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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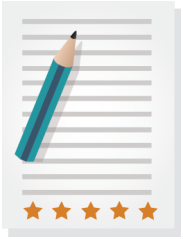
Updated 4/13/2025 v.20.1



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

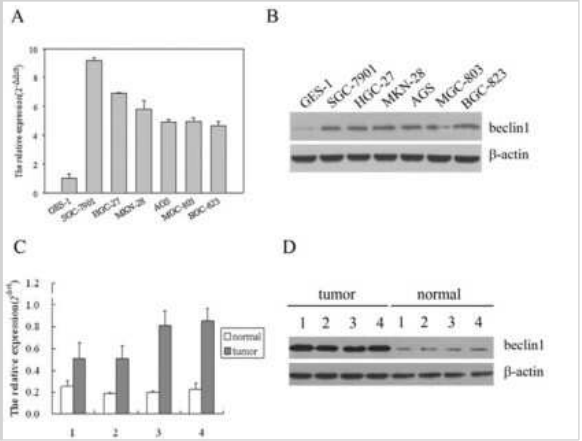
Host	Rabbit
Gene ID	8678
Gene Symbol	BECN1
Species	Human, Mouse, Rat
Immunogen	Internal synthetic peptide to human Beclin 1, within residues 1-100 [UniProt# Q14457].

Product Application Details

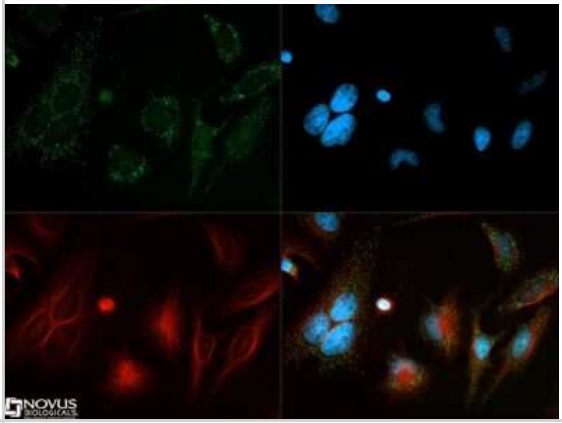
Applications	Western Blot, Simple Western, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Knockdown Validated
Recommended Dilutions	Western Blot 1:500-1:2000, Simple Western 1:50, Flow Cytometry 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
Application Notes	See Simple Western Antibody Database for Simple Western validation: Tested in HeLa lysate at 1.0 mg/ml; separated by size; antibody dilution of 1:50.

Images

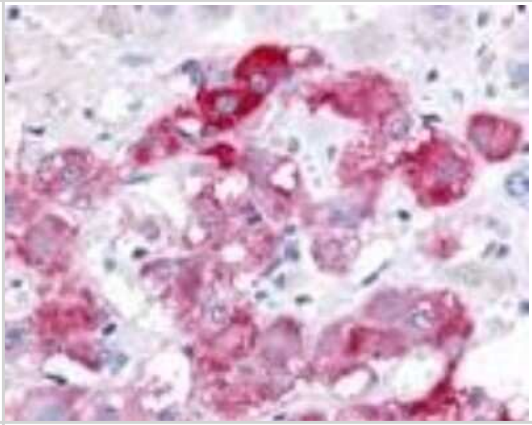
Western Blot: Beclin 1 Antibody - BSA Free [NB500-249] - Expression of Beclin 1 mRNA and protein in cells and tissues. (A) Beclin 1 mRNA expression level up-regulated in gastric cancer cell lines compared with normal gastric epithelial cells by reverse transcription-PCR. (B) Beclin 1 protein expression 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.



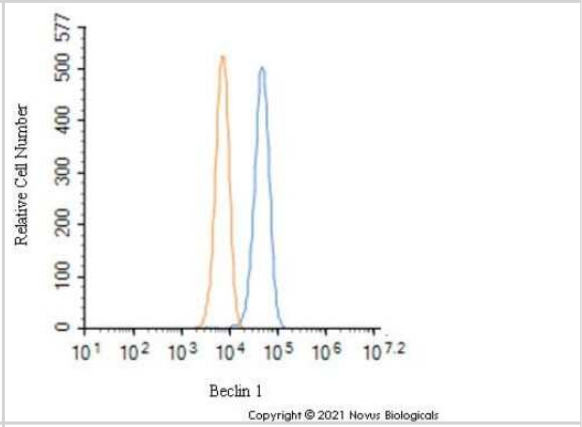
Immunocytochemistry/Immunofluorescence: Beclin 1 Antibody - BSA Free [NB500-249] - Beclin 1/ATG6 Antibody [NB500-249] - Beclin 1 antibody was tested in HeLa cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).



