting and lyophilisation steps are performed in a sterile environment.

### Stability and Storage
- Shipped at Room Temperature. Store at Room Temperature.
- Information available upon request.
- This product is an active protein and may elicit a biological response in vivo, handle with caution.

### Reconstitution
- Reconstitute with Phosphate Buffered Saline. Reconstituted protein stable at -80°C for 12 months or 4°C for 1 week. Lyophilized contents may appear as either a translucent film or a white powder. This variance does not affect the quality of the product.

### General Info

#### Function
- Cytokine that binds to TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR. It is mainly secreted by macrophages and can induce cell death of certain tumor cell lines. It is potent pyrogen causing fever by direct action or by stimulation of interleukin-1 secretion and is implicated in the induction of cachexia. Under certain conditions it can stimulate cell proliferation and induce cell differentiation.

#### Involvement in disease
- Genetic variations in TNF are a cause of susceptibility psoriatic arthritis (PSORAS) [MIM:607507]. PSORAS is an inflammatory, seronegative arthritis associated with psoriasis. It is a heterogeneous disorder ranging from a mild, non-destructive disease to a severe, progressive, erosive arthropathy. Five types of psoriatic arthritis have been defined: asymmetrical oligoarthritis characterized by primary involvement of the small joints of the fingers or toes; asymmetrical arthritis which involves the joints of the extremities; symmetrical polyarthritis characterized by a rheumatoidlike pattern that can involve hands, wrists, ankles, and feet; arthritis mutilans, which is a rare but deforming and destructive condition; arthritis of the sacroiliac joints and spine (psoriatic spondylitis).

#### Sequence similarities
- Belongs to the tumor necrosis factor family.

#### Post-translational modifications
- The soluble form derives from the membrane form by proteolytic processing.
- The membrane form, but not the soluble form, is phosphorylated on serine residues.
- Dephosphorylation of the membrane form occurs by binding to soluble TNFRSF1A/TNFR1.
- O-glycosylated; glycans contain galactose, N-acetylgalactosamine and N-acetylneuraminic acid.

#### Cellular localization
- Secreted and Cell membrane.
Wild-type A549 control cells or IP-10 knockout A549 cells (ab266969), grown to 40% confluency, were stimulated with Recombinant Human Interferon gamma protein (ab259377) at 100 ng/ml and Recombinant human TNF alpha protein (ab259410) at 10 ng/ml or vehicle control for 16 or 32 hours.

THP-1 cells, grown to 40% confluency, were stimulated with Recombinant Human Interferon gamma protein (ab259377) at 200 ng/ml and LPS at 50 ng/mL or vehicle control for 24 hours.

The concentrations of IP-10 (CXCL10) in cell culture supernatants were measured in duplicate and interpolated from the IP-10 standard curves using Human IP-10 ELISA Kit (ab173194). IP-10 from vehicle control samples were measured in undiluted supernatants and the treated samples were diluted 200 times. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2).

Fully biologically active when compared to standard. The ED50 as determined by the dose-dependant Killing/apoptosis of L-929 cells is 0.71 ng/mL corresponding to a Specific Activity of 1.41 x 10^6 IU/mg.
SDS-PAGE analysis of ab259410.

Sandwich ELISA - Recombinant human TNF alpha protein standard curve.

Background subtracted standard curve using Human TNF alpha Antibody Pair - BSA and Azide free (ab241791) and Recombinant human TNF alpha protein (Active) (ab259410) in sandwich ELISA. The ELISA was performed using the components of the corresponding SimpleStep® kit, which uses the same antibody pair with a different formulation and format.

Flow cytometry overlay histogram showing wild-type A549 (green line) and VCAM1 knockout A549 cells (red line, ab273758), treated with 10 ng/ml TNF-alpha for 16 h (left) and untreated (right), stained with ab103173. The cells were incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab103173) (1x10^6 in 100μl at 0.2μg/ml) for 30 min at 4°C.

