abcam

Product datasheet

Recombinant human TNF alpha protein (Active) ab259410

5 References 9 Images

Description

Product name	Recombinant human TNF alpha protein (Active)
Biological activity	Fully biologically active when compared to standard. The ED_{50} as determined by the dose-dependant Killing/apoptosis of L-929 cells is 0.71ng/mL corresponding to a Specific Activity of 1.41 x 10^6 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	VRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLA NGVELRDNQLVV PSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVN LLSAIKSP CQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPD YLDFAESGQV YFGIIAL
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

	Cell Culture
	Functional Studies
	Mass Spectrometry
	HPLC
	SDS-PAGE
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

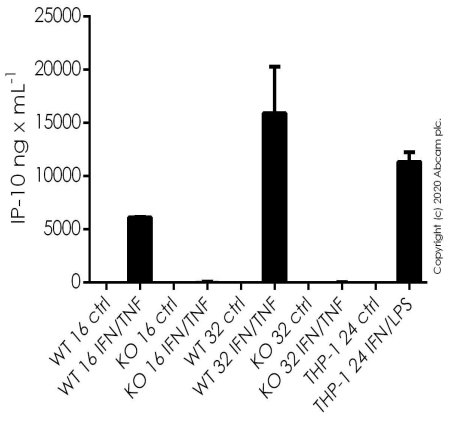
Preparation and Storage

Stability and Storage	Shipped at Room Temperature. Store at Room Temperature.
	pH: 6.00 Constituents: 0.727% Dibasic monohydrogen potassium phosphate, 0.248% Monobasic dihydrogen potassium phosphate, 10.26% Trehalose
	Buffer lyophilized from.
	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 -80C for 12 months or 4C 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.

Images

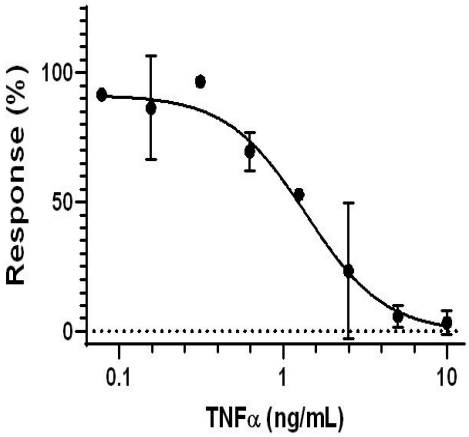


Sandwich ELISA - Recombinant human TNF alpha protein (Active) (ab259410)

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.

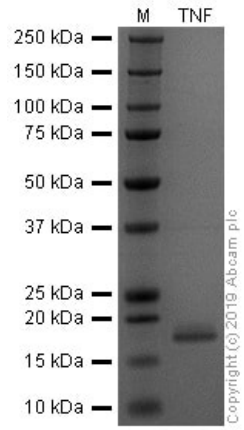
THP-1 cells, grown to 40% confluency, were stimulated with Recombinant Human Interferon gamma protein (**ab259377**) at 200 ng/ml and LPS at 50 ng/mL or vehicle control for 24 hours.

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).



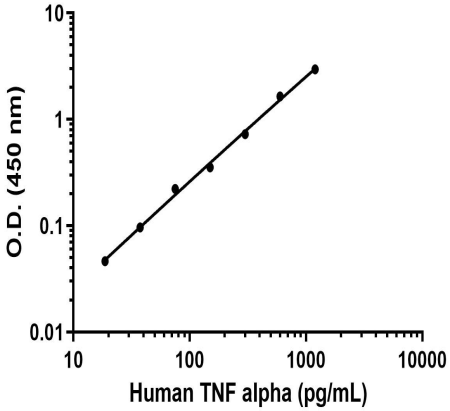
Functional Studies - Recombinant human TNF alpha protein (Active) (ab259410)

Fully biologically active when compared to standard. The ED_{50} 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 - Recombinant human TNF alpha protein (Active) (ab259410)

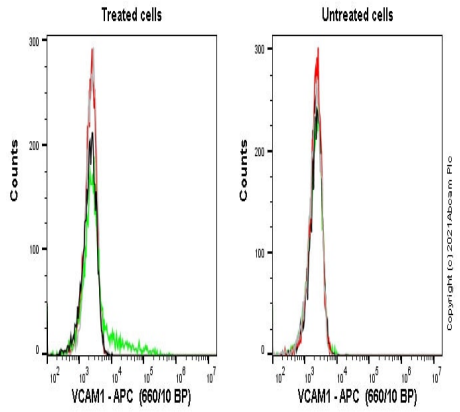
SDS-PAGE analysis of ab259410.



