Product Datasheet

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

Unit Size: 0.1 mg

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

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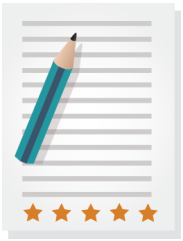


NB100-56565

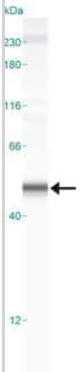
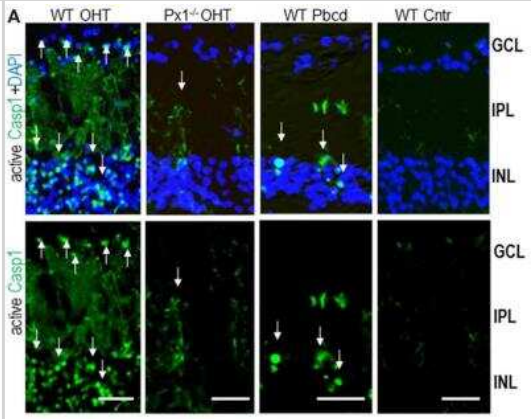
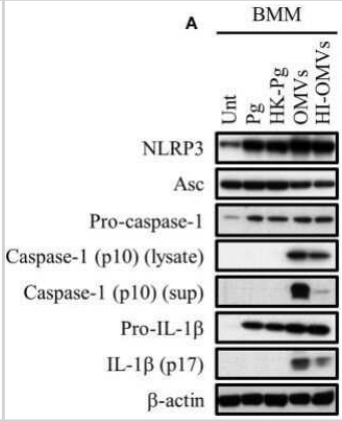
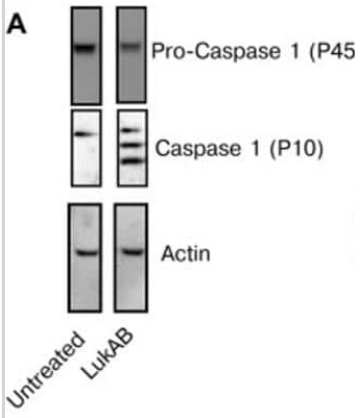
Caspase-1 Antibody (14F468) - BSA Free

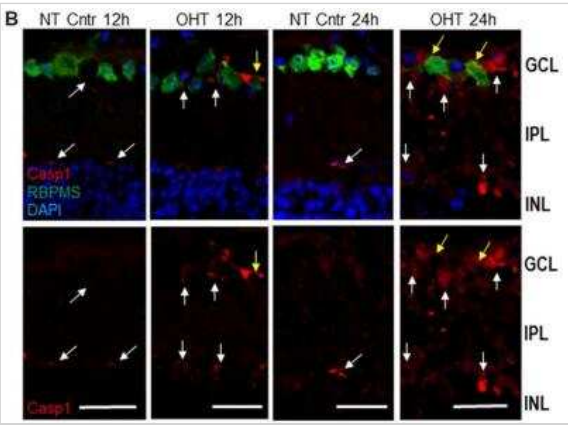
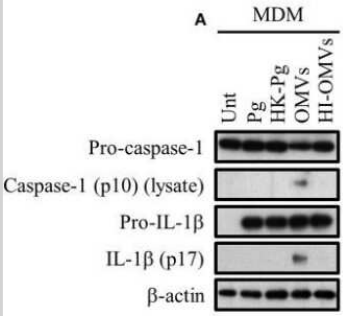
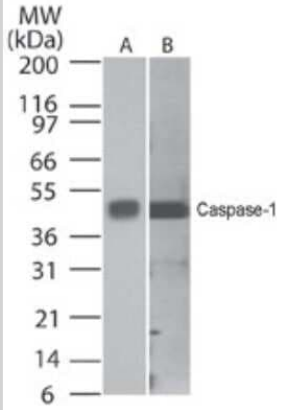
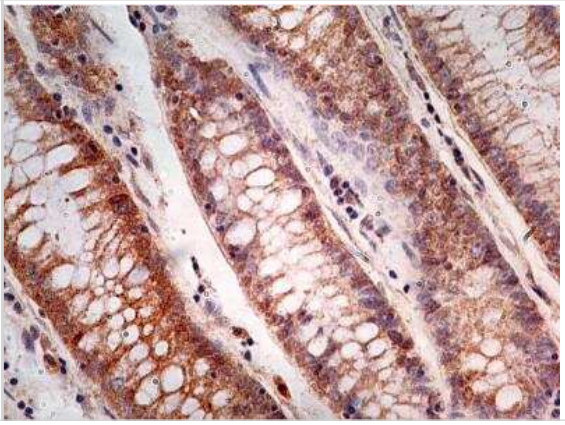
Product Information	
Unit Size	0.1 mg
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	14F468
Preservative	0.02% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein G purified
Buffer	PBS
Target Molecular Weight	45.2 kDa

