abcam

ab225576

Anti-TNF alpha antibody [EPR20972] - BSA and Azide free

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR20972
Purification technique	Affinity purification Protein A
Specificity	The protein level of TNF alpha in normal samples is very weak. The TNF alpha expression must be stimulated.
Concentration	1.015 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Mouse
Dilution info	-
Notes	-

Expected

Species	Human, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Human, Mouse
Dilution info	-
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

ICC/IF

Tested

Species	Mouse
Dilution info	-
Notes	-

Expected

Species	Human, Rat
Dilution info	Use at an assay dependent concentration.

Notes	-

Flow Cyt (Intra)

Tested

Species	Mouse
Dilution info	-
Notes	-

Expected

Species	Human, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

IHC-P

Tested

Species	Mouse
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Rat
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Expected

Species	Human
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information TNF

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. Impairs regulatory T-cells (Treg) function in individuals with rheumatoid arthritis via FOXP3 dephosphorylation. Up-regulates the expression of protein phosphatase 1 (PP1), which dephosphorylates the key 'Ser-418' residue of FOXP3, thereby inactivating FOXP3 and rendering Treg cells functionally defective (PubMed:23396208). Key mediator of cell death in the anticancer action of BCG-stimulated neutrophils in combination with DIABLO/SMAC mimetic in the RT4v6 bladder cancer cell line (PubMed:16829952, PubMed:22517918, PubMed:23396208). Induces insulin resistance in adipocytes via inhibition of insulin-induced IRS1 tyrosine phosphorylation and insulin-induced glucose uptake. Induces GKAP42 protein degradation in adipocytes which is partially responsible for TNF-induced insulin resistance (By similarity). Plays a role in angiogenesis by inducing VEGF production synergistically with IL1B and IL6 (PubMed:12794819). Promotes osteoclastogenesis and therefore mediates bone resorption (By similarity). The TNF intracellular domain (ICD) form induces IL12 production in dendritic cells.

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab225576 is the carrier-free version of ab215188.

Patented technology

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?

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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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.

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.

Compatibility
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.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

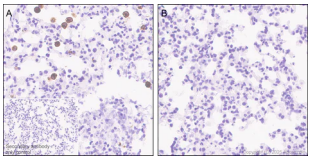
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

10 product images

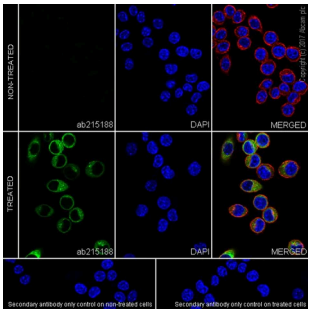


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TNF alpha antibody [EPR20972] - BSA and Azide free (ab225576)

TNF alpha Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-TNF alpha antibody

This data was developed using ab215188, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of formalin fixed paraffin embedded mouse lung labelling TNF alpha with ab215188 at a concentration of 1/1000 dilution. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins. The primary antibody Anti-TNF alpha antibody [EPR20972] was incubated for 30 mins at room temperature. Sections were counterstained with Hematoxylin. Positive staining on mouse lung treated with LPS (1ug/ml for 16 hours) and BFA (1ug/ml for 16h hours) (Image A) and no staining on wild type mouse lung (Image B).



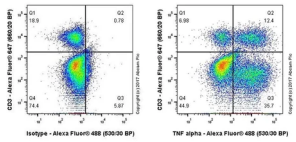
Immunocytochemistry/ Immunofluorescence - Anti-TNF alpha antibody [EPR20972] - BSA and Azide free (ab225576)

TNF alpha Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-TNF alpha antibody

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized RAW 264.7 (mouse macrophage cell line transformed with Abelson murine leukemia virus) cells, untreated or treated with 100 ng/ml lipopolysaccharides (LPS) for 7 hours with addition of 1 µg/ml brefeldin A (BFA) for the last 3 hours. labeling TNF alpha with ab215188 at 1/100 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Cytoplasmic staining was increased on RAW 264.7 cells when treated with 100 ng/ml lipopolysaccharides (LPS) for 7 hours with addition of 1 µg/ml brefeldin A (BFA) for the last 3 hours.
The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab215188).

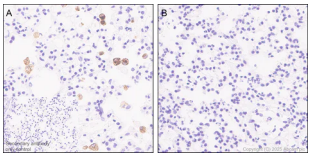


Flow Cytometry (Intracellular) - Anti-TNF alpha antibody [EPR20972] - BSA and Azide free (ab225576)

TNF alpha Flow Cytometry (Intracellular) staining using rabbit Anti-TNF alpha antibody

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 0.1% Tween-20 permeabilized mouse splenocytes treated with 20 ng/ml PMA, 1 µg/ml Ionomycin and 10 µM Brefeldin A for 6 hours labeling TNF alpha with ab215188 at 1/600 dilution (right panel) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (left panel). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/2000 dilution was used as the secondary antibody.
Cells were surface stained with anti-mouse CD3, fixed with 4% PFA for 10 minutes, then permeabilized with 0.1% Tween-20 and intracellular stained with anti-rabbit IgG and ab215188.
TNF alpha is mainly expressed in T cells (CD3+ population) while only a small population of CD3- cells can express TNF-alpha.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab215188).

