Human IL-1β/IL-1F2 Antibody

Monoclonal Mouse IgG_1 Clone # 2805

Catalog Number: MAB601

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human IL-1β/IL-1F2 in sandwich ELISAs and Western blots. In sandwich ELISAs, less than 4% cross-reactivity with recombinant rat (rr) IL-1β and less than 0.1% with recombinant porcine (rp) IL-1β, recombinant human IL-1α, rpIL-1α, rrIL-1α, recombinant mouse (rm) IL-1α, and rmIL-1β is observed.
Source	Monoclonal Mouse IgG_1 Clone # 2805
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	*E. coli*-derived recombinant human IL-1β/IL-1F2
Endotoxin Level	<0.10 EU per 1 µg of the antibody by the LAL method.
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

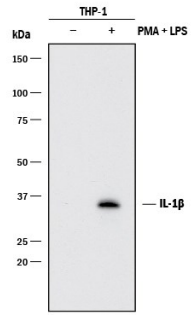
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	1 µg/mL	See Below
Immunocytochemistry	8-25 µg/mL	See Below
Simple Western	10 µg/mL	TF-1 human erythroleukemic cell line
Human IL-1β/IL-1F2 Sandwich Immunoassay		**Reagent**
ELISA Capture	2-8 µg/mL	Human IL-1β/IL-1F2 Antibody (Catalog # MAB601)
ELISA Detection	0.1-0.4 µg/mL	Human IL-1β/IL-1F2 Biotinylated Antibody (Catalog # BAF201)
Standard		Recombinant Human IL-1β/IL-1F2 (Catalog # 201-LB)
Neutralization	Measured by its ability to neutralize IL-1β/IL-1F2-induced proliferation in the D10.G4.1 mouse helper T cell line. The Neutralization Dose (ND_{50}) is typically 0.05-0.2 µg/mL in the presence of 0.05 ng/mL Recombinant Human IL-1β/IL-1F2.	

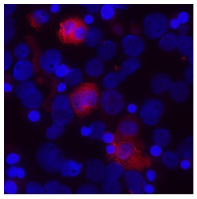
DATA

Western Blot

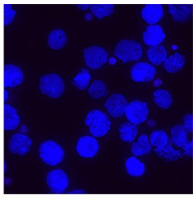


Detection of Human IL-1β/IL-1F2 by Western Blot. Western blot shows lysates of THP-1 human acute monocytic leukemia cell line untreated (-) or treated (+) with 200 nM PMA for 24 hours and 10 µg/mL LPS and 3 hours. PVDF membrane was probed with 1 µg/mL of Mouse Anti-Human IL-1ß/IL-1F2 Monoclonal Antibody (Catalog # MAB601) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # Catalog # HAF018). A specific band was detected for IL-1ß/IL-1F2 at approximately 36 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



Positive (treated THP-1 cells)



Negative (THP-1 WT cells)

Detection of IL-1β/IL-1F2 in THP-1 Human Cell Line. IL-1β/IL-1F2 was detected in immersion fixed THP-1 human acute monocytic leukemia cell line using Mouse Anti-Human IL-1β/IL-1F2 Monoclonal Antibody (Catalog # MAB601) at 25 µg/ml for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to the cytoplasm of THP-1 cells treated with 200nM PMA for 24 hours then 10ug/mL LPS for 24 hours. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

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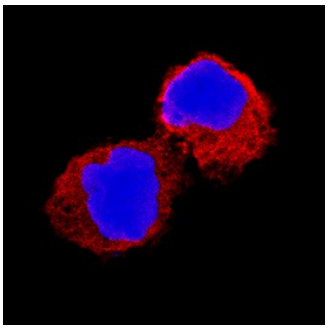
Human IL-1β/IL-1F2 Antibody

Monoclonal Mouse IgG_1 Clone # 2805

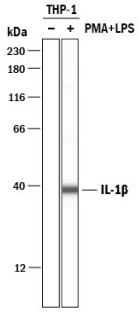
Catalog Number: MAB601

Immunocytochemistry



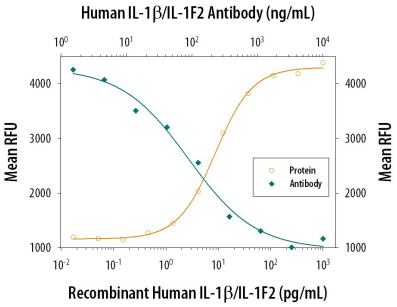
IL-1β/IL-1F2 in Human PBMCs. IL-1ß/IL-1F2 was detected in immersion fixed human peripheral blood mononuclear cells (PBMCs) using Mouse Anti-Human IL-1ß/IL-1F2 Monoclonal Antibody (Catalog # MAB601) at 8 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for Fluorescent ICC Staining of Non-adherent Cells.

