Product Datasheet

LC3B Antibody
NB100-2220

Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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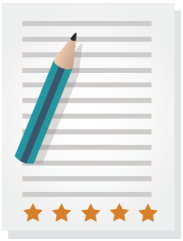
Updated 4/25/2019 v.20.1



NB100-2220

LC3B Antibody

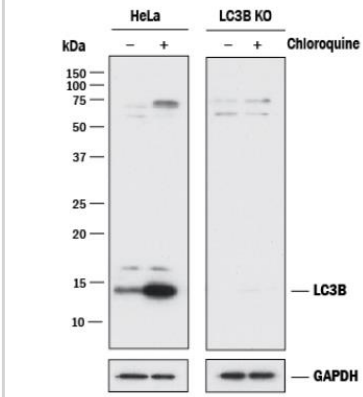
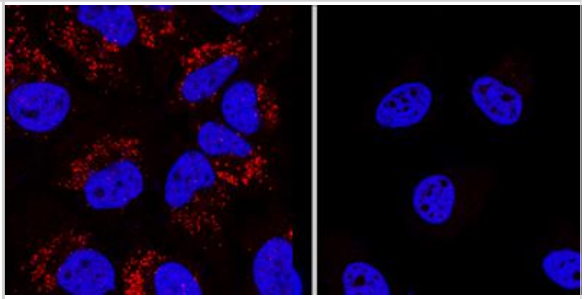
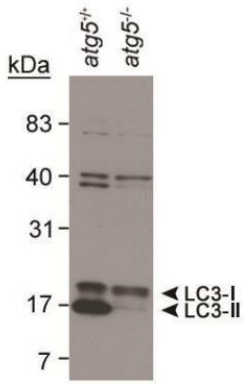
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

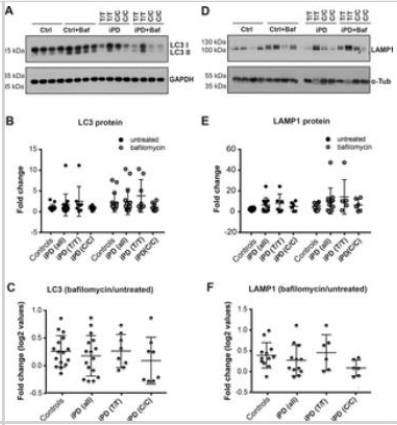
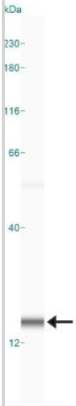
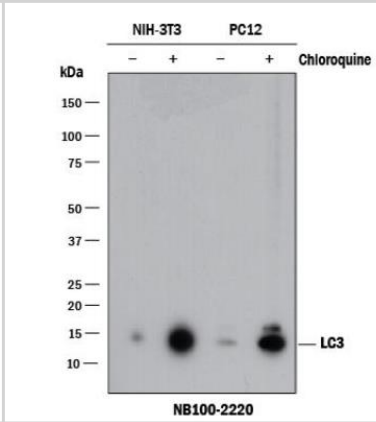
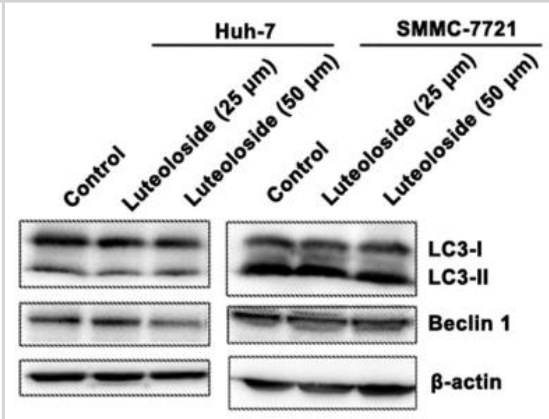
Product Description	
Host	Rabbit
Gene ID	81631
Gene Symbol	MAP1LC3B
Species	Human, Mouse, Rat, Porcine, Avian, Bacteria, Bovine, Canine, Invertebrate, Monkey, Primate, Golden Syrian Hamster, Zebrafish
Reactivity Notes	Bovine reactivity reported in scientific literature (PMID: 24895572). Primate reactivity reported in scientific literature (PMID: 25142602). Canine reactivity reported in scientific literature (PMID: 25839646). Avian reactivity reported in scientific literature (PMID: 29546310). The mouse detection has been reported to be weaker than the human. Immunogen sequence has 84% homology to Xenopus. Invertebrate reactivity reported in scientific literature (PMID: 26716072). Monkey reactivity reported in scientific literature (PMID: 30324853). Guinea pig reactivity reported by customer review.
Marker	Autophagosome Marker
Immunogen	A synthetic peptide made to an N-terminal portion of the human LC3B protein sequence (between residues 1-100). [UniProt# Q9GZQ8]