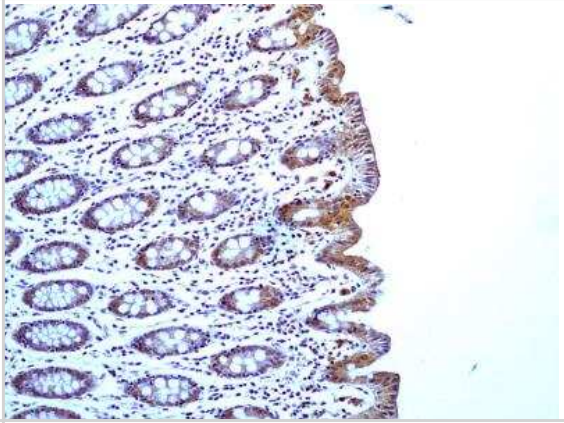
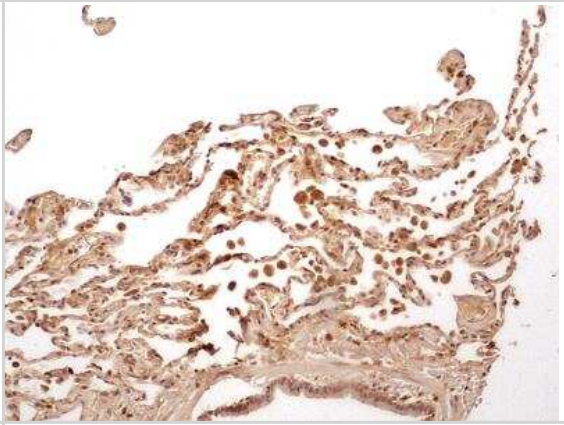
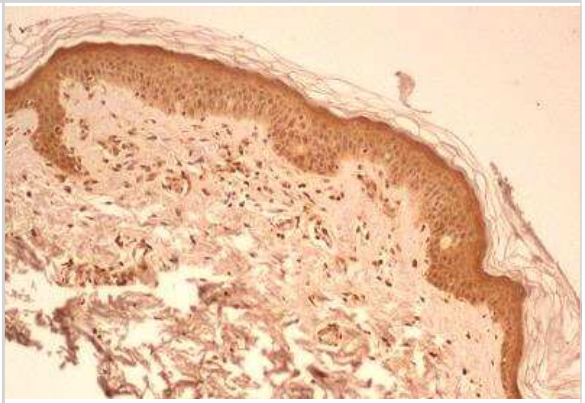
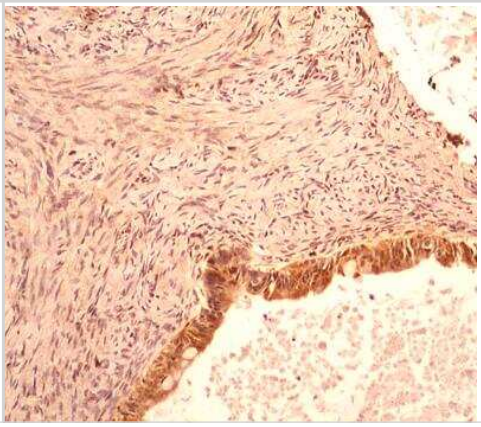
Product Description	
Host	Mouse
Gene Symbol	CASP1
Species	Human, Mouse, Rat
Reactivity Notes	Immunogen's sequence similarity with other species: Porcine/Pig (85%), Equine/Horse (80%), Canine (70%). Rat reactivity reported in scientific literature (PMID: 22133203).
Specificity/Sensitivity	Caspase-1 Antibody (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].

