

Product datasheet

Recombinant human TNF alpha protein ab9642

3 References 7 Images

Overview

Product name	Recombinant human TNF alpha protein
Protein length	Full length protein

Description

Nature	Recombinant
Source	Escherichia coli
Amino Acid Sequence	
Accession	P01375
Species	Human
Sequence	<pre> VRSSSRTPSD KPVAVHVVANP QAEGQLQWLN RRANALLANG VELRDNQLVV PSEGLYLIYS QVLFKGGQCP STHVLLTHTI SRIAVSYQTK VNLLSAIKSP CQRETPEGAE AKPWYEPYL GGVFQLEKGD RLSAEINRPD YLDFAESGQV YFGIAL </pre>
Molecular weight	17 kDa
Amino acids	77 to 233
Additional sequence information	aa 77 to 233 refers to the full length mature form (soluble).

Specifications

Our [Abpromise guarantee](#) covers the use of **ab9642** in the following tested applications.

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

Biological activity	The ED ₅₀ , as determined by the cytolysis of murine L929 cells in the presence of Actinomycin D, is ≤ 0.05 ng/mL, corresponding to a specific activity of ≥ 2 x 10 ⁷ units/mg.
Applications	<ul style="list-style-type: none"> Functional Studies Sandwich ELISA HPLC SDS-PAGE
Endotoxin level	< 1.000 Eu/μg

Purity	> 98 % SDS-PAGE. >98% by HPLC analyses. Sterile filtered.
Form	Lyophilised
Additional notes	Lots prior to June 2015 contain 0.036% Tris (including lots GR157466-11, -12 and -13).

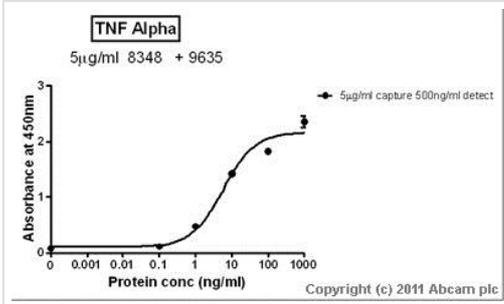
Preparation and Storage

Stability and Storage	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle. Constituents: 0.049% Sodium phosphate, 0.12% Sodium chloride This product is an active protein and may elicit a biological response in vivo, handle with caution.
Reconstitution	Reconstitute with dH2O to make a final concentration between 0.1 to 1.0 mg/ml.

General Info

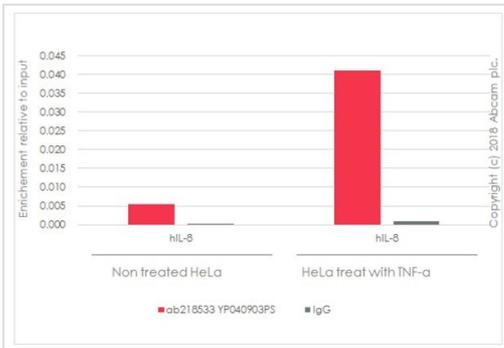
Function	Cytokine that binds to TNFRSF1A/TNFR1 and TNFRSF1B/TNFR2. It is mainly secreted by macrophages and can induce cell death of certain tumor cell lines. It is potent pyrogen causing fever by direct action or by stimulation of interleukin-1 secretion and is implicated in the induction of cachexia. 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 (ab9642)

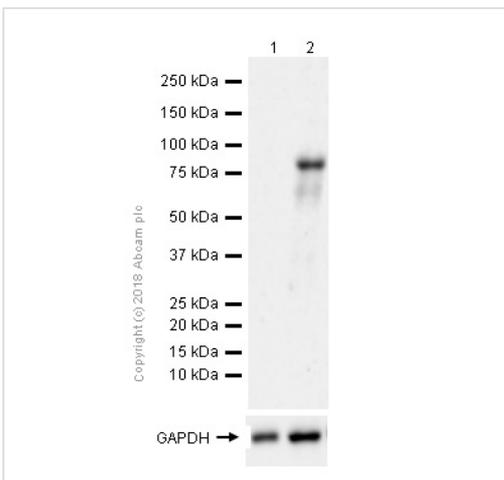
Standard curve for TNF alpha (Analyte: [ab9642](#)); dilution range 1µg/ml to 1µg/ml using Capture Antibody [Mouse monoclonal \[2C8\]](#) to TNF alpha ([ab8348](#)) at 5µg/ml and Detector Antibody [Rabbit polyclonal to TNF alpha \(ab9635\)](#) at 0.5µg/ml.