Product Application Details	
Applications	Western Blot, Simple Western, ELISA, Flow Cytometry, Immunoblotting, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Proximity Ligation Assay, Chromatin Immunoprecipitation (ChIP), Knockout Validated
Recommended Dilutions	Western Blot, Simple Western 1:50, Flow Cytometry, ELISA, Immunohistochemistry 1:200 - 1:400, Immunocytochemistry/Immunofluorescence 1:200, Immunoprecipitation 20ug/500ug of protein, Immunohistochemistry-Paraffin 1:200 - 1:400, Immunohistochemistry-Frozen, Immunoblotting, Proximity Ligation Assay, Chromatin Immunoprecipitation (ChIP), Knockout Validated

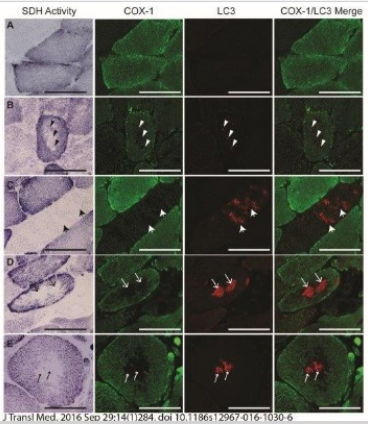
Application Notes	This LC3B antibody is tested for ICC/IF, IHC on paraffin embedded sections, IP and Western blot. Use in IHC on frozen sections reported in scientific literature (PMID: 20008275). Use in immunoblotting reported in scientific literature (PMID 28559895). Use in ELISA reported in scientific literature (PMID 20503249). Use in ChIP reported in scientific literature (PMID28431247). Use in FLOW reported in scientific literature (PMID: 27980456). In Western blot, bands are seen at ~17 and 19 kDa corresponding to LC3-II and LC3-I. In some cases a non-specific band is seen at ~21 kDa in mouse protein. In ICC/IF, cytoplasmic staining was observed in HeLa cells. Use in Proximity Ligation Assay reported in scientific literature (PMID 27219062). In Simple Western only 10 - 15 uL of the recommended dilution is used per data point.

Images

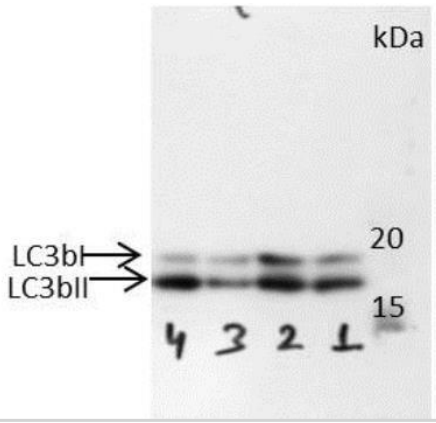
Knockout Validated: LC3B Antibody [NB100-2220] - Lysates of HeLa parental cell line and LC3B knockout HeLa cell line (KO) untreated (-) or treated (+) with 50 uM Chloroquine for 18 hours. PVDF membrane was probed with 0.5 ug/mL of Rabbit Anti-LC3B Polyclonal Antibody (Catalog # NB100-2220) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog# HAF008). A specific band was detected for LC3B at 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.	
Knockout Validated: LC3B Antibody [NB100-2220] - 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: LC3B Antibody [NB100-2220] - Detection of LC3B in mouse ES cell lysates. Atg5-/- ES cells from Dr. Noboru Mizushima [Mizushima, N. et al. J. Cell Biol. 152 (2001)] Photo courtesy of Dr. Beth Levine, UT SW Medical Center.	