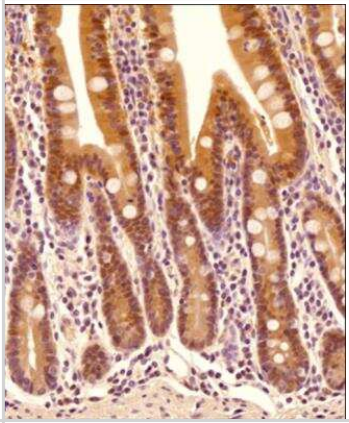
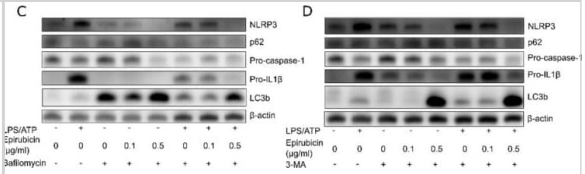
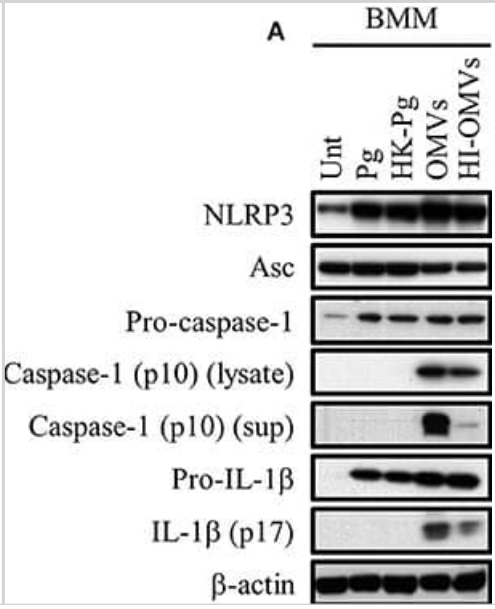
Product Application Details	
Applications	Western Blot, Simple Western, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 0.5-2 ug/ml, Simple Western 1:50, Immunohistochemistry 1:100 - 1:500, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry-Paraffin 1:10-1:500, Immunohistochemistry-Frozen reported in scientific literature (PMID 30930743)
Application Notes	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. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. Separated by Size-Wes, Sally Sue/Peggy Sue.