Simple Western



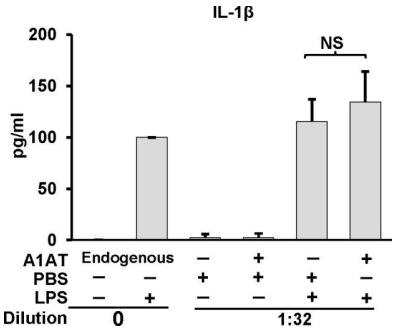
Detection of Human IL-1β/IL-1F2 by Simple Western™. Simple Western lane view shows lysates of THP-1 human acute monocytic leukemia cell line untreated (-) or treated (+) with 200 nm PMA and 10 ug/ml LPS for 24 hrs and 3 hrs, respectively, and loaded at 0.2 mg/mL. A specific band was detected for IL-1β/IL-1F2 at approximately 38 kDa (as indicated) using 10 µg/mL of Mouse Anti-Human IL-1β/IL-1F2 Monoclonal Antibody (Catalog # MAB601) . This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

Neutralization



Cell Proliferation Induced by IL-1β/IL-1F2 and Neutralization by Human IL-1β/IL-1F2 Antibody. Recombinant Human IL-1ß/IL-1F2 (Catalog # Catalog # 201-LB) stimulates proliferation in the the D10.G4.1 mouse helper T cell line in a dose-dependent manner (orange line). Proliferation elicited by Recombinant Human IL-1ß/IL-1F2 (0.05 ng/mL) is neutralized (green line) by increasing concentrations of Mouse Anti-Human IL-1ß/IL-1F2 Monoclonal Antibody (Catalog # MAB601). The ND_{50} is typically 0.05-0.2 µg/mL.

ELISA



Detection of Human IL-1 beta/IL-1F2 by ELISA Effect of A1AT on whole blood IL-1β release. IL-1β production in whole blood cultures in response to LPS (1.0 µg/ml) was performed in the presence of endogenous A1AT (i.e., undiluted) or exogenously added A1AT (2 mg/ml) in blood diluted 1:32 with RPMI. Whole blood cultures were incubated for 18 h. After incubation, plasma supernatants were removed, and IL-1β quantified by ELISA and expressed as mean ± SD for three donors. The diluted sample result was corrected for the dilution. NS indicates no significant difference. Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0117330), licensed under a CC-BY license. Not internally tested by R&D Systems.

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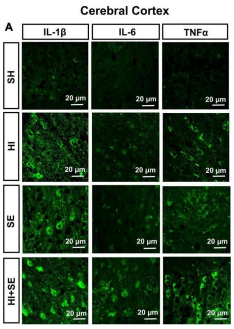
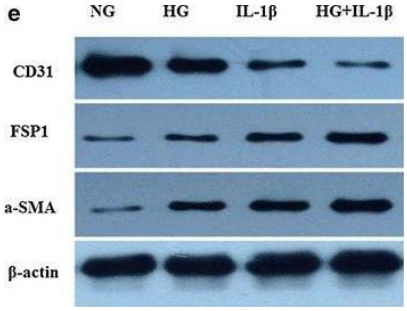
Immunohistochemistry



Detection of Mouse IL-1 beta/IL-1F2 by Immunohistochemistry
Inflammatory response induced by HI injury and maternal SE. Representative images of immunofluorescence staining of inflammatory cytokines IL-1β, IL-6, and TNFα in the cerebral cortex (A) and the hippocampus (E). The immunofluorescence intensity of IL-1β (B,F), IL-6 (C,G), and TNFα (D,H) in the cerebral cortex and hippocampus. Results are presented as mean ± SEM. *P < 0.05, **P < 0.01, n = 4, analyzed by two-way ANOVA followed by post hoc Turkey tests. SH, from sham exposed dams with sham surgery; HI, hypoxic-ischemic injury; SE, from smoke exposed dams with sham surgery; HI + SE, from smoke exposed dams with hypoxic-ischemic injury. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35250486), licensed under a CC-BY license. Not internally tested by R&D Systems.