Western Blot: LC3B Antibody [NB100-2220] - Autophagy Investigation. (A) Representative WBs of LC3B-I, LC3B-II and GAPDH loading control in DANs derived from 4 iPD patients and 4 healthy controls. (B) WBs from three independent experiments were quantified by densitometry and LC3B-II normalized to a loading control. Grouping of data by healthy vs iPD as well as iPD stratified for T/T and C/C genotype. (C) Ratio of LC3B-II (normalized to loading control) following bafilomycin treatment/untreated. Citation: Marrone L, Bus C, Schondorf D, Fitzgerald JC, Kubler M, Schmid B, et al. (2018) Generation of iPSCs carrying a common LRRK2 risk allele for in vitro modeling of idiopathic Parkinson's disease. PLoS ONE 13(3): e0192497. https://doi.org/10.1371/journal.pone.0192497	
Simple Western: LC3B Antibody [NB100-2220] - Image shows a specific band for LC3B in 0.5 mg/mL of Neuro2A lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	
Western Blot: LC3B Antibody [NB100-2220] - Lysates of mouse NIH3T3 and rat PC-12 cell lines untreated (-) or treated (+) with Chloroquine. PVDF membrane was probed with 0.5 ug/mL rabbit anti-LC3B polyclonal Antibody (NB100-2220, Novus Biologicals), followed by 1:2000 dilution of goat anti-rabbit IgG secondary antibody.	
Western Blot: LC3B Antibody [NB100-2220] - Analysis of LC3B in Huh-7 and SMMC-7721 cells using anti-LC3B antibody. Image from verified customer review.	

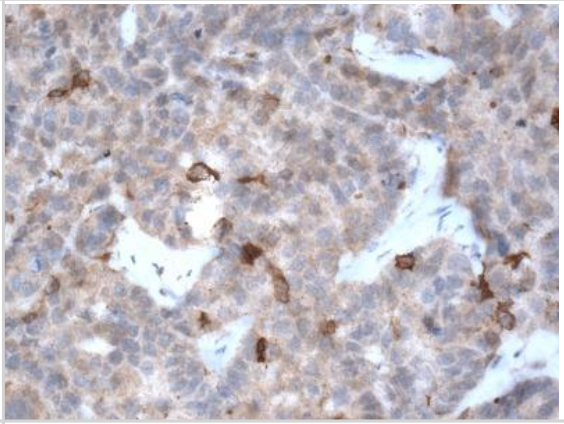
Immunohistochemistry-Frozen: LC3B Antibody [NB100-2220] - LC3 accumulation in muscle fibers from patients with PAD (row B - E). Normal fibers from a Non-PAD sample (row A). Scale bar = 100 uM. Image from verified customer review.



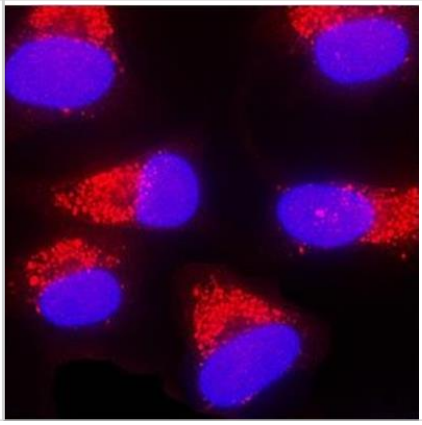
Western Blot: LC3B Antibody [NB100-2220] - Analysis using the Biotin conjugate of NB100-2220. Image from verified customer review.