Sandwich ELISA - Recombinant human TNF alpha protein (Active) (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.

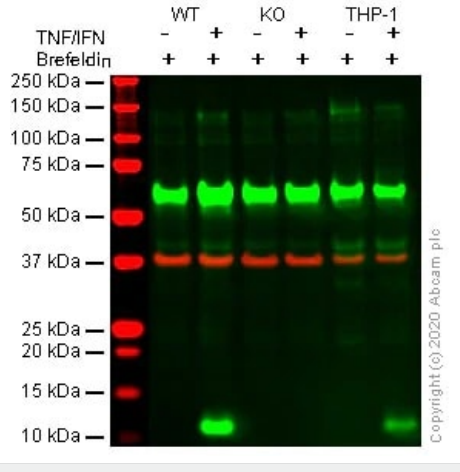


Flow Cytometry - Recombinant human TNF alpha protein (Active) (ab259410)

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-γ (**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-γ (**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-γ (**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

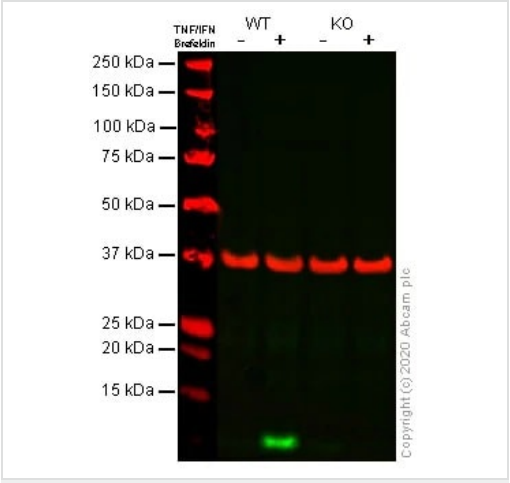
Lysates/proteins at 30 µg per lane.

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.



Western blot - Recombinant human TNF alpha protein (Active) (ab259410)

All lanes : Anti-IP10 antibody [EPR7850] (**ab137018**) at 1/500 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) for 32 hours, 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**) (100 ng/ml, 32 h) and TNF-alpha (ab259410) (10 ng/ml) for 32 hours, and Brefeldin A (**ab120299**)-treated (5ug/ml for the last 6h) cell lysate

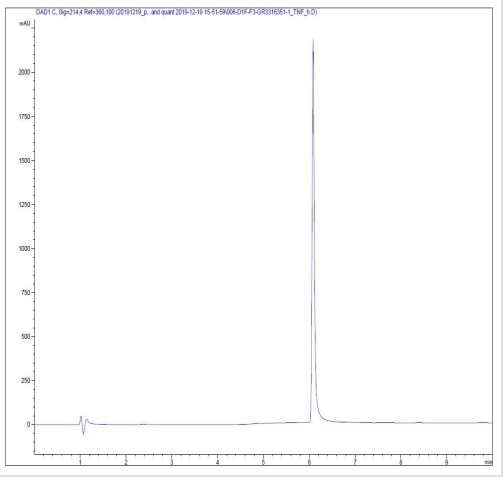
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Observed band size: 11 kDa

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

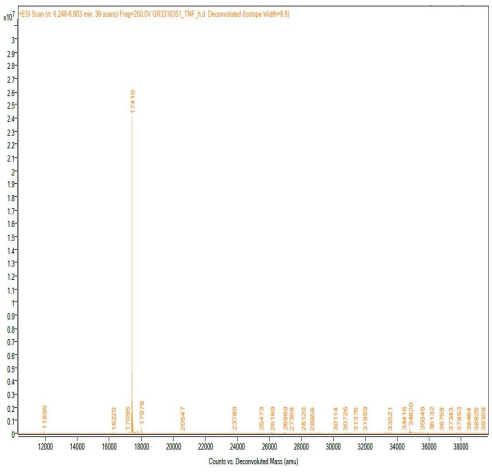
ab137018 was shown to react with IP10 in A549 wild-type cells in western blot with loss of signal observed in IP10 knockout cell line **ab266969** (IP10 knockout cell lysate **ab256886**). A549 wild-type and IP10 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent 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.



HPLC - Recombinant human TNF alpha protein (Active) (ab259410)

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.



Mass Spectrometry - Recombinant human TNF alpha protein (Active) (ab259410)

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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