abcam

Product datasheet

Anti-TNF alpha antibody [EPR21753-109] ab205587

重组 RabMAb

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概述

产品名称	Anti-TNF alpha抗体[EPR21753-109]
描述	兔单克隆抗体[EPR21753-109] to TNF alpha
宿主	Rabbit
特异性	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.
经测试应用	**适用于:** ICC/IF, WB, IP, Flow Cyt (Intra) **不适用于:** IHC-P
种属反应性	**与反应:** Mouse, Rat
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: Rat splenocytes treated with 20ng/ml PMA, 1µg/ml Ionomycin and 10µM BFA for 6h whole cell lysate; RAW 264.7 treated with 100ng/ml LPS for 3h, then together with 300ng/ml BFA for another 3h, whole cell lysate. ICC/IF: RAW 264.7 cells. Flow: RAW 264.7 cells. IP: Rat splenocytes treated with 20ng/ml PMA, 1µg/ml Ionomycin and 10µM BFA for 6h, whole cell lysate
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 long term. Avoid freeze / thaw cycle.
存储溶液	pH: 7.2

	Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	EPR21753-109
同种型	IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab205587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。

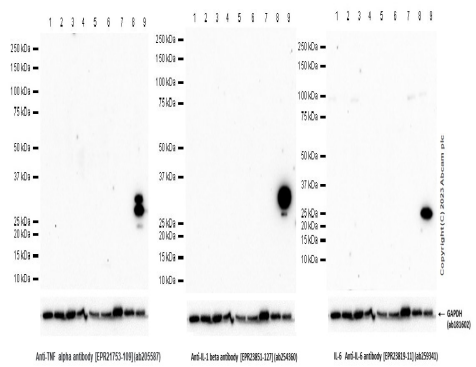
应用	Ab评论	说明
ICC/IF		1/100.
WB		1/1000. Predicted molecular weight: 26 kDa.
IP		1/50.
Flow Cyt (Intra)		1/100.

应用说明 Is unsuitable for 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 [EPR21753-109] (ab205587)

All lanes : Anti-TNF alpha antibody [EPR21753-109] (ab205587) at 1/1000 dilution (**ab254360**:1:1000 dilution (0.5 ug/ml) **ab259341**:1:1000 dilution (0.5 ug/ml))

Lane 1 : Rat cerebral cortex tissue lysate
Lane 2 : Rat cerebellum tissue lysate
Lane 3 : Rat hippocampus tissue lysate
Lane 4 : Rat spinal cord tissue lysate
Lane 5 : Rat small intestine tissue lysate
Lane 6 : Rat kidney tissue lysate
Lane 7 : Rat skin tissue lysate
Lane 8 : RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate
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

Lysates/proteins at 20 µg per lane.

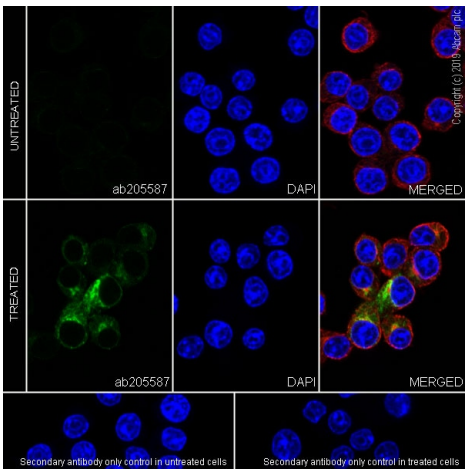
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 26 kDa
Observed band size: 23, 26 kDa

Exposure time: 180 seconds

Blocking/Diluting buffer and concentration: 5% NFDM/TBST

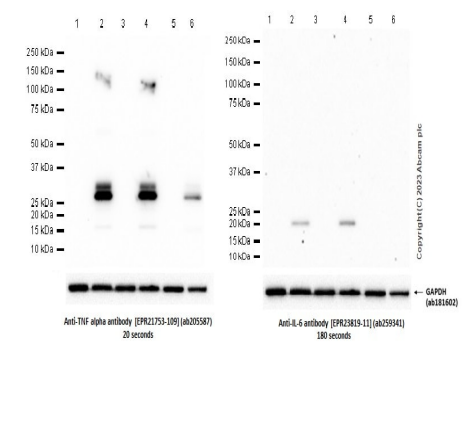
Normal tissues express undetectable level of TNF alpha, IL-1 beta and IL-6 proteins.
IL-1 beta and IL-6 could be a good control validating the increased TNF alpha level after drug treatment.