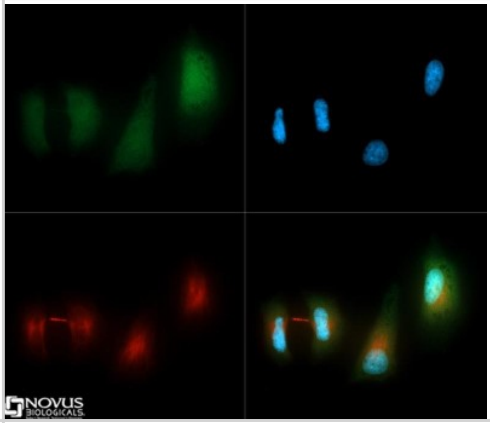
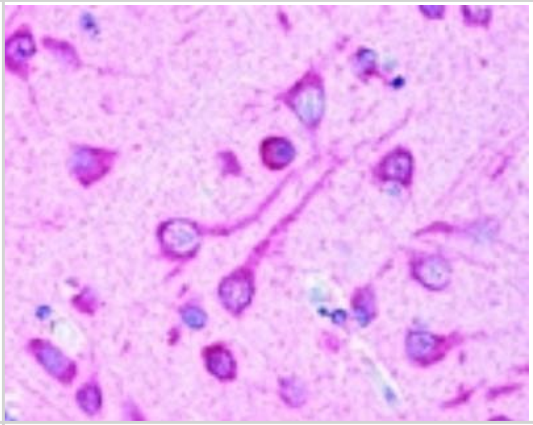
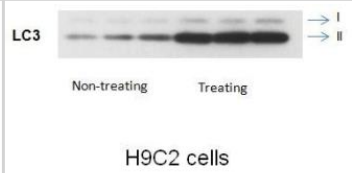
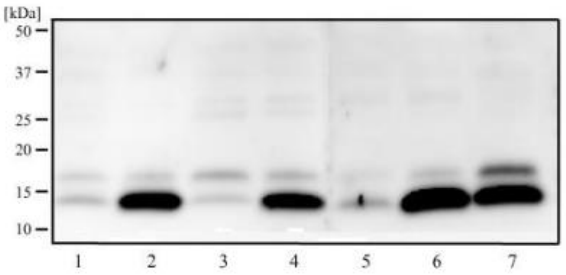


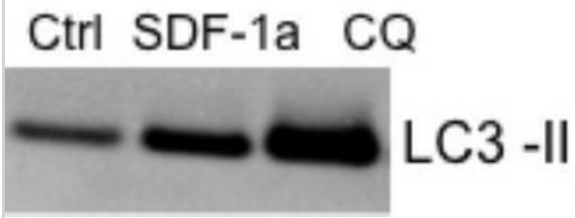
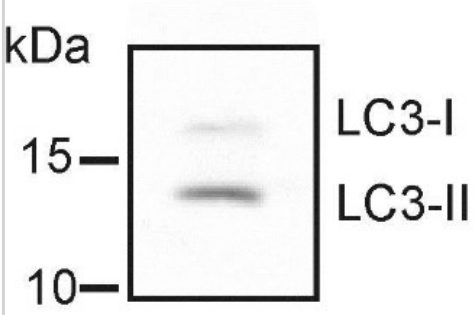
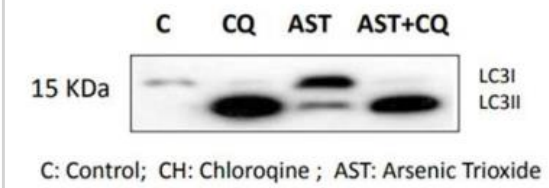
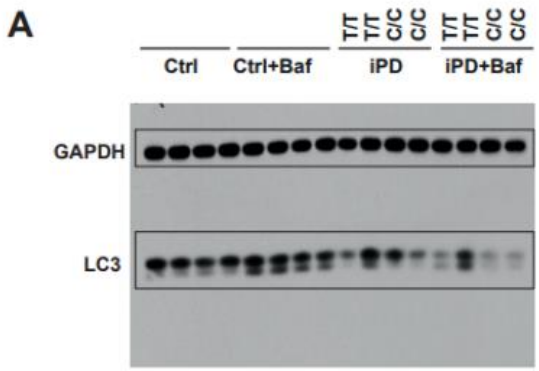
Immunohistochemistry-Paraffin: LC3B Antibody [NB100-2220] - Human ovarian Cancer tissue stained using heat mediated antigen retrieval in pH 6.0 citrate buffer at 1:200 dilution. Image provided by verified customer review.



