abcam

ab205587

Anti-TNF alpha antibody [EPR21753-109]

Anti-TNF alpha antibody [EPR21753-109] (ab205587) is a rabbit monoclonal antibody detecting TNF alpha in **Western Blot, Flow Cytometry (Intra), IP, ICC/IF**. Suitable for **Mouse, Rat**.

- Biophysical QC for unrivalled batch-batch consistency
- Over 40 publications

Lab Essentials | Recombinant | RabMAb | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR21753-109
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. IL-1 beta and IL-6 could be good controls validating the increased TNF alpha level after drug treatment.
Concentration	0.696 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Rat
Dilution info	1/50
Notes	-

Expected

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

WB

Tested

Species	Mouse
Dilution info	1/1000
Notes	-

Species	Rat
Dilution info	1/1000
Notes	-

IHC-P

Tested

Species	Mouse
Dilution info	1/1000
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Not recommended

Species	Rat
Dilution info	-

Notes	-

ICC/IF

Tested

Species	Mouse
Dilution info	1/100
Notes	-

Expected

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

Flow Cyt (Intra)

Tested

Species	Mouse
Dilution info	1/100
Notes	-

Expected

Species	Rat
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 (By similarity). 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 (PubMed:25586176). Plays a role in angiogenesis by inducing VEGF production synergistically with IL1B and IL6 (By similarity). Promotes osteoclastogenesis and therefore mediates bone resorption (PubMed:32741026). The TNF intracellular domain (ICD) form induces IL12 production in dendritic cells.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

What is this antibody validated in?
Anti-TNF alpha antibody [EPR21753-109] (ab205587) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Immunoprecipitation (IP), Immunocytochemistry/immunofluorescence (ICC/IF) in Mouse, Rat samples.

What is the molecular weight of TNF alpha?
Anti-TNF alpha [EPR21753-109] (ab205587) specifically detects a band for TNF alpha (UniProt: P16599) at a molecular weight of 26kDa.

Trusted by the scientific community
Anti-TNF alpha [EPR21753-109] (ab205587) was first used in a scientific publication in 2019 and has been cited over 40 times in peer-reviewed journals.

Other related products
We have a range of other formats of antibody clone [EPR21753-109] also available for your convenience: ab205587, Carrier free - ab252382, Alexa Fluor® 488 - ab283321, Alexa Fluor® 647 - ab300093, APC - ab317476, PE - ab322526, FITC - ab323680

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.

Trial sizes available!
Test your antibody or perform pre-screening before committing to a larger quantity. Sold in 20µl. Discover our selection of trial-size antibodies.

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

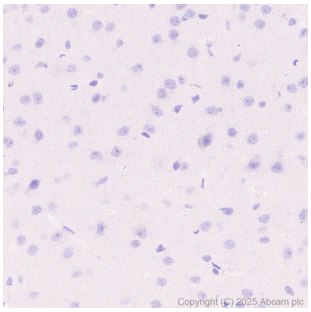
We do not recommend this combination. It is not covered by our product promise.

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.

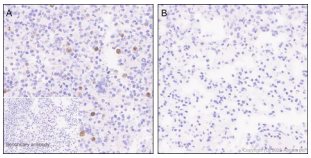
9 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TNF alpha antibody [EPR21753-109] (ab205587)

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

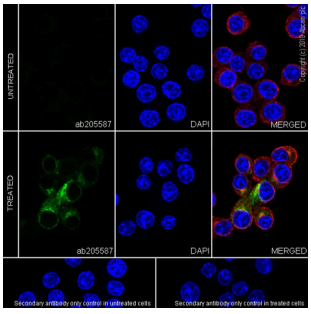
Immunohistochemical analysis of formalin fixed paraffin embedded mouse cerebrum labelling TNF alpha with ab205587 at a concentration of 1/1000 dilution. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins. The primary antibody Anti-TNF alpha antibody [EPR21753-109] was incubated for 30 mins at room temperature. Sections were counterstained with Hematoxylin. Negative control: no staining on mouse cerebrum.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TNF alpha antibody [EPR21753-109] (ab205587)

