abcam

ab283818

Anti-IL-1 beta antibody [RM1009]

Anti-IL-1 beta antibody [RM1009] (ab283818) is a rabbit recombinant multiclonal antibody detecting IL-1 beta in **Western Blot, Flow Cytometry, IP, IHC-P**. Suitable for **Human, Mouse, Rat**.

- KO validated for confirmed specificity
- Biophysical QC for unrivalled batch-batch consistency
- Over 30 publications

Lab Essentials | Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Multiclonal
Immunogens	The exact immunogen used to generate this antibody is proprietary information.
Clone number	RM1009
Specificity	IL-1 beta is not present under homeostatic conditions, and it is induced and secreted only upon inflammatory signals. Stimulation may be required for the detection of IL-1β, no matter in precursor or mature form (PMID: 22019906). IL-1β is a secreted protein, it is recommended to treat cells with anti-secretion reagents (e.g. Brefeldin A) when test IL-1β using whole cell lysate in WB. Please navigate to the Product Protocol section for specificity details in Chinese.
Concentration	0.521 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ICC

Tested

Species	Mouse
Dilution info	1/50
Notes	-

Species	Human
Dilution info	1/50
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

IP

Tested

Species	Mouse
Dilution info	1/30
Notes	-

Species	Human
Dilution info	1/30
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt

Tested

Species	Mouse
Dilution info	1/500
Notes	-

Species	Human
Dilution info	1/500
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/1000
Notes	-

Species	Rat
Dilution info	1/1000
Notes	-

Species	Human
Dilution info	1/1000
Notes	-

IHC-P

Tested

Species	Mouse

Dilution info	1/500
Notes	-

Not recommended

Species	Human, Rat
Dilution info	-
Notes	-

Target data

See full target information IL1B

Function	Potent pro-inflammatory cytokine (PubMed:10653850, PubMed:12794819, PubMed:28331908, PubMed:3920526). 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 (PubMed:3920526). Promotes Th17 differentiation of T-cells. Synergizes with IL12/interleukin-12 to induce IFNG synthesis from T-helper 1 (Th1) cells (PubMed:10653850). Plays a role in angiogenesis by inducing VEGF production synergistically with TNF and IL6 (PubMed:12794819). Involved in transduction of inflammation downstream of pyroptosis: its mature form is specifically released in the extracellular milieu by passing through the gasdermin-D (GSDMD) pore (PubMed:33377178, PubMed:33883744). Acts as a sensor of S.pyogenes infection in skin: cleaved and activated by pyogenes SpeB protease, leading to an inflammatory response that prevents bacterial growth during invasive skin infection (PubMed:28331908).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Product Specifications
Anti-IL-1 beta antibody [RM1009] (ab283818) is a recombinant rabbit multiclonal antibody which is comprised of multiple monoclonal antibodies targeting different epitopes and recapitulating the performance of a polyclonal. Each component clone was developed by Abcam using patented rabbit monoclonal antibody technology.
Anti-IL-1 beta antibody [RM1009] (ab283818) is validated for use in Flow Cyt, ICC, IHC-P, IP and WB in human, mouse and rat samples.
Anti-IL-1 beta antibody [RM1009] (ab283818) specifically detects IL-1 beta/ Interleukin-1 beta (UniProt ID: P01584; Molecular weight: 31kDa) and is sold in a convenient trial size to enable initial testing (20 µL) and larger sizes for subsequent scaling up experiments (100 µL and 1 mL).

Quality and Validation
Abcams high quality manufacturing and validation processes ensure anti-IL-1 beta antibody [RM1009] (ab283818) has high sensitivity and specificity alongside high lot-to-lot consistency and reproducibility.
The specificity of anti-IL-1 beta antibody [RM1009] (ab283818) has been confirmed by testing in knockout samples.

Related Products
Conjugation-ready, carrier free format available for antibody clone RM1009 - ab283822.
What are recombinant multiclonals?
Recombinant multiclonals are a mixture of recombinant antibodies co-expressed from a library of heavy and light chains. They offer several advantages including:

- The sensitivity of polyclonal antibodies by recognising multiple epitopes
- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

View our range of recombinant multiclonal antibodies.