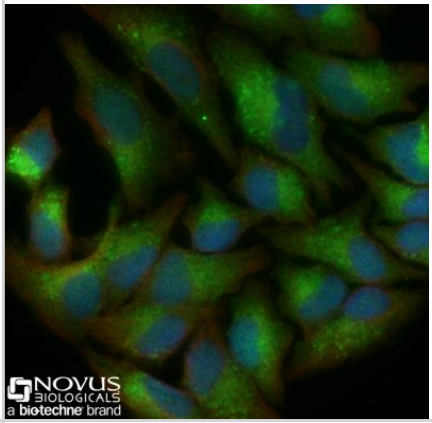
Immunocytochemistry/Immunofluorescence: LC3B Antibody [NB100-2220] - LC3B detected in immersion fixed HeLa human cervical epithelial carcinoma cell line treated with Chloroquine using 1 ug/mL rabbit anti-LC3B polyclonal (NB100-2220, Novus Biologicals). Cells were stained using donkey anti-rabbit IgG-NL557 and counterstained with DAPI (blue).



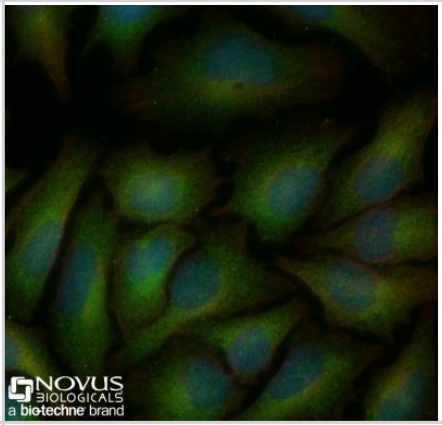
Immunocytochemistry/Immunofluorescence: LC3B Antibody [NB100-2220] - LC3B antibody was tested in HeLa cells with DyLight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and DyLight 550 (red).	
Immunohistochemistry: LC3B Antibody [NB100-2220] - Analysis using the Biotin conjugate of NB100-2220. Staining of brain, cerebral cortex, neurons with cell processes.	
Western Blot: LC3B Antibody [NB100-2220] - Analysis of LC3 in H9C2 cells.	
Western Blot: LC3B Antibody [NB100-2220] - Western blot analysis of HeLa (1), HeLa + CQ (2), SHSY5Y (3), SHSY5Y +CQ (4), A431 (5), A431 +CQ (6) and Ntera2 (7) using LC3 antibody at 2 ug/mL.	

Western Blot: LC3B Antibody [NB100-2220] - Analysis using the DyLight 550 conjugate of NB100-2220. Detection of LC3 in pancreatic cancer cells.	
Western Blot: LC3B Antibody [NB100-2220] - Detection of LC3B in mouse HL-1 cell lysate. Image from verified customer review.	
Western Blot: LC3B Antibody [NB100-2220] - Detection of LC3I and LC3II in mouse cochlea cell line SV-K1. Cells were treated with chloroquine (1 uM), and As2O3 (1 uM) for 24 hrs. Image from verified customer review.	
Western Blot: LC3B Antibody [NB100-2220] - Detection of LC3B. Normalized protein levels by the indicated housekeeping protein. iPD genotypes are reported. Blots show protein levels in the presence or absence of a specified treatment. Baf = bafilomycin, Rot = rotenone, Val = valinomycin, CZC = CZC-25146 Citation: Marrone L, Bus C, Schondorf D, Fitzgerald JC, Kubler M, Schmid B, et al. (2018) Generation of iPSCs carrying a common LRRK2 risk allele for in vitro modeling of idiopathic Parkinson's disease. PLoS ONE 13(3): e0192497. https://doi.org/10.1371/journal.pone.0192497	