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

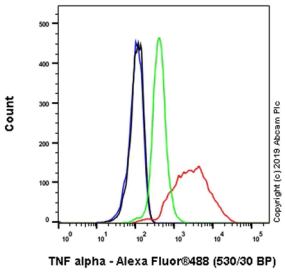
Immunohistochemical analysis of formalin fixed paraffin embedded mouse lung labelling TNF alpha with ab205587 at a concentration of 1/1000 dilution. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins. The primary antibody Anti-TNF alpha antibody [EPR21753-109] 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 [EPR21753-109] (ab205587)

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

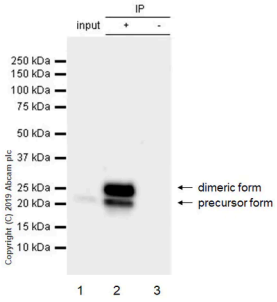
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) (+/- treatment with 100ng/ml LPS for 3h, then together with 300ng/ml BFA for another 3h) cells labeling TNF alpha with ab205587 at 1/100 dilution, followed by a AlexaFluor®488 Goat anti-Rabbit secondary (ab150077) at 1/1000 dilution (Green). Confocal image showing cytoplasmic staining in RAW 264.7 cells treated with 100ng/ml LPS for 3h, then together with 300ng/ml BFA for another 3h. The Nuclear counterstain is DAPI (Blue). Tubulin was stained using an Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594, ab195889) at 1/200 dilution.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is AlexaFluor®488 Goat anti-Rabbit secondary (ab150077) at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-TNF alpha antibody [EPR21753-109] (ab205587)

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

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed 90% methanol-permeabilized RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) treated with 100ng/ml LPS for 3h, then together with 300ng/ml BFA for another 3h (Red) / Untreated control (Green), compared to an unlabeled control (cells without incubation with primary antibody and secondary antibody, Blue) and an isotype control- Rabbit monoclonal IgG (ab172730, Black). A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077)at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-TNF alpha antibody [EPR21753-109] (ab205587)

TNF alpha was immunoprecipitated from 0.35 mg rat splenocytes (treated with 20ng/ml PMA, 1µg/ml Ionomycin and 10µM BFA for 6h) whole cell lysate with ab205587 at 1/50 dilution. Western blot was performed on the immunoprecipitate using ab205587 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366) was used for detection at 1/5000 dilution.
Lane 1: Rat splenocytes (treated with 20ng/ml PMA, 1µg/ml Ionomycin and 10µM BFA for 6h) whole cell lysate 10µg (input).

Lane 2: ab205587 IP in rat splenocytes (treated with 20ng/ml PMA, 1µg/ml Ionomycin and 10µM BFA for 6h) whole cell lysate.

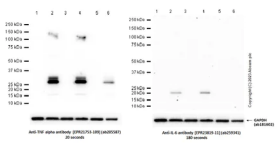
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab205587 in rat splenocytes (treated with 20ng/ml PMA, 1µg/ml Ionomycin and 10µM BFA for 6h) whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.
Exposure time: 30 seconds.

The MW observed is consistent with what has been described in the literature (PMID: 9933416).

All lanes:
Immunoprecipitation - Anti-TNF alpha antibody [EPR21753-109] (ab205587)

Predicted band size: 25 kDa



Western blot - Anti-TNF alpha antibody [EPR21753-109] (ab205587)

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

Blocking/Diluting buffer and concentration: 5% NFDM/TBST.
IL-1 beta and IL-6 could be a good control validating the increased TNF alpha level after drug treatment.

Lanes 1 - 6:
Western blot - Anti-TNF alpha antibody [EPR21753-109] (ab205587) at 1/1000 dilution

Lanes 1 - 6:
Western blot - Anti-TNF alpha antibody [EPR21753-109] - BSA and Azide free (ab252382) at 1/1000 dilution

Lanes 1, 3 and 5:
Untreated NR8383 (rat lung macrophage (alveolar)) whole cell lysate at 20 µg

Lane 2:
NR8383 (rat lung macrophage (alveolar)) treated with 100ng/ml lipopolysaccharide (LPS) for 4h then add 1000ng/ml BFA for another 3h whole cell lysate at 20 µg

Lane 4:
NR8383 (rat lung macrophage (alveolar)) treated with 100ng/ml lipopolysaccharide (LPS) for 3h then add 300ng/ml BFA for another 3h whole cell lysate at 20 µg