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

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

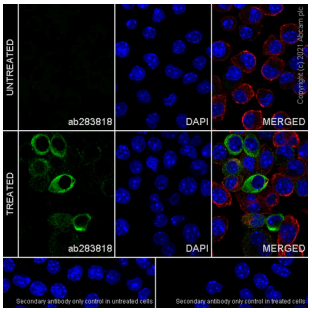
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

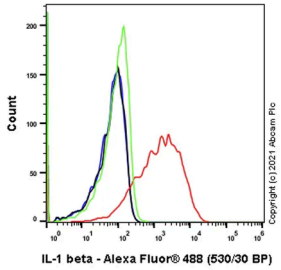
15 product images



Immunocytochemistry - Anti-IL-1 beta antibody [RM1009] (ab283818)

IL-1 beta Immunocytochemistry staining using rabbit Anti-IL-1 beta antibody

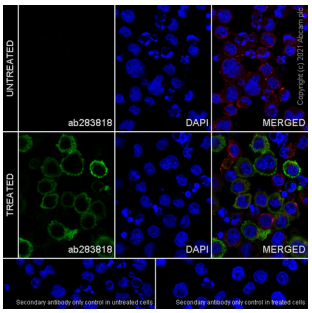
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized J774A.1 cells labelling IL-1 beta with ab283818 at 1/50 (10.58 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2 ug/ml) dilution (Green). Confocal image showing cytoplasmic staining in J774A.1 cells treated with Lipopolysaccharides (100 ng/ml) for 4h, with the addition of brefeldin A (1 ug/ml) for the last 3 hours. ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 (2.5 ug/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue).
Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/ml) dilution.



Flow Cytometry (Intracellular) - Anti-IL-1 beta antibody [RM1009] (ab283818)

IL-1 beta Flow Cytometry (Intracellular) staining using rabbit Anti-IL-1 beta antibody

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized U-937 (Human histiocytic lymphoma monocyte) treated with 100nM PMA for 2days then 1µg/ml LPS treated for 13h and add 5µg/ml BFA for another 4h (Red) / U-937 treated with 100nM PMA for 2days (Green) cells labelling IL-1 beta with ab283818 at 1/500 dilution (0.1ug) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/2000 dilution was used as the secondary antibody.



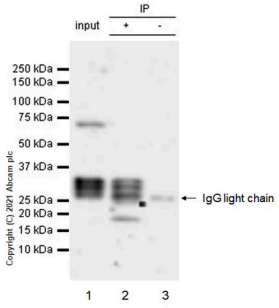
Immunocytochemistry - Anti-IL-1 beta antibody [RM1009] (ab283818)

IL-1 beta Immunocytochemistry staining using rabbit Anti-IL-1 beta antibody

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized U-937 cells labelling IL-1 beta with ab283818 at 1/50 (10.58 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2 ug/ml) dilution (Green). Confocal image showing cytoplasmic staining in U-937 cells treated with Phorbol-12-

myristate-13-acetate (100 nM) for 2 days, then replaced it with Lipopolysaccharides (1 µg/ml) for 13 h, with the addition of Brefeldin A (5 µg/ml) for the last 4 hours. ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 (2.5ug/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2ug/ml) dilution.



Immunoprecipitation - Anti-IL-1 beta antibody [RM1009] (ab283818)

IL-1 beta was immunoprecipitated from 0.35 mg U-937 (Human histiocytic lymphoma monocyte) treated with 100nM PMA for 2 days, then 1µg/ml LPS for 13h and add 5µg/ml BFA for another 4 h, whole cell lysate 10ug with ab283818 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab283818 at 1/1000 dilution. VeriBlot for IP secondary antibody (HRP) (ab131366) was used at 1/5000 dilution.
Lane 1: U-937 (Human histiocytic lymphoma monocyte) treated with 100nM PMA for 2 days, then 1µg/ml LPS for 13h and add 5µg/ml BFA for another 4 h, whole cell lysate 10ug

Lane 2: ab283818 IP in U-937 treated with 100nM PMA for 2 days, then 1µg/ml LPS for 13h and add 5µg/ml BFA for another 4 h, whole cell lysate

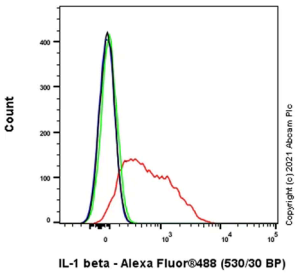
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab283818 in U-937 treated with 100nM PMA for 2 days, then 1µg/ml LPS for 13h and add 5µg/ml BFA for another 4 h, whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

