

## Product datasheet

### Anti-TNF alpha antibody [EPR20972] ab215188

KO VALIDATED Recombinant RabMAb

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#### Overview

<b>Product name</b>	Anti-TNF alpha antibody [EPR20972]
<b>Description</b>	Rabbit monoclonal [EPR20972] to TNF alpha
<b>Host species</b>	Rabbit
<b>Specificity</b>	The protein level of TNF alpha in normal samples is very weak. The TNF alpha expression must be stimulated.
<b>Tested applications</b>	<b>Suitable for:</b> WB, ICC/IF, IP, Flow Cyt (Intra)
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Human
<b>Immunogen</b>	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
<b>Positive control</b>	WB: RAW 264.7 treated with lipopolysaccharides (LPS) with addition of brefeldin A (BFA) whole cell lysate; THP-1 differentiated with TPA overnight, then treated with LPS for 7 hours with addition of BFA for the last 3 hours, whole cell lysate. ICC/IF: RAW 264.7 cells treated with LPS with addition of BFA. Flow Cyt (intra): Mouse splenocytes treated with 20ng/ml PMA, 1Åµg/ml Ionomycin and 10ÅµM BFA. IP: RAW 264.7 treated with LPS with addition of BFA whole cell lysate
<b>General notes</b>	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> <li>- High batch-to-batch consistency and reproducibility</li> <li>- Improved sensitivity and specificity</li> <li>- Long-term security of supply</li> <li>- Animal-free production</li> </ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb® patents</a>.</p>

#### Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	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.
<b>Storage buffer</b>	pH: 7.2 Preservative: 0.01% Sodium azide

	Constituents: 0.05% BSA, 40% Glycerol, PBS
<b>Purity</b>	Protein A purified
<b>Clonality</b>	Monoclonal
<b>Clone number</b>	EPR20972
<b>Isotype</b>	IgG

## Applications

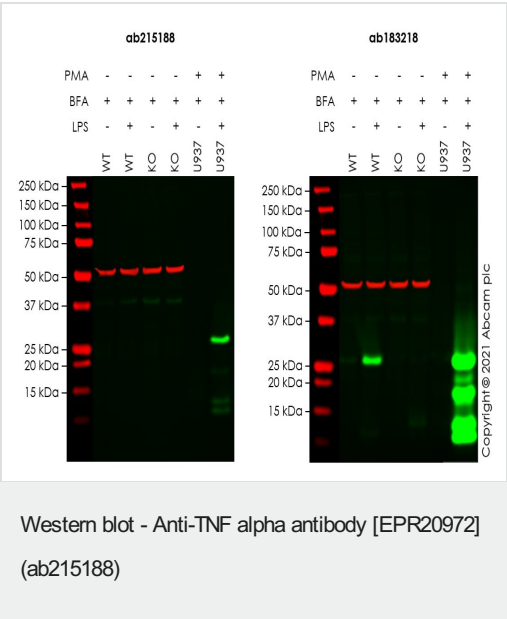
**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab215188 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
<b>WB</b>		1/1000. Detects a band of approximately 33, 26, 17 kDa (predicted molecular weight: 26 kDa). We recommend <b>ab183218</b> to detect TNF alpha in Western blot, as it is more sensitive than ab215188
<b>ICC/IF</b>		1/100.
<b>IP</b>		1/30.
<b>Flow Cyt (Intra)</b>		1/600. <b>ab172730</b> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

## Target

<b>Function</b>	Cytokine that binds to TNFRSF1A/TNFR1 and TNFRSF1B/TNFR. 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.
<b>Involvement in disease</b>	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).
<b>Sequence similarities</b>	Belongs to the tumor necrosis factor family.
<b>Post-translational modifications</b>	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.

Images



**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.

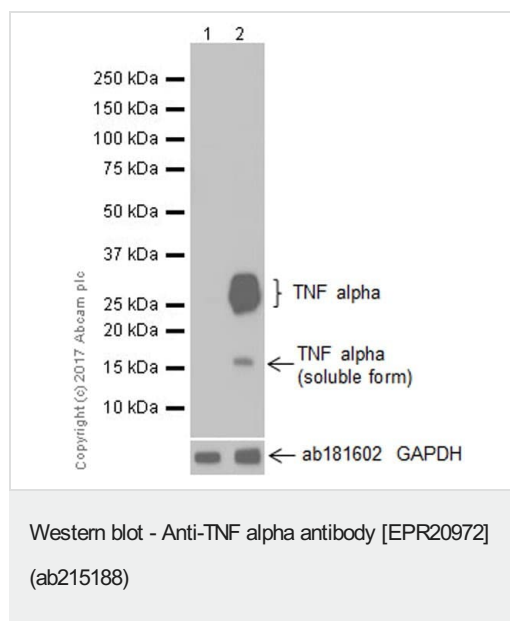
Performed under reducing conditions.