Immunocytochemistry/ Immunofluorescence - Anti-TNF alpha antibody [EPR21753-109] (ab205587)

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.



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

All lanes : Anti-TNF alpha antibody [EPR21753-109] (ab205587) at 1/1000 dilution (**ab259341** 1:1000 dilution)

Lanes 1 & 3 & 5 : Untreated NR8383 (rat lung macrophage (alveolar)) whole cell lysate
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
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
Lane 6 : NR8383 (rat lung macrophage (alveolar)) treated with 1000ng/ml lipopolysaccharide (LPS) for 2h whole cell lysate

Lysates/proteins at 20 µg per lane.

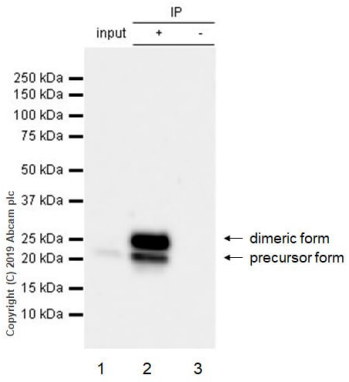
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 26 kDa
Observed band size: 23, 26,26 kDa

Exposure time: 20 seconds

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.



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.

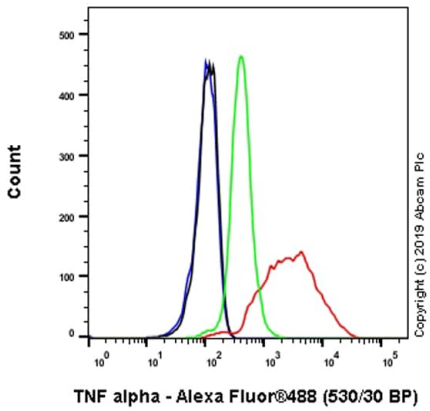
Lane 1: Rat splenocytes (treated with 20ng/ml PMA, 1µg/ml Ionomycin and 10µM BFA for 6h) whole cell lysate 10µg (input).

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.

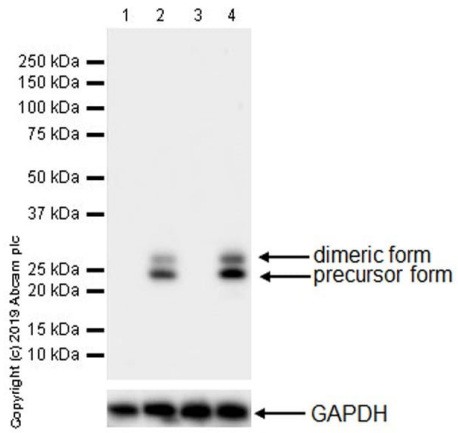
Blocking and dilution buffer and concentration: 5% NFDM/TBST.
Exposure time: 30 seconds.

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



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

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.



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

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

Lane 1 : Untreated rat splenocytes whole cell lysate
Lane 2 : Rat splenocytes treated with 20ng/ml PMA, 1µg/ml Ionomycin and 10µM BFA for 6h whole cell lysate
Lane 3 : Untreated RAW 264.7(mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate
Lane 4 : RAW 264.7 treated with 100ng/ml LPS for 3h, then together with 300ng/ml BFA for another 3h whole cell lysate

Lysates/proteins at 20 µg per lane.

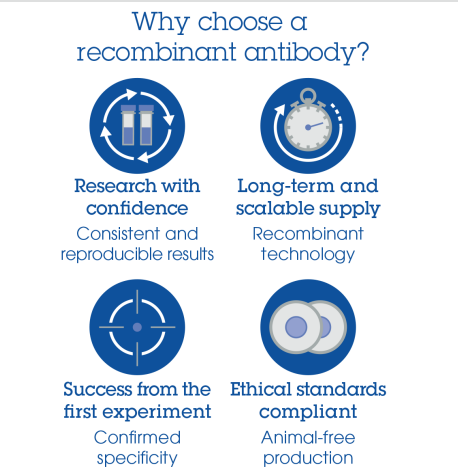
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 26 kDa

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)



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