All lanes:
Immunoprecipitation - Anti-IL-1 beta antibody [RM1009] (ab283818) at 1/1000 dilution

Predicted band size: 30 kDa

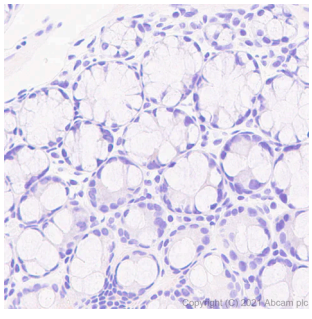
Observed band size: 17.5 kDa, 28 kDa, 31 kDa



Flow Cytometry (Intracellular) - Anti-IL-1 beta antibody [RM1009] (ab283818)

IL-1 beta Flow Cytometry (Intracellular) staining using rabbit Anti-IL-1 beta antibody

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized J774A.1 (Mouse reticulum cell sarcoma monocyte macrophage) treated with 100ng/ml LPS treated for 4h and add 1µg/ml BFA for another 3h (Red) / Untreated control (Green) cells labelling IL-1 beta with ab283818 at 1/500 dilution (0.1ug) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/2000 dilution was used as the secondary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IL-1 beta antibody [RM1009] (ab283818)

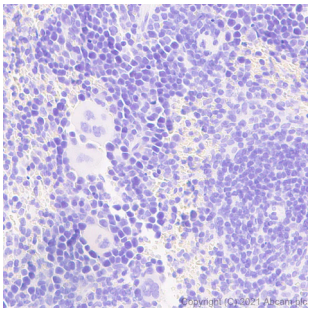
IL-1 beta Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-IL-1 beta antibody

Immunohistochemical analysis of paraffin-embedded Mouse colon tissue labelling IL-1 beta with ab283818 at 1/500 (1.058 ug/ml) followed by a ready to use LeicaDS9800 (Bond™, Polymer Refine

Detection). The section was incubated with ab283818 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.
Negative control: no staining on mouse colon.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™, Polymer Refine Detection).

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.



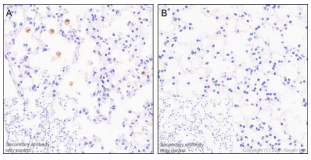
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IL-1 beta antibody [RM1009] (ab283818)

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

Immunohistochemical analysis of paraffin-embedded Mouse spleen tissue labelling IL-1 beta with ab283818 at 1/500 (1.058 ug/ml) followed by a ready to use LeicaDS9800 (Bond™, Polymer Refine Detection). The section was incubated with ab283818 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.
Negative control: no staining on mouse spleen.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™, Polymer Refine Detection).

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.

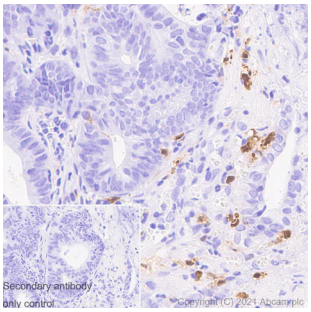


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IL-1 beta antibody [RM1009] (ab283818)

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

Immunohistochemical analysis of paraffin-embedded Mouse lung tissue labelling IL-1 beta with ab283818 at 1/500 (1.058 ug/ml) followed by a ready to use LeicaDS9800 (Bond™, Polymer Refine Detection). Positive staining on mouse lung treated with LPS (1ug/ml for 16 hours) and BFA (1ug/ml for 16h hours) (Image A) and no staining on control mouse lung was observed (Image B). The section was incubated with ab283818 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin. Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™, Polymer Refine Detection).

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins

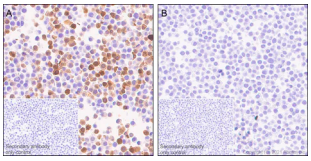


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IL-1 beta antibody [RM1009] (ab283818)

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

Immunohistochemical analysis of paraffin-embedded Mouse colon carcinom tissue labelling IL-1 beta with ab283818 at 1/500 (1.058 ug/ml) followed by a ready to use LeicaDS9800 (Bond™, Polymer Refine Detection). Positive staining on the lamina propria in mouse colon carcinoma. The section was incubated with ab283818 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin. Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™, Polymer Refine Detection).

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.

