Product datasheet

Anti-TNF alpha antibody ab66579

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, Rat, Human
Predicted to work with: Cat, Chimpanzee, Baboon
Immunogen: Synthetic peptide conjugated to KLH derived from within residues 1 - 100 of Human TNF alpha. Read Abcam’s proprietary immunogen policy (Peptide available as ab66578.)
Positive control: This antibody gave a positive signal in the following LPS stimulated lysates: THP1, Raw264.7 IF/ICC: PMA/Befreldin/LPS treated RAW246.7 cell line.

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: Preservative: 0.02% Sodium Azide
Constituents: 1% BSA, PBS, pH 7.4
Purity: Immunogen affinity purified
Clonality: Polyclonal
Isotype: IgG

Applications

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.
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 a 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.

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).

Belongs to the tumor necrosis factor family.

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-acetylenuraminic acid.

Secreted and Cell membrane.

### Application

- **WB**: Use a concentration of 1 µg/ml. Detects a band of approximately 26 kDa (predicted molecular weight: 25 kDa). Can be blocked with Human TNF alpha peptide (ab66578). Abcam recommends using 3% Milk as the blocking agent.

- **ICC/IF**: Use a concentration of 1 µg/ml.
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) (ab97051) at 1/10000 dilution

Performed under reducing conditions.

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

why is the actual band size different from the predicted?

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.

ab66579 stained RAW246.7 cells. The cells were treated with PMA for 24 hours, LPS for 6 hours and Brefeldin for the final 3 hours. The cells were then 4% formaldehyde fixed for 10 minutes at room temperature and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1hour at room temperature to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab66579 at 1µg/ml) overnight at +4°C. The secondary antibody (pseudo-colored green) was Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (ab150081) used at a 1/1000 dilution for 1hour at room temperature. Alexa Fluor® 594 WGA was used to label plasma membranes (pseudo-colored red) at a 1/200 dilution for 1hour at room temperature. DAPI was used to stain the cell nuclei (pseudo-colored blue) at a concentration of 1.43µM for
1 hour at room temperature.

**Please note:** All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"

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