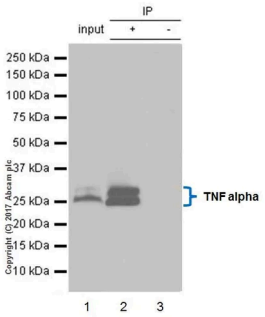


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TNF alpha antibody [EPR20972] - BSA and Azide free (ab225576)

TNF alpha Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-TNF alpha antibody

This data was developed using ab215188, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of formalin fixed paraffin embedded rat lung labelling TNF alpha with ab215188 at a concentration of 1/1000 dilution. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins. The primary antibody Anti-TNF alpha antibody [EPR20972] was incubated for 30 mins at room temperature. Sections were counterstained with Hematoxylin. Positive staining on rat lung treated with LPS (1ug/ml for 16 hours) and BFA (1ug/ml for 16h hours) (Image A) and no staining on wild type rat lung (Image B).



Immunoprecipitation - Anti-TNF alpha antibody [EPR20972] - BSA and Azide free (ab225576)

TNF alpha was immunoprecipitated from 0.35 mg of RAW 264.7 (mouse macrophage cell line transformed with Abelson murine leukemia virus) treated with 100 ng/ml lipopolysaccharides (LPS) for 7 hours with addition of 1 µg/ml brefeldin A (BFA) for the last 3 hours, whole cell lysate with ab215188 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab215188 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.
Lane 1: RAW 264.7 treated with 100 ng/ml lipopolysaccharides (LPS) for 7 hours with addition of 1 µg/ml brefeldin A (BFA) for the last 3 hours, whole cell lysate 10 µg (Input).

Lane 2: ab215188 IP in RAW 264.7 treated with 100 ng/ml lipopolysaccharides (LPS) for 7 hours with addition of 1 µg/ml brefeldin A (BFA) for the last 3 hours, whole cell lysate (+).

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab215188 in RAW 264.7 treated with 100 ng/ml lipopolysaccharides (LPS) for 7 hours with addition of 1 µg/ml brefeldin A (BFA) for the last 3 hours, whole cell lysate (-).

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

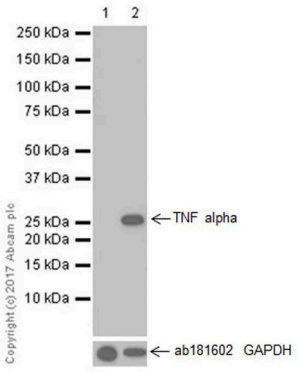
Exposure time: 3 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab215188).

All lanes:
Immunoprecipitation - Anti-TNF alpha antibody [EPR20972] (ab215188)

Predicted band size: 25 kDa

Observed band size: 26 kDa, 33 kDa



Western blot - Anti-TNF alpha antibody [EPR20972] - BSA and Azide free (ab225576)

TNF alpha Western blot staining using rabbit Anti-TNF alpha antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab215188).
Blocking/Dilution buffer: 5% NFDM/TBST.

All lanes:
Western blot - Anti-TNF alpha antibody [EPR20972] (ab215188) at 1/1000 dilution

Lane 1:
THP-1 (human monocytic leukemia cell line) differentiated with 100 nM TPA overnight, whole cell lysate at 10 µg

Lane 2:
THP-1 (human monocytic leukemia cell line) differentiated with 100 nM TPA overnight, then treated with 100 ng/ml lipopolysaccharides (LPS) for 7 hours with addition of 1 µg/ml brefeldin A (BFA) for the last 3 hours, whole cell lysate at 10 µg

Secondary

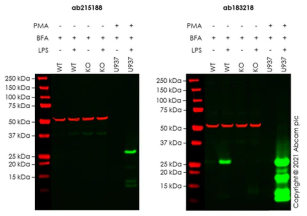
All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Developed using the ECL technique.

Predicted band size: 25 kDa

Observed band size: 26 kDa

Exposure time: 3min



Western blot - Anti-TNF alpha antibody [EPR20972] - BSA and Azide free (ab225576)

TNF alpha Western blot staining using rabbit Anti-TNF alpha antibody

This Western blot image is a comparison between ab215188 and ab183218 tested under the same conditions. While ab215188 is suitable for WB for some samples, ab183218 was found to be more sensitive. False colour image of Western blot: Anti-TNF alpha antibody [EPR20972] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab215188 was shown to bind specifically to TNF alpha. A band was observed at 27 kDa in treated U937 cell lysates with no signal observed at this size without treatment. No signal was observed in wild-type THP-1 cell lysates or in TNF knockout cell line ab273761 (knockout cell lysate ab275507) with ab215188. However, a band was observed at 27 kDa in treated wild-type THP-1 cell lysates with ab183218. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with primary antibodies overnight at 4 Â°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDyeÂ® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDyeÂ® 680RD) preabsorbed (ab216776) at 1/20000 dilution.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab215188).

