

Product Datasheet

Caspase-1 Antibody (14F468) - BSA Free NB100-56565SS

Unit Size: 0.025 mg

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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NB100-56565SS

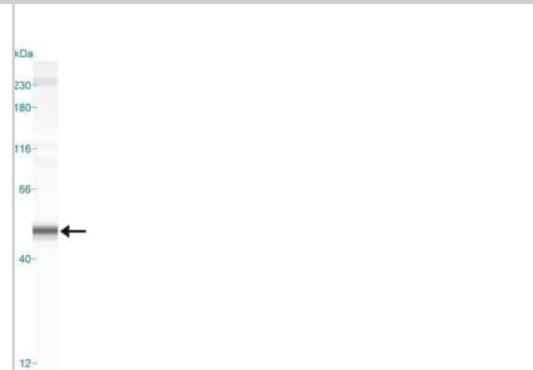
Caspase-1 Antibody (14F468) - BSA Free

Product Information	
Unit Size	0.025 mg
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	14F468
Preservative	0.02% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein G purified
Buffer	PBS
Target Molecular Weight	45.2 kDa
Product Description	
Description	Novus Biologicals Mouse Caspase-1 Antibody (14F468) - BSA Free (NB100-56565) is a monoclonal antibody validated for use in IHC, WB, ELISA, ICC/IF and Simple Western. Anti-Caspase-1 Antibody: Cited in 70 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Mouse
Gene ID	834
Gene Symbol	CASP1
Species	Human, Mouse, Rat
Reactivity Notes	Immunogen's sequence similarity with other species: Porcine/Pig (85%), Equine/Horse (80%), Canine (70%). Rat reactivity reported in scientific literature (PMID: 22133203).
Specificity/Sensitivity	Caspase-1 Antibody (14F468) will recognize full-length Caspase-1 and cleaved Caspase-1 forms that retain amino acids 371-390 of the Caspase-1 protein.
Immunogen	Caspase-1 Antibody (14F468) was developed against two synthetic peptides from the human Caspase-1 protein (amino acids 371-390 and 31-45) [UniProt P29466].
Product Application Details	
Applications	Western Blot, Simple Western, Immunohistochemistry-Paraffin, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen
Recommended Dilutions	Western Blot 0.5-2 ug/ml, Simple Western 1:50, Immunohistochemistry 1:100 - 1:500, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry-Paraffin 1:10-1:500, Immunohistochemistry-Frozen reported in scientific literature (PMID 30930743)
Application Notes	<p>Staining of formalin-fixed tissues is enhanced by boiling tissue sections in 10 mM sodium citrate buffer, pH 6.0 for 10-20 min followed by cooling at RT for 20 min.</p> <p>In Simple Western only 10 - 15 uL of the recommended dilution is used per data point.</p> <p>See Simple Western Antibody Database for Simple Western validation: Tested in HeLa lysate 1.0 mg/mL, separated by Size, antibody dilution of 1:50, apparent MW was 51 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue.</p>

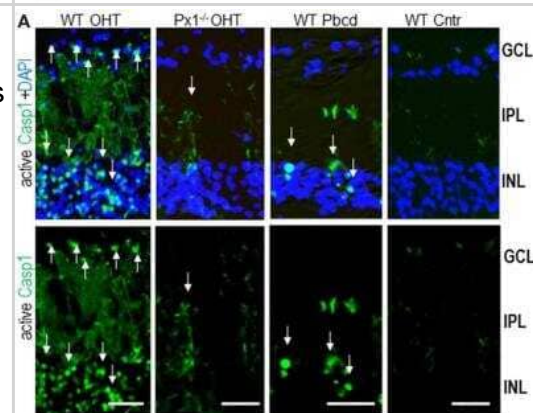


Images

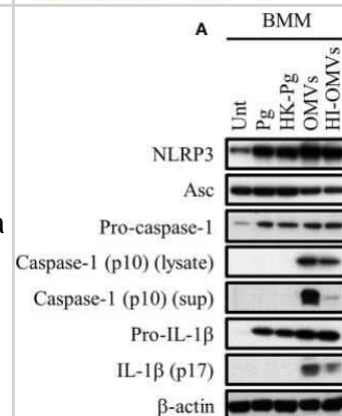
Simple Western lane view shows a specific band for Caspase 1 in 1.0 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



