Product Datasheet

Beclin 1 Antibody - BSA Free
NB500-249

Unit Size: 0.1 ml

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

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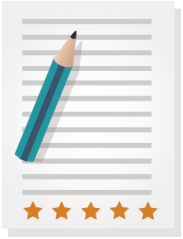
Updated 7/1/2024 v.20.1



NB500-249

Beclin 1 Antibody - BSA Free

Product Information

Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

Product Description

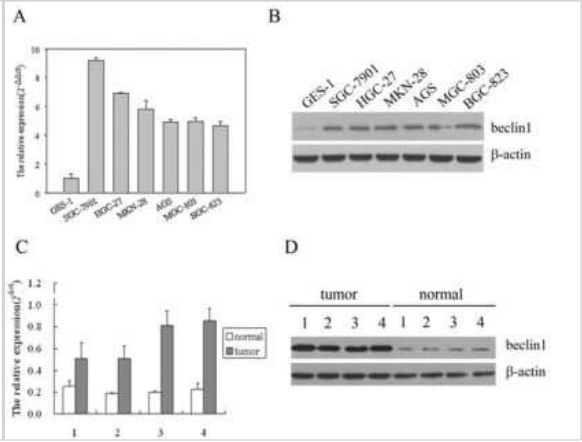
Host	Rabbit
Gene ID	8678
Gene Symbol	BECN1
Species	Human, Mouse, Rat
Immunogen	Internal synthetic peptide to human Beclin 1, within residues 1-100 [UniProt# Q14457].

Product Application Details

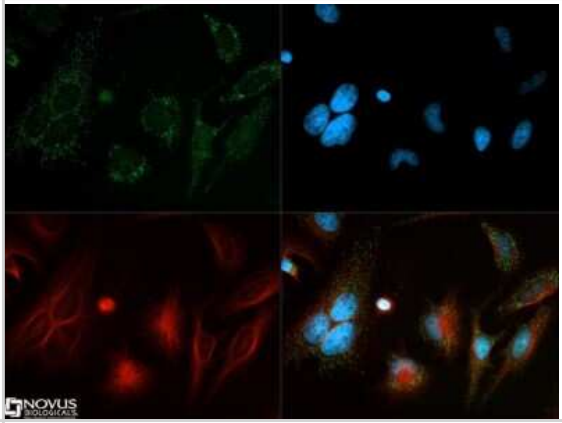
Applications	Western Blot, Simple Western, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Knockdown Validated
Recommended Dilutions	Western Blot 1:500-1:2000, Simple Western 1:50, Flow Cytometry 2-10 ug/ml, Immunohistochemistry 1:400, Immunocytochemistry/ Immunofluorescence 1:50-1:200, Immunoprecipitation 1:80, Immunohistochemistry-Paraffin 1:400, Immunohistochemistry-Frozen 1:400, Knockdown Validated

Images

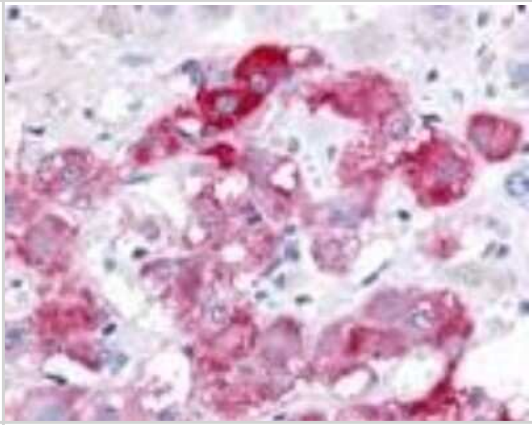
Western Blot: Beclin 1 Antibody - BSA Free [NB500-249] - Expression of Beclin 1 mRNA and protein in cells and tissues. (A) Beclin 1 mRNA expression level up-regulated in gastric cancer cell lines compared with normal gastric epithelial cells by reverse transcription-PCR. (B) Beclin 1 protein expression level up-regulated in gastric cancer cell lines compared with normal gastric epithelial cells by western blotting. (C) Beclin 1 mRNA expression is elevated in primary gastric tumors compared with paired gastric adjacent noncancerous tissues by reverse transcription-PCR. (D) Beclin 1 protein expression is elevated in primary gastric tumors compared with paired gastric adjacent noncancerous tissues by western blotting. Image collected and cropped by CiteAb from the following publication (//doi.org/10.1371/journal.pone.0045968) licensed under a CC-BY license.