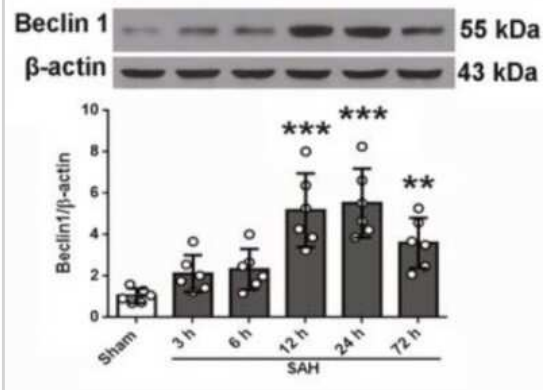
Immunohistochemistry: Beclin 1 Antibody - BSA Free [NB500-249] - Detection of Beclin 1 (red) in Pheochromocytes of the Adrenal Medulla 40x.

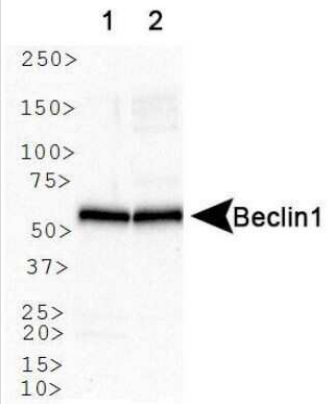
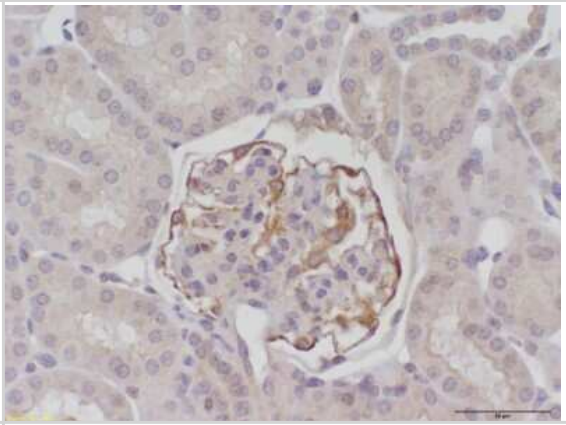
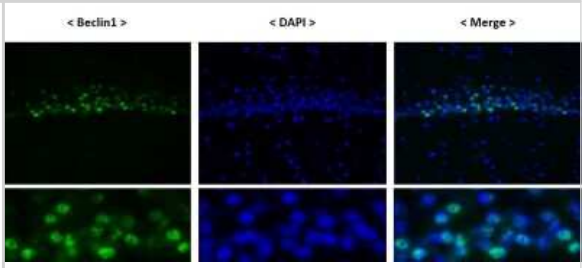
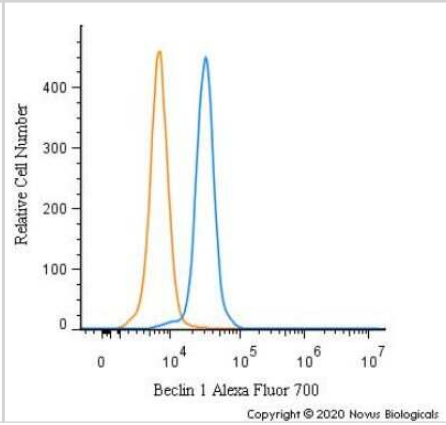


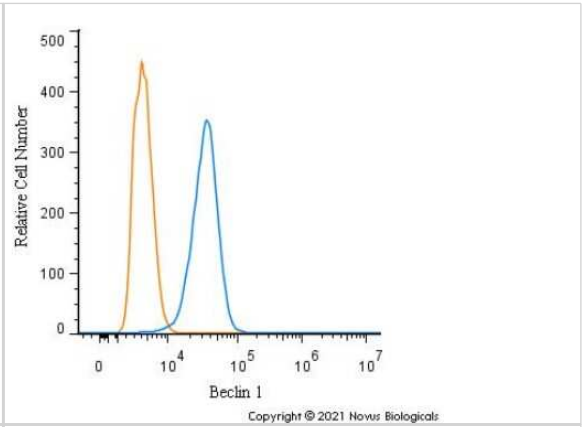
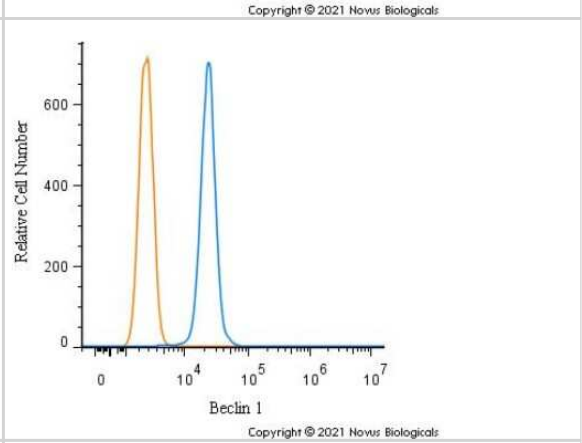
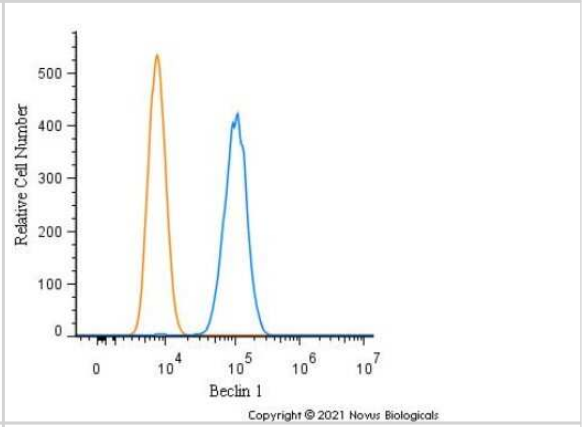
Flow Cytometry: Beclin 1 Antibody - BSA Free [NB500-249] - An intracellular stain was performed on U-87MG cells with Beclin 1 Antibody NB500-249 (blue) and a matched isotype control NBP2-24891 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an 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).



