abcam

Product datasheet

Anti-TNF alpha antibody [EPR20972] - BSA and Azide free ab225576

KO 評価済 リコンビナント RabMAb®

1 References   画像数 5

製品の概要

製品名	Anti-TNF alpha antibody [EPR20972] - BSA and Azide free
製品の詳細	Rabbit monoclonal [EPR20972] to TNF alpha - BSA and Azide free
由来種	Rabbit
特異性	The protein level of TNF alpha in normal samples is very weak. The TNF alpha expression must be stimulated.
アプリケーション	**適用あり:** Flow Cyt (Intra), WB, ICC/IF, IP
種交差性	**交差種:** Mouse, Human
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
ポジティブ·コントロール	ICC/IF: RAW 264.7 cells treated with LPS with addition of BFA.
特記事項	ab225576 is the carrier-free version of **ab215188**.

Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

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.

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.

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**.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C. Do Not Freeze.
バッファー	pH: 7.2 Constituent: PBS
キャリア・フリー	はい
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPR20972
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee　**Abpromise保証は、**次のテスト済みアプリケーションにおけるab225576の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 33, 26, 17 kDa (predicted molecular weight: 26 kDa).
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.

ターゲット情報

機能	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.
関連疾患	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).

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配列類似性	Belongs to the tumor necrosis factor family.
翻訳後修飾	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.
細胞内局在	Secreted and Cell membrane.

画像

Western blot - Anti-TNF alpha antibody [EPR20972] - BSA and Azide free (ab225576)
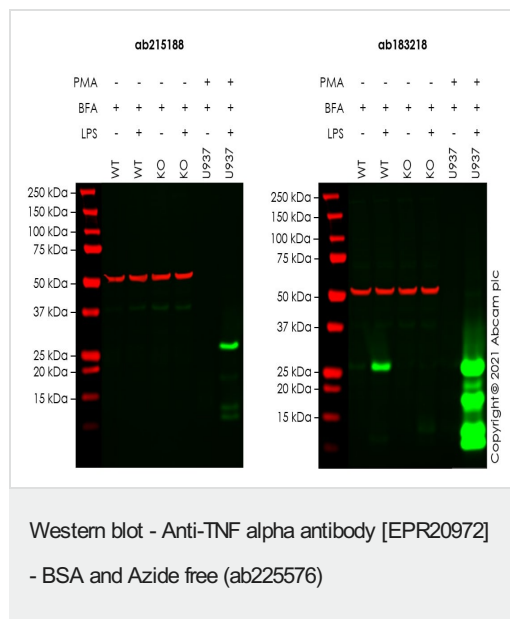
All lanes : Anti-TNF alpha antibody [EPR20972] (**ab215188**) at 1/1000 dilution

Lane 1 : Wild-type THP-1 control: Brefeldin A (5 ug/mL, 4 h) cell lysate

Lane 2 : Wild-type treated THP-1: LPS (100 ng/mL, 16 h), Brefeldin A (5 ug/mL, last 4 h) cell lysate

Lane 3 : TNF alpha knockout THP-1 control: Brefeldin A (5 ug/mL, 4 h) cell lysate

Lane 4 : TNF alpha knockout THP-1 treated: LPS (100 ng/mL, 16 h), Brefeldin A (5 ug/mL, last 4 h) cell lysate

Lane 5 : U937 control: PMA (10 mM, 2 days), Brefeldin A (5 ug/mL, last 4 h) cell lysate

Lane 6 : U937 treated: PMA (10 mM, 2 days), LPS (1 ug/mL, last 16 h), Brefeldin A (5 ug/mL, last 4 h) cell lysate

Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Predicted band size: 26 kDa
Observed band size: 27 kDa

This Western blot image is a comparison between **ab215188** and **ab183218** tested under the same conditions. While **ab215188** is suitable for WB for some samples, **ab183218** was found to be more sensitive. False colour image of Western blot: Anti-TNF alpha antibody [EPR20972] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab215188** was shown to bind specifically to TNF alpha. A band was observed at 27 kDa in treated U937 cell lysates with no signal observed at this size

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without treatment. No signal was observed in wild-type THP-1 cell lysates or in TNF knockout cell line **ab273761** (knockout cell lysate **ab275507**) with **ab215188**. However, a band was observed at 27 kDa in treated wild-type THP-1 cell lysates with **ab183218**. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with primary antibodies overnight at 4 Â°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye$^{Â®}$ 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye$^{Â®}$ 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab215188**).
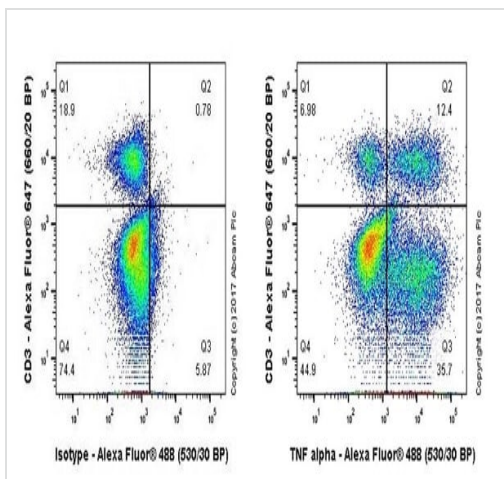

