Product datasheet

Anti-IL1 beta antibody ab9722

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Overview

Product name  Anti-IL1 beta antibody
Description  Rabbit polyclonal to IL1 beta
Host species  Rabbit
Tested applications  Suitable for: WB, ELISA, Neutralising, IHC-P, ICC, IHC-Fr, IHC-FoFr, ICC/IF
Species reactivity  Reacts with: Mouse, Rat, Human
Immunogen  Full length protein aa 118-269. Full length mature protein minus the propeptide from aa 1-117.
Database link: P10749
Positive control  FFPE mouse kidney tissue sections

Properties

Form  Lyophilised: Reconstitute with 200µl of sterile water.
Storage buffer  PBS, pH 7.4, no preservative, sterile filtered
Purity  Immunogen affinity purified
Clonality  Polyclonal
Isotype  unknown
Light chain type  unknown

Applications

Our Abpromise guarantee covers the use of ab9722 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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<tbody>
<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent dilution. To detect mIL-1b 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 mIL-1b is 1.5 - 3.0 ng/lane, under either reducing or non-reducing conditions.</td>
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</tbody>
</table>
### Function

Potent proinflammatory cytokine. Initially discovered as the major endogenous pyrogen, induces prostaglandin synthesis, neutrophil influx and activation, T-cell activation and cytokine production, B-cell activation and antibody production, and fibroblast proliferation and collagen production. Promotes Th17 differentiation of T-cells.

### Tissue specificity

Expressed in activated monocytes/macrophages (at protein level).

### Sequence similarities

Belongs to the IL-1 family.

### Post-translational modifications

Activation of the IL1B precursor involves a CASP1-catalyzed proteolytic cleavage. Processing and secretion are temporarily associated.

### Cellular localization

Cytoplasm, cytosol. Lysosome. Secreted, exosome. Cytoplasmic vesicle, autophagosome. Secreted. The precursor is cytosolic. In response to inflammasome-activating signals, such as ATP for NLRP3 inflammasome or bacterial flagellin for NLRC4 inflammasome, cleaved and secreted. IL1B lacks any known signal sequence and the pathway(s) of its secretion is(are) not yet fully understood (PubMed:24201029). On the basis of experimental results, several unconventional secretion mechanisms have been proposed. 1. Secretion via secretory lysosomes: a fraction of CASP1 and IL1B precursor may be incorporated, by a yet undefined mechanism, into secretory lysosomes that undergo Ca(2+)-dependent exocytosis with release of mature IL1B (PubMed:15192144). 2. Secretory autophagy: IL1B-containing autophagosomes may fuse with endosomes or multivesicular bodies (MVBs) and then merge with the plasma membrane releasing soluble IL1B or IL1B-containing exosomes (PubMed:24201029). However, autophagy impacts IL1B production at several levels and its role in secretion is still controversial. 3. Secretion via exosomes: ATP-activation of P2RX7 leads to the formation of MVBs containing exosomes with entrapped IL1B, CASP1 and other inflammasome components. These MVBs undergo exocytosis with the release of exosomes. The release of soluble IL1B occurs after the lysis of exosome membranes (By similarity). 4. Secretion by microvesicle shedding: activation of the ATP receptor P2RX7 may induce an immediate shedding of membrane-derived microvesicles containing IL1B and possibly inflammasome components. The cytokine is then

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<tr>
<td>ELISA</td>
<td>Use at an assay dependent dilution. To detect mIL-1b 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 mIL-1b.</td>
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<td>Neutralising</td>
<td>Use at an assay dependent dilution. To yield one-half maximal inhibition [ND50] of the biological activity of mIL-1b (50 pg/ml), a concentration of 100 - 150 ng/ml of this antibody is required.</td>
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<tr>
<td>IHC-P</td>
<td>Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.</td>
<td></td>
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<tr>
<td>ICC</td>
<td>1/100.</td>
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<tr>
<td>IHC-Fr</td>
<td>Use at an assay dependent dilution. PubMed: 18420712Acetone fixed.</td>
<td></td>
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<tr>
<td>IHC-FoFr</td>
<td>Use at an assay dependent concentration.</td>
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</tr>
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### Notes

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- **IHC-FoFr**
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- **ICC/IF**
  - Use at an assay dependent concentration.

### Application

- **ELISA**
- **Neutralising**
- **IHC-P**
- **ICC**
- **IHC-Fr**
- **IHC-FoFr**
- **ICC/IF**

### Target

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released in the extracellular compartment after microvesicle lysis (PubMed:11728343). 5. Release by translocation through permeabilized plasma membrane. This may occur in cells undergoing pyroptosis due to sustained activation of the inflammasome (By similarity). These mechanisms may not be not mutually exclusive.

Images

- **ab9722 staining IL1 beta in murine bone marrow-derived macrophages by Immunocytochemistry/Immunofluorescence.**
  > Cells are immortalised murine bone marrow-derived macrophages stably transfected with GFP-LC3 (green) to visualise autophagosomes. The cells were fixed in paraformaldehyde, permeabilised in 0.01% Triton X-100 and then blocked using 5% serum for 1 hour at 20°C. Samples were then incubated with primary antibody at 1/200 for 1 hour at 20°C. The secondary antibody used was a goat anti-rabbit IgG conjugated to Alexa Fluor® 568 (red) used undiluted.

- **ab9722 staining IL1 beta in rat brain tissue sections by Immunohistochemistry (PFA perfusion fixed frozen sections).**
  > Tissue was fixed with paraformaldehyde and blocked with 10% serum for 1 hour at 25°C. Samples were incubated with primary antibody (1/100 in diluent) for 10 hours at 25°C. An AlexaFluor®555-conjugated donkey anti-goat IgG polyclonal (1/300) was used as the secondary antibody.
ab9722 staining IL1 beta (green) in murine macrophage cells by Immunocytochemistry/Immunofluorescence.

Cells were fixed with formaldehyde, permeabilized with 0.1% Triton X-100 + 3% BSA and blocked with 3% BSA for 3 hours at 25°C. Samples were incubated with primary antibody (1/100 in 1% BSA in PBS) for 1 hour at 25°C. A FITC-conjugated goat anti-rabbit polyclonal IgG (1/100) was used as the secondary antibody. Nuclei were stained with DAPI (blue).

IHC image of IL1 beta staining in mouse kidney formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol B. 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 ab9722, 1µg/ml, for 15 mins at room temperature. A goat anti-rabbit biotinylated secondary antibody was used to detect the primary, and visualized using an HRP conjugated ABC 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.
Immunohistochemical analysis of PFA perfusion fixed frozen mouse brain, staining IL1 beta (green) with ab9722 at 0.5 µg/ml. A peroxidase conjugated secondary antibody was used and staining was detected using a fluorescein Tyramide Signal Amplification (TSA™) reagent.

Sandwich ELISA detecting IL1 beta using ab9722 at a concentration of 0.1 µg/ml.

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