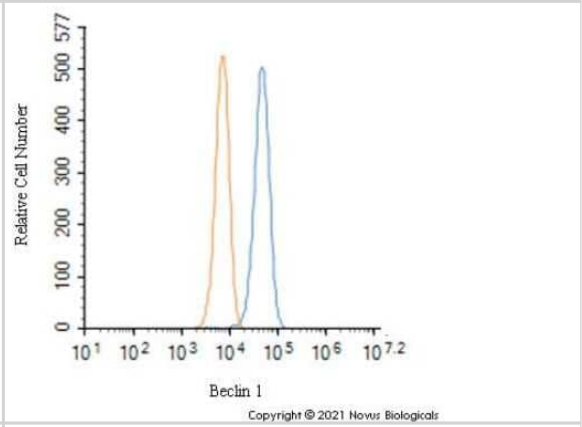
Immunocytochemistry/Immunofluorescence: Beclin 1 Antibody - BSA Free [NB500-249] - Beclin 1/ATG6 Antibody [NB500-249] - Beclin 1 antibody was tested in HeLa cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).



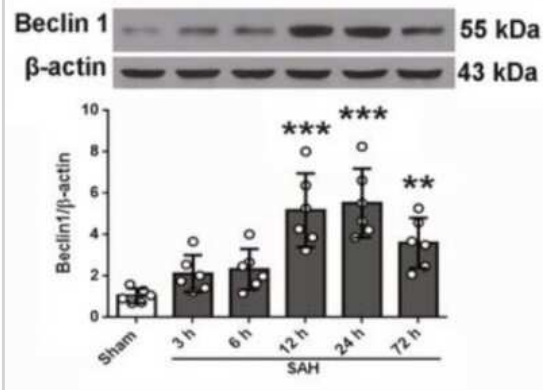
Immunohistochemistry: Beclin 1 Antibody - BSA Free [NB500-249] - Detection of Beclin 1 (red) in Pheochromocytes of the Adrenal Medulla 40x.

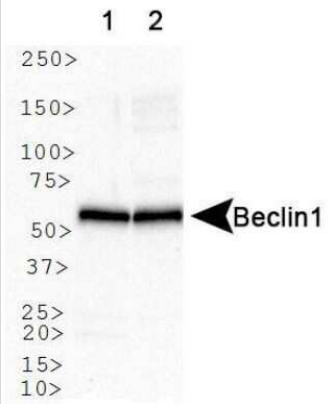
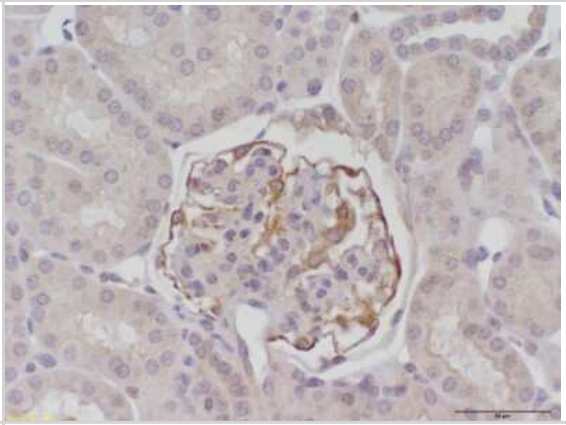
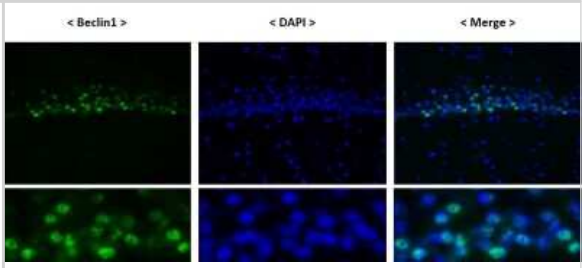
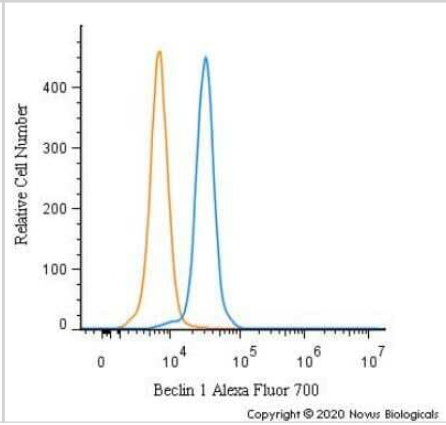


