abcam

ab252382

Anti-TNF alpha antibody [EPR21753-109] - BSA and Azide free

Recombinant | RabMAb

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	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	1.03 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Rat
Dilution info	-
Notes	-

Expected

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

WB

Expected

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

IHC-P

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Mouse
Dilution info	-
Notes	-

Expected

Species	Rat

Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Mouse
Dilution info	-
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	Blue Ice
Appropriate short-term storage conditions	+4°C

Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab252382 is the carrier-free version of ab205587.

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

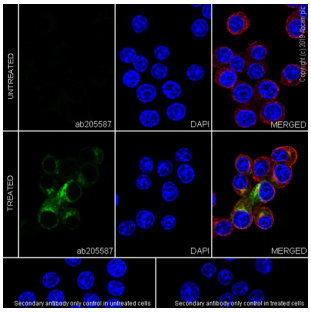
We do not recommend this combination. It is not covered by our product promise.

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.

5 product images

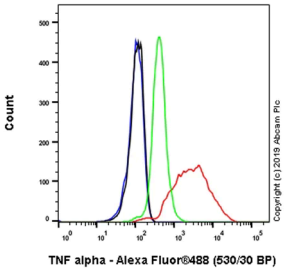


Immunocytochemistry/ Immunofluorescence - Anti-TNF alpha antibody [EPR21753-109] - BSA and Azide free (ab252382)

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.

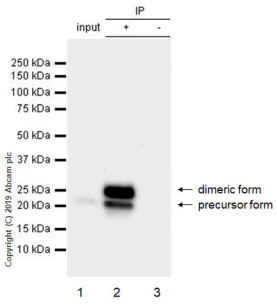
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab205587).



Flow Cytometry (Intracellular) - Anti-TNF alpha antibody [EPR21753-109] - BSA and Azide free (ab252382)

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.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab205587).



Immunoprecipitation - Anti-TNF alpha antibody [EPR21753-109] - BSA and Azide free (ab252382)

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.

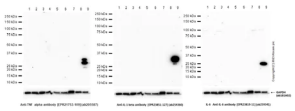
Blocking and dilution buffer and concentration: 5% NFDM/TBST.
Exposure time: 30 seconds.

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab205587).

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

Predicted band size: 25 kDa



Western blot - Anti-TNF alpha antibody [EPR21753-109] - BSA and Azide free (ab252382)

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 (ab205587).
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

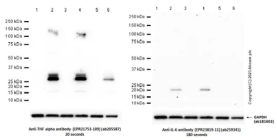
Lane 9:
RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) treated with 100ng/ml lipopolysaccharide (LPS) for 4h then add 1000ng/ml BFA for another 3h whole cell lysate at 20 µg

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



Western blot - Anti-TNF alpha antibody [EPR21753-109] - BSA and Azide free (ab252382)

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 (ab205587).
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

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