ChIP - Recombinant human TNF alpha protein (ab9642)

Chromatin was prepared from HeLa (human epithelial cell line from cervix adenocarcinoma) cells treated with and without 20 ng/ml TNF- α ([ab9642](#)) for 60 minutes according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25 µg of chromatin, 5 µg of [ab218533](#) (red), and 20 µl of Protein A/G sepharose beads. 5 µg of rabbit normal IgG was added to the beads control (gray). The immunoprecipitated DNA was quantified by real time PCR (SYBR green approach).

The ChIP data are consistent with the literature (PMID: 16135789).



Western blot - Recombinant human TNF alpha protein (ab9642)

All lanes : Anti-TNFAIP3 antibody [EPR2663] ([ab92324](#)) at 1/5000 dilution

Lane 1 : WEHI-3 (Mouse leukemia lymphoblast) whole cell lysate

Lane 2 : WEHI-3 treated with 20 ng/ml TNF alpha ([ab9642](#)) for 6 h

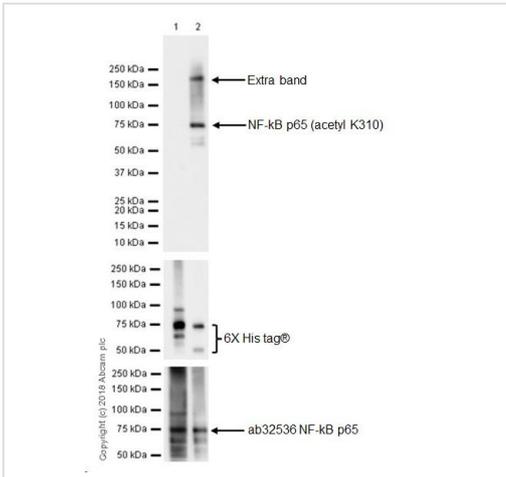
Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/20000 dilution

Observed band size: 80 kDa

[why is the actual band size different from the predicted?](#)



Western blot - Recombinant human TNF alpha protein (ab9642)

All lanes : Anti-NF-kB p65 (acetyl K310) antibody [EPR21781] - ChIP Grade ([ab218533](#)) at 1/2000 dilution

Lane 1 : HEK-293 transfected with NF-kB p65 expression vector containing a myc-His-tag®, whole cell lysate

Lane 2 : HEK-293 transfected with NF-kB p65 and p300 (aa1287-1663) expression vectors containing a myc-His-tag®, then treated with 20 ng/ml TNF-alpha (ab9642) for 60 minutes, whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

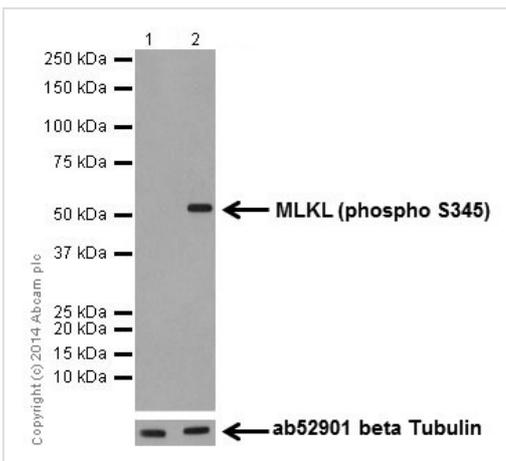
All lanes : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/100000 dilution

Observed band size: 70 kDa [why is the actual band size different from the predicted?](#)

Exposure time: 37 seconds

Blocking/Dilution buffer: 5% NFDm/TBST.

NF-kB p65 (acetyl K310) expression is induced by TNF-α and p300 acetyltransferases (PMID: 20160011, PMID: 12456660, PMID: 16135789).



Western blot - Recombinant human TNF alpha protein (ab9642)

All lanes : Anti-MLKL (phospho S345) antibody [EPR9515(2)] ([ab196436](#)) at 1/1000 dilution

Lane 1 : Untreated L-929 (Mouse connective tissue fibroblast cells) whole cell lysate

Lane 2 : L-929 whole cell lysate treated with 20 ng/ml TNF alpha (ab9642), 100 nM Smac mimetic, and 20 µM z-VAD ([ab120382](#)) for 8 h and then harvested.