**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 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 °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<sup>®</sup> 800CW) preabsorbed ([ab216773](#)) and Goat anti-Mouse IgG H&L (IRDye<sup>®</sup> 680RD) preabsorbed ([ab216776](#)) at 1/20000 dilution.



**All lanes :** Anti-TNF alpha antibody [EPR20972] (ab215188) 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 :** Untreated 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

Lysates/proteins at 10 µg per lane.

### Secondary

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

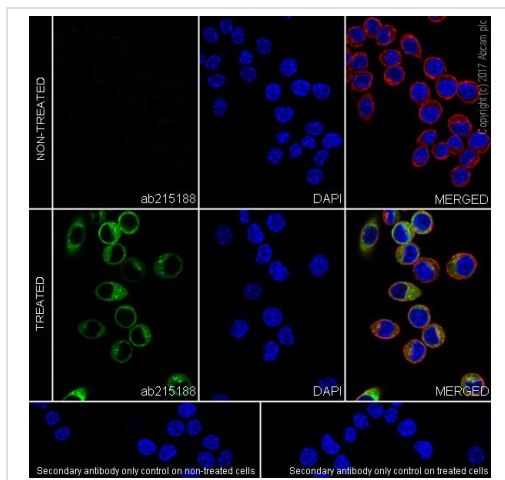
Developed using the ECL technique.

**Predicted band size:** 26 kDa

**Observed band size:** 17,26,33 kDa

**Exposure time:** 15 seconds

Blocking/Dilution buffer: 5% NFDm/TBST.

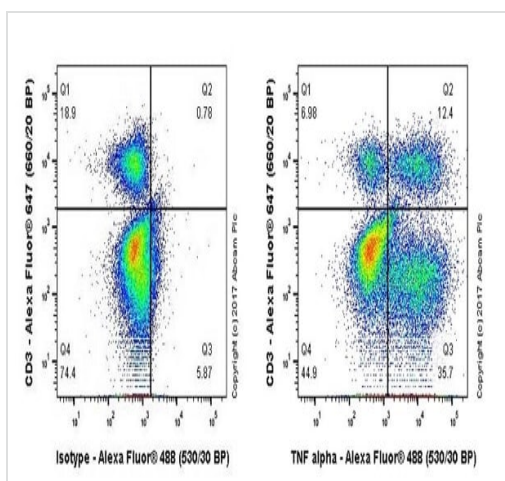


Immunocytochemistry/ Immunofluorescence - Anti-TNF alpha antibody [EPR20972] (ab215188)

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.

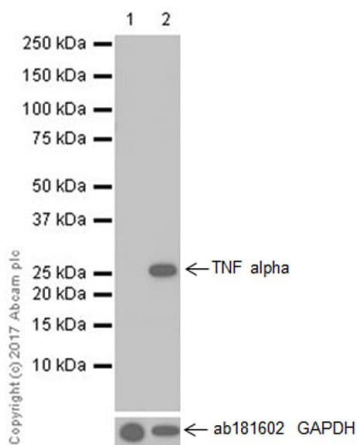
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) ([ab150077](#)) secondary antibody at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-TNF alpha antibody [EPR20972] (ab215188)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 0.1% Tween-20 permeabilized mouse splenocytes treated with 20ng/ml PMA, 1 µg/ml Ionomycin and 10µM Brefeldin A for 6 hours labeling TNF alphawith 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.



Western blot - Anti-TNF alpha antibody [EPR20972] (ab215188)

**All lanes :** Anti-TNF alpha antibody [EPR20972] (ab215188) at 1/1000 dilution

**Lane 1 :** THP-1 (human monocytic leukemia cell line) differentiated with 100 nM TPA overnight, whole cell lysate

**Lane 2 :** THP-1 (human monocytic leukemia cell line) differentiated with 100 nM TPA overnight, then 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

Lysates/proteins at 10 µg per lane.

## Secondary

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/50000 dilution

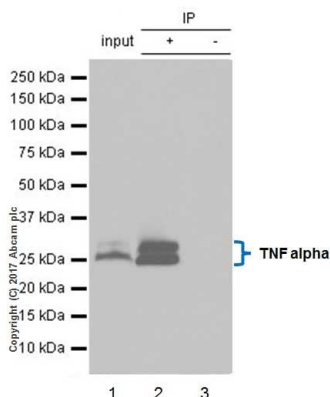
Developed using the ECL technique.

**Predicted band size:** 26 kDa

**Observed band size:** 26 kDa

**Exposure time:** 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Immunoprecipitation - Anti-TNF alpha antibody [EPR20972] (ab215188)

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 1/10,000 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.

Why choose a recombinant antibody?

 <p><b>Research with confidence</b> Consistent and reproducible results</p>	 <p><b>Long-term and scalable supply</b> Recombinant technology</p>
 <p><b>Success from the first experiment</b> Confirmed specificity</p>	 <p><b>Ethical standards compliant</b> Animal-free production</p>

Anti-TNF alpha antibody [EPR20972] (ab215188)

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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