

# Product Datasheet

## Autophagy Antibody Pack

### NB910-94877

Unit Size: 6 Vials

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

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**NB910-94877**

## Autophagy Antibody Pack

Product Information	
<b>Unit Size</b>	6 Vials
<b>Concentration</b>	Concentration of individual antibodies may be found on the vial label. If unlisted please contact technical services.
<b>Storage</b>	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
<b>Buffer</b>	See individual datasheets

Product Description	
<b>Description</b>	This pack contains 1 vial each of: NB100-2220 (25 uL), NB110-87318 (25 uL), NB110-53818 (25 uL), NB110-56893 (25 uL) and NB110-82384 (25 uL).
<b>Gene ID</b>	8678
<b>Gene Symbol</b>	BECN1
<b>Species</b>	Human, Mouse, Rat, Porcine, Alligator, Bovine, Primate
<b>Reactivity Notes</b>	See individual datasheets of components for their validated species
<b>Specificity/Sensitivity</b>	Autophagy Antibodies (Beclin, LC3, ATG5, ATG9, ATG16L1)
<b>Immunogen</b>	See individual datasheets
<b>Kit Components</b>	NB110-56893: ATG9A Antibody - BSA Free, NB110-87318: Beclin 1 Antibody - BSA Free, NB100-2220: LC3B Antibody - BSA Free, HAF008: Goat anti-Rabbit IgG Secondary Antibody [HRP], NB110-82384: ATG16L1 Antibody - BSA Free, NB110-53818: ATG5 Antibody - BSA Free

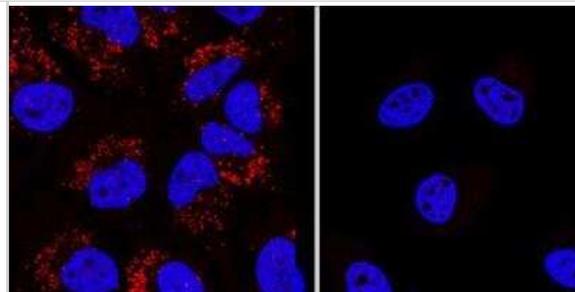
Product Application Details	
<b>Applications</b>	Western Blot, Simple Western, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, Knockdown Validated
<b>Recommended Dilutions</b>	Western Blot, Simple Western, Flow Cytometry, Immunohistochemistry, Immunocytochemistry/ Immunofluorescence, Immunoprecipitation, Immunohistochemistry-Paraffin, Knockdown Validated
<b>Application Notes</b>	See individual datasheets of components for their validated applications

**Images**

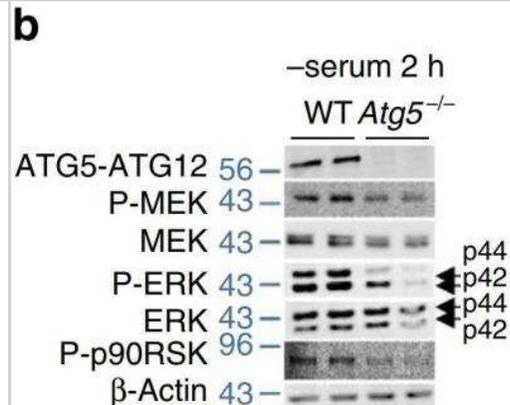
Western Blot: Autophagy Antibody Pack [NB910-94877] - Lysates of HeLa parental cell line and LC3B knockout HeLa cell line (KO) untreated (-) or treated (+) with 50 uM Chloroquine for 18 hours. PVDF (Polyvinylidene difluoride) membrane was probed with 0.5 ug/mL of Rabbit Anti-LC3B Polyclonal Antibody (Catalog # NB100-2220) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog# HAF008). A specific band was detected for LC3B at a molecular weight of approximately 15 kDa (as indicated) in the parental HeLa cell line, but is not detectable in the knockout HeLa cell line. GAPDH is shown as a loading control. This experiment was conducted under reducing conditions.



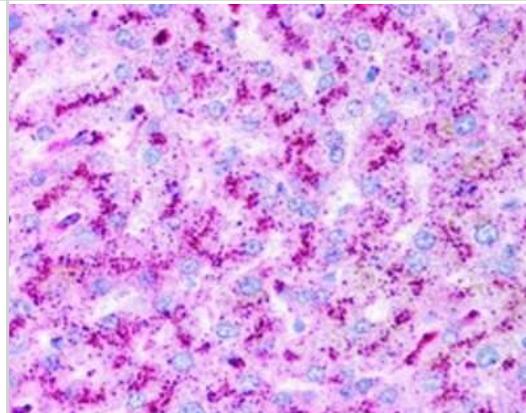
Immunocytochemistry/ Immunofluorescence: Autophagy Antibody Pack [NB910-94877] - LC3B was detected in immersion fixed Cloroquine treated HeLa cells (left) but was not detected in LC3B knockout HeLa cells (right) using rabbit anti-human LC3B polyclonal antibody (Catalog #NB100-2220) at 0.3 ug/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated anti-Rabbit IgG Secondary Antibody (red; Catalog # NL004) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm.