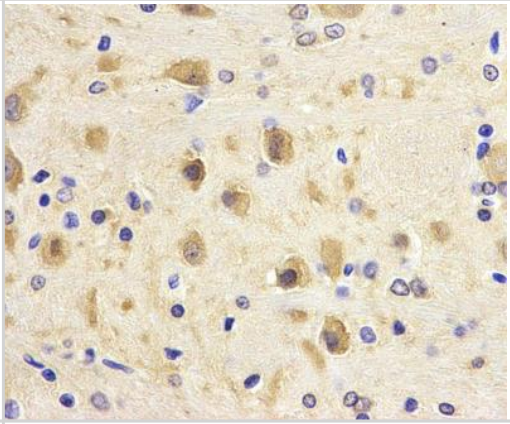
Immunocytochemistry/Immunofluorescence: LC3B Antibody [NB100-2220] - HeLa cells were treated with 50 uM CQ overnight, fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.05% Triton X-100. The cells were incubated with anti-LC3B at 2 ug/mL overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:500 dilution. Alpha tubulin (DM1A) NB100-690 was used as a co-stain at a 1:1000 dilution and detected with an anti-mouse DyLight 550 (Red) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



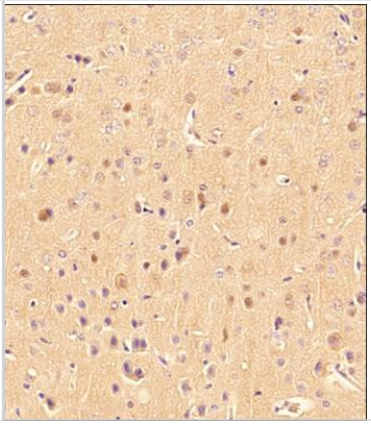
Immunocytochemistry/Immunofluorescence: LC3B Antibody [NB100-2220] - Untreated HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.05% Triton X-100. The cells were incubated with anti-LC3B at 2 ug/mL overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:500 dilution. Alpha tubulin (DM1A) NB100-690 was used as a co-stain at a 1:1000 dilution and detected with an anti-mouse DyLight 550 (Red) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



Immunohistochemistry: LC3B Antibody [NB100-2220] - Rat brain tissue section. Image from verified customer review.



Immunohistochemistry-Paraffin: LC3B Antibody [NB100-2220] - FFPE tissue section of mouse brain using 1:200 dilution of rabbit anti-LC3B antibody. The specific signal of LC3 was detected using HRP-conjugated secondary antibody with DAB reagent, and nuclei of cells were counterstained using hematoxylin. This LC3B antibody generated a low to moderate levels of cytoplasmic staining in the glial cells. The neurons depicted a moderate to strong staining for LC3 in their cytoplasm.



Publications

Chai M, Jiang M, Vergnes L et al. Hair regeneration by small molecules that activate autophagy bioRxiv Apr 18 2018 12:00AM

Wei X, Zhang H, Wei G et al. Honokiol Protects against Anti-B1-Adrenergic Receptor Autoantibody-Induced Myocardial Dysfunction via Activation of Autophagy. Oxidative Medicine and Cellular Longevity Jul 18 2018 12:00AM [PMID: 30116474] (WB, Rat)

Negoita F, Blomdahl J, Wasserstrom S et al. PNPLA3 variant M148 causes resistance to starvation-mediated lipid droplet autophagy in human hepatocytes. J. Cell. Biochem. Sep 1 2018 12:00AM [PMID: 30171718] (WB, ICC/IF, Human)

