abcam

Product datasheet

Anti-TNF alpha antibody [EPR19147] ab183218

KO VALIDATED Recombinant RabMAb

★★★★★ 1 Abreviews 58 References 7 Images

Overview

Product name	Anti-TNF alpha antibody [EPR19147]
Description	Rabbit monoclonal [EPR19147] to TNF alpha
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:** WB, IP, ELISA, ICC/IF
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 whole cell lysate, TPA pretreated RAW 264.7 whole cell lysate and U937 (human histocytic lymphoma cell line) cell lysate. ICC/IF: RAW264.7 cells. IP: TPA pretreated THP-1 (Human monocytic leukemia cell line) whole cell lysate.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR19147
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab183218 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
WB		1/1000 - 1/2000. **1/1000 dilution:** Detects a band of approximately 26 kda (predicted molecular weight: 25 kDa).
IP		1/40.
ELISA		Use at an assay dependent concentration.
ICC/IF	★★★★★ (1)	1/5000.

Target

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.

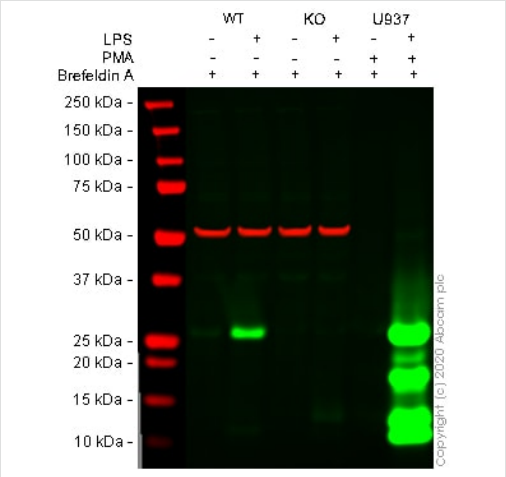
Involvement in disease Genetic variations in TNF are a cause of susceptibility psoriatic arthritis (PSORAS) [MIM:607507]. PSORAS is an inflammatory, seronegative arthritis associated with psoriasis. It is a heterogeneous disorder ranging from a mild, non-destructive disease to a severe, progressive, 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



Western blot - Anti-TNF alpha antibody [EPR19147] (ab183218)

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

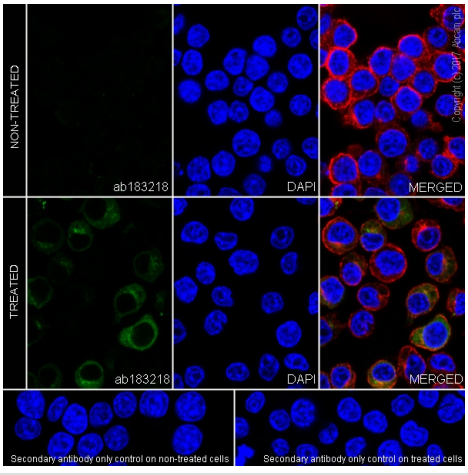
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Observed band size: 26 kDa

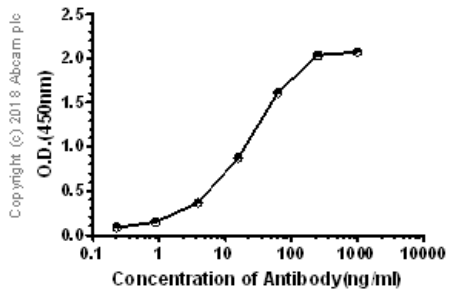
Lanes 1 - 6: Merged signal (red and green). Green - ab183218 observed at 26 kDa. Red - loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

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.



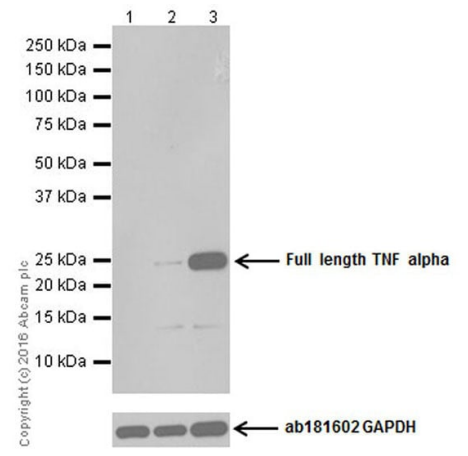
Immunocytochemistry/ Immunofluorescence - Anti-TNF alpha antibody [EPR19147] (ab183218)

Immunocytochemistry/ Immunofluorescence analysis of RAW264.7(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.



