abcam

Product datasheet

Anti-IL-1 beta antibody ab2105

21 Abreviews 54 References 5 Images

Overview

Product name	Anti-IL-1 beta antibody
Description	Rabbit polyclonal to IL-1 beta
Host species	Rabbit
Tested applications	**Suitable for:** WB, IP, ELISA, IHC-P, RIA, IHC-Fr, Functional Studies, Flow Cyt, Neutralising, ICC/IF, IHC-FoFr
Species reactivity	**Reacts with:** Human **Predicted to work with:** Non human primates
Immunogen	Recombinant 153 aa human IL-1beta produced in E.coli. MW of recombinant IL-1beta 17kDa, with the N-terminal amino acid at position alanine 117. This is the cleavage site generated by the IL-1beta converting enzyme (ICE, capase-1).
General notes	Immunohistochemistry 1/100 to 1/200 (paraffin or cryofixation can be used; 1/100 for staining intracellular IL-1beta). ELISA 1/200 to 1/1000 (this antibody is best used as the second antibody with a monoclonal as a capture antibody). Radio immunoassay 1/8000 Neutralization of IL-1beta activity in bioassays 1/100 (>4 hours incubation, normal rabbit IgG as negative control) FACS analysis - caution, F(c) domain of rabbit IgG may interact with cells non-specifically.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.
Storage buffer	pH: 7.20 Constituents: 0.42% Potassium phosphate, 0.87% Sodium chloride
Purity	IgG fraction
Purification notes	IgG from whole rabbit serum purified by DEAE fractionation.
Clonality	Polyclonal
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab2105** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

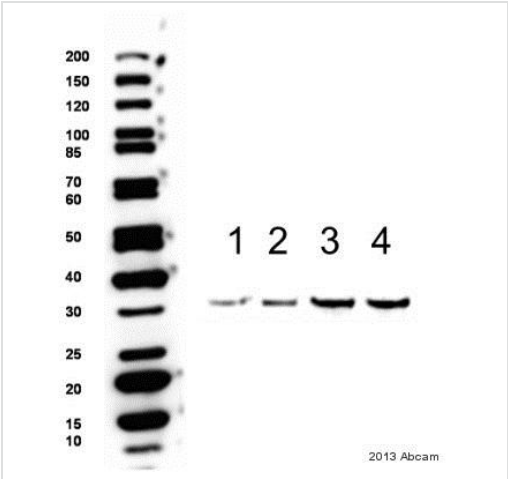
Application	Abreviews	Notes
WB	★★★★☆	Use at an assay dependent concentration. 1/1000 - 1/2000 (antigen - supernatants or lysates of 2 x 106 endotoxin-stimulated human PBMC. Denatured 31kDa precursor IL-1beta will be recognized, but often migrates as a 35 kDa band).
IP		Use at an assay dependent concentration. 1/400 - 1/800 (pre-clearing with a non-specific rabbit IgG is helpful to reduce background).
ELISA	★★★★☆	1/500 - 1/2000.
IHC-P	★★★★☆	1/100 - 1/200.
RIA		1/8000.
IHC-Fr	★★★★☆	1/100 - 1/200.
Functional Studies		Use at an assay dependent concentration.
Flow Cyt	★★★★☆	Use at an assay dependent concentration. F(c) domain of rabbit IgG may interact with cells non-specifically! ab171870 - Rabbit polyclonal IgG, is suitable for use as an isotype control with this antibody.
Neutralising		1/100. Neutralization of IL-1beta activity in bioassays 1/100 (>4 hours incubation, normal rabbit IgG as negative control). ab2105 does not neutralize the biological activity of murine, rat or rabbit IL1 beta.
ICC/IF	★★★★★	Use at an assay dependent concentration.
IHC-FoFr		Use at an assay dependent concentration. PubMed: 28726778

Target

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 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



Western blot - Anti-IL-1 beta antibody (ab2105)

This image is courtesy of an anonymous Abreview

All lanes : Anti-IL-1 beta antibody (ab2105) at 1/1000 dilution

Lane 1 : Human peripheral blood mononuclear cell lysate - PBMC's unstimulated
Lane 2 : Human peripheral blood mononuclear cell lysate - monocytes unstimulated
Lane 3 : Human peripheral blood mononuclear cell lysate - monocytes stimulated with LPS
Lane 4 : Human peripheral blood mononuclear cell lysate - positive control (rh IL-1 beta protein)

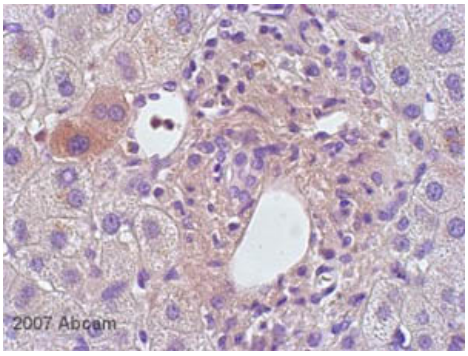
Lysates/proteins at 25 µg per lane.