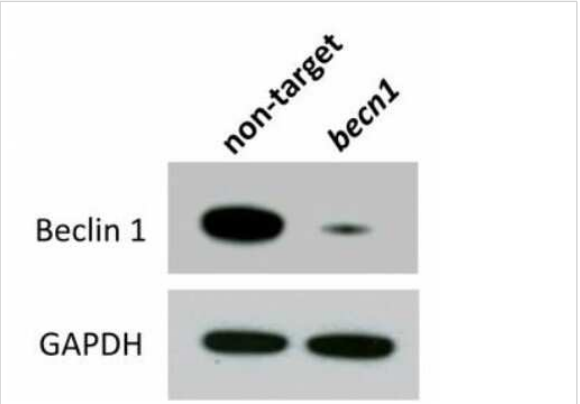
Western Blot: Beclin 1 Antibody - BSA Free [NB500-249] - Representative Western blot images and quantitative analyses of Beclin 1 from the left hemisphere of rat brains at different time points after SAH. Sample size is 36, n = 6 per group. Data were presented as mean +/- SD. F = 12.37 for Beclin 1. *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.



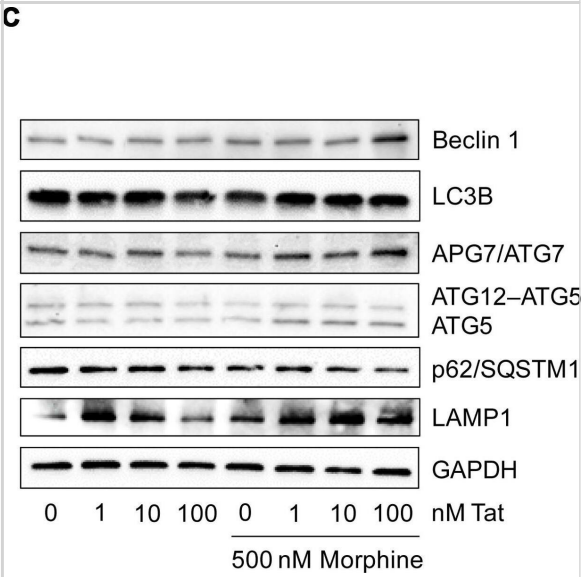
Western Blot: Beclin 1 Antibody - BSA Free [NB500-249] - Analysis of Belclin1. Lane 1: human brain. Lane 2: mouse brain.	
Immunohistochemistry-Paraffin: Beclin 1 Antibody - BSA Free [NB500-249] - Beclin 1/ATG6 Antibody [NB500-249] - Analysis of Beclin1 in mouse kidney. Image courtsey of product review 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.	

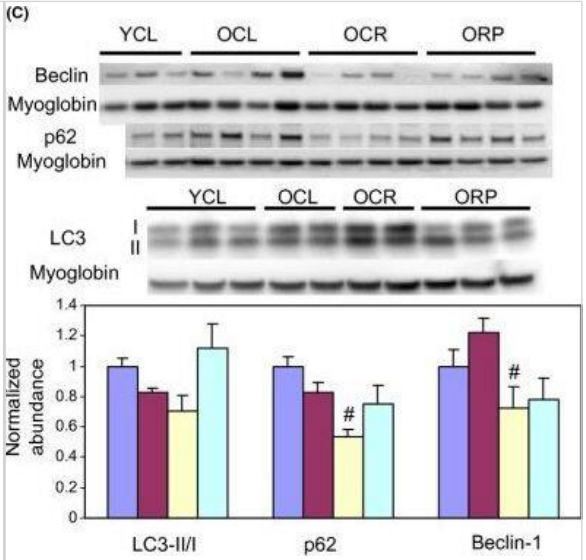
Western Blot: Beclin 1 Antibody - BSA Free [NB500-249] - siRNA knockdown of becn1 (Beclin 1). Lysates from OKP7 cells treated with non-targeting siRNA (non-target) or an siRNA pool directed against Beclin 1 (becn1) were analyzed by Western blot for Beclin 1 production. GAPDH was used as a loading control. Image collected and cropped by CiteAb from the following publication (//doi.org/10.1371/journal.ppat.1003394) licensed under a CC-BY license.