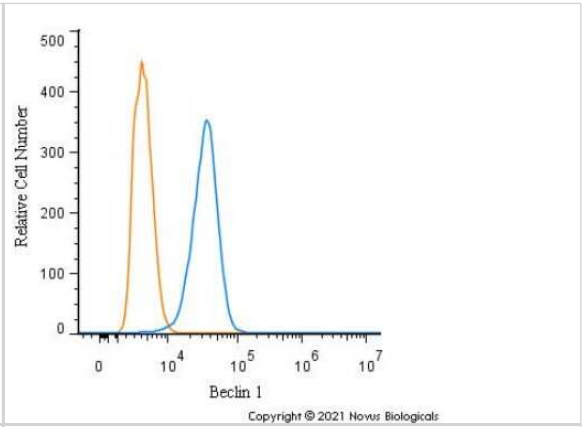
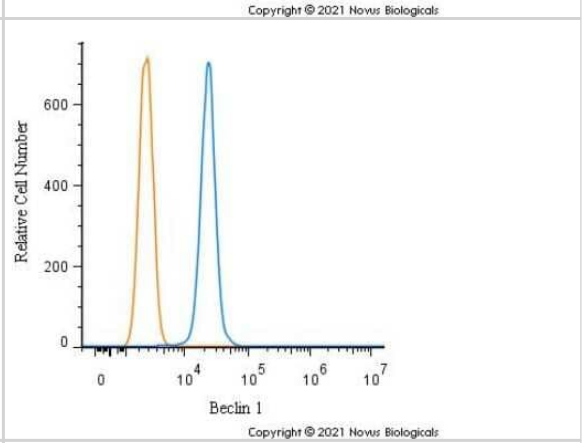
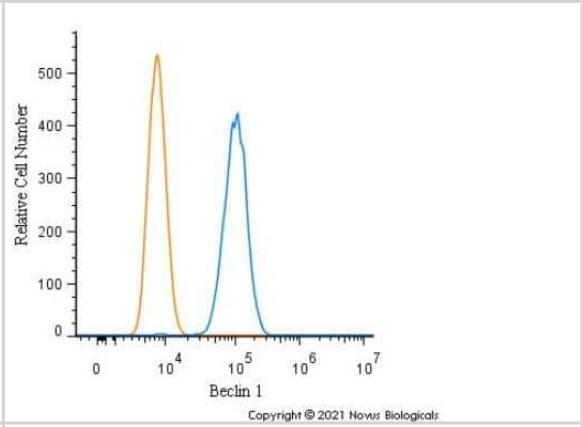
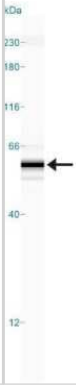
Flow Cytometry: Beclin 1 Antibody - BSA Free [NB500-249] - An intracellular stain was performed on U-87MG cells with Beclin 1 Antibody NB500-249 (blue) and a matched isotype control NBP2-24891 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (SA5-10033, Thermo Fisher).