Isotype control antibody mouse IgG1κ Allophycocyanin was used at the same concentration and conditions as the primary antibody (wild-type A549 - black line VCAM knockout A549 - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 40 mW Red laser (638nm) and 660/10 bandpass filter.
Western blot - Recombinant human TNF alpha protein (Active) (ab259410)

All lanes: Anti-IP10 antibody [EPR20764] (ab214668) at 1/1000 dilution

Lane 1: Wild-type A549 Brefeldin A (ab120299)-treated (5ug/ml, 6h) cell lysate

Lane 2: Wild-type A549 IFN-y (ab259377) (100 ng/ml, 32 h) and TNF-alpha (ab259410) (10 ng/ml, 32h), and Brefeldin A (ab120299)-treated (5ug/ml for the last 6h) cell lysate

Lane 3: IP10 knockout A549 Brefeldin A (ab120299)-treated (5ug/ml, 6h) cell lysate

Lane 4: IP10 knockout A549 IFN-y (ab259377) (100ng/ml, 32h) and TNF-alpha (ab259410) (10ng/ml, 32h), and Brefeldin A (ab120299)-treated (5ug/ml for the last 6h) cell lysate

Lane 5: THP-1 Brefeldin A (ab120299)-treated (5ug/ml, 6h) cell lysate

Lane 6: THP-1 IFN-y (ab259377) (200ng/ml, 24h) and LPS (50ng/ml, 24h)-treated for 24 hours, and Brefeldin A (ab120299)-treated (5ug/ml for the last 6h) cell lysate

Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Observed band size: 11 kDa

Lanes 1 - 6: Merged signal (red and green). Green - ab214668 observed at 11 kDa. Red - loading control ab8245 (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab214668 was shown to react with IP10 in wild-type A549 cells in western blot with loss of signal observed in IP10 knockout cell line ab266971 (knockout cell lysate ab256888). Wild-type and IP10 knockout A549 cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with ab214668 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (ab216772) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.
All lanes: Anti-IP10 antibody [EPR7850] (ab137018) at 1/500 dilution

Lane 1: Wild-type A549 Brefeldin A (ab120299)-treated (5µg/ml, 6h) cell lysate
Lane 2: Wild-type A549 IFN-γ (ab259377) (100 ng/ml, 32 h) and TNF-alpha (ab259410) (10 ng/ml) for 32 hours, and Brefeldin A (ab120299)-treated (5µg/ml for the last 6h) cell lysate
Lane 3: IP10 knockout A549 Brefeldin A (ab120299)-treated (5µg/ml, 6h) cell lysate
Lane 4: IP10 knockout A549 IFN-γ (ab259377) (100 ng/ml, 32 h) and TNF-alpha (ab259410) (10 ng/ml) for 32 hours, and Brefeldin A (ab120299)-treated (5µg/ml for the last 6h) cell lysate

Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

**Observed band size:** 11 kDa

**Lanes 1 - 4:** Merged signal (red and green). Green - ab137018 observed at 11 kDa. Red - loading control ab8245 (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab137018 was shown to react with IP10 in A549 wild-type cells in western blot with loss of signal observed in IP10 knockout cell line ab266969 (IP10 knockout cell lysate ab256886). A549 wild-type and IP10 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with ab137018 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 500 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (ab216772) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.
Purity: 100%

The spectrum was recorded using a 1260 Infinity II HPLC system with DAD and a MabPac RP column (3.0x100 mm, 4 µm). 5 µL of purified protein was injected and the gradient run from 80 % water:TFA (99.9:0.1 v/v) and 20 % acetonitrile:water:TFA (90:9.9:0.1 v/v/v) to 20 % water:TFA (99.9:0.1 v/v) and 80 % acetonitrile:water:TFA (90:9.9:0.1 v/v/v) within 3 minutes followed by an isocratic step for another 3 min. Flow rate was 0.5 mL/min and the column compartment temperature was 50 °C.

M + 0.2 Da (calc. mass 17409.8 Da)

The spectrum was recorded with a 6545XT AdvanceBio LC/Q-TOF (Agilent Technologies) and a MabPac RP column (42.1x50 mm, 4 µm, Thermo Scientific). 5 µL of purified protein was injected and the gradient run from 85 % water:FA (99.9:0.1 v/v) and 15 % acetonitrile:FA (90:9.9:0.1 v/v/v) to 55 % water:FA (99.9:0.1 v/v) and 45 % acetonitrile:FA (90:9.9:0.1 v/v/v) within 3 minutes followed by an isocratic step for another 2.5 min. Flow rate was 0.4 mL/min and the column compartment temperature was 60 °C. Data was analysed and deconvoluted using the Bioconfirm software (Agilent Technologies).

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