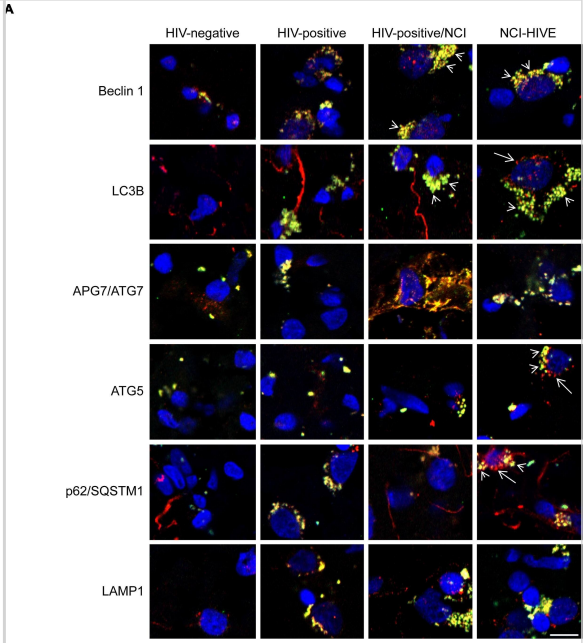
Differences in neuronal autophagy & dendrite varicosity following HIV-1 Tat protein & morphine treatment. (A) Representative images of neurons transfected with a fluorescent reporter plasmid to monitor autophagic flux at 8 h following the indicated treatments. GFP (green) & GFP + mRFP (yellow) fluorescence are observed prior to the fusion of autophagosomes with lysosomes whereas only mRFP (red) fluorescence is present in post-fusion autolysosomes. DIC, differential interference contrast microscopy image. DAPI (blue) staining indicates cell nuclei. (B) Quantification of autolysosomes (red puncta) from (A). F(3,13) = 8.756, p = 0.0019; □p < 0.05 when compared to all other groups. (C) Western blotting analysis of the indicated autophagy associated protein levels at 24 h following the indicated treatments. GAPDH was used as a loading control. Blots are representative of three independent experiments. (D) Quantification of dendrite beading from (A). F(3,77) = 6.429, p = 0.0006; □p < 0.05 when compared to control cells. Error bars show the SEM. Image collected & cropped by CiteAb from the following publication (http://journal.frontiersin.org/Article/10.3389/fmicb.2015.00653/abstract), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



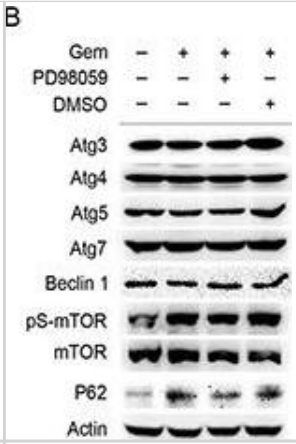
Metabolic profiling & biochemical assay. (A) Relative abundance of the substrates in the glycolytic pathway & TCA cycle in ORP compared to OCL by targeted metabolic profiling. When compared with OCL heart, ORP hearts have significantly lower glucose-6-phosphate & fructose-6-phosphate (both are glycolytic metabolites), & significantly higher a-ketoglutarate, fumarate, malate, & citrate (all are TCA cycle metabolites). *P < 0.05 compared with OCL. See Table S6 for numerical data. (B) A schematic diagram summarizing the changes in metabolism by rapamycin in old heart. (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. (D) Both CR & RP significantly reduce the age-dependent increase in protein carbonyls (nmol mL−1). #P < 0.05 compared with OCL. (E). Both CR & RP significantly reduce the age-dependent increase in protein ubiquitination.*P < 0.05 compared with YCL & #P < 0.05 compared with OCL. n = 3–8. G6P: glucose 6-phosphate; G1P: glucose 1-phosphate; F6P: fructose 6-phosphate; F1P: fructose 1-phosphate; F16BP: fructose 1,6-bisphosphate; F26BP: fructose 2,6-biphosphate; G3P: glyceraldehyde 3-phosphate; DHAP:dihydroxyacetone phosphate; 2(3)-PGA: 2- or 3-phosphoglycerate; & PEP: phosphoenolpyruvate. Isomers of same molecular weight, that is, G6P versus G1P, F6P versus F1P, & F16BP versus F26BP, were not distinguishable by the LC-MS/MS-based metabolic profiling method. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/24612461), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