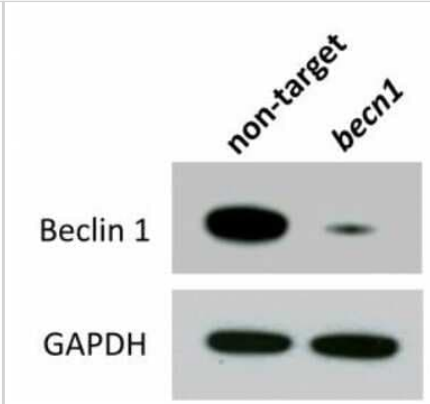
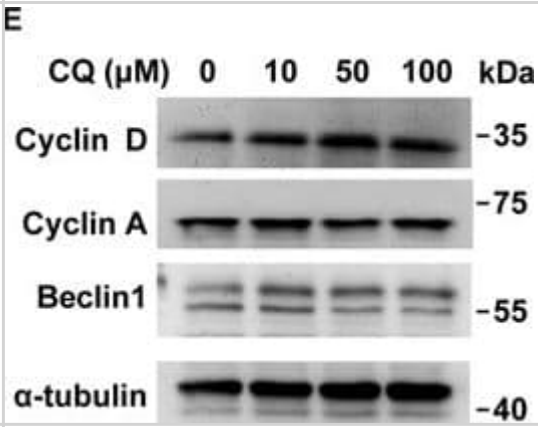
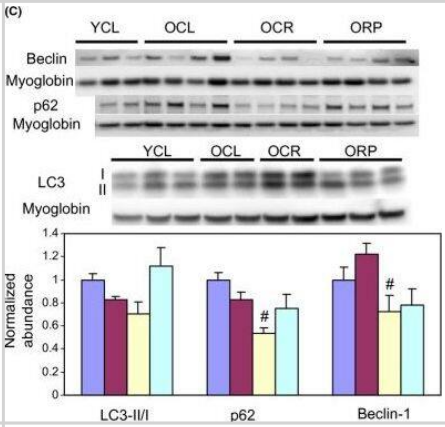
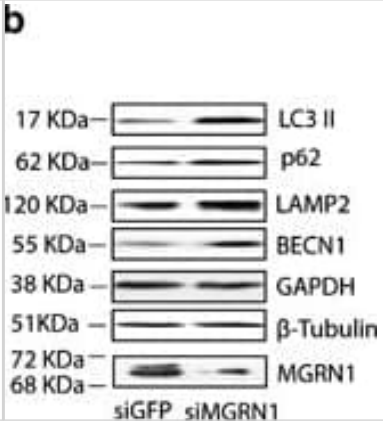


Western Blot: Beclin 1 Antibody - BSA Free [NB500-249] - Representative Western blot images and quantitative analyses of Beclin 1 from the left hemisphere of rat brains at different time points after SAH. Sample size is 36, n = 6 per group. Data were presented as mean +/- SD. F = 12.37 for Beclin 1. *P < .05, ** P < .01, ***P < .001 vs Sham group. SAH, subarachnoid hemorrhage. Image collected and cropped by CiteAb from the following publication (//pubmed.ncbi.nlm.nih.gov/31436915/) licensed under a CC-BY license.

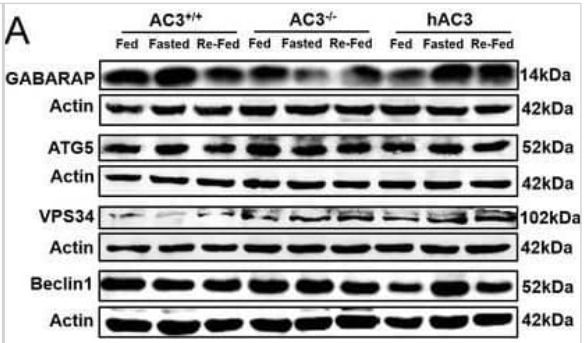


Western Blot: Beclin 1 Antibody - BSA Free [NB500-249] - Analysis of Belclin1. Lane 1: human brain. Lane 2: mouse brain.	
Immunohistochemistry-Paraffin: Beclin 1 Antibody - BSA Free [NB500-249] - Beclin 1/ATG6 Antibody [NB500-249] - Analysis of Beclin1 in mouse kidney. Image courtsey of product review submitted by Kelly Hudkins.	
Immunohistochemistry-Frozen: Beclin 1 Antibody - BSA Free [NB500-249] - Merged immunostaining of frozen section of Rat brain tissue. Image from verified customer review.	
Flow Cytometry: Beclin 1 Antibody - BSA Free [NB500-249] - An intracellular stain was performed on HeLa cells with NB500-249AF700 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 700.	