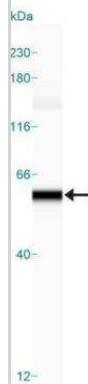
Western Blot: Autophagy Antibody Pack [NB910-94877] - ERK phosphorylation in *Atg5*<sup>-/-</sup> cells depends on nutrient availability. Serum-deprived *Atg5*<sup>-/-</sup> MEFs display decreased ERK and p90RSK phosphorylation. Immunoblots for the indicated proteins in total lysates from 2 h serum-deprived WT and *Atg5*<sup>-/-</sup> MEFs. The bars represent mean±s.e.m. \*P<0.05, \*\*\*P<0.001 compared with Con; Student's t-test, n=3. Image collected and cropped by CiteAb from the following publication ([//www.nature.com/articles/ncomms3799](http://www.nature.com/articles/ncomms3799)), licensed under a CC-BY license. ATG antibody (NB110-53818) used.



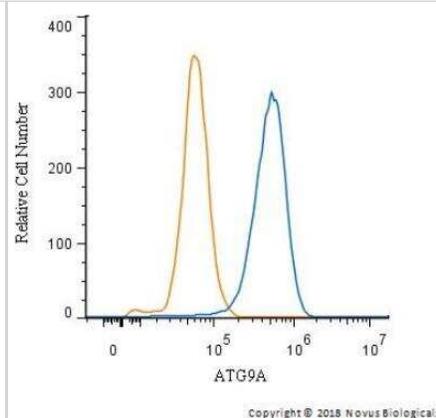
Immunohistochemistry: Autophagy Antibody Pack [NB910-94877] - Staining of ATG5 using NB110-53818 in human hepatocytes (liver) at 2.5ug/ml. 40X magnification.



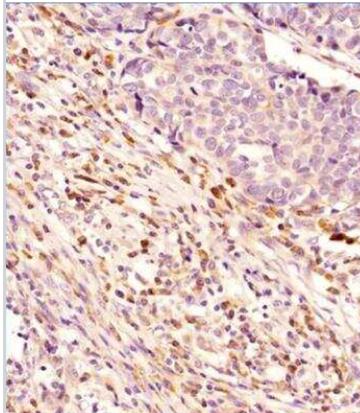
Simple Western: Autophagy Antibody Pack [NB910-94877] - Lane view shows a specific band for Beclin using NB110-87318 in 1.0 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



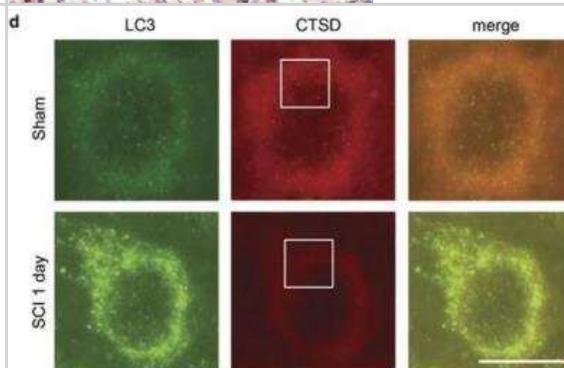
**Flow Cytometry: Autophagy Antibody Pack [NB910-94877]** - An intracellular stain was performed on HeLa Cells with NB110-56893 to detect ATG9 and a matched isotype control. Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG APC-conjugated Secondary Antibody (R&D Systems, F0111).image 5 of 5previousnextclose



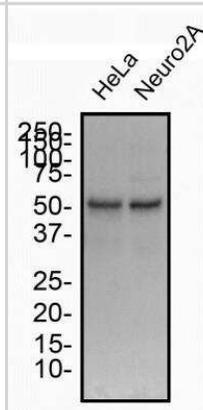
**Immunohistochemistry: Autophagy Antibody Pack [NB910-94877]** - Analysis of a FFPE tissue section of human breast cancer using Beclin 1 antibody (NB110-87318) at 1:300 dilution, followed by a HRP labeled secondary antibody and DAB. Nuclei of the cells were counterstained with hematoxylin. The signal is strongest in a subset of stromal cells, likely the cancer associated fibroblasts.



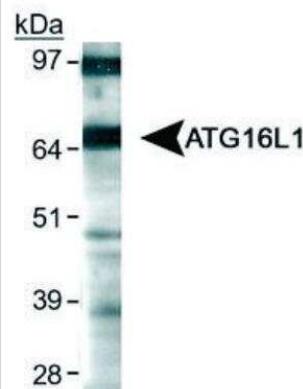
**Immunohistochemistry: Autophagy Antibody Pack [NB910-94877]** - SCI leads to lysosomal dysfunction. Representative high magnification images ( x 60) of LC3 (NB100-2220, green) and CTSD (red) staining in VH of gray matter from sham and day 1 SCI animals. Decrease in number and intensity of CTSD puncta (lysosomes) and increase in LC3 puncta (autophagosomes) is apparent after SCI. Scale bar is 10 um. Image collected and cropped by CiteAb from the following publication (<http://www.nature.com/articles/cddis2014527>) licensed under a CC-BY license.



