# abcam

# Product datasheet

# Anti-TNF alpha antibody ab66579

★★★★★ 10 Abreviews 128 References 2 Images

Overview

Product name Anti-TNF alpha antibody

**Description** Rabbit polyclonal to TNF alpha

Host species Rabbit

**Specificity** Ab66579 has been batch tested in Western Blot using LPS stimulated lysates. Abcam does not

recommend this product for use in IHC. Please contact Abcam Scientific Support for more

information.

Tested applications Suitable for: WB, ICC/IF

Species reactivity Reacts with: Mouse, Human

Predicted to work with: Rat, Cat, Chimpanzee, Baboon

**Immunogen** Synthetic peptide corresponding to Human TNF alpha aa 1-100 conjugated to keyhole limpet

haemocyanin.

(Peptide available as ab66578)

**Positive control** This antibody gave a positive signal in the following LPS stimulated lysates: THP1, RAW 264.7

ICC/IF: PMA/Befreldin/LPS treated RAW 264.7 cell line.

General notes

The Life Science industry has been in the grips of a reproducibility crisis for a number of years.

Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

**Properties** 

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -

80°C. Avoid freeze / thaw cycle.

Storage buffer pH: 7.40

Preservative: 0.02% Sodium azide

Constituent: PBS

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our

scientific support team who will be happy to help.

**Purity** Immunogen affinity purified

**Clonality** Polyclonal

**Isotype** IgG

## **Applications**

#### The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab66579 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
WB	<b>★★★★</b> <u>(5)</u>	Use a concentration of 1 µg/ml. Detects a band of approximately 26 kDa (predicted molecular weight: 25 kDa).  Abcam recommends using 3% Milk as the blocking agent.
ICC/IF	<b>★★★★ (2)</b>	Use a concentration of 1 µg/ml.

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**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.

Involvement in disease

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).

Sequence similarities

Belongs to the tumor necrosis factor family.

Post-translational modifications

The soluble form derives from the membrane form by proteolytic processing.

The membrane form, but not the soluble form, is phosphorylated on serine residues.

 $\label{thm:prop} \mbox{Dephosphorylation of the membrane form occurs by binding to soluble TNFRSF1A/TNFR1.}$ 

O-glycosylated; glycans contain galactose, N-acetylgalactosamine and N-acetylneuraminic acid.

Cellular localization

Secreted and Cell membrane.

# **Images**



Western blot - Anti-TNF alpha antibody (ab66579)

All lanes: Anti-TNF alpha antibody (ab66579) at 1 µg/ml

**Lane 1 :** THP1 cells treated with PMA (negative control)

Lane 2: THP1 cells treated with PMA+LPS+Brefeldin A

Lane 3: Raw264.7 cells treated with PMA (negative control)

Lane 4: Raw264.7 cells treated with PMA+LPS+Brefeldin A

Lysates/proteins at 20 µg per lane.

# **Secondary**

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/10000 dilution

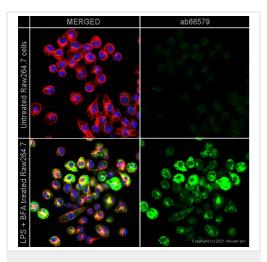
Performed under reducing conditions.

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

**Additional bands at:** 28 kDa (possible glycosylated form), 50 kDa, 75 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 2 minutes

Mature THP-1 and Raw264.7 cells were treated with LPS (1 ug/ml) for 6 hours to stimulate production of TNF alpha. Brefeldin A (300 ng/ml) was added in the final 3 hours of incubation to inhibit golgi traffic.



Immunocytochemistry/ Immunofluorescence - Anti-TNF alpha antibody (ab66579)

ab66579 staining TNF alpha in Raw264.7 cells. The cells were treated with LPS for 7 hours and Brefeldin A (ab120299) for the final 3 hours. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Tween for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab66579 at 1µg/ml and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with ab150081, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and ab150120, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Also suitable in cells fixed with 4% paraformaldehyde (10 min).

Image was acquired with a confocal microscope (Leica-Microsystems TCS SP8) and a single confocal section is shown.

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

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