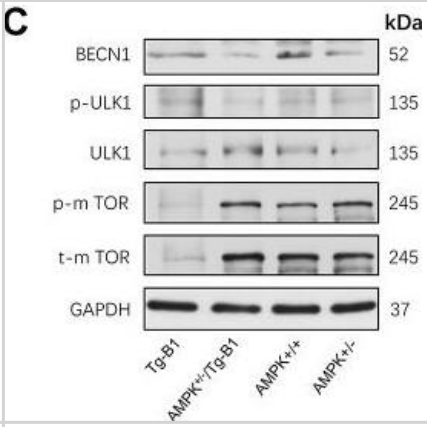
Flow Cytometry: Beclin 1 Antibody - BSA Free [NB500-249] - An intracellular stain was performed on HepG2 cells with Beclin 1 Antibody NB500-249 (blue) and a matched isotype control NBP2-24891 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1.0 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (SA5-10033, Thermo Fisher).	
Flow Cytometry: Beclin 1 Antibody - BSA Free [NB500-249] - An intracellular stain was performed on THP-1 cells with Beclin 1 Antibody NB500-249 (blue) and a matched isotype control NBP2-24891 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1.0 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (SA5-10033, Thermo Fisher).	
Flow Cytometry: Beclin 1 Antibody - BSA Free [NB500-249] - An intracellular stain was performed on Neuro2a cells with Beclin 1 Antibody NB500-249 (blue) and a matched isotype control NBP2-24891 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1.0 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (SA5-10033, Thermo Fisher).	
Simple Western: Beclin 1 Antibody - BSA Free [NB500-249] - Image shows a specific band for Beclin1 in 1.0 mg/mL of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	

Knockdown Validated: Beclin 1 Antibody - BSA Free [NB500-249] - siRNA knockdown of becn1 (Beclin 1). Lysates from OKP7 cells treated with non-targeting siRNA (non-target) or an siRNA pool directed against Beclin 1 (becn1) were analyzed by Western blot for Beclin 1 production. GAPDH was used as a loading control. Image collected and cropped by CiteAb from the following publication (//doi.org/10.1371/journal.ppat.1003394) licensed under a CC-BY license.	
Inhibition of lysosomes leads to G1 arrest by inactivating cyclin E/CDK2 complex. (E,F) Whole cell extracts of CQ-treated TM3 cells at the concentration of 10, 50, or 100 uM are analyzed by immunoblot with antibodies against Beclin1, cyclin D, cyclin A, cyclin E1, CDK2, phosphorylated CDK2 at Thr160 (pCDK2) and alpha-tubulin. Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/s41598-017-00393-4), licensed under a CC-BY licence.	
Metabolic profiling and biochemical assay. (C) Western blots of autophagic markers show no significant change of LC3 II/I, p62, or beclin-1 in cardiac aging. However, OCR has significantly lower p62 than that in OCL. #P < 0.05 compared with OCL. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/24612461), licensed under a CC-BY licence.	
Compromised function of MGRN1 affects markers of late endocytosis/lysosomes and autophagy. (b) HeLa cells similarly treated as in (a) were lysed and immunoblotted for autophagy and lysosomal proteins. The levels of GAPDH and beta-tubulin serve as loading controls. The blots are representative of at least three experiments. Efficiency of knockdown was checked using anti-MGRN1 antibody. Note that the antibody used against LC3, detects only endogenous LC3 II. Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/cddis2015257), licensed under a CC-BY licence.	

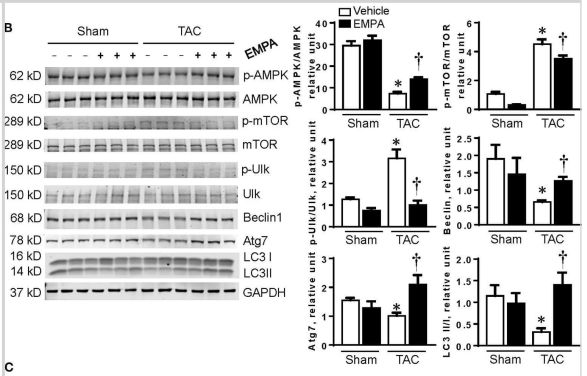
GABARAP interacts with AC3 via LIRs of AC3 in a ciliary expression-dependent manner. A-E) WB (A) and densitometric quantification of the expression of GABARAP (B), ATG5 (C), VPS34 (D), and Beclin1(E) in the hypothalami of AC3+/+, AC3-/-, and hAC3 mice (n = 3 mice per group). Actin served as the loading control. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/34783461), licensed under a CC-BY licence.