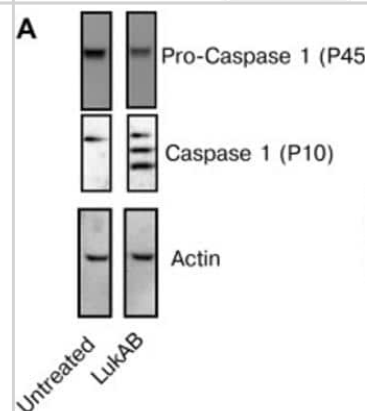
Activity of Casp1 in OHT-injured and normotensive control eyes. (A) Casp1 was detected by intraocular injection FLICA660-labeled substrate (green) in vivo 24 h after injury. Bright labeling (arrows) is evident in cells in the GCL and inner nuclear layer (INL) layers of the OHT-challenged retinas, a diffuse labeling of cell processes located in the IPL. Casp1 activity is diminished in *Panx1*^{-/-} (*Px1*^{-/-} OHT) retinas and WT retinas treated with probenecid (WT/Pbcd) at 12 h postinjury. Image collected and cropped by CiteAb from the following publication (<https://www.frontiersin.org/article/10.3389/fnmol.2019.00036/full>), licensed under a CC-BY license.



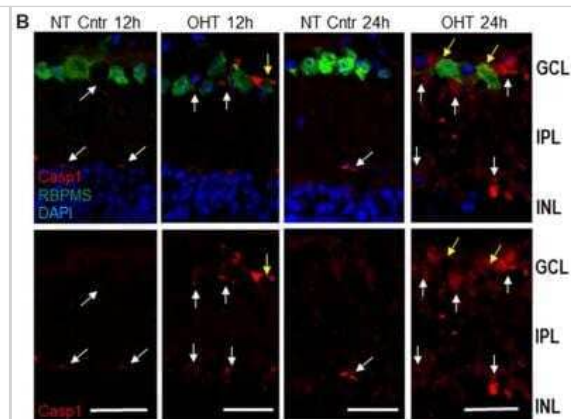
P. gingivalis and its OMVs differentially induce inflammasome signaling and pyroptosis in murine macrophages. BMM were infected as before (2 h at MOI of 25:1, see Materials and Methods) with viable *P. gingivalis* (Pg), heat-killed-Pg (HK-Pg), OMVs, or heat-inactivated-OMVs (HI-OMVs) and the activation of inflammasome components in the lysates [or supernatants (sup) where indicated] measured after 24 h by Western blot; beta-actin serves as a loading control throughout. Western blot data are representative of at least three independent experiments. Image collected and cropped by CiteAb from the following publication (<https://journal.frontiersin.org/article/10.3389/fcimb.2017.00351/full>), licensed under a CC-BY license.



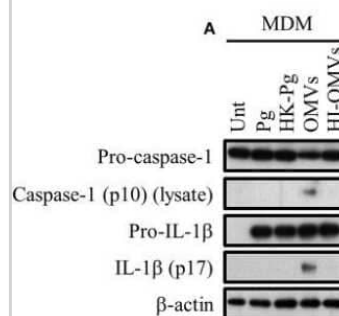
LukAB is a potent activator of Caspase 1. THP1 cells were intoxicated with 50 ng/mL LukAB for 1 hour and cell lysates were analyzed by immunoblot for Caspase 1 cleavage, which indicates activation. Image collected and cropped by CiteAb from the following publication (<https://doi.org/10.1371/journal.ppat.1004970>) licensed under a CC-BY license.



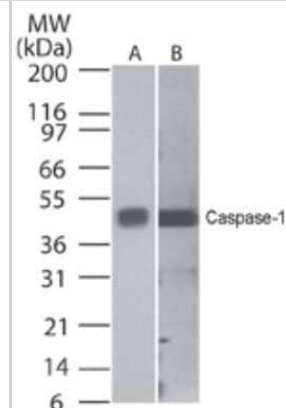
Activity of Casp1 in OHT-injured and normotensive control eyes. (B) The analysis of the Casp1 immunolabeling (red, white arrows) in normotensive (NT control) and injured (OHT) retinas at 12 h and 24 h postinjury. Yellow arrows denote Casp1 colocalization with RGCs (RBPMS +, green) as well as with other cells at 24 h post-OHT. Bar, 25 μ m. Image collected and cropped by CiteAb from the following publication (<https://www.frontiersin.org/article/10.3389/fnmol.2019.00036/full>), licensed under a CC-BY license.