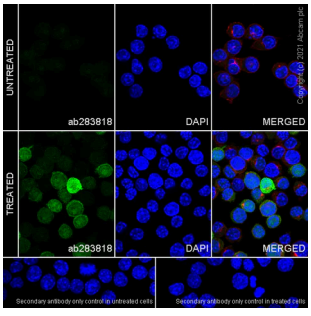


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IL-1 beta antibody [RM1009] (ab283818)

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

Immunohistochemical analysis of paraffin-embedded RAW 264.7 cells labelling IL-1 beta with ab283818 at 1/500 (1.058 ug/ml) followed by a ready to use LeicaDS9800 (Bond™, Polymer Refine Detection). Positive staining on RAW 264.7 cells treated with LPS (100ng/ml for 4+3 hours) added BFA (1µg/ml for 3 hours) (Image A) and no staining on control RAW 264.7 (Image B). The section was incubated with ab283818 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin. Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™, Polymer Refine Detection).

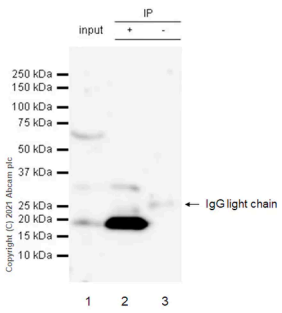
Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins



Immunocytochemistry - Anti-IL-1 beta antibody [RM1009] (ab283818)

IL-1 beta Immunocytochemistry staining using rabbit Anti-IL-1 beta antibody

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized RAW 264.7 cells labelling IL-1 beta with ab283818 at 1/50 (10.58 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2 ug/ml) dilution (Green). Confocal image showing cytoplasmic and nuclear staining in RAW 264.7 cells transfected with Lipopolysaccharides (100 ng/ml) for 4h, with the addition of brefeldin A (1 ug/ml) for the last 3 hours. (PMID:18490713, PMID:18939951). ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 (2.5ug/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue).
Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/ml) dilution.



Immunoprecipitation - Anti-IL-1 beta antibody [RM1009] (ab283818)

This data was developed using ab283818, the same antibody clone in a different buffer formulation.IL-1 beta was immunoprecipitated from 0.35 mg RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) treated with 100ng/ml LPS for 4h and add 1ug/ml BFA for another 3 h whole cell lysate 10ug with ab283818 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab283818 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(ab131366) was used at 1/5000 dilution.
Lane 1: RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) treated with 100ng/ml LPS for 4h and add 1ug/ml BFA for another 3 h whole cell lysate 10ug

Lane 2: ab283818 IP in RAW 264.7 treated with 100ng/ml LPS for 4h and add 1ug/ml BFA for another 3 h whole cell lysate

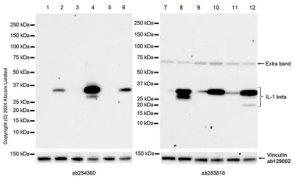
Lane 3:Rabbit monoclonal IgG (ab172730) instead of ab283818 in RAW 264.7 treated with 100ng/ml LPS for 4h and add 1ug/ml BFA for another 3 h whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time:

All lanes:
Immunoprecipitation - Anti-IL-1 beta antibody [RM1009] (ab283818)

Predicted band size: 30 kDa



Western blot - Anti-IL-1 beta antibody [RM1009] (ab283818)

IL-1 beta Western blot staining using rabbit Anti-IL-1 beta antibody

Expression of IL-1 beta is induced by LPS treatment. 31-kDa precursor IL-1 beta, 28- and 17.5-kDa proteolytically cleaved IL-1 beta are observed. The expression pattern and molecular weight observed is consistent with what has been described in the literature (PMID: 8446594, 19559631).

Compared with ab254360, ab283818 has higher affinity. For better using ab254360, we recommend loading higher amount of human/rat lysate or using lower antibody dilution.

