

Anti-TNF alpha antibody [EPR19147] - Low endotoxin, Azide free ab222500

KO VALIDATED Recombinant RabMAb

5 Images

Overview

Product name	Anti-TNF alpha antibody [EPR19147] - Low endotoxin, Azide free
Description	Rabbit monoclonal [EPR19147] to TNF alpha - Low endotoxin, Azide free
Host species	Rabbit
Specificity	We recommend ab183218 to detect TNF alpha in Western blot, as it is more sensitive than ab215188 . Stimulation is required for the detection of TNF alpha in most samples.
Tested applications	Suitable for: IP, ICC/IF, ELISA, WB
Species reactivity	Reacts with: Mouse, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: TPA pretreated THP-1 treated with 100 ng/ml LPS for 7 hours and TPA pretreated THP-1 treated with 100 ng/ml LPS for 4 hours, then added 1 µg/ml BFA for 3 hours whole cell lysates; TPA pretreated RAW 264.7, TPA pretreated RAW 264.7 treated with 100 ng/ml LPS for 7 hours and TPA pretreated RAW 264.7 treated with 100 ng/ml LPS for 4 hours, then added 1 µg/ml BFA for 3 hours whole cell lysates. IP: TPA pretreated THP-1 treated with 100 ng/ml LPS for 4 hours, then added 1 µg/ml BFA for 3 hours cell lysate.
General notes	<p>ab222500 is the carrier-free version of ab183218.</p> <p>Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.</p> <p>This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.</p> <p>Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.</p> <p>This product is compatible with the Maxpar[®] Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p>

Our **Low endotoxin, azide-free formats** have low endotoxin level (≤ 1 EU/ml, determined by the LAL assay) and are free from azide, to achieve consistent experimental results in functional assays.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR19147
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab222500 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IP		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
ELISA		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration.

Target

Function	Cytokine that binds to TNFRSF1A/TNFR1 and TNFRSF1B/TNFR. 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.

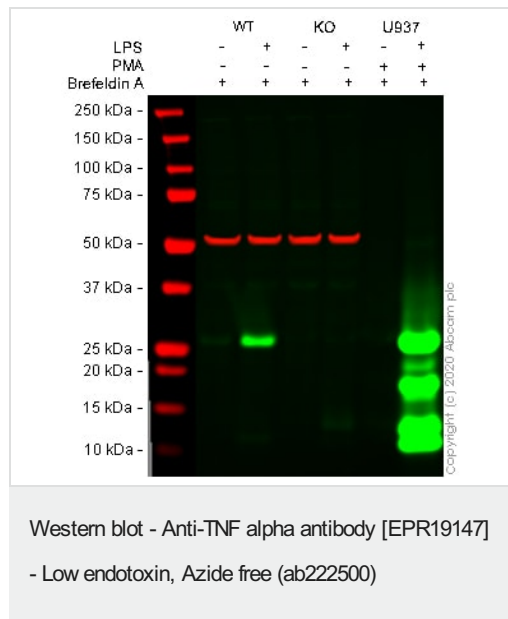
Dephosphorylation of the membrane form occurs by binding to soluble TNFRSF1A/TNFR1.

O-glycosylated; glycans contain galactose, N-acetylgalactosamine and N-acetylneuraminic acid.

Cellular localization

Secreted and Cell membrane.

Images



All lanes : Anti-TNF alpha antibody [EPR19147] ([ab183218](#)) at 1/1000 dilution

Lane 1 : Wild-type THP-1 Brefeldin A ([ab120299](#)) treated (5 µg/ml, 4 h) cell lysate

Lane 2 : Wild-type THP-1 LPS treated (100 ng/ml, 16 h) and Brefeldin A ([ab120299](#)) treated (5 µg/ml, 4 h) cell lysate

Lane 3 : TNF alpha knockout THP-1 Brefeldin A ([ab120299](#)) treated (5 µg/ml, 4 h) cell lysate

Lane 4 : TNF alpha knockout THP-1 LPS treated (100 ng/ml, 16 h) and Brefeldin A ([ab120299](#)) treated (5 µg/ml, 4 h) cell lysate

Lane 5 : U937 PMA treated (10 mM, 2 days) plus 16 h no treatment and Brefeldin A ([ab120299](#)) treated (5 µg/ml, 4 h) cell lysate

Lane 6 : U937 PMA treated (10 mM, 2 days) and LPS treated (1 µg/ml, 16 h) plus Brefeldin A ([ab120299](#)) treated (5 µg/ml, 4 h) cell lysate

Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

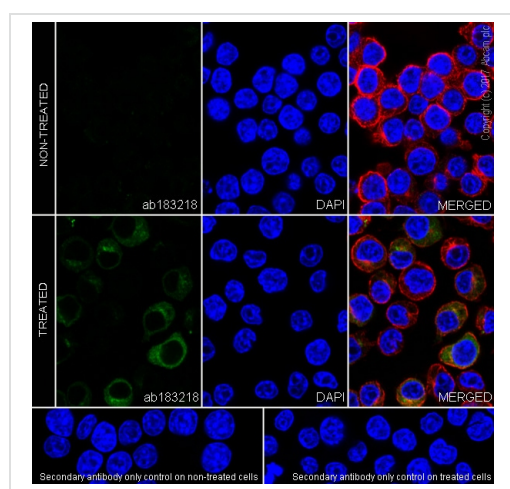
Observed band size: 26 kDa

This data was developed using the same antibody clone in a different buffer formulation ([ab183218](#)).