Western Blot



Detection of Human IL-1 beta/IL-1F2 by Western Blot
Effect of high glucose and IL-1β alone or in combination on the protein expressions of CD31, FSP1 and α-SMA in HAECs. a–d HAECs were incubated for 48 h with NG and HG. Mannitol was used as a control for hyperosmolarity. Representative western blots (a) and quantitative determinations of CD31, FSP1 and α-SMA protein levels (b–d) are presented. e–h HAECs were treated for 48 h with NG, HG, IL-1β (10 ng/ml)and HG in the presence of the IL-1β (10 ng/ml). Representative western blots (E) and quantitative determinations of CD31, FSP1 and α-SMA protein levels (f–h) are presented. The data are expressed as the mean ± SD. Experiments were repeated at least three times. NG normal glucose (5.5 mM), HG high glucose (30 mM), MN 5.5 mM glucose + 24.5 mM mannitol, IL-1β (10 ng/ml), HG + IL-1β: high glucose (30 mM) + IL-1β (10 ng/ml) *P < 0.05 vs. MN or NG, **P < 0.01 vs. NG, #P < 0.05 vs. HG Image collected and cropped by CiteAb from the following publication (https://www.cardiab.com/content/15/1/42), licensed under a CC-BY license. Not internally tested by R&D Systems.

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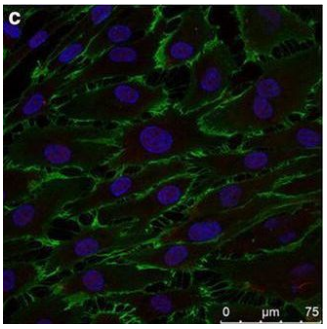
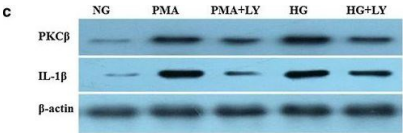
Immunocytochemistry/ Immunofluorescence



Detection of Human IL-1 beta/IL-1F2 by Immunocytochemistry/Immunofluorescence The influence of high glucose or IL-1β on immunofluorescence of CD31 and FSP1 in HAECs. Representative immunofluorescence images showing CD31 (green), FSP1 (red) labeling and DAPI (blue) stains nuclei. a Normal ECs monolayers displayed a cobble stone morphology. b A merge of the three images revealed some cells populations that acquired a spindle-shaped morphology and lost CD31 expression (white arrow). c HAECs exposure to IL-1β alone for 48 h acquired a spindle-shaped morphology. d High glucose and IL-1β in combination resulted in decreased CD31 (the left white arrow) and increased FSP1staining (the right arrow). a normal glucose (5.5 mM) group, b high glucose (30 mM) group for 48 h; c treatment with a normal glucose (5.5 mM) + IL-1β (10 ng/ml) treatment for 48 h, d treatment with a high glucose (30 mM) + IL-1β (10 ng/ml) treatment for 48 h. Scale bar, 75 µm Image collected and cropped by CiteAb from the following publication (https://www.cardiab.com/content/15/1/42), licensed under a CC-BY license. Not internally tested by R&D Systems.

Western Blot



Detection of Human IL-1 beta/IL-1F2 by Western Blot Effects of PKCβ on high glucose induced IL-1β up-regulation. Confluent cultures of HAECs were exposed to NG, HG, PMA (30 nM) and HG in the presence of the selective PKCβ inhibitors (LY317615, 0.3 µM) for 48 h. Real-time PCR analyses showed mRNA expression of PKCβ and IL-1β (a, b). Representative western blots (c) and quantitative determinations of PKCβ and IL-1β (d, e) are presented. The data are expressed as the mean ± SD. Experiments were repeated at least three times. NG normal glucose (5.5 mM), HG high glucose (30 mM), PMA (30 nM): phorbol 12-myristate13-acetate; LY (0.3 uM): LY317615; *P < 0.05 vs.NG, **P < 0.01 vs. NG, #P < 0.05 vs. HG or PMA Image collected and cropped by CiteAb from the following publication (https://www.cardiab.com/content/15/1/42), licensed under a CC-BY license. Not internally tested by R&D Systems.

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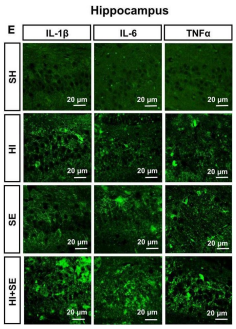
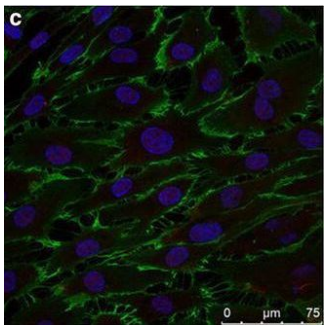


Detection of Mouse IL-1 beta/IL-1F2 by Immunohistochemistry Inflammatory response induced by HI injury and maternal SE. Representative images of immunofluorescence staining of inflammatory cytokines IL-1β, IL-6, and TNFα in the cerebral cortex (A) and the hippocampus (E). The immunofluorescence intensity of IL-1β (B,F), IL-6 (C,G), and TNFα (D,H) in the cerebral cortex and hippocampus. Results are presented as mean ± SEM. *P < 0.05, **P < 0.01, n = 4, analyzed by two-way ANOVA followed by post hoc Turkey tests. SH, from sham exposed dams with sham surgery; HI, hypoxic-ischemic injury; SE, from smoke exposed dams with sham surgery; HI + SE, from smoke exposed dams with hypoxic-ischemic injury. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35250486), licensed under a CC-BY license. Not internally tested by R&D Systems.

