# abcam

## Product datasheet

## Anti-IL-1 alpha antibody [EPR25263-27] ab300499

Recombinant RobMAb

## 7 Images

#### Overview

**Product name** Anti-IL-1 alpha antibody [EPR25263-27]

**Description** Rabbit monoclonal [EPR25263-27] to IL-1 alpha

**Host species** Rabbit

**Tested applications** Suitable for: WB, IHC-P, Flow Cyt (Intra), ICC/IF

Unsuitable for: IP

Reacts with: Mouse Species reactivity

Does not react with: Rat, Human

Recombinant fragment. This information is proprietary to Abcam and/or its suppliers. **Immunogen** 

Positive control WB: RAW264.7 treated with 10µg/ml lipopolysaccharide, whole cell lysate. ICC/IF: RAW 264.7

> (mouse abelson murine leukemia virus-induced tumor macrophages), THP-1 (human monocytic leukemia monocytes). IHC-P: Mouse lung treated with lipopolysaccharides. Flow Cyt (Intra): RAW264.7 cells treated with 10ug/ml LPS, mouse bone marrow cells treated with 5ug/ml LPS.

**General notes** This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb patents**.

#### **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 long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

**Purity** Protein A purified

**Clonality** Monoclonal

Clone number EPR25263-27

**Isotype** IgG

#### **Applications**

The Abpromise guarantee Our Abpromise guarantee covers the use of ab300499 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		1/1000.
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Flow Cyt (Intra)		1/500.
ICC/IF		1/50.

**Application notes** Is unsuitable for IP.

#### **Target**

**Function** Produced by activated macrophages, IL-1 stimulates thymocyte proliferation by inducing IL-2

release, B-cell maturation and proliferation, and fibroblast growth factor activity. IL-1 proteins are involved in the inflammatory response, being identified as endogenous pyrogens, and are reported to stimulate the release of prostaglandin and collagenase from synovial cells.

Sequence similarities Belongs to the IL-1 family.

**Domain** The similarity among the IL-1 precursors suggests that the amino ends of these proteins serve

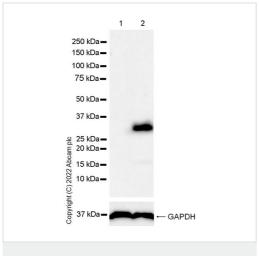
some as yet undefined function.

Cellular localization Secreted. The lack of a specific hydrophobic segment in the precursor sequence suggests that IL-

1 is released by damaged cells or is secreted by a mechanism differing from that used for other

secretory proteins.

#### **Images**



Western blot - Anti-IL-1 alpha antibody [EPR25263-27] (AB300499)

**All lanes :** Anti-IL-1 alpha antibody [EPR25263-27] (ab300499) at 1/1000 dilution

**Lane 1 :** Untreated RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate

**Lane 2 :** RAW264.7 treated with  $10\mu g/ml$  lipopolysaccharide (LPS) for 7 hours, whole cell lysate

Lysates/proteins at 40 µg per lane.

### Secondary

**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

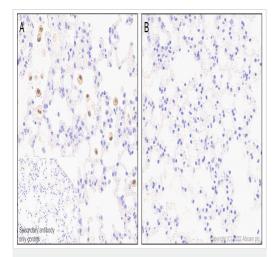
Observed band size: 30 kDa

Exposure time: 59 seconds

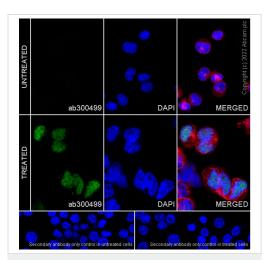
Blocking / Diluting buffer and concentration: 5% NFDM/TBST

The expression level of IL-1 alpha was upregulated by LPS  $\,$ 

stimulation (PMID: 25870118)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-IL-1 alpha antibody
[EPR25263-27] (AB300499)



Immunocytochemistry/ Immunofluorescence - Anti-IL-1 alpha antibody [EPR25263-27] (ab300499)

Immunohistochemical analysis of paraffin-embedded Mouse lung treated tissue labeling IL-1 alpha with ab300499 at 1/500 dilution (1.05 μg/mL) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection kit). Positive staining on mouse lung induced by LPS+BFA (A) and no staining on untreated mouse lung (B). The section was incubated with ab300499 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND<sup>®</sup> RX instrument. Counterstained with Hematoxylin.