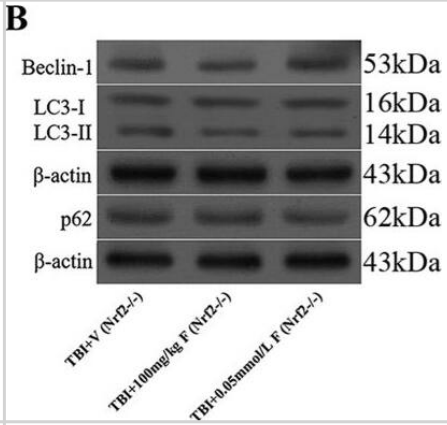
Autophagy associated protein immunoreactivity in HIV-infected brain tissue. (A) Representative images from five randomly selected fields of cells each examined in duplicate frontal lobe white matter sections for the indicated subject groups. The indicated proteins were labeled red & microglia with the cell-type-specific marker Iba1 (green). Blue staining indicates cell nuclei. Arrow heads indicate examples of higher Iba1 immunoreactivity whereas arrows indicate more focal (punctal) vs. diffuse (filamentous) patterns of autophagy associated protein expression. Scale bar = 10 μm. (B) Quantification of relative Iba1 immunoreactivity from (A). F(3,20) = 6.450, p = 0.0031; □p < 0.05 when compared to all other subject groups. Error bars show the SEM for the average values of 2–6 regions from each subject group across the six autophagy associated proteins examined. (C) Quantification of the indicated autophagy associated protein relative immunoreactivity from (A). Beclin 1: F(3,12) = 11.29, p = 0.0008; LC3B: F(3,12) = 1.994, p = 0.1687; APG7/ATG7: F(3,12) = 84.20, p = < 0.0001; ATG5: F(3,12) = 6.218, p = 0.0086; p62/SQSTM1: F(3,12) = 87.04, p = < 0.0001; LAMP1: F(3,12) = 8.317, p = 0.0029. □p < 0.05 when compared to HIV-negative; #p < 0.05 when compared to HIV-positive; & Ωp < 0.05 when compared to HIV-positive/NCI subjects. Error bars show the SEM for four regions from each subject group. Image collected & cropped by CiteAb from the following publication (http://journal.frontiersin.org/Article/10.3389/fmicb.2015.00653/abstract), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



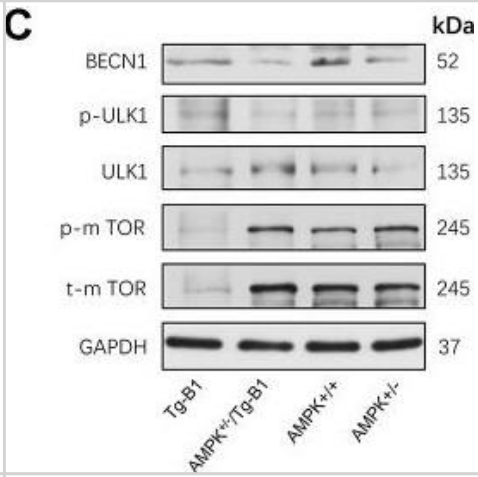
Western Blot: Beclin 1 Antibody - BSA Free [NB500-249] - Excessive activation of P62-mediated autophagic degradation by the phosphorylated ERα-ERK cascades may lead to the autophagic cell death induced by gemcitabine in ER positive MCF-7 cells. B. MCF-7 cells treated by gemcitabine, gemcitabine+ PD98059 (30 μmol/L, added before gemcitabine treatment for 1 h) or DMSO for 48 h. Then total cell lysates subjected to immunoblot analysis with indicated antibodies. The data represented a typical experiment conducted 3times with similar results. Image collected & cropped by CiteAb from the following publication (https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.10363), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



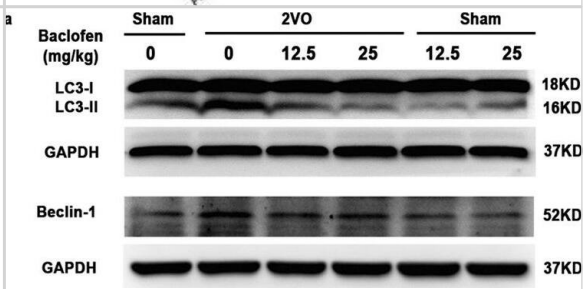
Western Blot: Beclin 1 Antibody - BSA Free [NB500-249] - Fucoxanthin failed to suppress oxidative stress & activate autophagy in Nrf2−/− mice following TBI.Fucoxanthin treatment had no effect on change the level of MDA & the activity of GPx (A) & the expression of Beclin-1, LC3 & p62 (B) in Nrf2−/− mice compared to the vehicle-treated group. n = 6 per group. @p > 0.05 versus TBI + vehicle group. β-actin was used as a loading control. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28429775), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



