abcam

Product datasheet

Anti-TNF alpha antibody [EPR22598-212] ab255275

敲除验证 重组 RabMAb

8 References 6 图像

概述

产品名称	Anti-TNF alpha抗体[EPR22598-212]
描述	兔单克隆抗体[EPR22598-212] to TNF alpha
宿主	Rabbit
经测试应用	**适用于:** WB, IP, Flow Cyt (Intra) **不适用于:** ICC/IF or IHC-P
种属反应性	**与反应:** Mouse, Human
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: THP-1 (treated with 80nM TPA / 100 ng/ml lipopolysaccharide / 300ng/ml Brefeldin A) and RAW 264.7 (treated with 100 ng/ml lipopolysaccharide / 300 ng/ml Brefeldin A) whole cell lysates; Wild-type THP-1 LPS treated (100 ng/ml, 16 h) and Brefeldin A treated (5 Âµg/ml, 4 h) cell lysate. IP:THP-1 (treated with 80nM TPA / 100 ng/ml lipopolysaccharide / 300ng/ml Brefeldin A) whole cell lysate. Flow Cyt (intra): Human PBMC (treated with 50 ng/ml PMA/ 250 ng/ml ionomycon / 300 ng/ml Brefeldin A).
常规说明	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**.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
存储溶液	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol (glycerin, glycerine), 0.05% BSA, PBS

纯度	Protein A purified
克隆	单克隆
克隆编号	EPR22598-212
同种型	IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab255275于以下的经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

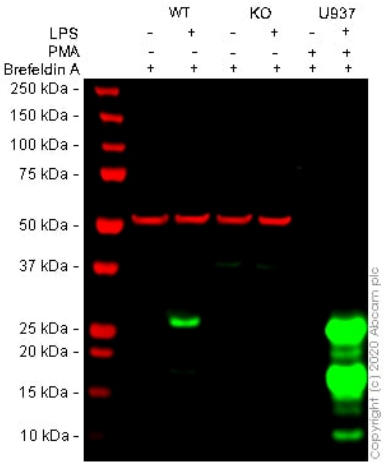
应用	Ab评论	说明
WB		1/1000. Detects a band of approximately 25 kDa (predicted molecular weight: 25 kDa).
IP		1/30.
Flow Cyt (Intra)		1/700.

应用说明 Is unsuitable for ICC/IF or IHC-P.

靶标

功能	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.
疾病相关	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).
序列相似性	Belongs to the tumor necrosis factor family.
翻译后修饰	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.
细胞定位	Secreted and Cell membrane.

图片



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

All lanes : 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
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

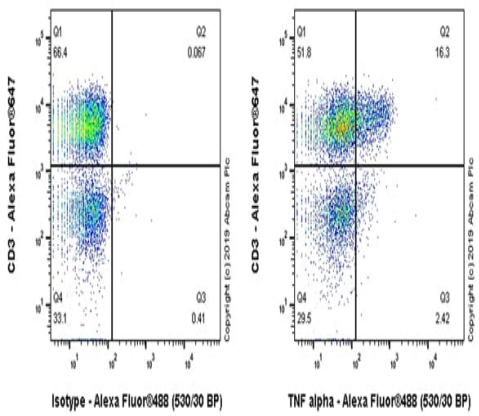
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Predicted band size: 25 kDa
Observed band size: 26 kDa

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.

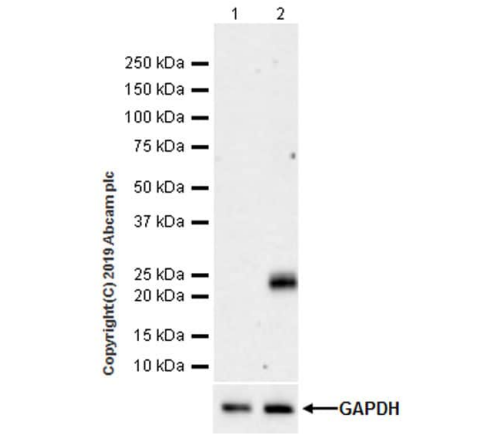


Flow Cytometry (Intracellular) - Anti-TNF alpha antibody [EPR22598-212] (ab255275)

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).



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

All lanes : 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
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

Lysates/proteins at 20 µg per lane.

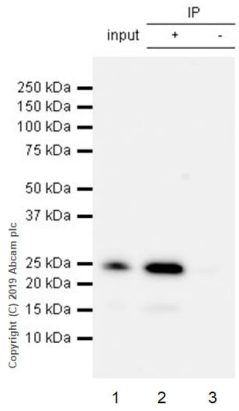
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 25 kDa
Observed band size: 25 kDa

Exposure time: 3 minutes

Blocking and dilution buffer: 5% NFDM/TBST.

The expression profile observed is consistent with what has been described in the literature (PMID: 9657756).

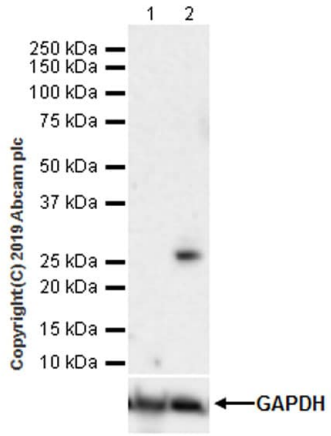


Immunoprecipitation - Anti-TNF alpha antibody [EPR22598-212] (ab255275)

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.



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

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

Lane 1 : Untreated RAW 264.7 (mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate
Lane 2 : RAW 264.7 (treated with 100 ng/ml lipopolysaccharide (LPS) for 6 hours with addition of 300 ng/ml Brefeldin A (BFA) for the last 3 hours) whole cell lysate

Lysates/proteins at 20 µg per lane.

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

Predicted band size: 25 kDa
Observed band size: 25 kDa

Exposure time: 3 minutes

Blocking and dilution buffer: 5% NFDM/TBST.

The expression profile observed is consistent with what has been described in the literature (PMID: 9657756).

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

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