Flow Cytometry (Intracellular) - Anti-TNF alpha antibody [EPR20972] - BSA and Azide free (ab225576)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 0.1% Tween-20 permeabilized mouse splenocytes treated with 20 ng/ml PMA, 1 µg/ml Ionomycin and 10 µM Brefeldin A for 6 hours labeling TNF alpha with **ab215188** at 1/600 dilution (right panel) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (**ab172730**) (left panel). Goat Anti-Rabbit IgG H&L (Alexa Fluor$^®$ 488) at 1/2000 dilution was used as the secondary antibody.

Cells were surface stained with anti-mouse CD3, fixed with 4% PFA for 10 minutes, then permeabilized with 0.1% Tween-20 and intracellular stained with anti-rabbit IgG and **ab215188**. TNF alpha is mainly expressed in T cells (CD3+ population) while only a small population of CD3- cells can express TNF-alpha.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab215188**).
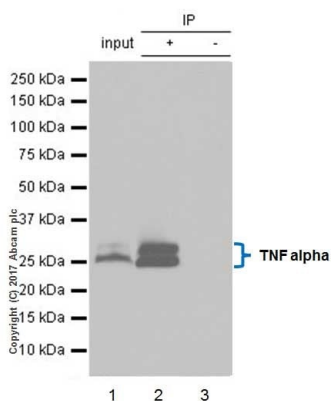
Immunoprecipitation - Anti-TNF alpha antibody [EPR20972] - BSA and Azide free (ab225576)

TNF alpha was immunoprecipitated from 0.35 mg of RAW 264.7 (mouse macrophage cell line transformed with Abelson murine leukemia virus) treated with 100 ng/ml lipopolysaccharides (LPS) for 7 hours with addition of 1 µg/ml brefeldin A (BFA) for the last 3 hours, whole cell lysate with **ab215188** at 1/30 dilution. Western blot was performed from the immunoprecipitate using **ab215188** at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10000 dilution.

Lane 1: RAW 264.7 treated with 100 ng/ml lipopolysaccharides (LPS) for 7 hours with addition of 1 µg/ml brefeldin A (BFA) for the last 3 hours, whole cell lysate 10 µg (Input).
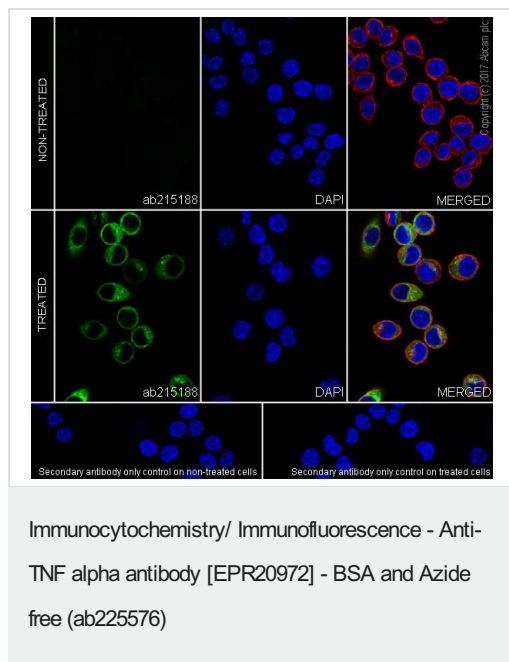
Lane 2: **ab215188** IP in RAW 264.7 treated with 100 ng/ml lipopolysaccharides (LPS) for 7 hours with addition of 1 µg/ml brefeldin A (BFA) for the last 3 hours, whole cell lysate (+).

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab215188** in RAW 264.7 treated with 100 ng/ml lipopolysaccharides (LPS) for 7 hours with addition of 1 µg/ml brefeldin A (BFA) for the last 3 hours, whole cell lysate (-).

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab215188**).

Immunocytochemistry/ Immunofluorescence - Anti-TNF alpha antibody [EPR20972] - BSA and Azide free (ab225576)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized RAW 264.7 (mouse macrophage cell line transformed with Abelson murine leukemia virus) cells, untreated or treated with 100 ng/ml lipopolysaccharides (LPS) for 7 hours with addition of 1 µg/ml brefeldin A (BFA) for the last 3 hours. labeling TNF alpha with **ab215188** at 1/100 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Cytoplasmic staining was increased on RAW 264.7 cells when treated with 100 ng/ml lipopolysaccharides (LPS) for 7 hours with addition of 1 µg/ml brefeldin A (BFA) for the last 3 hours.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody 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 (**ab215188**).

Anti-TNF alpha antibody [EPR20972] - BSA and Azide free (ab225576)

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