Anti-TNF alpha antibody [2C8] ab8348

Overview

Product name: Anti-TNF alpha antibody [2C8]

Description: Mouse monoclonal [2C8] to TNF alpha

Host species: Mouse

Specificity: This antibody recognises human TNF-alpha. It is effective in inhibition of the biological activity of recombinant and natural TNF-alpha.

Tested applications: Suitable for: Flow Cyt, Neutralising, ELISA, IHC-P, IHC-Fr, ICC/IF, WB, Sandwich ELISA

Species reactivity: Reacts with: Mouse, Human

Immunogen: Other Immunogen Type corresponding to Human TNF alpha. Human recombinant tumor necrosis factor of alpha type.

Positive control: This antibody gave a positive result when used in the following formaldehyde fixed cell lines: A431

General notes: Abcam is committed to meeting high quality standards of ethical manufacturing and has decided to discontinue this product by June 2020 as it has been generated by the ascites method. We are sorry for any inconvenience this may cause. We suggest ab1793 or ab183218 as possible replacements.

Properties

Form: Liquid

Storage instructions: Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.

Storage buffer: pH: 7.40
Preservative: 0.09% Sodium azide
Constituent: PBS

Purity: Ascites

Purification notes: Purified from ascites.

Clonality: Monoclonal

Clone number: 2C8

Myeloma: x63-Ag8.653

Isotype: IgG1

Light chain type: unknown
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. 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.

Applications

Our Abpromise guarantee covers the use of ab8348 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
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<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tr>
<td>Flow Cyt</td>
<td>Use 1µg for 10⁶ cells. ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.</td>
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<tr>
<td>Neutralising</td>
<td>Use a concentration of 5 µg/ml.</td>
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<tr>
<td>ELISA</td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>IHC-P</td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>IHC-Fr</td>
<td>Use a concentration of 10 - 20 µg/ml.</td>
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<tr>
<td>ICC/IF</td>
<td>Use a concentration of 10 µg/ml.</td>
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<tr>
<td>WB</td>
<td>Use at an assay dependent concentration. Predicted molecular weight: 25 kDa.</td>
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<tr>
<td>Sandwich ELISA</td>
<td>Use a concentration of 5 µg/ml. Can be paired for Sandwich ELISA with Rabbit polyclonal to TNF alpha (ab9635). Use this antibody as Capture at 5µg/ml with ab9635 as Detection.</td>
<td></td>
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</tbody>
</table>
All lanes: Anti-TNF alpha antibody [2C8] (ab8348) (Undiluted)

All lanes: Human skeletal muscle (gastrocnemius) tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes: Alexa Fluor® 555-conjugated Goat anti-mouse IgG polyclonal at 1/5000 dilution

Performed under reducing conditions.

Predicted band size: 25 kDa

Observed band size: 28 kDa

why is the actual band size different from the predicted?

Additional bands at: 38 kDa (possible non-specific binding), 41 kDa (possible non-specific binding), 65 kDa (possible non-specific binding)

Exposure time: 2 minutes

CON+ = Patient with increased TNF alpha levels on ELISA of muscle homogenate.

POS = Diseased patient with suspected increased TNF alpha levels in muscle.

Standard curve for TNF alpha (Analyte: ab9642); dilution range 1pg/ml to 1µg/ml using Capture Antibody Mouse monoclonal [2C8] to TNF alpha (ab8348) at 5µg/ml and Detector Antibody Rabbit polyclonal to TNF alpha (ab9635) at 0.5µg/ml.
ab8348 staining TNF alpha in Human skeletal muscle (gastrocnemius) tissue sections by Immunohistochemistry (Formalin/PFA-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 10% serum for 10 minutes at 25°C; antigen retrieval was by heat mediation in a Tris buffer (pH 8). Samples were incubated with primary antibody (1/200) for 16 hours at 4°C. An undiluted HRP-conjugated Rabbit anti-mouse IgG polyclonal was used as the secondary antibody.

Overlay histogram showing THP1 cells stained with ab8348 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab8348, 1µg/1x10⁶ cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, 2µg/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in THP1 cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

Western blot - Anti-TNF alpha antibody [2C8] (ab8348)

**All lanes**: Anti-TNF alpha antibody [2C8] (ab8348) at 1/500 dilution

**All lanes**: Mouse liver whole tissue lysate

Lysates/proteins at 75 µg per lane.

Developed using the ECL technique.

Performed under reducing conditions.

**Predicted band size**: 25 kDa

**Observed band size**: 28 kDa why is the actual band size different from the predicted?
Additional bands at: 30 kDa (possible non-specific binding), 50 kDa (possible non-specific binding), 65 kDa (possible non-specific binding)

Exposure time: 15 minutes

Immunocytochemistry/ Immunofluorescence - Anti-TNF alpha antibody [2C8] (ab8348)

ICC/IF image of ab8348 stained A431 cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab8348 at 10µg/ml overnight at +4°C. The secondary antibody (green) was DyLight® 488 goat anti- mouse (ab96879) IgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TNF alpha antibody [2C8] (ab8348)

Ab8348 staining human tonsil. Staining is localized to the plasma membrane and cytoplasm.

Left panel: with primary antibody at 2 ug/ml. Right panel: isotype control.

Sections were stained using an automated system (Dako PT Link), at room temperature. Sections were rehydrated and antigen retrieved with the Dako 3-in-1 antigen retrieval buffer, EDTA pH 9.0. Slides were peroxidase blocked in 3% H2O2 in methanol for 10 minutes. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 minutes and detected with Dako Envision Flex amplification kit for 30 minutes. Colorimetric detection was completed with diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX.

Please note that for manual staining we recommend to optimize the primary antibody concentration and incubation time (overnight incubation), and amplification may be required.

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