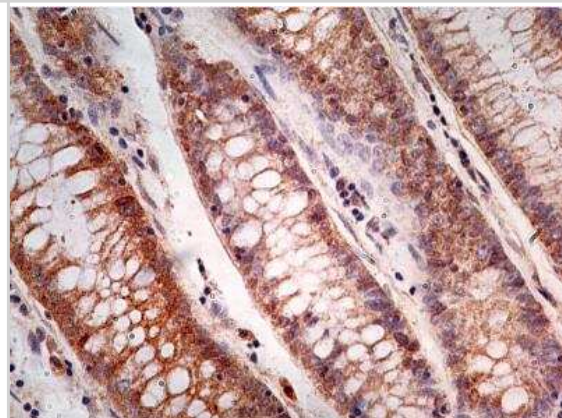
P. gingivalis and its OMVs differentially induce inflammasome signaling and pyroptosis in human macrophages. MDM were infected as before (2 h at MOI of 25:1, see Materials and Methods) with viable *P. gingivalis* (Pg), heat-killed-Pg (HK-Pg), OMVs, or heat-inactivated-OMVs (HI-OMVs) and the activation of inflammasome components in the lysates was measured after 24 h by Western blot; beta-actin serves as a loading control throughout. Western blot data are representative of at least three independent experiments. Image collected and cropped by CiteAb from the following publication (<https://journal.frontiersin.org/article/10.3389/fcimb.2017.00351/full>), licensed under a CC-BY license.



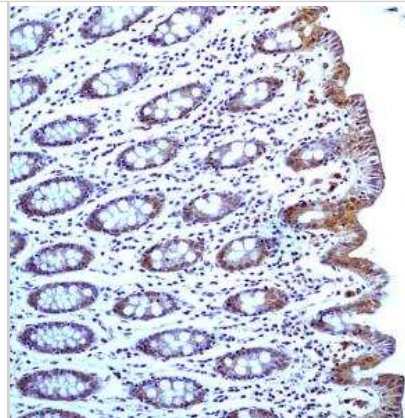
Analysis of Caspase-1 using a Caspase-1 monoclonal antibody. Human HeLa (A) and mouse NIH3T3 lysate probed with Caspase-1 antibody at 0.5 μ g/ml and 2 μ g/ml, respectively.



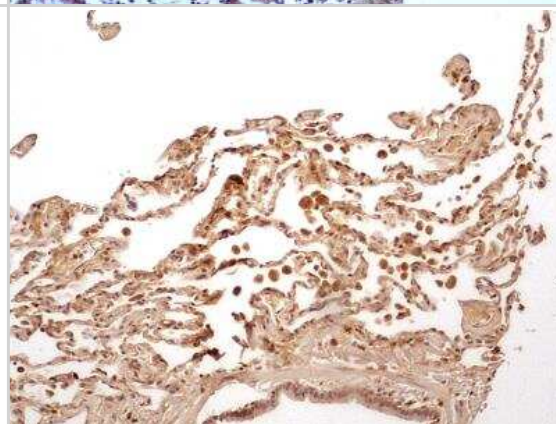
Adenocarcinoma of the rectum stained with Caspase-1 antibody (5 μ g/ml), peroxidase-conjugate and DAB chromogen. Staining of formalin-fixed tissues is enhanced by boiling tissue sections in 10 mM sodium citrate buffer, pH 6.0 for 10-20 min followed by cooling at RT for 20 min.



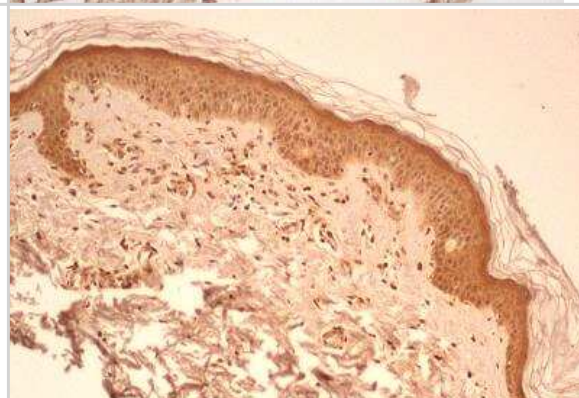
Detection of Caspase-1 protein in a section of normal human colon using 5 ug/ml concentration of Caspase 1 antibody (clone 14F468). Distinct cytoplasmic staining along with some nuclear positivity was observed in crypts/mucosa, and staining was found to be more intense in the absorptive columnar epithelial cells. [10X Magnification]



