Anti-IL-1 beta Antibody [A7F9]

HA601002

Product Type:	Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, ELISA, IF-Cell
Molecular Wt:	Predicted band size: 31 kDa
Clone number:	A7F9

Description: The protein encoded by this gene is a member of the interleukin 1 cytokine family. This cytokine is produced by activated macrophages as a proprotein, which is proteolytically processed to its active form by caspase 1 (CASP1/ICE). This cytokine is an important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis. The induction of cyclooxygenase-2 (PTGS2/COX2) by this cytokine in the central nervous system (CNS) is found to contribute to inflammatory pain hypersensitivity. Similarly, IL-1B has been implicated in human osteoarthritis pathogenesis. Patients with severe Coronavirus Disease 2019 (COVID-19) present elevated levels of pro-inflammatory cytokines such as IL-1B in bronchial alveolar lavage fluid samples. The lung damage induced by the Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is to a large extent, a result of the inflammatory response promoted by cytokines such as IL-1B. This gene and eight other interleukin 1 family genes form a cytokine gene cluster on chromosome 2.

Immunogen: Recombinant protein within Human IL-1 beta aa 117-269 / 269.

Positive control: THP-1 treated with 80nM TPA overnight then replaced with 100ng/mL LPS for 6 hours add 300ng/mL BFA for last 3 hours cell lysate, RAW264.7 treated with 80nM TPA overnight then replaced with 100ng/mL LPS for 6 hours add 300ng/mL BFA for last 3 hours cell lysate, HeLa cells treated with 80nM TPA overnight then replaced with 100ng/mL LPS for 6 hours add 300ng/mL BFA for last 3 hours.

Subcellular location: Extracellular exosome, Secreted, Lysosome, Cytosol.

Database links: SwissProt: P01584 Human | P10749 Mouse | Q63264 Rat

Recommended Dilutions:

WB	1:1,000-1:2,000
ELISA	1:20,000
IF-Cell	1:100

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

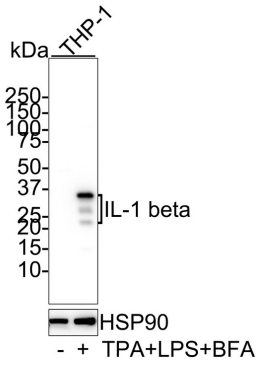

Fig1: Western blot analysis of IL-1 beta on different lysates with Mouse anti-IL-1 beta antibody (HA601002) at 1/1,000 dilution.

Lane 1: THP-1 cell lysate
Lane 2: THP-1 treated with 80nM TPA overnight then replaced with 100ng/mL LPS for 6 hours add 300ng/mL BFA for last 3 hours cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 31 kDa
Observed band size: 31/28/20 kDa

Exposure time: 5 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA601002) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

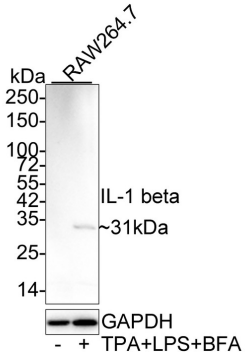

Fig2: Western blot analysis of IL-1 beta on different lysates with Mouse anti-IL-1 beta antibody (HA601002) at 1/2,000 dilution.

Lane 1: RAW264.7 cell lysate
Lane 2: RAW264.7 treated with 80nM TPA overnight then replaced with 100ng/mL LPS for 6 hours add 300ng/mL BFA for last 3 hours cell lysate

Lysates/proteins at 30 µg/Lane.

Predicted band size: 31 kDa
Observed band size: 31 kDa

Exposure time: 3 minutes 25 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA601002) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

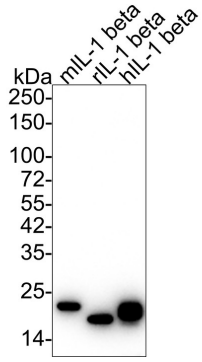

Fig3: Western blot analysis of IL-1 beta on different proteins with Mouse anti-IL-1 beta antibody (HA601002) at 1/2,000 dilution.

Lane 1: Recombinant mouse IL-1 beta
Lane 2: Recombinant rat IL-1 beta
Lane 3: Recombinant human IL-1 beta

Lysates/proteins at 50 ng/Lane.

Exposure time: 30 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA601002) at 1/2,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

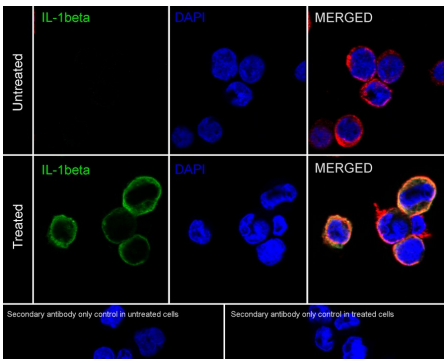

Fig4: Immunocytochemistry analysis of HeLa cells treated with 80nM TPA overnight then replaced with 100ng/mL LPS for 6 hours add 300ng/mL BFA for last 3 hours labeling IL-1 beta with Mouse anti-IL-1 beta antibody (HA601002) at 1/100 dilution.

Cells were fixed in 100% precooled methanol for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Mouse anti-IL-1 beta antibody (HA601002) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

beta Tubulin (ET1602-4, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) were used as the secondary antibody at 1/1,000 dilution.

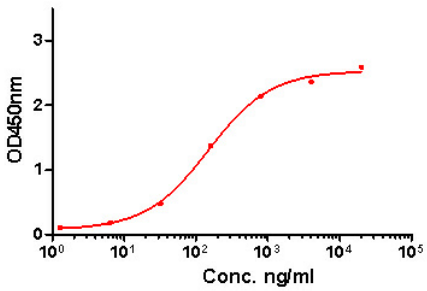

Fig5: IL-1 beta Antibody (HA601002) in indirect ELISA.

Indirect ELISA analysis of IL-1 beta was performed by coating wells of a 96-well plate with 50 µl per well of IL-1 beta antigen diluted in carbonate/bicarbonate buffer, at a concentration of 1 µg/mL overnight at 4℃. Wells of the plate were washed, blocked with StartingBlock blocking buffer, and incubated with 50 µl per well of a mouse IL-1 beta monoclonal antibody starting at a concentration of 20 µg/mL and serially diluting it to a concentration of 1.28 ng/mL for 2 hours at room temperature. The plate was washed and incubated with 50 µl per well of an HRP-conjugated goat anti-mouse IgG secondary antibody at a dilution of 1:10,000 for one hour at room temperature. Detection was performed using an Ultra TMB Substrate for 5 minutes at room temperature in the dark. The reaction was stopped with sulfuric acid and absorbances were read on a spectrophotometer at 450 nm.

Background References

1. Zou L. et. al. HO-1 induced autophagy protects against IL-1 beta-mediated apoptosis in human nucleus pulposus cells by inhibiting NF-kappaB. Aging (Albany NY). 2020 Feb
2. Libby P. Interleukin-1 Beta as a Target for Atherosclerosis Therapy: Biological Basis of CANTOS and Beyond. J Am Coll Cardiol. 2017 Oct