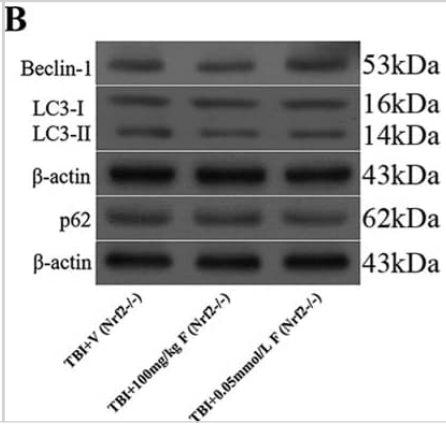
AMPK knockout interrupts and decreases apoptosis in the cochlea. (C) Western blot results show changes in autophagy-related proteins in the cochleae of aging mice. There is a remarkable decline of mTOR signaling (Tg-B1 vs. AMPK+/-/Tg-B1, p<0.0001; Tg-B1 vs. WT, p=0.0001) and more Beclin-1 (Tg-B1 vs. AMPK+/-/Tg-B1, p<0.0001, one-way ANOVA followed by Bonferroni post-test) expressed in the cochleae of Tg-B1 mice. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32240104), licensed under a CC-BY licence.



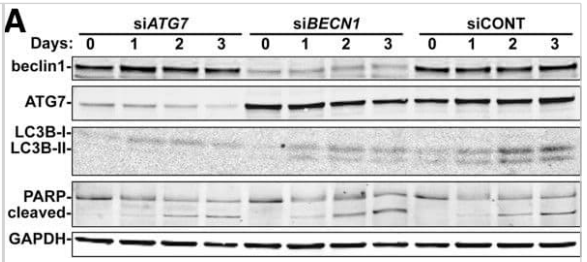
The p53-dependent regulation of ET-1 in RCC4 cells. (a) RCC4 cells were transfected with 10 nM siRNA to p53 or nonsilencing control (NSC) duplex for 24 h before addition of 2 μM CPT (+) or DMSO vehicle control (−) for a further 24 h. Panels, whole-cell lysates were assayed by western blot for p53 protein. Actin was used as a loading control. Graph, mRNA expression of ET-1 by real-time quantitative PCR relative to GAPDH. Mean±S.E. of duplicate values of one representative experiment is shown. (b) RCC4 cells were transfected with 10 nM siRNA to p53, HIF-1α (H1), or HIF-2α (H2) or NSC duplex for 24 h. Panels, whole-cell lysates were assayed by western blot for p53, HIF-1α and HIF-2α proteins. Tubulin was used as a loading control. Graph, conditioned media were harvested and secreted protein levels of ET-1 were determined by ELISA and normalized to cell number. Mean±S.E. of duplicate values of one representative experiment is shown. *P<0.05, t test compared with control Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/24136229), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



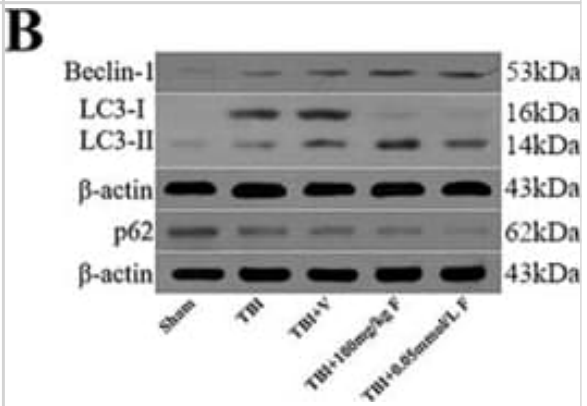
Detection of PGAM in KLN-205 cells with antibodies directed to whole PGAM protein or to C-terminal peptide of PGAMA. control conditions (scan parameters in red channel were set to emphasize nucleolar staining with propidium iodide – PI) B. RNase-treated cells. Bar=15 μm. Image collected and cropped by CiteAb from the following open publication (https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.4044), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