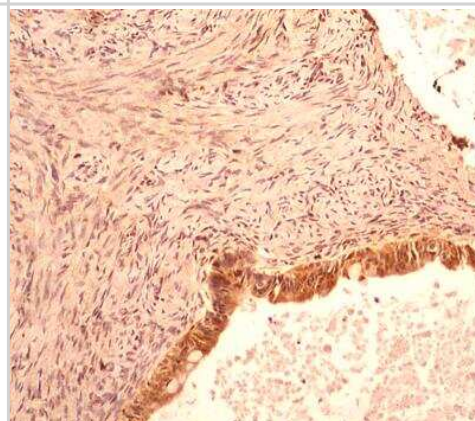
Normal lung from human using 5 ug/ml concentration of Caspase 1 antibody (clone 14F468). In this representative lung section, different type of cells including pseudostratified columnar epithelium of bronchiole and the simple squamous epithelium of alveoli may be seen to develop immunoreactivity for Caspase 1. [10X Magnification]



Normal skin from human using 5 ug/ml concentration of Caspase 1 antibody (clone 14F468). Strong cytoplasmic/nuclear staining developed in all the epidermal cells, blood vessels and some cells of the dermal connective tissues layer. [10X Magnification]



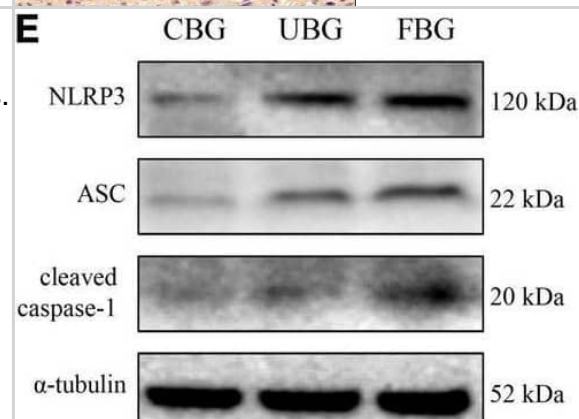
Detection of Caspase-1 in a section of human ovarian cancer using 5 ug/ml concentration of Caspase 1 antibody (clone 14F468).



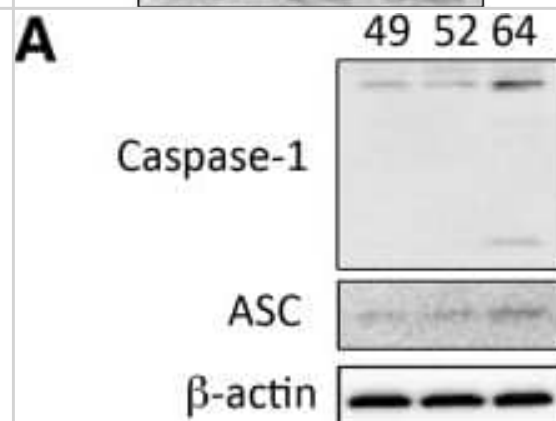
Tissue section of human intestine using Caspase-1 antibody (clone 14F468) at 5ug/ml concentration (1:200 dilution). The primary antibody binding to Caspase 1 in cells was detected using HRP conjugated anti-Mouse secondary antibody with DAB reagent, and the sections were further counterstained with hematoxylin for labeling cellular nuclei. This Caspase 1 antibody generated a diffused but specific cytoplasmic staining in columnar epithelia cells of villi, and a few cells depicted nuclear staining also. Only a subset of connective tissue cells in lamina propria depicted positivity (cytoplasmic) for this protein.



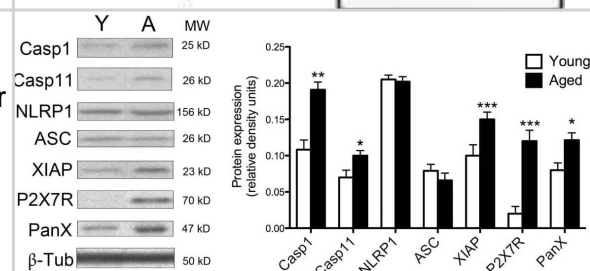
Western Blot: Caspase-1 Antibody (14F468) - BSA Free [NB100-56565] - Glucose fluctuations promoted the activation of NLRP3 inflammasome. (A) Immunofluorescence staining of NLRP3-ASC interaction in rat hearts. The heart sections were labeled with anti-NLRP3 (red), anti-ASC (green), & DAPI (blue) (n = 3 per group). (B–D) The mRNA levels of NLRP3, IL-1 β & IL-18 in rat hearts of the three groups (n = 4 per group). (E–H) The protein expressions of NLRP3, ASC & cleaved caspase-1 in rat hearts of the three groups (n = 4 per group). Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/35592403>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