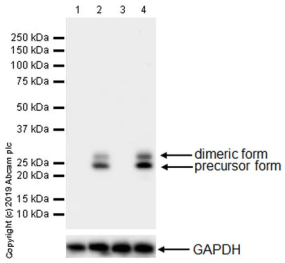
Lane 6:
NR8383 (rat lung macrophage (alveolar)) treated with 1000ng/ml lipopolysaccharide (LPS) for 2h whole cell lysate at 20 µg

Secondary

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

Observed band size: 23 kDa, 26 kDa

Exposure time: 20s



Western blot - Anti-TNF alpha antibody [EPR21753-109] (ab205587)

Blocking/Diluting buffer and concentration: 5% NFDM/TBST
Exposure time: 20 seconds

The expression profile/molecular weight observed is consistent with what has been described in the literature (PMID: 9933416)

All lanes:
Western blot - Anti-TNF alpha antibody [EPR21753-109] (ab205587) at 1/1000 dilution

Lane 1:
Untreated rat splenocytes whole cell lysate at 20 µg

Lane 2:
Rat splenocytes treated with 20ng/ml PMA, 1µg/ml Ionomycin and 10µM BFA for 6h whole cell lysate at 20 µg

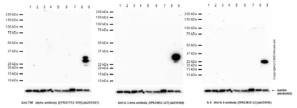
Lane 3:
Untreated RAW 264.7(mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate at 20 µg

Lane 4:
RAW 264.7 treated with 100ng/ml LPS for 3h, then together with 300ng/ml BFA for another 3h whole cell lysate at 20 µg

Secondary

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

Predicted band size: 25 kDa



Western blot - Anti-TNF alpha antibody [EPR21753-109] (ab205587)

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

Blocking/Diluting buffer and concentration: 5% NFDM/TBST.
Normal tissues express undetectable level of TNF alpha, IL-1 beta and IL-6 proteins.

IL-1 beta and IL-6 could be a good control validating the increased TNF alpha level after drug treatment.

All lanes:
Western blot - Anti-TNF alpha antibody [EPR21753-109] (ab205587) at 1/1000 dilution

Lane 1:
Rat cerebral cortex tissue lysate at 20 µg

Lane 2:
Rat cerebellum tissue lysate at 20 µg

Lane 3:
Rat hippocampus tissue lysate at 20 µg

Lane 4:
Rat spinal cord tissue lysate at 20 µg

Lane 5:
Rat small intestine tissue lysate at 20 µg

Lane 6:
Rat kidney tissue lysate at 20 µg

Lane 7:
Rat skin tissue lysate at 20 µg

Lane 8:
RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate at 20 µg

Lane 9:
RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) treated with 100ng/ml lipopolysaccharide (LPS) for 4h then add 1000ng/ml BFA for another 3h whole cell lysate at 20 µg

Secondary

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

Observed band size: 23 kDa, 26 Da

Exposure time: 180s

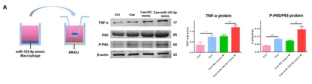


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Western blot - Anti-TNF alpha antibody [EPR21753-109] (ab205587)

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

TNF alpha western blot using anti-TNF alpha antibody [EPR21753-109] ab205587. Publication image and figure legend from Tang, D. S., Cao, F., et al., 2022, Front Immunol, PubMed 35911777.

ab205587 was used in this publication in western blot. This may not be the same as the application(s) guaranteed by Abcam. For a full list of applications guaranteed by Abcam for ab205587 please see the product overview.

Macrophages overexpressing miR-183-5p aggravate AC damage. (A) Representative western blot and quantification of P-P65/P65 and TNF-α levels in ACs from the four groups: Ctrl, Cae, Cae+NC-mimic M, and Cae+miR-183-5p mimic M. (B) Trypsinogen activation assay in ACs from the above groups. The percentage of positive cells (white arrow) was calculated to quantify the degree of trypsinogen activation. (C) Representative flow cytometry results for necrosis in ACs from the above groups. All experiments were repeated three times. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Ctrl: PBS-treated acinar cells, Cae: caerulein-treated acinar cells, Cae+NC-mimic: M caerulein-treated acinar cells cocultured with macrophages transfected with the NC-mimic, Cae+miR-183-5p mimic M: caerulein-treated acinar cells cocultured with macrophages transfected with the miR-183-5p mimic.

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