Immunocytochemistry/ Immunofluorescence



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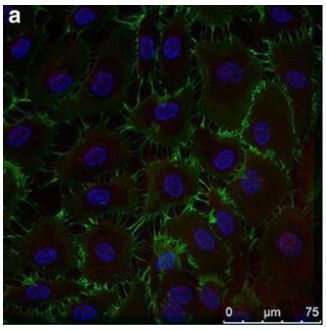
Immunocytochemistry/ Immunofluorescence



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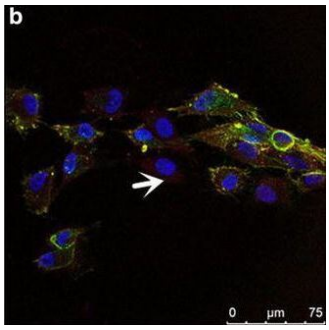
Immunocytochemistry/ Immunofluorescence



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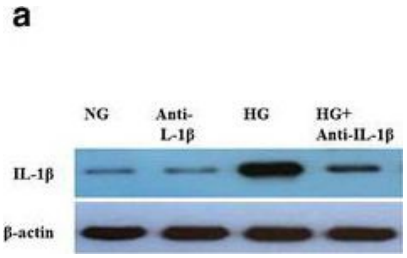
Western Blot



Detection of Human IL-1 beta/IL-1F2 by Western Blot The influence of blocking IL-1β treatment on the protein expressions of CD31, FSP1, a-SMA, and IL-1β. (a–f) HAECs were incubated for 48 h with anti-IL-1β antibodies (1000 ng/ml) in the presence of NG or HG. (a1–f1) We performed gene-silencing experiments using transfection with siRNA specific for IL-1β. The protein expressions of IL-1β, CD31, FSP1 and α-SMA were assessed by western blotting. The data are expressed as the mean ± SD. Experiments were repeated at least three times. NG normal glucose (5.5 mM), HG high glucose (30 mM). Anti-IL-1β: anti-IL-1β antibodies (1000 ng/ml). *P < 0.05 vs. NG or anti-IL-1β, #P < 0.05 vs. HG or HG +Vehicle Image collected and cropped by CiteAb from the following publication (https://www.cardiab.com/content/15/1/42), licensed under a CC-BY license. Not internally tested by R&D Systems.

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PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS. For liquid material, refer to CoA for concentration.
Shipping	Lyophilized product is shipped at ambient temperature. Liquid small pack size (-SP) is shipped with polar packs. Upon receipt, store immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

IL-1 is a name that designates two pleiotropic cytokines, IL-1α (IL-1F1) and IL-1β (IL-1F2, IL1B), which are the products of distinct genes. IL-1α and IL-1β are structurally related polypeptides that share approximately 21% amino acid (aa) identity in human. Both proteins are produced by a wide variety of cells in response to inflammatory agents, infections, or microbial endotoxins. While IL-1α and IL-1β are regulated independently, they bind to the same receptor and exert identical biological effects. IL-1 RI binds directly to IL-1α or IL-1β and then associates with IL-1 R accessory protein (IL-1 R3/IL-1 R AcP) to form a high-affinity receptor complex that is competent for signal transduction. IL-1 RII has high affinity for IL-1β but functions as a decoy receptor and negative regulator of IL-1β activity. IL-1ra functions as a competitive antagonist by preventing IL-1α and IL-1β from interacting with IL-1 RI. Intracellular cleavage of the IL-1 beta precursor by Caspase-1/ICE is a key step in the inflammatory response. The 17 kDa molecular weight mature human IL-1β shares 96% aa sequence identity with rhesus and 67%-78% with canine, cotton rat, equine, feline, mouse, porcine, and rat IL-1β. IL-1β functions in a central role in immune and inflammatory responses, bone remodeling, fever, carbohydrate metabolism, and GH/IGF-I physiology. IL-1 beta dysregulation is implicated in many pathological conditions including sepsis, rheumatoid arthritis, inflammatory bowel disease, acute and chronic myelogenous leukemia, insulin-dependent diabetes mellitus, atherosclerosis, neuronal injury, and aging-related diseases.

PRODUCT SPECIFIC NOTICES

This product is covered by one or more patents, including US Patent # 5,681,933.

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