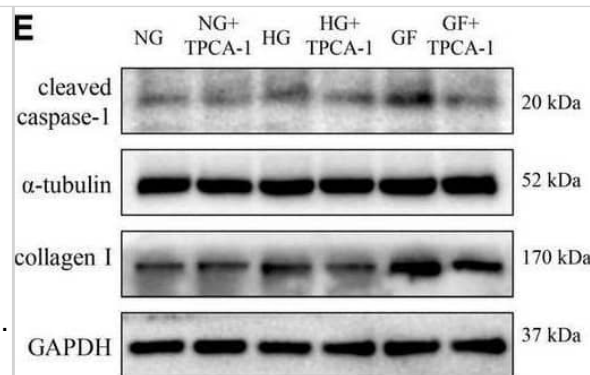
Western Blot: Caspase-1 Antibody (14F468) - BSA Free [NB100-56565] - Caspase-1 expression increases with age in human fibroblasts: a Immunoblot analysis of caspase-1 & ASC in human fibroblasts obtained from a donor at the ages of 49, 52 & 64 y/o. b Immunocytochemistry image of human fibroblasts stained for caspase-1 (green) & Phalloidin (red) from a donor at three different ages (49, 52 & 64 y/o). Bar graph: 100 μ m Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/30473634>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



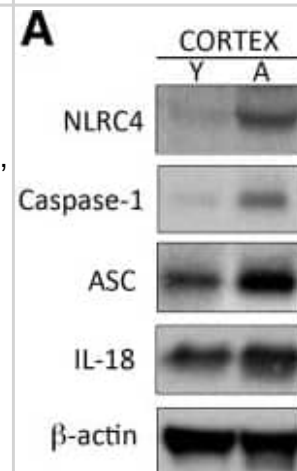
Western Blot: Caspase-1 Antibody (14F468) - BSA Free [NB100-56565] - Aging alters expression of NLRP1 inflammasome components. Representative immunoblots & densitometric analysis of immunoblots for caspase-1 (Casp1), caspase-11 (Casp11), NLRP1, ASC, cleaved XIAP, P2X7 receptor (P2X7R) & pannexin-1 (PanX1) in brain lysates of young (Y) & aged (A) animals. Protein levels of cleaved caspase-1 & -11 are higher in aged animals compared to young. Protein levels of NLRP1 & ASC did not change with age whereas P2X7 receptor, the pannexin-1 protein & the cleaved fragment of XIAP are higher in aged animals than in young animals. β -Tubulin was used as an internal standard & control for protein loading. Data are presented as mean \pm SEM. *p < 0.05, **p < 0.01, ***p < 0.005 compared with young. n = 6 per group. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/22133203>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



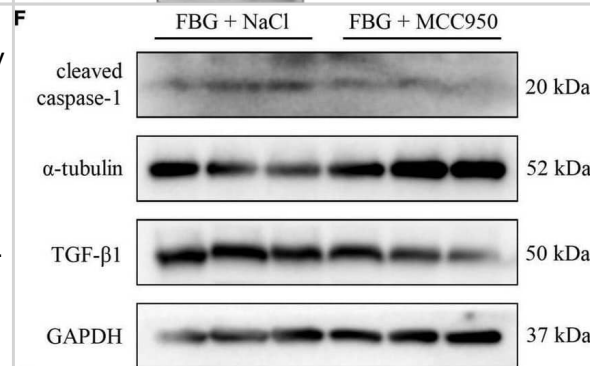
Western Blot: Caspase-1 Antibody (14F468) - BSA Free [NB100-56565]
 - Role of NF- κ B in glucose fluctuation-induced inflammasome-related myocardial fibrosis. (A) Immunofluorescence staining of NF- κ B/p65 nucleation (n = 3 per group). (B,C) The phosphorylation level of NF- κ B/p65 in rat hearts of the three groups (n = 4 per group). (D) Immunofluorescence staining of intracellular NLRP3–ASC interaction in the GF & GF + TPCA-1 groups. The NRCFs were labeled with anti-NLRP3 (red), anti-ASC (green), & DAPI (blue) (n = 3 per group). (E–G) After application of TPCA-1 (0.5 μ M), the protein expressions of cleaved caspase-1 & collagen I were measured by western blot (n = 3 per group). Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/35592403>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