Images

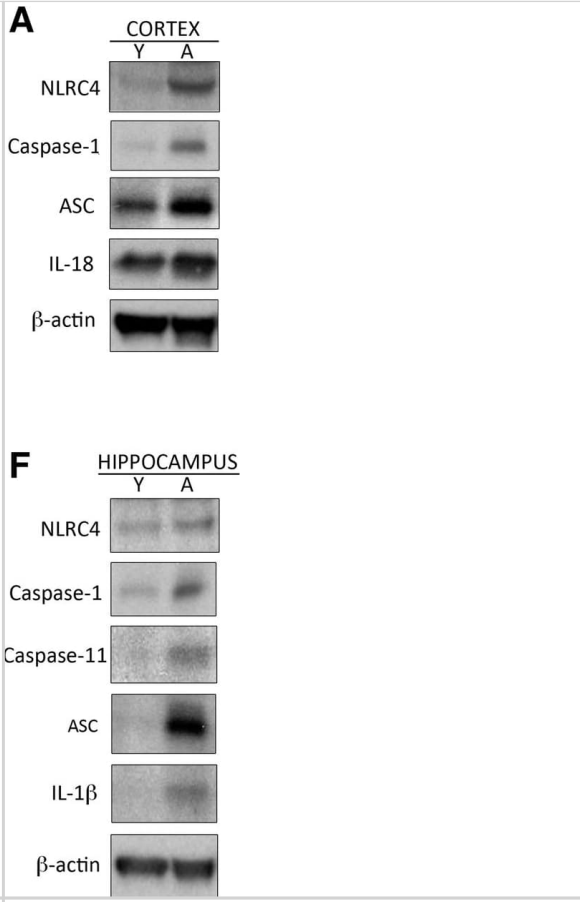
Simple Western lane view shows a specific band for Caspase 1 in 1.0 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	
Activity of Casp1 in OHT-injured and normotensive control eyes. (A) Casp1 was detected by intraocular injection FLICA660-labeled 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 (http://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 (//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 um. 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 (http://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 ug/ml and 2 ug/ml, respectively.	
Adenocarcinoma of the rectum stained with Caspase-1 antibody (5 ug/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.	

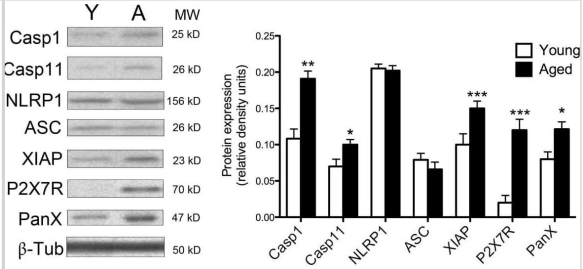
Detection of Caspase-1 protein in a section of normal human colon using 5 ug/ml concentration of Caspase 1 antibody (clone 14F468). Distinct cytoplasmic staining along with some nuclear positivity was observed in crypts/mucosa, and staining was found to be more intense in the absorptive columnar epithelial 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.	
Epirubicin suppresses the NLRP3 inflammasome in presence of autophagy inhibitors. (A) LPS/ATP-induced IL-1beta secretion from PM in the absence or presence of bafilomycin and 3-MA is shown. Means +/- SEM from 10 (IL-1beta) independent experiments are shown. (B) Representative immunoblot of LC3b after epirubicin and LPS/ATP treatments. (C,D) Representative immunoblots of inflammasome and 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 and NLRP3 activation by ATP (1 mM, 1 h). Whole cell lysates were subjected to SDS-PAGE (10% gel) and processed for immunoblot analysis with specific antibodies. Here, beta-actin served as loading control. Statistical tests: one-sample t-test (A) ** $p < 0.01$, *** $p < 0.001$. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31905600), licensed under a CC-BY licence.	
Prolonged hypoxia increases glutamine metabolism in PDAC cells, and HIF□2α can promote non□canonical glutamine metabolism in chronic hypoxic conditions. (A) Time course of glutamine consumption at 1%, 3% or 20% O2, each time data point is an average of triplicate experiments. (B) Panc□1 and Capan□2 were incubated for 48 hrs at 1%, 3% or 20% O2, GLS1, GOT1 and GOT2 protein were measured by Western blot. β□Actin was used as loading control. (C) Panc□1 and Capan□2 were incubated for 48 hrs at 1%, 3% or 20% O2, GLS1, GOT1 and GOT2 mRNA were measured by qRT□PCR. Data are presented as mean ± S.D. from three independent experiments. (D and F) Si□HIF□2α□transfected Panc□1 and Capan□2 cultured at 1% or 3%O2 for 48 hrs. The level of HIF□2α and glutamine metabolism enzymes mRNA and protein were determined by qRT□PCR (mRNA) and Western blot (protein), and β□actin was used as loading control. Data are presented as mean ± S.D. from three independent experiments. *P < 0.05, **P < 0.01. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/28544376), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

