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Datasheet for 209-401-301

IL-1 Beta Antibody

Overview

Description:	Anti-Human IL-1ß (RABBIT) Antibody - 209-401-301
Item No.:	209-401-301
Size:	1 mg
Applications:	ELISA, IHC, WB, Functional Assay
Reactivity:	Human
Host Species:	Rabbit

Product Details

Background:	IL-1 beta (also known as Interleukin-1 beta, IL-1ß and catabolin) is 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. IL-1ß is a monomeric secreted protein that may be released by damaged cells or is secreted by a mechanism differing from that used for other secretory proteins.
Synonyms:	rabbit anti-IL-1 beta Antibody, rabbit anti-interleukin-1 beta antibody, IL-1 beta, Interleukin-1 beta, IL-1ß, catabolin
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	IL1B
Reactivity:	Human
Immunogen Type:	Recombinant Protein

Immunogen:	This antibody was prepared by repeated immunizations with recombinant human IL-1ß produced in E.coli. The MW of the recombinant 153 aa IL-1ß was 17 kDa with the N-terminal amino acid at position alanine 117. This cleavage site is generated by the IL-1ß converting enzyme (ICE, capase-1).
Purity/Specificity:	This is an IgG preparation of whole rabbit serum purified by DEAE fractionation. This antibody is primarily directed against mature, 17,000 MW human IL-1ß and is useful in determining its presence in various assays. In general, this antibody also detects primate IL-1ß in the same formats using similar dilutions. The antiserum does not recognize human IL-1α. In ELISA formats and other immunoreactive assays, this antibody will recognize 10% of the non-denatured (native) precursor 31,000 MW IL-1ß containing samples but will primarily detect all of the 17,000 MW mature molecule. However, in immunoblot analysis of natural cell products or human body fluids, the usual procedure of hearing the sample in SDS with or without reducing agents will facilitate denaturing of the 31,000 MW IL- 1ß precursor molecule. Denatured 31,000 precursor IL-1ß will be recognized by this antibody but often migrates as a 35,000 MW band. This is due to the unfolding of the denatured precursor IL-1ß exposing epitopes not exposed in the natural state. In immunoblots, depending on the number of cells, the antibody detects the 17,000 MW band in supernatants as well as a 35,000 MW band representing the 31,000 MW IL-1ß precursor in lysates.
Relevant Links:	• NCBI - P01584.2 • UniProtKB - P01584 • GeneID - 3553

Application Details

Tested Applications:	ELISA, IHC, WB
Suggested Applications:	Functional Assay (Based on references)

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Application Note:	Anti-Human IL-1ß has been tested for use in ELISA, immunohistochemistry, immunoblotting. This antibody is suitable for neutralizations, radioimmunoassays, flow cytometry, and immunoprecipitation. It recognizes the 17,000 MW mature IL-1ß. For immunoblots, typically, IL-1ß is detected from supernatants or lysates of 2 x 10E6 endotoxin-stimulated peripheral blood mononuclear cells (PBMC). PBMC are stimulated for 24 hours with 1% (v/v) serum plus 10 ng/mL E.coli LPS. For immunoprecipitation pre-clearing the preparation with a non-specific Rabbit IgG (p/n 011-001-297) to reduce background is suggested. For immunohistochemistry either paraffin fixation or cryofixation can be used for sample preparation to stain intracellular IL-1ß. For ELISA use HRP Conjugated Anti-Rabbit IgG [H&L] (Goat) (611-1302) for detection. In ELISA formats this antibody is best used as the second antibody in combination with a monoclonal antibody as a capture antibody. This antibody is also useful for neutralization of human and primate IL-1ß activity in bioassays. It does not neutralize the biological activity IL-1α. It does not neutralize the biological activity of murine, rat or rabbit IL-1ß. For neutralization, it is recommended to incubate the sample with a dilution of the antibody for at least 4 hours before being tested. A control of similarly diluted normal rabbit IgG is recommended. This antibody can be used for FACS analysis. Caution should be exhibited as the F(c) domain of the rabbit IgG molecule may interact with cells non-specifically.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:500 - 1:2,000
FC:	User Optimized
IHC:	1:100 - 1:200
IP:	1:400 - 1:800
Neutralization:	1:100
WB:	1:1,000