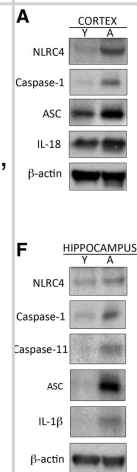
Western Blot: Caspase-1 Antibody (14F468) - BSA Free [NB100-56565]
 - Inflammasome proteins are elevated in the cytosolic fraction of aged mice: a Representative image of immunoblot analyses of inflammasome proteins in the cytosolic fraction of the cortex of young (Y) & aged (A) mice. Quantification of immunoblot analysis of NLRC4 (b), caspase-1 (c), ASC (d), IL-18 (e) in the cortex. a Representative image of immunoblot analyses of inflammasome proteins in the cytosolic fraction of the hippocampus of young (Y) & aged (A) mice. Quantification of immunoblot analysis of NLRC4 (g), caspase-1 (h), caspase-11 (i), ASC (j), IL-1 β (k) in the hippocampus. Data presented as mean \pm SEM. N = 5 per group. *p < 0.05 Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/30473634>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



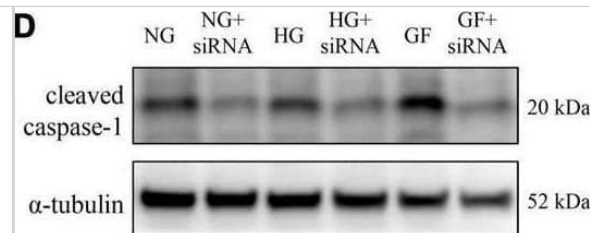
Western Blot: Caspase-1 Antibody (14F468) - BSA Free [NB100-56565]
 - NLRP3 inhibition with MCC950 reversed myocardial fibrosis induced by glucose fluctuations. (A) Representative images of hematoxylin & eosin (HE) staining for myocardial tissue in the FBG + NaCl & FBG + MCC950 groups (n = 3 per group). (B–C) Representative images of myocardial fibrosis & fibrosis area (%) in the FBG + NaCl & FBG + MCC950 groups (n = 4 per group). (D–K) The protein expressions of NLRP3, cleaved caspase-1, TGF- β 1, collagen I & collagen III in the FBG + NaCl & FBG + MCC950 groups (n = 6 per group). Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/35592403>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



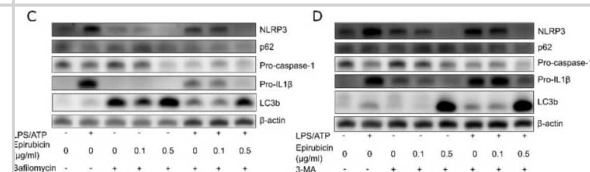
Western Blot: Caspase-1 Antibody (14F468) - BSA Free [NB100-56565]
 - Inflammasome proteins are elevated in the cytosolic fraction of aged mice: a Representative image of immunoblot analyses of inflammasome proteins in the cytosolic fraction of the cortex of young (Y) & aged (A) mice. Quantification of immunoblot analysis of NLRC4 (b), caspase-1 (c), ASC (d), IL-18 (e) in the cortex. a Representative image of immunoblot analyses of inflammasome proteins in the cytosolic fraction of the hippocampus of young (Y) & aged (A) mice. Quantification of immunoblot analysis of NLRC4 (g), caspase-1 (h), caspase-11 (i), ASC (j), IL-1 β (k) in the hippocampus. Data presented as mean \pm SEM. N = 5 per group. *p < 0.05 Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/30473634>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



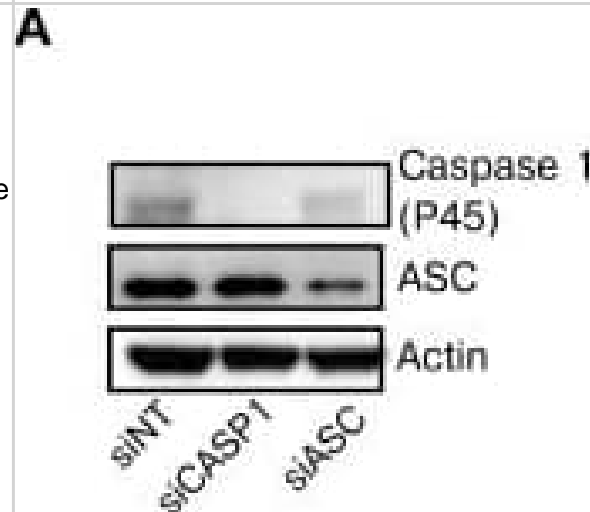
Western Blot: Caspase-1 Antibody (14F468) - BSA Free [NB100-56565]
 - Role of inflammasome in glucose fluctuation-induced myocardial fibrosis. (A) Immunofluorescence staining of intracellular NLRP3–ASC interaction examined by confocal microscopy. The NRCFs were labeled with anti-NLRP3 (red), anti-ASC (green), & DAPI (blue) (n = 3 per group). (B–G) After siRNA targeting NLRP3 transfection, the protein expressions of NLRP3, cleaved caspase-1 & collagen I were measured by western blot (n = 3 per group). (H) Representative images of immunofluorescence staining of intracellular α -SMA in the NG, HG, GF, & GF + siRNA groups (n = 3 per group). Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/35592403>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