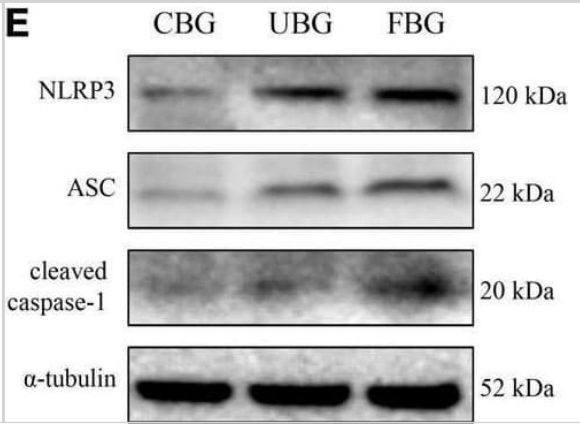
Compound MI-743 causes intracellular 8-oxo-dG accumulation and DNA damage.a MGC-803, HGC-27 and GES-1 cells were treated with DMSO or 5 μM MI-743 for 48 h. Intracellular 8-oxo-dG was stained with Cy3-conjugated avidin. DNA was counterstained with 4,6-diamidino-2-phenylindole (DAPI). Images were acquired at ×100 magnification by a Nikon Eclipse TE 2000-S fluorescence microscope. At least three independent experiments were performed for each group. b MGC-803, HGC-27 and GES-1 cells were treated with DMSO or 10 μM MI-743 for 48 h and run in alkaline comet assay. Pictures were originally captured at ×40 magnification. H2O2 was used as positive control. c Tail moment was calculated by CometScore software. Three individual experiments were performed for each group. d–f Western blot analysis of the protein levels of MTH1, MUTYH, OGG1, p21, ATMpS1981 and p53pS15 in MGC-803, HGC-27 and GES-1 cells, treated with increasing concentrations of MI-743 (0, 1, 2, 4, 8 and 12 μM). g, h Densitometry shows relative protein expression normalized for GAPDH. Data are representative of three independent experiments. i–k Western Blot analysis of the protein levels of MTH1, MUTYH, OGG1, p21, ATMpS1981 and p53pS15 of protein lysates, isolated from MGC-803, HGC-27 and GES-1 cells, which were treated with 12 μM MI-743 for 0, 12, 24, 36 and 48 h. l, m Densitometry shows relative protein expression normalized for GAPDH. Data are presented as means ± SD. Three individual experiments were performed for each group. *P < 0.05, **P < 0.01, ***P < 0.001 as compared with the controls Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/31164636), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

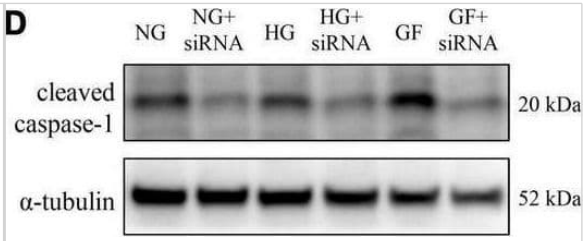
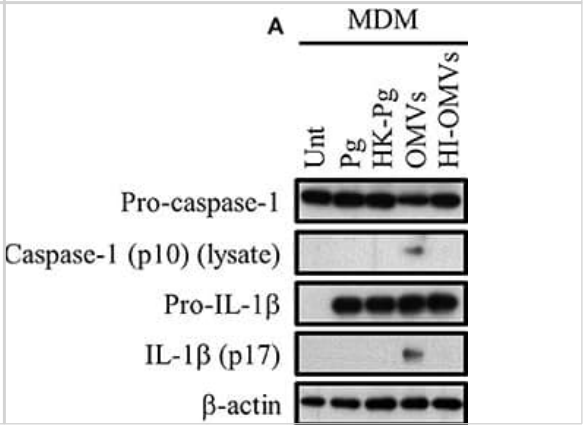