ELISA - Anti-TNF alpha antibody [EPR19147] (ab183218)

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



Western blot - Anti-TNF alpha antibody [EPR19147] (ab183218)

All lanes : Anti-TNF alpha antibody [EPR19147] (ab183218) at 1/2000 dilution

Lane 1 : TPA pretreated THP-1 (Human monocytic leukemia cell line) whole cell lysate
Lane 2 : TPA pretreated THP-1 (Human monocytic leukemia cell line) whole cell lysate treated with 100 ng/ml LPS for 7 hours
Lane 3 : 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

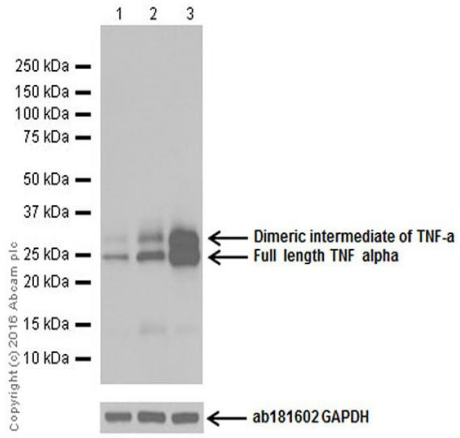
Lysates/proteins at 20 µg per lane.

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

Observed band size: 26 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer : 5% NFDM/TBST.



Western blot - Anti-TNF alpha antibody [EPR19147] (ab183218)

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

Lane 1 : TPA pretreated RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate
Lane 2 : TPA pretreated RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate treated with 100 ng/ml LPS for 7 hours
Lane 3 : TPA pretreated RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate treated with 100 ng/ml LPS for 4 hours, then added 1 µg/ml BFA for 3 hours

Lysates/proteins at 20 µg per lane.

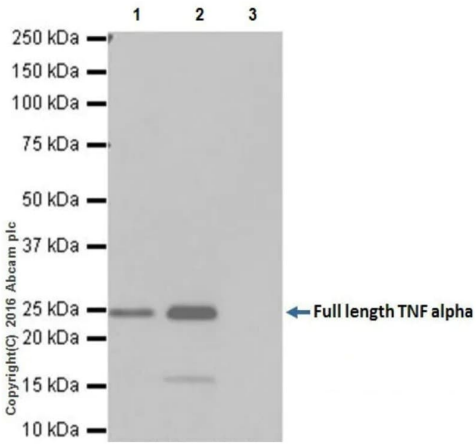
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Observed band size: 26,33 kDa

Exposure time: 3 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The 33kDa band probably is the dimeric intermediate form that has been described in the literature (PMID: 9933416).



Immunoprecipitation - Anti-TNF alpha antibody [EPR19147] (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.

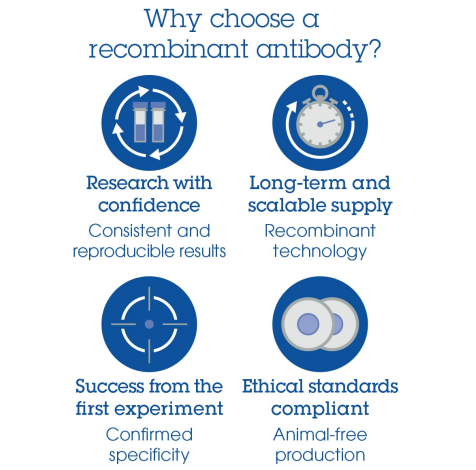
Lane 1: TPA pretreated THP-1 whole cell lysate treated with 100 ng/ml LPS for 4 hours, then added 1 µg/ml BFA for 3 hours 10µg (Input).

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.



Anti-TNF alpha antibody [EPR19147] (ab183218)

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