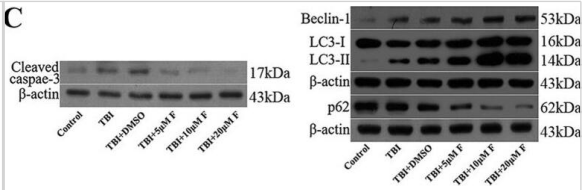
Western Blot: Beclin 1 Antibody - BSA Free [NB500-249] - AMPK knockout interrupts & decreases apoptosis in cochlea. (C) WB results show changes in autophagy-related proteins in cochleae of aging mice. There is a remarkable decline of mTOR signaling (Tg-B1 vs. AMPK +/−/Tg-B1, p<0.0001; Tg-B1 vs. WT, p=0.0001) & more Beclin-1 (Tg-B1 vs. AMPK+/−/Tg-B1, p<0.0001, one-way ANOVA followed by Bonferroni post-test) expressed in cochleae of Tg-B1 mice. (D) The histograms of WB analyses show knockouts of AMPK relieve the ROS-induced autophagic stress in Tg-B1 mice. Analysis performed by using Image J software & one-way ANOVA followed by Bonferroni post-test. * P<0.05, ** P<0.01, ***P<0.001, **** P<0.0001; n=3 per group. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32240104), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



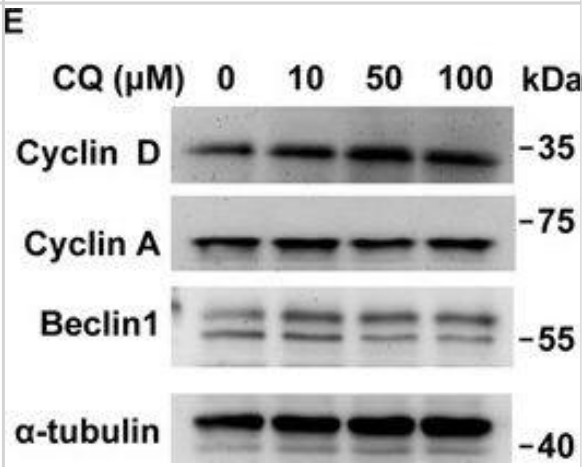
Western Blot: Beclin 1 Antibody - BSA Free [NB500-249] - Baclofen reversed the changes of protein markers characteristic for autophagy in hippocampal CA1 area under chronic cerebral hypoperfusion.(a–c) Five weeks after induction of hypoperfusion, p-mTOR was significantly decreased, & LC3-II, Beclin 1, atg5 & atg7 were significantly increased, & baclofen could reverse the changes of these proteins expression. Treatment with baclofen at 12.5 mg/kg & 25 mg/kg in sham-operated rats did not change the expression of LC3-II, mTOR, p-mTOR, Beclin 1, atg5 & atg7 compared with sham-operated rats (n = 4 in each group). Blots shown have been cropped to fit space requirements & run under the same experimental conditions. *P < 0.05 & **P < 0.01 vs sham-operated rats; ##P < 0.01 vs 2VO rats. Image collected & cropped by CiteAb from the following publication (https://www.nature.com/articles/srep14474), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



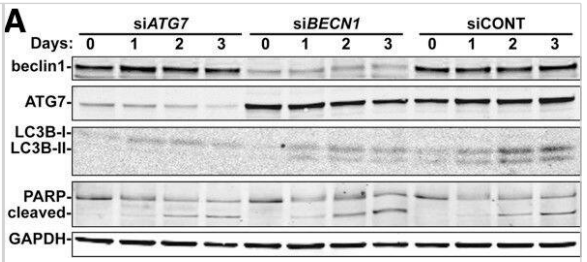
Western Blot: Beclin 1 Antibody - BSA Free [NB500-249] - Fucoxanthin protected primary cultured neurons from TBI.(A) Primary cortical neurons were subjected to scratch injury & then treated with 5, 10 or 20 μM fucoxanthin or DMSO for 1 day. The LDH release assay was used to evaluate cell viability. The percentage of survival cells significantly decreased after TBI compared to the control group. Fucoxanthin treatment significantly increased survival cells after TBI. (B) Fucoxanthin repressed the production of ROS in primary cultured cells after TBI. Cells were subjected to scratch injury & subsequently treated with 100 μM edaravone or 5, 10 or 20 μM fucoxanthin or DMSO for 1 day. Then cells were incubated with DCFH-DA & subjected to fluorescence spectrophotometer analysis. The intracellular ROS was significantly increased after TBI compared to the sham group, & administration of edaravone or fucoxanthin significantly repressed ROS production as compared to the TBI + DMSO group. (C) Fucoxanthin inhibited apoptosis & activated autophagy in primary cultured neurons. Primary cortical neurons were subjected to scratch injury & then treated with 5, 10 or 20 μM fucoxanthin or DMSO for 1 day, the expression of cleaved caspase-3, Beclin-1, LC3 & p62 was measured by western blot. Fucoxanthin significantly decreased the expression of cleaved caspase-3 & p62 while increased the expression of Beclin-1 & LC3-II. Data are presented as mean ± SEM, n = 6 per group; *p < 0.05, **p < 0.01, ***p < 0.001 versus control group; #p < 0.05, ##p < 0.01, ###p < 0.001 versus TBI + DMSO group; @p > 0.05 versus TBI + DMSO group. β-actin was used as a loading control. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28429775), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