Formulation

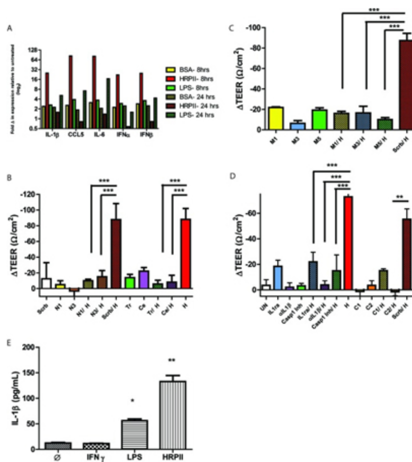
Physical State:	Liquid (sterile filtered)
Concentration:	1.1 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	None
Stabilizer:	None

Shipping & Handling

Shipping Condition:	Dry Ice

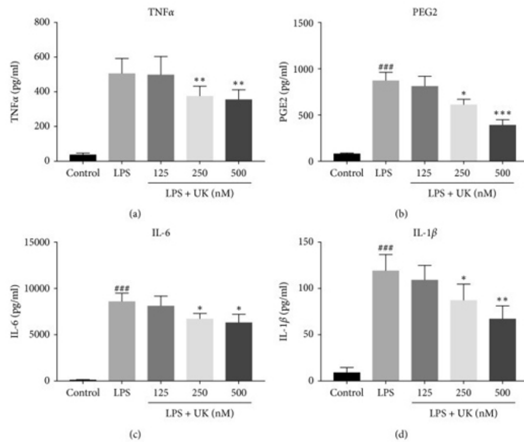
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

Images



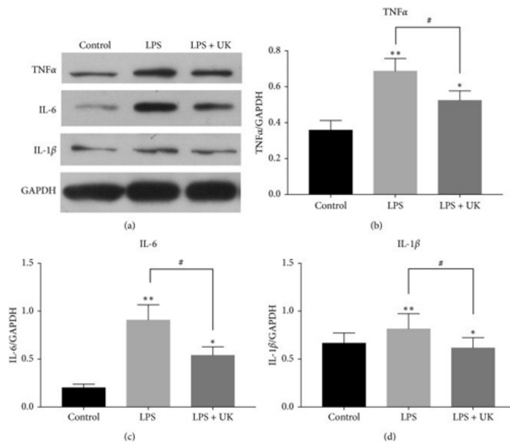
Neutralization

HRPII activates an inflammatory pathway in human cerebral microvascular endothelial cells. (A) qRT-PCR of chemokine/cytokine mRNA levels of hCMEC/D3 cells treated with 25 µg HRPII or BSA for 8 h and 24 h. (B) TEER measurements for in vitro hCMEC/D3 barriers transfected with shRNAs for NFκB (N1 and N3) or a scrambled control (Scrb) for 36 h or incubated with inhibitors for NFκB, celastrol (Ce), and triptolide (tr) for 2 h prior to addition of HRPII (H; 10 µg). Data are mean values ± SEM of results from 6 to 8 replicates pooled from three independent experiments. ***, P < 0.0001 (by one-way ANOVA). (C) TEER measurements for in vitro barriers transfected with shRNAs to MyD88 (M1 and M3 and M5) or a scrambled control (Scrb) for 36 h prior to addition of recombinant purified HRPII (10 µg). Data are mean ± SEM of results from 6 to 8 replicates pooled from 3 independent experiments. ***, P < 0.0001 (by one-way ANOVA). Results of assessment of knockdown levels are shown in Fig. S2 in the supplemental material. (D) TEER measurements for in vitro barriers transfected with shRNAs for caspase-1 (C1 and C2) or a scrambled control (Scrb) for 36 h or with IL-1Ra (500 ng), anti-IL-1β (αIL-1β) (25 ng), or the caspase-1 inhibitor YVAD-CMK (80 µM) (C1 Inh) for 1 h prior to treatment with recombinant purified HRPII (10 µg; H). Data are mean values ± SEM of results from 6 to 8 replicates pooled from four independent experiments. ***, P < 0.001 (by one-way ANOVA); **, P < 0.05 (by one-way ANOVA). (E) Quantitative ELISA for cleaved IL-1β from cell lysates. Cells were treated for 24 h with HRPII (10 µg), LPS (3 µg/ml), or IFN-γ (100 ng/ml) or left untreated. Data represent results from three biological replicates, each performed in triplicate. *, P = 0.0002; **, P = 0.0005 (compared to untreated control by unpaired t test). Fig 3. PMID: 27273825