ATG7/LC3-II regulates ERK phosphorylation.(a) Atg7−/− livers display decreased ERK phosphorylation. Immunoblots for the indicated proteins in liver homogenate (Hom) and cytosolic (Cyt) fractions of control (Con) and liver-specific Atg7−/− mice are shown. The bars represent mean ±s.e.m. *P<0.05, **P<0.01, ***P<0.001 compared with Con; Student's t-test, n=4. (b) Atg7−/− livers display decreased ERK2 dimers. Immunoblots show the indicated proteins in Hom from Con and Atg7−/− livers. The bars represent mean±s.e.m. *P<0.05, ***P<0.001 compared to Con; Student's t-test, n=4–5. Arrows indicate p42 monomers and dimers. (c) Atg7−/− brown adipose tissues (BAT) display decreased ERK phosphorylation. Immunoblots for indicated proteins in Hom from Con and Atg7−/−BAT. The bars represent mean±s.e.m. **P<0.01 compared with Con; Student's t-test, n=4–6. (d) Atg7−/− livers exhibit decreased nuclear ERK phosphorylation. Immunoblots show the indicated proteins in homogenate (Hom, lane 1) and nuclear fractions (lanes 2–5) from Con and Atg7−/− livers. The bars represent mean±s.e.m. ***P<0.001 compared with Con; Student's t-test, n=4–5. (e) LC3 C terminus glycine-deleted (ΔG) mutants exhibit decreased ERK phosphorylation. Immunoblots for indicated proteins in total lysates from CFP-LC3- and CFP-LC3ΔG-transfected NIH/3T3 cells exposed or not to EGF (10 min). The bars represent mean±s.e.m. ***P<0.001 compared with Con; Student's t-test, n=8. Image collected and cropped by CiteAb from the following open publication (https://www.nature.com/articles/ncomms3799), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



GamGFP marks DSBs in mammalian cells and is inhibited by Ku.(A) GamGFP co-localizes with 53BP1 on laser-induced DNA breaks. (B) Ku inhibits recruitment of GamGFP to laser-induced damage, live cells. (C and D) Ku inhibits recruitment of GamGFP, fixed cells. Mean ± SEM of three experiments, n >25 cells each. (E) GamGFP forms IR-induced foci in Ku80-defective MEFs. (F) Zoomed image from E. (G) IR-induced foci containing Gam only, 53BP1 only or both Gam and 53BP1 (>2600 total foci counted in three independent experiments). Error bars, SEM. Scale bars = 5 μm.DOI:\nhttps://dx.doi.org/10.7554/eLife.01222.012GamGFP marks DSBs in mammalian cells.(A) Live analysis of GamGFP localization to laser-induced DNA damage. Hela cells producing GamGFP were laser damaged along the cell track indicated by the red line at 0 min (m) and images were taken at the indicated times as shown. (B) GamGFP co-localizes with γH2AX in fixed Hela cells.DOI: \nhttps://dx.doi.org/10.7554/eLife.01222.013Ku inhibits GamGFP recruitment at DSBs independently of non-homologous end joining.Cells lacking either Ku80 or LigIV are defective in non-homologous end joining (NHEJ), yet the presence of Ku still inhibits recruitment of GamGFP to laser-induced DSBs even in NHEJ-defective cells, and thus independently of the cell's ability to complete NHEJ. Whereas it could have been possible that reduced GamGFP recruitment in the presence of Ku was caused by reduced persistence of DSBs due to their repair by NHEJ, our data show instead that Ku inhibits recruitment independently of successful NHEJ and imply that Ku binding to DSEs itself is inhibitory.DOI:\nhttps://dx.doi.org/10.7554/eLife.01222.014 Image collected and cropped by CiteAb from the following open publication (https://elifesciences.org/articles/01222), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Karaman E, Onder GO, Goktepe O et al. Protective Effects of Boric Acid Taken in Different Ways on Experimental Ovarian ?schemia and Reperfusion Biological trace element research 2023-09-25 [PMID: 37743417]

Yang D, Wu X, Wang W et al. Ciliary Type III Adenylyl Cyclase in the VMH Is Crucial for High-Fat Diet-Induced Obesity Mediated by Autophagy Advanced Science 2022-01-01 [PMID: 34783461] (WB)

Li X, Flynn ER, do Carmo JM et al. Direct Cardiac Actions of Sodium-Glucose Cotransporter 2 Inhibition Improve Mitochondrial Function and Attenuate Oxidative Stress in Pressure Overload-Induced Heart Failure Frontiers in Cardiovascular Medicine 2022-05-12 [PMID: 35647080] (WB)