Lanes 1 - 6:
Western blot - Anti-IL-1 beta antibody [EPR23851-127] (ab254360) at 1/1000 dilution

Lanes 7 - 12:
Western blot - Anti-IL-1 beta antibody [RM1009] (ab283818)

Lanes 1 and 7:
Untreated THP-1 (human monocytic leukemia monocyte) whole cell lysate at 20 µg

Lanes 2 and 8:
THP-1 treated with 100ng/ml lipopolysaccharide (LPS) for 3h whole cell lysate at 20 µg

Lanes 3 and 9:
Untreated RAW 264.7 (mouse abelson murine leukemia virus-induced tumor macrophage) whole cell lysate at 20 µg

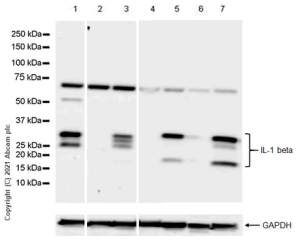
Lanes 4 and 10:
RAW 264.7 treated with 100 ng/ml LPS and 300 ng/ml Brefeldin A (ab193369) for 4 hours, whole cell lysate at 20 µg

Lanes 5 and 11:
Untreated NR8383 (rat lung macrophage (alveolar)) whole cell lysate at 20 µg

Lanes 6 and 12:
NR8383 treated with 100 ng/ml LPS for 7 hours and 1 µg/ml Brefeldin A (ab193369) for the last 3 hours, whole cell lysate at 20 µg

Observed band size: 17.5 kDa, 28 kDa, 31 kDa

Exposure time: 102s



Western blot - Anti-IL-1 beta antibody [RM1009] (ab283818)

IL-1 beta Western blot staining using rabbit Anti-IL-1 beta antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST
Expression of IL-1 beta is induced by LPS treatment. 31-kDa precursor IL-1 beta, 28- and 17.5-kDa proteolytically cleaved IL-1 beta are observed.

The expression pattern and molecular weight observed is consistent with what has been described in the literature (PMID: 8446594, 19559631)

Exposure time: Lane 1-3: 3 min ; Lane 4-7: 70 seconds

All lanes:
Western blot - Anti-IL-1 beta antibody [RM1009] (ab283818) at 1/1000 dilution

Lane 1:
U-87 MG (Human glioblastoma-astrocytoma epithelial cell) whole cell lysate at 20 µg

Lane 2:
U-937 (Human histiocytic lymphoma monocyte) whole cell lysate at 20 µg

Lane 3:
U-937 treated with 100nM PMA for 2 days, then 1µg/ml LPS for 13h and add 5µg/ml BFA for another 4 h whole cell lysate at 20 µg

Lane 4:
RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate at 20 µg

Lane 5:
RAW 264.7 treated with 100ng/ml LPS for 4h and add 1µg/ml BFA for another 3 h whole cell lysate at 20 µg

Lane 6:
NR8383 (Rat lung macrophage (alveolar)) whole cell lysate at 20 µg

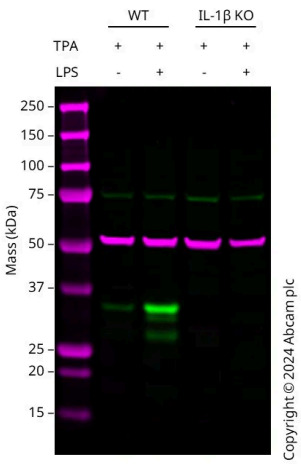
Lane 7:
NR8383 treated with 100ng/ml LPS for 4h and add 1µg/ml BFA for another 3 h whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 30 kDa

Observed band size: 17.5 kDa, 28 kDa, 31 kDa



Western blot - Anti-IL-1 beta antibody [RM1009] (ab283818)

IL-1 beta Western blot staining using rabbit Anti-IL-1 beta antibody

Western blot: Anti-IL1B antibody [RM1009] (ab283818) staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab283818 was shown to bind specifically to IL1B. A band was observed at 34 kDa in wild-type THP-1 cell lysates with no signal observed at this size in IL1B knockout cell line. To generate this image, wild-type and IL1B knockout THP-1 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.
All lanes:
Western blot - Anti-IL-1 beta antibody [RM1009] (ab283818) at 1/1000 dilution

Lane 1:
Wild-type THP-1 Vehicle control: TPA (80 nM overnight) then LPS (0 ng/mL, 6 h) cell lysate at 20 µg

Lane 2:
Wild-type THP-1 TPA (80 nM overnight) then LPS (100 ng/mL, 6 h) cell lysate at 20 µg

Lane 3:
IL-1b knockout THP-1 Vehicle control: TPA (80 nM overnight) then LPS (0 ng/mL, 6 h) cell lysate at 20 µg

Lanes 3 - 4:
Western blot - Human IL1B knockout THP-1 cell line (ab273762)

Lane 4:
IL-1b knockout THP-1 TPA (80 nM overnight) then LPS (100 ng/mL, 6 h) cell lysate at 20 µg

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