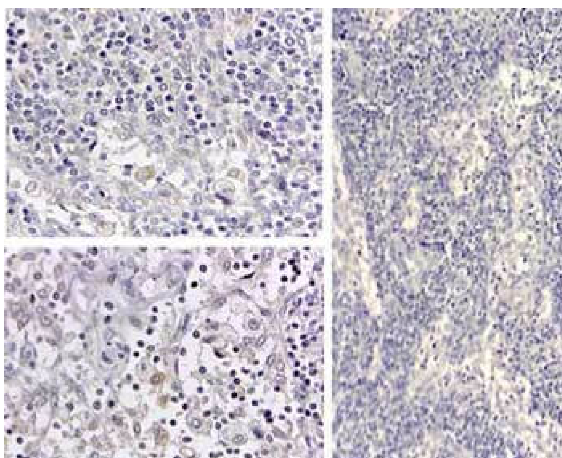
Western Blot

The effect of UK on the expressions of proinflammatory cytokines in LPS- stimulated BV-2 cells. Cells were pretreated with UK (125 nM, 250 nM, and 500 nM) for 12 h and with LPS (1000 ng/mL) treatment for another 12 h. The production of TNFα, PGE2, IL-6, and IL-1β was measured by ELISA kits. ###p < 0.001 versus the control group, �p < 0.05 versus the LPS-stimulated group, ��p < 0.01 versus the control group, and ���p < 0.001 versus the LPS-stimulated group, n = 3. Fig 2. PMID: 31428213



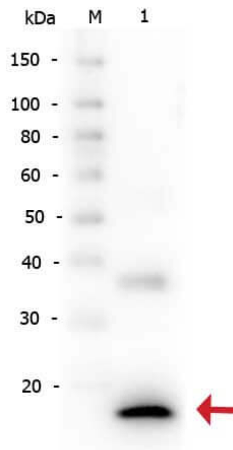
Western Blot

The effect of UK on the protein expressions of proinflammatory cytokines by western blot analysis. Cells were pretreated with UK (500 nM) for 12 h and with LPS (1000 ng/mL) treatment for another 12 h. The production of TNFα, IL-6, and IL-1β was analyzed by western blot. #p < 0.05 versus the LPS-stimulated group, �p < 0.05 versus the control group, and ��p < 0.01 versus the control group, n = 3. Fig 3. PMID: 31428213



Immunohistochemistry

Immunohistochemistry of Human IL1 beta antibody. Tissue: human medullary lymph node. Fixation: formalin fixed paraffin embedded. Antigen retrieval: user optimized. Primary antibody: Human IL1 beta antibody. Secondary antibody: Peroxidase goat anti-rabbit at 1:10,000 for 45 min at RT. Localization: cytoplasm. Staining: Close up of medullary lymph node: positive staining in the cytoplasm of circulating macrophages. Neg Ctr (far right) of normal rabbit IgG with pH 6.2 at 40X.



Western Blot

Western Blot of Rabbit anti-Human IL-1ß antibody. Lane 1: Human IL-1ß. Load: 5 ng per lane. Primary antibody: Human IL-1ß antibody at 1:2,000 for overnight at 4°C. Secondary antibody: Peroxidase rabbit secondary antibody at 1:40,000 for 30 min at RT. Block: Blocking Buffer for Fluorescent Western Blotting (MB-070) for 30 min at RT. Predicted/Observed size: 17 kDa, 17 kDa for Human IL-1ß. Other band(s): Unspecific band at ~35 kDa.

References

- Zhao Z et al. Human Urinary Kallidinogenase Reduces Lipopolysaccharide-Induced Neuroinflammation and Oxidative Stress in BV-2 Cells. *Pain Res Manag.* (2019)
- Pal et al. Plasmodium falciparum Histidine-Rich Protein II Compromises Brain Endothelial Barriers and May Promote Cerebral Malaria Pathogenesis. *mBio* (2016)

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