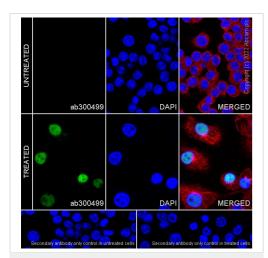
Secondary antibody only control: PBS was used instead of primary antibody, followed by a ready to use secondary antibody
LeicaDS9800 (Bond™ Polymer Refine Detection kit).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized THP-1 (human monocytic leukemia monocyte) cells labeling IL-1 alpha with ab300499 at 1/50 (10.5 µg/ml) dilution, followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) preadsorbed antibody at 1/1000 2 µg/mL dilution (Green). Confocal image showing increased nuclear staining in THP-1 cells treated with Phorbol-12-myristate-13-acetate (50 nM) for 24 h. is observed.

<u>ab195889</u> Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor<sup>®</sup> 594) was used to counterstain tubulin at 1/200 (2.5  $\mu$ g/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is  $\underline{ab150081}$  Goat Anti-Rabbit lgG H&L (Alexa Fluor 488) preadsorbed at 1/1000 2µg/mL dilution.

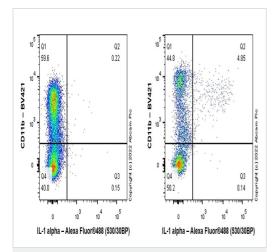


Immunocytochemistry/ Immunofluorescence - Anti-IL-1 alpha antibody [EPR25263-27] (ab300499)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized RAW 264.7 (mouse abelson murine leukemia virus-induced tumor macrophage) cells labeling IL-1 alpha with ab300499 at 1/50 (10.5  $\mu g/ml$ ) dilution, followed by ab150081 Goat Anti-Rabbit lgG H&L (Alexa Fluor  $^8$  488) preadsorbed antibody at 1/1000 2 $\mu g/mL$  dilution (Green). Confocal image showing increased nuclear staining in Raw 264.7 cells treated with lipopolysaccharide (10  $\mu g/ml$ ) for 24 h (PMID:25870118) is observed.

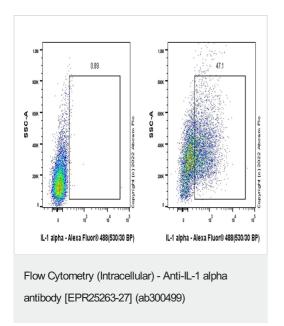
**ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor<sup>®</sup> 594) was used to counterstain tubulin at 1/200 (2.5 μg/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is <a href="mailto:ab150081">ab150081</a> Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) preadsorbed at 1/1000 2µg/mL dilution.

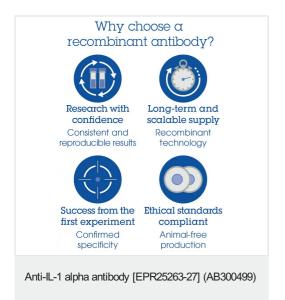


Flow Cytometry (Intracellular) - Anti-IL-1 alpha antibody [EPR25263-27] (ab300499)

Flow Cytometry (Intracellular) analysis of mouse bone marrow cells treated with 5  $\mu$ g/ml LPS for 7 hours (Right) / Untreated control (Left) labelling IL-1 alpha with ab300499 at a 1/500 dilution. Cells were fixed with 2% paraformaldehyde and permeabilized using True-Nuclear buffer. A Goat Anti-Rabbit lgG (Alexa Fluor<sup>®</sup> 488, (ab150081)) was used as the secondary antibody at a 1/2000 dilution. Cells were surface stained with BV421 conjugated CD11b, then fixed with PFA followed by intracellularly stained with ab300499.



Flow Cytometry (Intracellular) analysis of RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) treated with 10 µg/ml LPS for 24 hours (Right) / Untreated control (Left) IL-1 alpha with ab300499 at a 1/500 dilution. Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. A Goat Anti-Rabbit IgG (Alexa Fluor® 488, (ab150081)) was used as the secondary antibody at a 1/2000 dilution. The expression level of IL-1 alpha was upregulated by LPS stimulation (PMID: 25870118).



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