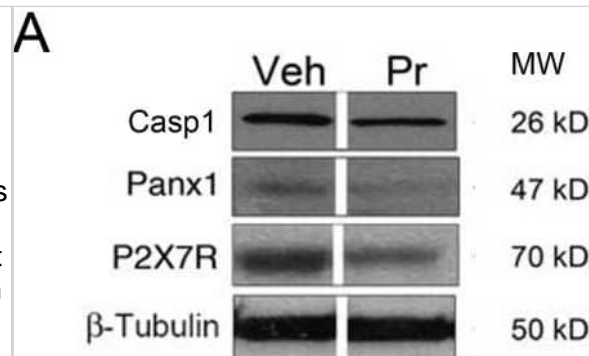
Western Blot: Caspase-1 Antibody (14F468) - BSA Free [NB100-56565]
 - Epirubicin suppresses the NLRP3 inflammasome in presence of autophagy inhibitors. (A) LPS/ATP-induced IL-1 β secretion from PM in the absence or presence of bafilomycin & 3-MA is shown. Means \pm SEM from 10 (IL-1 β) independent experiments are shown. (B) Representative immunoblot of LC3b after epirubicin & LPS/ATP treatments. (C,D) Representative immunoblots of inflammasome & autophagy components after inhibition with (C) bafilomycin or (D) 3-MA in PM whole cell lysates. Cells were treated with indicated concentrations of epirubicin treatment over 24 h, followed by LPS (10 ng/mL) in the absence or presence of bafilomycin (300 nM) or 3-MA (10 mM) for 3 h & NLRP3 activation by ATP (1 mM, 1 h). Whole cell lysates were subjected to SDS-PAGE (10% gel) & processed for immunoblot analysis with specific antibodies. Here, β -actin served as loading control. Statistical tests: one-sample t-test (A) ** p < 0.01, *** p < 0.001. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/31905600>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: Caspase-1 Antibody (14F468) - BSA Free [NB100-56565]
 - Genetic or pharmacologic disruption of Caspase 1 blocks LukAB-induced cytokine secretion but not cell death. (A) THP1 cells were transfected with siRNA against Caspase 1, ASC or a non-targeting sequence. Cell lysates were collected & analyzed by immunoblot to confirm knockdown of pro-Caspase 1 & ASC. (B) THP1 siRNA cells were incubated with propidium iodide & intoxicated with 50 ng/mL LukAB for 1 hour then analyzed by flow cytometry. (C & D) THP1 siRNA cells were either primed with LTA (500ng/mL) (C) or untreated (D) then intoxicated with LukAB (50ng/mL) for 1 hr before culture supernatants were analyzed for release of IL-1 β (C) or IL-18 (D). (E & F) Primary CD14+ human monocytes were incubated with the indicated concentration of z-VYAD-FMK or VX-765 for 30 minutes. Primary monocytes were incubated in the presence of propidium iodide (E) then intoxicated with LukAB (50ng/mL) & analyzed by flow cytometry. (F) Primary monocytes were intoxicated with LukAB (50ng/mL) & culture supernatants were analyzed for release of IL-18. Propidium iodide staining & IL-18 secretion are reported as a fraction of measurement in primary cells not treated with inhibitor. Bars represent the mean \pm standard error of the mean for at least two independent experiments, each performed in triplicate. Asterisks indicate significance at a p-value of \leq 0.05 by Tukey's multiple comparisons post-test for 1-way or 2-way ANOVA, as appropriate. Image collected & cropped by CiteAb from the following publication (<https://dx.plos.org/10.1371/journal.ppat.1004970>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: Caspase-1 Antibody (14F468) - BSA Free [NB100-56565] - Probenecid reduces protein expression of NLRP1 inflammasome & ameliorates spatial learning deficits in aged rats. (A) Representative immunoblots of cleaved caspase-1, pannexin1 & P2X7R in hippocampal lysates of vehicle (Veh)-treated & probenecid (Pr)-treated 18-month-old rats. β -tubulin was used as an internal control. (B) Densitometric analysis of immunoblots from brain lysates of cleaved caspase-1 (Casp1), P2X7 receptor (P2X7R), & pannexin1 (PanX1). (C-D) Aged animals underwent behavioral testing following either probenecid or vehicle treatment. (C) In a hippocampal-dependent spatial learning task via Morris water maze, latency to platform was measured on days 1-3 & 8-10. Probenecid-treatment improved latency to platform measured on the final day of testing (D) Mean path length was determined on day 10 of testing & probenecid-treated rats demonstrated significantly shorter mean path lengths than vehicle-treated controls. Drug treatment was administered twice daily for 3 days (days 7-9). Data are presented as mean \pm SEM * $p < 0.05$, ** $p < 0.005$ compared to vehicle. N = 6-8/per group. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/22133203>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Chi W, Hua X, Chen X et al. Mitochondrial DNA oxidation induces imbalanced activity of NLRP3/NLRP6 inflammasomes by activation of caspase-8 and BRCC36 in dry eye. J. Autoimmun. 2017-02-23 [PMID: 28238526]