Nifedipine stimulated tremendous production of reactive oxygen species (ROS), and KIM-1 in 24 and 48 h. (a) Nifedipine 30 μM-treated group had induced a higher ROS (3.3-fold vs. control, p < 0.01) compared to H2O2 500 μM. (2.7-fold vs. control, p < 0.01). (b,c) Nifedipine 7.5, 15, and 30 μM-treated groups for 24 h (tubulin as internal control) had upregulated KIM-1 in dose dependent fashion (101%, 102%, p < 0.05, and 122%, p < 0.01 respectively) and reduced to 86%, 91%, and 80% in 48 h (actin as internal control), respectively. p-values ≤ 0.05 (marked as *) were considered statistically significant. In addition, p-values ≤ 0.01 are marked as **. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/30934807), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Simultaneous downregulation of multiple genes more effectively inhibits HCV replication: Huh-7.5 cells in a similar experimental setup as mentioned in Figure 1 were transfected with following combinations of siRNAs: La autoantigen + NS5B, La autoantigen + hVAP-A, La autoantigen + PSMA7, NS5B + hVAP-A, NS5B + PSMA7, and PSMA7 + hVAP-A. Downregulation of viral replication were determined by RT-PCR as shown in (a). (b) represents densitometry analysis of HCV NS5B downregulation. The data represent mean ± standard deviation. □P value < 0.05 versus control was considered statistically significant. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/27446609), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



The inositol-requiring enzyme 1 α (IRE1α)/ X-box-binding-protein 1 spliced isoform (XBP1s) arm of unfolded protein response (UPR) is induced by angiotensin II in VSMCs. (A–C) The rat aortic VSMCs infected with adenovirus encoding GRP78 or control GFP (100 moi) for 48 h were stimulated with 100 nM AngII (AII) for 1–6 h and immunoblotting was performed as indicated. (A) Representative blots from 4 independent experiments. (B) Signal intensity was used to calculate the expression ratio of XBP1s to GAPDH. (C) Signal intensity was used to calculate the IRE1α Ser724 phosphorylation ratio to the total IRE1α. The bars in the graphs show the mean ± SD from 4 independent experiments. * indicates $p < 0.05$. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/32679678), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
CIP2A siRNA knockdown caused G1 arrest and decreased Cdk1 and Cdk2 protein levels in E7-expressing cells(A) Flow cytometry of cells expressing 16E7 transfected with CIP2A siRNA for 48 hr, treated with DMSO control or bleomycin (10 μg/mL) for 24 hr, then stained with PI. G1, S and G2 phases are indicated. (B) Western blot analysis of Cdk1, Cdk2, Cyclin A2, Cdk4, Cdk6, Cyclin D1 protein levels in cells expressing HPV-16E7 transfected with CIP2A siRNA. Babe, vector control. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/25650660), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Publications

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] (WB, 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)

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

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] (WB, 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)

Luo T, Zhou X, Qin M et al. Corilagin Restrains NLRP3 Inflammasome Activation and Pyroptosis through the ROS/TXNIP/NLRP3 Pathway to Prevent Inflammation Oxidative medicine and cellular longevity 2022-10-17 [PMID: 36299604]

Zhao K, Li S, Chen J, Jin Q Inhibitory Effect of Trihydroxy Isoflavone on Neuronal Apoptosis in Natural Aging Rats Disease Markers 2022-08-21 [PMID: 36046381] (WB, Rat)

Zhang Z, Tan Q, Guo P et al. NLRP3 inflammasome-mediated choroid plexus hypersecretion contributes to hydrocephalus after intraventricular hemorrhage via phosphorylated NKCC1 channels Journal of neuroinflammation 2022-06-21 [PMID: 35729645] (WB, Rat)

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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