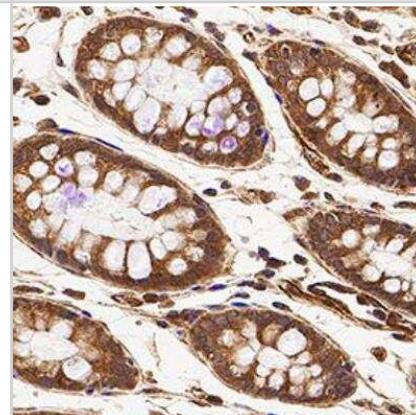
**Western Blot: Autophagy Antibody Pack [NB910-94877]** - Total protein from human HeLa and mouse Neuro2A cells was separated on a 12% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 2.0 ug/ml anti-Beclin-1 (NB110-87318) in blocking buffer and detected with an anti-rabbit HRP secondary antibody using chemiluminescence.



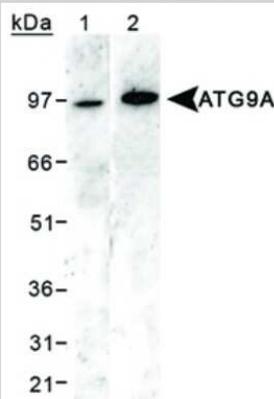
Western Blot: Autophagy Antibody Pack [NB910-94877] - Detection of ATG16L1 using NB110-60928 in HeLa whole cell extracts.



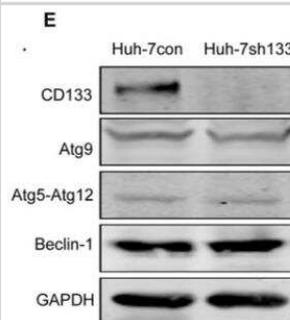
Immunohistochemistry: Autophagy Antibody Pack [NB910-94877] - IHC analysis of formalin fixed paraffin-embedded (FFPE) human colon cancer using ATG16L1 antibody (NB110-60928) at 1:500 on a Bond Rx autostainer (Leica Biosystems). The assay involved 20 minutes of heat induced antigen retrieval (HIER) using 10mM sodium citrate buffer (pH 6.0) and endogenous peroxidase quenching with peroxide block. The sections were incubated with primary antibody for 30 minutes and Bond Polymer Refine Detection (Leica Biosystems) with DAB was used for signal development followed by counterstaining with hematoxylin. Whole slide scanning and capturing of representative images was performed using Aperio AT2 (Leica Biosystems). Cytoplasmic staining of ATG16L1 was observed. Staining was performed by Histowiz.



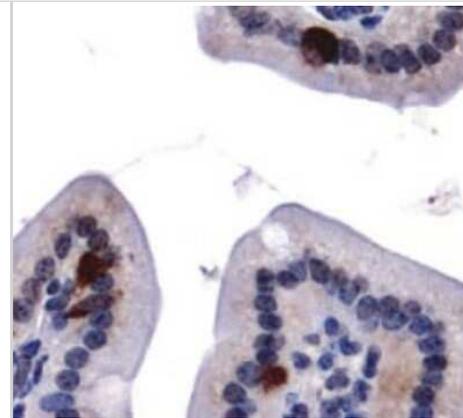
Western Blot: Autophagy Antibody Pack [NB910-94877] - Detection of ATG9A protein using NB110-56893 in HEK293 lysates. Lane 1: siRNA ATG9A knockdown Lane 2: wildtype ATG9A.



Western Blot: Autophagy Antibody Pack [NB910-94877] - CD133 promoted glucose uptake. E. Detection of autophagic genes in Huh-7con and Huh-7sh133 cells by Western blotting. Image collected and cropped by CiteAb from the following publication ([//dx.plos.org/10.1371/journal.pone.0056878](https://dx.plos.org/10.1371/journal.pone.0056878)) licensed under a CC-BY license. ATG9 detected using NB110-56893



Immunohistochemistry: Autophagy Antibody Pack [NB910-94877] -  
Detection of ATG9A (NB110-56893) in mouse intestine.



## Publications

Smith PY, Hernandez-Rapp J, Jolivette F et al. miR-132/212 deficiency impairs tau metabolism and promotes pathological aggregation in vivo. *Hum. Mol. Genet.* 2015-09-11 [PMID: 26362250] (WB, Mouse)



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### **Products Related to NB910-94877**

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H00008878-M01	p62/SQSTM1 Antibody (2C11) - Azide and BSA Free
AF835	Caspase-3 Antibody [Unconjugated] - Active
NB100-2331	LC3A Antibody - BSA Free
NB110-53818	ATG5 Antibody - BSA Free

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### **Limitations**

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

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