Secondary
All lanes : HRP-conjugated Goat anti-rabbit IgG polyclonal at 1/2000 dilution

Performed under reducing conditions.

Observed band size: 35 kDa
why is the actual band size different from the predicted?

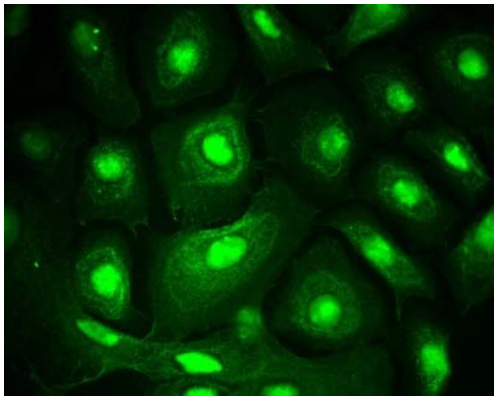
Exposure time: 10 minutes



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IL-1 beta antibody (ab2105)

This image is courtesy of an Abreview submitted by Miss Silke Vorwald

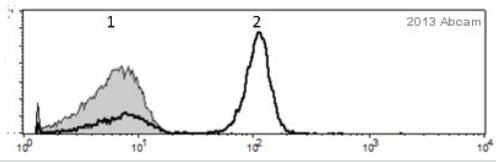
ab2105 at 1/20 staining human liver tissue sections by IHC-P. The cells were paraformaldehyde fixed and a heat mediated antigen retrieval step was performed. The tissue was incubated with the tissue overnight at room temperature. A biotinylated donkey anti-rabbit IgG was used as the secondary antibody and this was detected using streptavidin HRP.



Immunocytochemistry/ Immunofluorescence - Anti-IL-1 beta antibody (ab2105)

Image courtesy of an anonymous Abreview.

ab2105 staining IL-1 beta in human keratinocytes by Immunocytochemistry/ Immunofluorescence. Cells were fixed in methanol, permeabilized in 0,1% Saponin / PBS and then blocked using 4% BSA for 30 minutes at 25°C. Samples were then incubated with ab2105 at a 1/100 dilution for 2 hours at 4°C. An Alexa-fluor 488 conjugated goat anti-rabbit polyclonal was used as the secondary antibody at a 1/100 dilution.

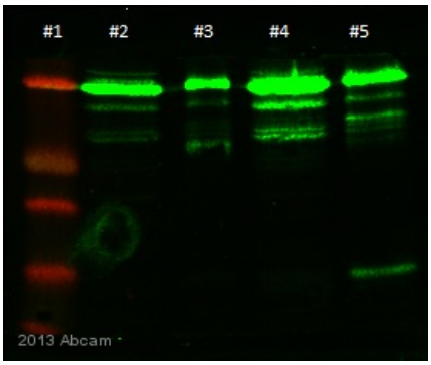


Flow Cytometry - Anti-IL-1 beta antibody (ab2105)

This image is courtesy of an anonymous Abreview

ab2105 staining IL-1 beta in Human monocytes by Flow Cytometry. Cells were fixed with paraformaldehyde and permeabilized with 0.1% saponin in PBS. The sample was incubated with the primary antibody (1/500 in 0.1% saponin + 1% FCS in PBS) for 45 minutes at 4°C. An Alexa Fluor® 488-conjugated Goat anti-rabbit IgG (1/100) was used as the secondary antibody.

1 - unstimulated monocytes. 2 - monocytes stimulated with LPS.



Western blot - Anti-IL-1 beta antibody (ab2105)

This image is courtesy of an anonymous Abreview

Lanes 2-5 : Anti-IL-1 beta antibody (ab2105) at 1/1000 dilution

Lane 1 : Ladder (top to bottom: p35, p25, p20, p17, p11)
Lane 2 : Human THP-1 leukemic monocyte cell lysate, no LPS at 20 µg
Lane 3 : Human THP-1 leukemic monocyte supernatant, no LPS at 20 µg
Lane 4 : Human THP-1 leukemic monocyte cell lysate, LPS stimulation at 20 µg
Lane 5 : Human THP-1 leukemic monocyte supernatant, LPS stimulation at 20 µg

Secondary
Lanes 2-5 : IRDye® 800CW-conjugated Donkey anti-rabbit IgG monoclonal at 1/5000 dilution

Performed under reducing conditions.

Observed band size: 17,30 kDa why is the actual band size different from the predicted?

Exposure time: 30 seconds

Both mature and pro-IL-1beta bands, p17 and p31 were detected by ab2105 in supernatant from THP-1 cells stimulated with LPS. Only pro-IL-1beta band (p31) was detected in supernatant from THP-1 cells not stimulated with LPS.

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