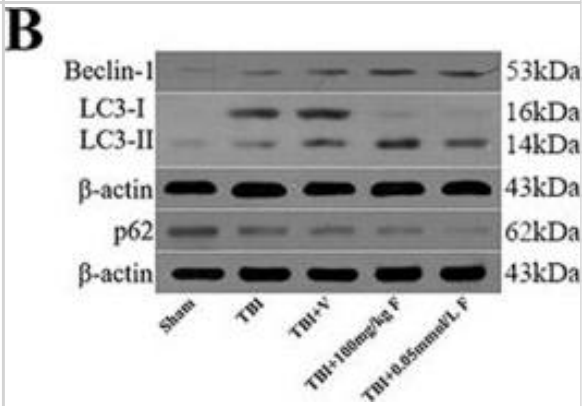
Western Blot: Beclin 1 Antibody - BSA Free [NB500-249] - Inhibition of lysosomes leads to G1 arrest by inactivating cyclin E/CDK2 complex. (A) CQ treatment (10, 50, or 100 μM, 24 h) leads to cell cycle G1 arrest. Quantitation of different cell cycle stages in TM3 cells in the presence or absence of CQ. (B–D) EdU incorporation & mitotic index are reduced in CQ-treated TM3 cells. (B) Immunostaining of EdU (red) & DAPI (blue) in scramble control (CTL) or CQ treated TM3 cells. Quantitation of EdU incorporation (C) or mitotic index (D) in scramble control (CTL) or CQ-treated TM3 cells. These results are mean +/− SD from three independent experiments; more than 1000 cells were counted in each individual group. (E–G) CQ inhibited cyclin E1 expression & CDK2 activation. (E,F) Whole cell extracts of CQ-treated TM3 cells at the concentration of 10, 50, or 100 μM are analyzed by immunoblot with antibodies against Beclin1, cyclin D, cyclin A, cyclin E1, CDK2, phosphorylated CDK2 at Thr160 (pCDK2) & α-tubulin. (G) Quantitation of relative intensity of cyclin E & pCDK2 in (F). *P < 0.05; **P < 0.01; ***P < 0.001. Image collected & cropped by CiteAb from the following publication (https://www.nature.com/articles/s41598-017-00393-4), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



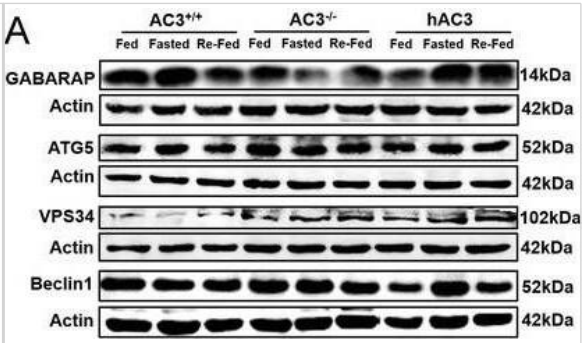
Western Blot: Beclin 1 Antibody - BSA Free [NB500-249] - 8-Cl-Ado-induces autophagic cell killing. (A) Western blot analysis of beclin1 & ATG7 levels in MCF-7 cells transfected with either a pool of control siRNA (siCONT), siRNA targeting the expression of the beclin1 gene (siBECN1), or targeting the expression of the ATG7 gene (siATG7). Immunoblot analysis of LC3B lipidation & PARP cleavage were assessed as markers of autophagosome formation & apoptosis, respectively. GAPDH was used as loading control. Flow cytometric analysis of cells transfected with siCONT, solid bars, siBECN1, hatched bars, or siATG7, checkered bars, treated with 10 μM 8-Cl-Ado & stained with (B) annexin V & PI, as well as (C) acridine orange. Effect of autophagy on 8-Cl-Ado-inhibiton of clonogenic survival. Cells transfected with (D) siCONT, ○, or siBECN1, ●, & with (E) siCONT, ○, or siATG7, ●, were treated with the indicated doses of 8-Cl-Ado for 3 days, washed with PBS, & cultured in fresh medium for 10 days. Colonies of >50 cells were counted under a dissecting microscope. Image collected & cropped by CiteAb from the following publication (https://jhoonline.biomedcentral.com/articles/10.1186/1756-8722-7-23), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