Madhu V, Boneski PK, Silagi E et al. Hypoxic Regulation of Mitochondrial Metabolism and Mitophagy in Nucleus Pulposus Cells Is Dependent on HIF-1?-BNIP3 Axis Journal of Bone and Mineral Research 2020-08-01 [PMID: 32251541] (ICC/IF)

Kshirsagar S, Alvir RV, Pradeepkiran JA et al. A Combination Therapy of Urolithin A+EGCG Has Stronger Protective Effects than Single Drug Urolithin A in a Humanized Amyloid Beta Knockin Mice for Late-Onset Alzheimer's Disease Cells 2022-08-27 [PMID: 36078067] (WB)

Wang C, Chen C, Lin M et al. TLR9 Binding to Beclin 1 and Mitochondrial SIRT3 by a Sodium-Glucose Co-Transporter 2 Inhibitor Protects the Heart from Doxorubicin Toxicity Biology 2020-10-29 [PMID: 33138323] (B/N)

Naskar M, Parekh VP, Abraham MA et al. ?-Hemolysin promotes uropathogenic E. coli persistence in bladder epithelial cells via abrogating bacteria-harboring lysosome acidification PLOS Pathogens 2023-05-11 [PMID: 37167325]

Zhang L, Lin Y, Bai W et al. Human umbilical cord mesenchymal stem cell-derived exosome suppresses programmed cell death in traumatic brain injury via PINK1/Parkin-mediated mitophagy CNS neuroscience & therapeutics 2023-03-08 [PMID: 36890626] (WB, Mouse)

Lisi L, Pizzoferrato M, Ciotti GMP et al. mTOR Inhibition Is Effective against Growth, Survival and Migration, but Not against Microglia Activation in Preclinical Glioma Models International journal of molecular sciences 2023-06-07 [PMID: 37372982] (WB, Human)

Details:
1:250 WB dilution

Medras ZJH, Mostafa YM, Ahmed AAM, El-Sayed NM Arctigenin improves neuropathy via ameliorating apoptosis and modulating autophagy in streptozotocin-induced diabetic mice CNS neuroscience & therapeutics 2023-05-11 [PMID: 37170684] (WB)

Popli P, Tang S, Chadchan SB et al. Beclin-1-dependent autophagy, but not apoptosis, is critical for stem-cell-mediated endometrial programming and the establishment of pregnancy Developmental cell 2023-04-04 [PMID: 37040770] (IHC, Mouse)

Falvo S, Latino D, Santillo A et al. Effects of a high-fat diet on rat epididymis Journal of experimental zoology. Part A, Ecological and integrative physiology 2023-04-03 [PMID: 37009779] (WB, Rat)

More publications at http://www.novusbio.com/NB500-249

Procedures

Immunoprecipitation protocol for Beclin 1 Antibody (NB500-249)

Immunoprecipitation Protocol:

1. Cells in 2x 75cm flasks (60% confluency) are scraped with 0.5ml of Tris lysis Buffer (50mM Tris, 150mM NaCl, 1mM EDTA, 100ug/ml PMSF, 1% triton).
2. Lyse 1h at 4C, with gentle agitation.
3. Centrifuge to clear the lysates.
4. 0.1 ml of lysate is kept aside for Western Blot experiments.
5. IP : Add 5ul of polyclonal beclin antibody (NB 500-249) to 0.4ml of lysate (1:80 dilution).
6. Incubate overnight at 4C, with gentle agitation.
7. Next day, add 60ul of protein A sepharose beads to the lysate.
8. Incubate for one hour at 4C.
9. Wash beads 3X with Tris lysis buffer.
10. Beads are re-suspended with 15ul of Laemmli buffer and boiled.
11. A SDS-PAGE gel is run and the proteins are transferred to a membrane.
12. The efficiency of IP is determined by using a monoclonal anti-beclin antibody.

Western Blot protocol for Beclin 1/ATG6 Antibody (NB500-249)

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 blocking buffer 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 manufacturer's instructions.

Immunohistochemistry Free-Floating Protocol for Beclin 1 Antibody (NB500-249)
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 at all times).

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.

Immunocytochemistry/Immunofluorescence Protocol for Beclin 1/ATG6 Antibody (NB500-249)
Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeablization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPi if required.

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Products Related to NB500-249

NBL1-07966	Beclin 1 Overexpression Lysate
NB500-249PEP	Beclin 1 Antibody Blocking Peptide
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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