abcam

ab255275

Anti-TNF alpha antibody [EPR22598-212]

Lab Essentials | Recombinant | RabMAb | KO Validated | 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	EPR22598-212
Purification technique	Affinity purification Protein A
Concentration	0.731 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	1/30
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

IHC-P

Not recommended

Species	Human
Dilution info	-
Notes	-

ICC/IF

Not recommended

Species	Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/700
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	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

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.

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.

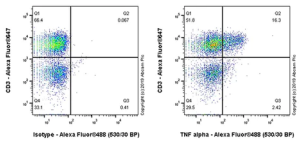
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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.

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4 product images

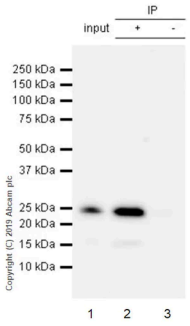


Flow Cytometry (Intracellular) - Anti-TNF alpha antibody [EPR22598-212] (ab255275)

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

Intracellular flow cytometric analysis of2% paraformaldehyde-fixed, 0.1% Tween 20 permeabilized Human peripheral blood mononuclear cell (PBMC) treated with 50ng/ml PMA, 250ng/ml ionomycin and 300ng/ml Brefeldin A for 16h, labeling TNF alpha with ab255275 at 1/700 dilution (Right) compared with Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (Left).
Goat Anti-Rabbit IgG Fc (Alexa Fluor® 488) preadsorbed (ab150097), at 1/5000 dilution was used as the secondary antibody.

Cells were surface stained with anti-CD3 conjugated to Alexa Fluor® 647. Then fixed with 2% PFA for 10min and intracellularly stained with rabbit IgG (Left) or ab236836 (Right).



Immunoprecipitation - Anti-TNF alpha antibody [EPR22598-212] (ab255275)

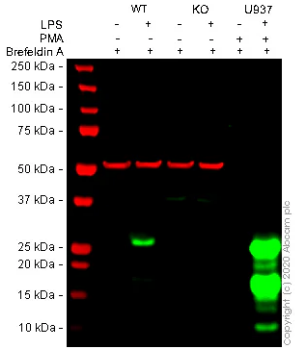
TNF alpha was immunoprecipitated from 0.35 mg of THP-1 (human monocytic leukemia cell line) (treated with 80nM TPA overnight, replaced the culture medium with 100 ng/ml Lipopolysaccharides (LPS) for 6 hours with addition of 300ng/ml Brefeldin A (BFA) for the last 3 hours) whole cell lysate with ab255275 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab255275 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used as secondary antibody at 1/5000 dilution.
Lane 1: THP-1 (treated as above) whole cell lysate 10 µg (Input).
Lane 2: ab255275 IP in THP-1 (treated as avove) whole cell lysate.
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab255275 in THP-1 (treated as above) whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.
Exposure time: 15 seconds.

All lanes:
Immunoprecipitation - Anti-TNF alpha antibody [EPR22598-212] (ab255275)

Predicted band size: 25 kDa

Observed band size: 25 kDa



Western blot - Anti-TNF alpha antibody [EPR22598-212] (ab255275)

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

Lanes 1 - 6: Merged signal (red and green). Green - ab255275 observed at 26 kDa. Red - loading control ab7291 (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.
ab255275 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 ab255275 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.

All lanes:
Western blot - Anti-TNF alpha antibody [EPR22598-212] (ab255275) at 1/1000 dilution

Lane 1:
Wild-type THP-1 Brefeldin A (ab120299) treated (5 µg/ml, 4 h) cell lysate at 30 µg

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 at 30 µg

Lane 3:
TNF alpha knockout THP-1 Brefeldin A (ab120299) treated (5 µg/ml, 4 h) cell lysate at 30 µg

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 at 30 µg

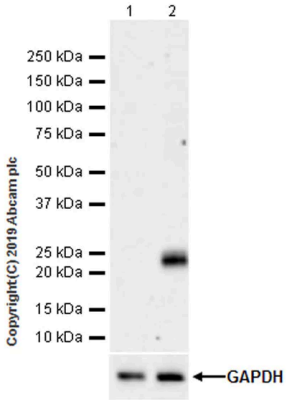
Lane 5:
U937 PMA treated (10 mM, 2 days) plus 16 h no treatment and Brefeldin A (ab120299) treated (5 µg/ml, 4 h) cell lysate at 30 µg

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 at 30 µg

Performed under reducing conditions.

Predicted band size: 25 kDa

Observed band size: 26 kDa



Western blot - Anti-TNF alpha antibody [EPR22598-212] (ab255275)

Blocking and dilution buffer: 5% NFDM/TBST.
The expression profile observed is consistent with what has been described in the literature (PMID: 9657756).

All lanes:
Western blot - Anti-TNF alpha antibody [EPR22598-212] (ab255275) at 1/1000 dilution

Lane 1:
THP-1 (human monocytic leukemia cell line) (treated with 80nM 12-O-Tetradecanoylphorbol-13-acetate (TPA) overnight) whole cell lysate at 20 µg

Lane 2:
THP-1 (treated with 80nM TPA overnight, replaced the culture medium with 100 ng/ml lipopolysaccharides (LPS) for 6 hours with addition of 300ng/ml Brefeldin A (BFA) for the last 3 hours) 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

Observed band size: 25 kDa

Exposure time: 3min

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