Lanes 1 - 6: Merged signal (red and green). Green - [ab183218](#) observed at 26 kDa. Red - loading control [ab7291](#) (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

[ab183218](#) was shown to react with TNF alpha in THP-1 wild-type

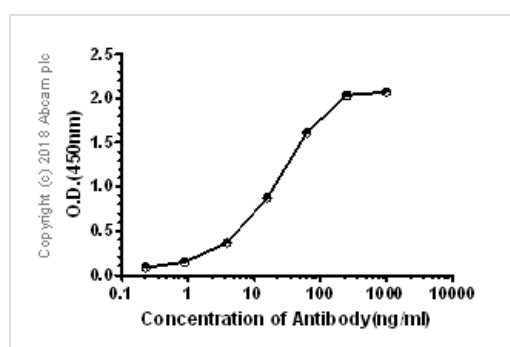
cells in Western blot with loss of signal observed in TNF knockout sample. Wild-type and TNF knockout THP-1 cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with **ab183218** and **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) 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® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-TNF alpha antibody [EPR19147] - Low endotoxin, Azide free (ab222500)

Immunocytochemistry/ Immunofluorescence analysis of RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) treated with 100ng/ml LPS for 7 h and 1µg/ml BFA for the last 3h cells labeling TNF alpha with purified **ab183218** at 1/5000 dilution (0.09 µg/ml). Cells were fixed in 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1/1000 dilution (2 µg/ml) dilution. DAPI was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.

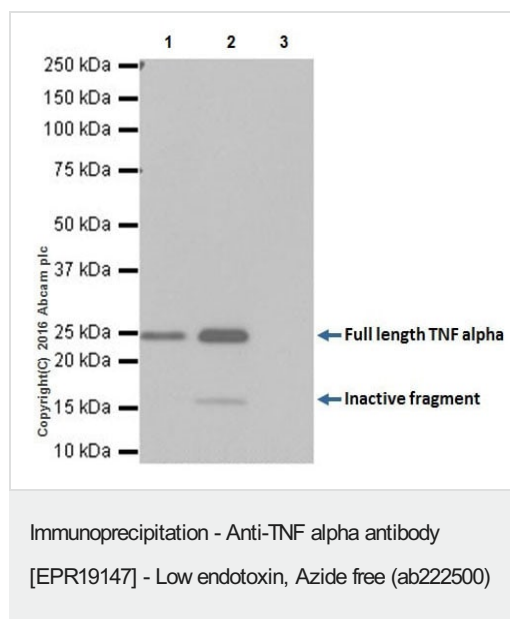
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab183218**).



ELISA - Anti-TNF alpha antibody [EPR19147] - Low endotoxin, Azide free (ab222500)

ELISA using **ab183218** between 0.1 and 1000 ng/ml to detect human TNF alpha peptide. Secondary used was Alkaline Phosphatase AffiniPure Goat Anti-Rabbit IgG (H+L) 1/2000.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab183218**).



TNF alpha was immunoprecipitated from 0.35 mg of TPA pretreated THP-1 (Human monocytic leukemia cell line) whole cell lysate treated with 100 ng/ml LPS for 4 hours, then added 1 µg/ml BFA for 3 hours with **ab183218** at 1/40 dilution. Western blot was performed from the immunoprecipitate using **ab183218** at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/1000 dilution.

Lane 1: TPA pretreated THP-1 whole cell lysate treated with 100 ng/ml LPS for 4 hours, then added 1 µg/ml BFA for 3 hours 10µg (Input).

Lane 2: **ab183218** IP in TPA pretreated THP-1 whole cell lysate treated with 100 ng/ml LPS for 4 hours, then added 1 µg/ml BFA for 3 hours.

Lane 3: Rabbit IgG, monoclonal [EPR25A]-Isotype Control (**ab172730**) instead of **ab183218** in TPA pretreated THP-1 whole cell lysate treated with 100 ng/ml LPS for 4 hours, then added 1 µg/ml BFA for 3 hours.

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

Exposure time: 1 second.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab183218**).

Why choose a recombinant antibody?

Research with confidence
Consistent and reproducible results

Long-term and scalable supply
Recombinant technology

Success from the first experiment
Confirmed specificity

Ethical standards compliant
Animal-free production

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