All lanes:

Western blot - Anti-TNF alpha antibody [EPR20972] (ab215188) at 1/1000 dilution

Lane 1:
Wild-type THP-1 control: Brefeldin A (5 ug/mL, 4 h) cell lysate at 30 µg

Lane 2:
Wild-type treated THP-1: LPS (100 ng/mL, 16 h), Brefeldin A (5 ug/mL, last 4 h) cell lysate at 30 µg

Lane 2:
Western blot - Human TNF knockout THP-1 cell line (ab273761)

Lane 3:
TNF alpha knockout THP-1 control: Brefeldin A (5 ug/mL, 4 h) cell lysate at 30 µg

Lane 4:
TNF alpha knockout THP-1 treated: LPS (100 ng/mL, 16 h), Brefeldin A (5 ug/mL, last 4 h) cell lysate at 30 µg

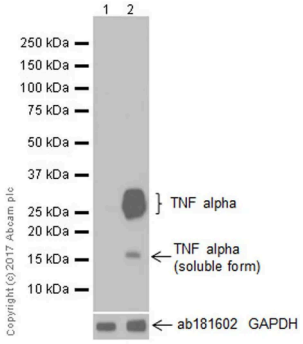
Lane 5:
U937 control: PMA (10 mM, 2 days), Brefeldin A (5 ug/mL, last 4 h) cell lysate at 30 µg

Lane 6:
U937 treated: PMA (10 mM, 2 days), LPS (1 ug/mL, last 16 h), Brefeldin A (5 ug/mL, last 4 h) cell lysate at 30 µg

Performed under reducing conditions.

Predicted band size: 25 kDa

Observed band size: 27 kDa



Western blot - Anti-TNF alpha antibody [EPR20972] - BSA and Azide free (ab225576)

TNF alpha Western blot staining using rabbit Anti-TNF alpha antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab215188).
Blocking/Dilution buffer: 5% NFDM/TBST.

All lanes:
Western blot - Anti-TNF alpha antibody [EPR20972] (ab215188) at 1/1000 dilution

Lane 1:
Untreated RAW 264.7 (mouse macrophage cell line transformed with Abelson murine leukemia virus), whole cell lysate at 10 µg

Lane 2:
RAW 264.7 (mouse macrophage cell line transformed with Abelson murine leukemia virus), treated with 100 ng/ml lipopolysaccharides (LPS) for 4 hours, 1µg/ml Brefeldin A (BFA) was then added for additional 3 hours, whole cell lysate at 10 µg

Secondary

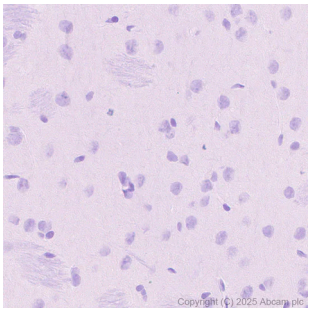
All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Developed using the ECL technique.

Predicted band size: 25 kDa

Observed band size: 17 kDa, 26 kDa, 33 kDa

Exposure time: 15s

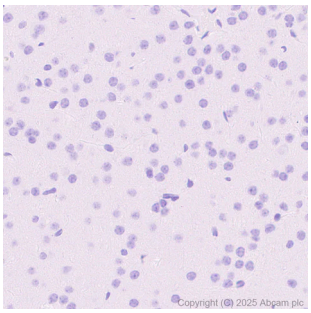


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TNF alpha antibody [EPR20972] - BSA and Azide free (ab225576)

TNF alpha Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Rat brain using rabbit Anti-TNF alpha antibody

This data was developed using ab215188, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of formalin fixed paraffin embedded rat brain labelling TNF alpha with ab215188 at a concentration of 1/1000 dilution. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins. The primary antibody Anti-TNF alpha antibody [EPR20972] was incubated for 30 mins at room temperature. Sections were counterstained with Hematoxylin. Negative control: no staining on rat brain.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TNF alpha antibody [EPR20972] - BSA and Azide free (ab225576)

TNF alpha Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of mouse brain using rabbit Anti-TNF alpha antibody

This data was developed using ab215188, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of formalin fixed paraffin embedded mouse brain labelling TNF alpha with ab215188 at a concentration of 1/1000 dilution. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins. The primary antibody Anti-TNF alpha antibody [EPR20972] was incubated for 30 mins at room temperature. Sections were counterstained with Hematoxylin. Negative control: no staining on mouse brain.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.