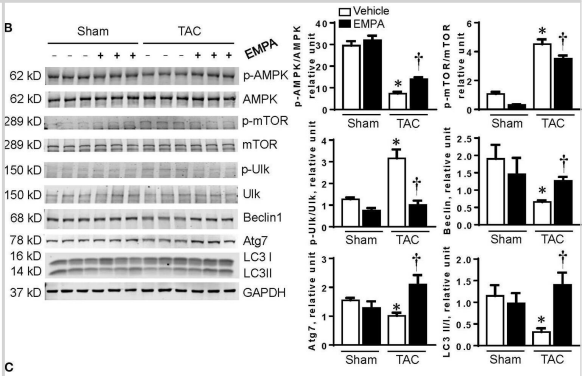
Western Blot: Beclin 1 Antibody - BSA Free [NB500-249] - Fucoxanthin activated autophagy after TBI.(A) Representative images of immunofluorescence for LC3 surrounding the injured cortex. LC3 punctate dots were observed in the cytoplasm by immunofluorescent staining of LC3 (red). Neuron cells & nuclei are labeled with NeuN (green) & DAPI (blue), respectively. Magnification: 40 x. Scale bar: 50 mm. (B) Mice brain tissues were collected 1 day after TBI in different groups, & the expression of LC3, Beclin-1 & p62 was measured by western blot. Fucoxanthin treatment significantly increased the level of LC3-II & Beclin-1 while decreasing the level of p62 after TBI. (C) 3-MA (400 nM) was injected i.c.v. 30 min before TBI. Mice were then subjected to TBI & treatment of fucoxanthin 30 min after TBI. Pretreatment with 3-MA significantly attenuated fucoxanthin-induced activation of autophagy & suppression of apoptosis & oxidative stress in the ipsilateral cortex. Data are presented as mean ± SEM, n = 6 per group; **p < 0.01, ***p < 0.001 versus sham group; #p < 0.05, ##p < 0.01 versus TBI + vehicle group; &&p < 0.01, &&&p < 0.001 versus TBI + fucoxanthin group. β-actin was used as a loading control. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28429775), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: Beclin 1 Antibody - BSA Free [NB500-249] - GABARAP interacts with AC3 via LIRs of AC3 in a ciliary expression□dependent manner. A□E) WB (A) & densitometric quantification of the expression of GABARAP (B), ATG5 (C), VPS34 (D), & Beclin1(E) in the hypothalami of AC3+/+, AC3−/−, & hAC3 mice (n = 3 mice per group). Actin served as the loading control. F) Representative IF co□staining with AC3 & GABARAP antibodies in the VMHs of WT mice. F′) A higher magnification of the boxed region. Scale bars: F) 20 μm; F′) 5 μm. G) Representative images showing the expression levels of GABARAP & AC3 in the VMHs of VMH pIFT88□AC3 KD mice & the controls. Scale bars: 20 μm. H) Schematic representation of GABARAP binding LIRs at aa488□aa493 & aa958□aa963 of AC3. I) Co□IP analysis of GABARAP & AC3 (WT), AC3 (LIR1 Mut), or AC3 (LIR2 Mut). LgG served as the negative control. J) Pull□down analysis of GABARAP & AC3. K) Co□IP analysis of GABARAP & AC3 (WT), AC3 (296 Mut), or AC3 (465 Mut). LgG served as the negative control. L) Schematic representation of AC3 regulating GABARAP. Data represent the mean ± SEM; *p < 0.05 & **p < 0.01; one□way ANOVA & Bonferroni pairwise comparisons. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/34783461), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: Beclin 1 Antibody - BSA Free [NB500-249] - EMPA treatment enhanced autophagy in failing hearts. (A) Transmission electron microscopy (TEM) showed autophagosomes in the hearts. Red arrows point to autophagosomes. (B) EMPA activated autophagy pathway by inhibiting mTOR pathway. (C) RT-PCR results showed that the mRNA expression of Beclin1, Atg7 & LC3 were increased by EMPA treatment after TAC. Results are expressed as mean ± SEM, n = 5–7, *p < 0.05 vs. corresponding sham group, †p < 0.05 vs. corresponding TAC vehicle group. One-way ANOVA & Tukey post hoc test. EMPA, empagliflozin; SEM, standard error of the mean; TAC, transverse aortic constriction; AMPK, AMP-activated protein kinase; mTOR, mammalian target of rapamycin; Ulk, Unc-51 like autophagy activating kinase; Atg7, autophagy related 7; LC3, light chain 3; GAPDH, glyceraldehyde 3-phosphate dehydrogenase. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35647080), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



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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

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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