**Product datasheet**

**Anti-TNF alpha antibody ab9635**

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**Overview**

**Product name**
Anti-TNF alpha antibody

**Description**
Rabbit polyclonal to TNF alpha

**Host species**
Rabbit

**Tested applications**
Suitable for: IHC-P, WB, ELISA, Neutralising, Sandwich ELISA

**Species reactivity**
Reacts with: Human

**Immunogen**
Recombinant fragment corresponding to Human TNF alpha aa 77-233. E.coli derived
Recombinant Human TNF-alpha
Database link: P01375

**Positive control**
WB: Recombinant human TNF alpha protein (ab9642), SW480 cell lysate. IHC-P: Human tonsil tissue.

**Properties**

**Form**
Lyophilised: Reconstitute with 200µl of sterile water.

**Storage instructions**

**Storage buffer**
PBS, pH 7.4, no preservative, sterile filtered

**Purity**
Immunogen affinity purified

**Clonality**
Polyclonal

**Isotype**
IgG

**Light chain type**
unknown

**Applications**

Our Abpromise guarantee covers the use of ab9635 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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</thead>
<tbody>
<tr>
<td>IHC-P</td>
<td></td>
<td>Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.</td>
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<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration. To detect TNF-alpha by Western Blot analysis this antibody can be used at a concentration of 0.1 - 0.2 µg/ml. Used in conjunction with compatible secondary reagents the detection limit for recombinant TNF-alpha is 1.5 - 3.0 ng/lane, under either reducing or non-reducing conditions.</td>
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<tr>
<td>ELISA</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration. Can be paired for ELISA with Mouse monoclonal to TNF alpha (ab9348). To detect TNF-alpha by direct ELISA (using 100µl/well antibody solution) a concentration of at least 0.5µg/ml of this antibody is required. This antigen affinity purified antibody, in conjunction with compatible secondary reagents, allows the detection of 0.2 - 0.4 ng/well of recombinant TNF-alpha.</td>
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<tr>
<td>Neutralising</td>
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<td>Use at an assay dependent concentration. To yield one-half maximal inhibition [ND50] of the biological activity of hTNF-alpha (0.5 ng/ml), a concentration of 0.08 - 0.10 µg/ml of this antibody is required.</td>
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<tr>
<td>Sandwich ELISA</td>
<td></td>
<td>Use a concentration of 0.5 µg/ml. Can be paired for Sandwich ELISA with Mouse monoclonal [2C8] to TNF alpha (ab8348). For sandwich ELISA, use this antibody as Detection at 0.5µg/ml with ab8348 as Capture.</td>
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**Target**

**Function**: Cytokine that binds to TNFRSF1A/TNFR1 and TNFRSF1B/TNFRBR. 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.

**Images**
Anti-TNF alpha antibody (ab9635) at 0.2 µg/ml + SW480 (Human colon adenocarcinoma cell line) Whole Cell Lysate at 10 µg

**Secondary**

Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

**Observed band size:** 26 kDa

*why is the actual band size different from the predicted?*

**Additional bands at:** 48 kDa. We are unsure as to the identity of these extra bands.

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.

IHC image of TNF alpha staining in human tonsil formalin fixed paraffin embedded tissue section, performed on a Leica Bond system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab9635, 1µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.
Western blot - Anti-TNF alpha antibody (ab9635) at 0.2 µg/ml + Recombinant
Human TNF alpha protein (ab55237) at 0.01 µg

Secondary
Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (ab97080) at
1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Exposure time: 10 seconds

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