Lysates/proteins at 10 µg per lane.

Secondary

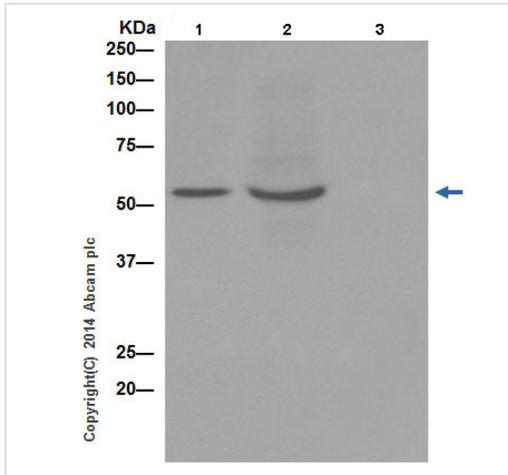
All lanes : Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Observed band size: 54 kDa [why is the actual band size different](#)

from the predicted?

Exposure time: 15 seconds

Blocking and dilution buffer: 5% NFDm/TBST.



Immunoprecipitation - Recombinant human TNF alpha protein (ab9642)

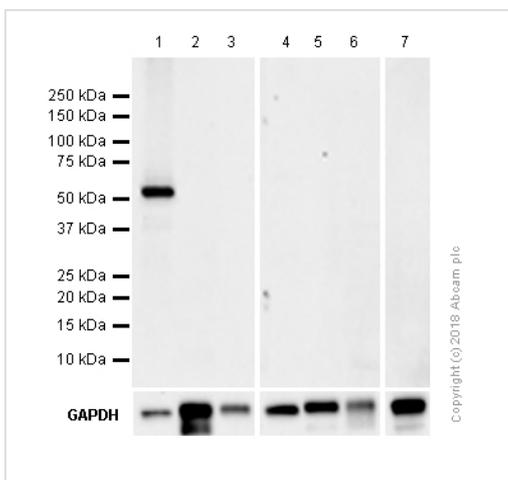
MLKL (phospho S345) was immunoprecipitated from 1mg of L-929 (Mouse connective tissue fibroblast cells) whole cell lysate treated with 20 ng/ml TNF alpha (ab9642) + 100 nM Smac mimetic + 20 μ M z-VAD compound (ab120382) for 8h using ab196436 at 1/150 dilution. Western blot was performed from the immunoprecipitate using ab196436 at 1/1000 dilution. Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG, was used as secondary antibody at 1/1500 dilution.

Lane 1: L-929 whole cell lysate treated with 20 ng/ml TNF alpha (ab9642) + 100 nM Smac mimetic+ 20 μ M z-VAD compound (ab120382) for 8h;10 μ g (Input).

Lane 2: ab196436 IP in L-929 whole cell lysate treated with 20 ng/ml TNF alpha (ab9642) + 100 nM Smac mimetic+ 20 μ M z-VAD compound (ab120382) for 8h.

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab196436 in L-929 whole cell lysate treated with 20 ng/ml TNF alpha (ab9642) + 100 nM Smac mimetic+ 20 μ M z-VAD compound (ab120382) for 8h.

Blocking and dilution buffer and concentration: 5% NFDm/TBST.



Western blot - Recombinant human TNF alpha protein (ab9642)

All lanes : Anti-MLKL (phospho S345) antibody [EPR9515(2)] (ab196436) at 1/1000 dilution

Lane 1 : L-929 treated with 20 ng/ml TNF alpha (ab9642), 100 nM Smac mimetic, and 20 μ M z-VAD (ab120382) for 8 h, whole cell lysate

Lane 2 : Mouse brain tissue lysate

Lane 3 : Mouse colon tissue lysate

Lane 4 : Mouse lung tissue lysate

Lane 5 : Mouse retina tissue lysate

Lane 6 : Mouse liver tissue lysate

Lane 7 : Raw264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate

Lysates/proteins at 20 μ g per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/20000 dilution

Observed band size: 54 kDa [why is the actual band size different from the predicted?](#)

Exposure time: 50 seconds

Blocking and diluting buffer: 5% NFDM/TBST.

MLKL pS345 is a trigger for necroptosis. It is only detectable in infection/cellular damaged (PMID:29229989) or aging tissue (PMID: 28807105) but not in normal tissues.

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