Zhang ZY, Dang SP, Li SS et al. Glucose Fluctuations Aggravate Myocardial Fibrosis via the Nuclear Factor- κ B-Mediated Nucleotide-Binding Oligomerization Domain-Like Receptor Protein 3 Inflammasome Activation Frontiers in Cardiovascular Medicine 2022-05-03 [PMID: 35592403]

Yue RZ, Li YJ, Su BH et al. Atorvastatin reduces contrast media-induced pyroptosis of renal tubular epithelial cells by inhibiting the TLR4/MyD88/NF- κ B signaling pathway BMC nephrology 2023-02-02 [PMID: 36732683]

Fei Gao, Dian Xiong, Zhaorui Sun, Jingbo Shao, Dong Wei, Shinan Nie ARC DPBNPs suppress LPS-induced acute lung injury via inhibiting macrophage pyroptosis and M1 polarization by ERK pathway in mice. International immunopharmacology 2024-04-10 [PMID: 38457983]

Spurlock M, An W, Reshetnikova G et al. Inflammasome-Dependent Dysfunction and Death of Retinal Ganglion Cells after Repetitive Intraocular Pressure Spikes preprints.org 2023-09-29 [PMID: 37998361] (ELISA, Mouse)

Chen JW, Shan TK, Wei TW et al. SIRT3-dependent mitochondrial redox homeostasis mitigates CHK1 inhibition combined with gemcitabine treatment induced cardiotoxicity in hiPSC-CMs and mice Archives of toxicology 2023-12-01 [PMID: 37798514] (Western Blot, Mouse)

Yang L, Lu P, Qi X et al. Metformin inhibits inflammatory response and endoplasmic reticulum stress to improve hypothalamic aging in obese mice iScience 2023-09-01 [PMID: 37860765] (WB, Mouse)

Li F, Wang C, Wang J et al. Resveratrol Attenuates Exercise-induced Acute Kidney Injury by Inhibiting NLRP3 Inflammasome-mediated Renal Tubular Pyroptosis Research Square 2023-07-24 (ICC/IF, WB, Rat)

Fu Y, Cao J, Wei X et al. Klotho alleviates contrast-induced acute kidney injury by suppressing oxidative stress, inflammation, and NF-KappaB/NLRP3-mediated pyroptosis International immunopharmacology 2023-05-01 [PMID: 37018977] (Western Blot, Human)

Sobrano Fais R, Menezes da Costa R, Carvalho Mendes A et al. NLRP3 activation contributes to endothelin-1-induced erectile dysfunction Journal of cellular and molecular medicine 2023-01-01 [PMID: 36515571] (WB, Mouse)

Details:

Dilution used in WB 1:500

Missiroli S, Perrone M, GafA R et al. PML at mitochondria-associated membranes governs a trimeric complex with NLRP3 and P2X7R that modulates the tumor immune microenvironment Cell death and differentiation 2022-11-30 [PMID: 36450825] (WB, Mouse)

Xie J, Zhu CL, Wan XJ et al. GSDMD-mediated NETosis promotes the development of acute respiratory distress syndrome European journal of immunology 2022-10-17 [PMID: 36250416] (WB, Mouse)

More publications at <http://www.novusbio.com/NB100-56565>



Procedures

Western Blot Protocol for Caspase-1 Antibody (NB100-56565)

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in 1% Non-fat milk in TBST and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers inst

Immunohistochemistry-Paraffin Protocol for Caspase-1 Antibody (NB100-56565)

Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:

Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer all the time).

Staining:

1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.





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Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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