Seibler P, Burbulla LF, Dulovic M et al. Iron overload is accompanied by mitochondrial and lysosomal dysfunction in WDR45 mutant cells. Brain Oct 1 2018 12:00AM [PMID: 30169597] (WB, Human)

Lopez A, Fleming A, Rubinsztein DC. Seeing is believing: methods to monitor vertebrate autophagy in vivo Drug Des Devel Ther Oct 2 2018 12:00AM [PMID: 30355753] (ICC/IF)

Li , Wang C, Li Z et al. Nano-sized Al2O3 particle-induced autophagy reduces osteolysis in aseptic loosening of total hip arthroplasty by negative feedback regulation of RANKL expression in fibroblasts Cell Death Dis Aug 14 2018 12:00AM [PMID: 30082761] (WB, Human)

Allen SP, Hall B, Castelli LM et al. Astrocyte adenosine deaminase loss increases motor neuron toxicity in amyotrophic lateral sclerosis Brain Mar 1 2019 12:00AM [PMID: 30698736] (WB, Mouse)

Shin JH, Park SJ, Jo DS et al. Down-regulated TMED10 in Alzheimer disease induces autophagy via ATG4B activation Autophagy Mar 1 2019 12:00AM [PMID: 30821607] (WB, Human)

Zhuang J, Lu J, Wang X et al. Purple sweet potato color protects against high-fat diet-induced cognitive deficits through AMPK-mediated autophagy in mouse hippocampus J. Nutr. Biochem. Mar 1 2019 12:00AM [PMID: 30616064] (WB, Mouse)

Lin YJ, Liang WM, Chen CJ et al. Network analysis and mechanisms of action of Chinese herb-related natural compounds in lung cancer cells Phytomedicine Mar 13 2019 12:00AM [PMID: 30901663] (WB, Human)

Wang Y, Shi P, Chen Q et al. Mitochondrial ROS promote macrophage pyroptosis by inducing GSDMD oxidation J Mol Cell Biol Mar 12 2019 12:00AM [PMID: 30860577] (WB, Human)

Li L, Liu Y, Li S et al. Signal regulatory protein alpha protects podocytes through promoting autophagic activity JCI Insight Mar 19 2019 12:00AM [PMID: 30888336] (WB, IHC-Fr, Human)

More publications at http://www.novusbio.com/NB100-2220

Procedures

Western Blot Protocol protocol specific for LC3 Antibody (NB100-2220)
Protocol: Inhibition of Autophagy and LC3B Antibody (NB100-2220) Western Blot

Materials

Chloroquine diphosphate (CQ) (10 mM) in dH2O
1X PBS
Sample buffer, 2X Laemmli buffer: 4% SDS, 5% 2-mercaptoethanol (BME), 20% glycerol, 0.004% bromophenol blue, 0.125 M Tris HCl, pH 6.8
RIPA buffer: 150 mM NaCl, 1% NP-40 or Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris-HCl, pH 8.0, 20 mM Tris-HCl, pH 7.5
1X Running Buffer: 25 mM Tris-base, 192 mM glycine, 0.1% SDS. Adjust to pH 8.3
1X Transfer buffer (wet): 25 mM Tris-base, 192 mM glycine, 20% methanol, Adjust to pH 8.3
TBS
TBST, TBS and 0.1% Tween
Blocking solution: TBST, 5% non-fat dry milk
rabbit anti-LC3B primary antibody (NB100-2220) in blocking buffer (~2 ug/mL)

Methods

Tip: For more information on Western Blotting, see our Western Blot handbook.

1. Grow cells (e.g. HeLa or Neuro2A) in vitro to semi-confluency (70-75%).

2. Add CQ to culture dishes to a final concentration of 50 uM and incubate overnight (16 hours). Remember to include an untreated sample as a negative control.
Note: Validated autophagy inducers should be included as positive controls.

3. Rinse cells with ice-cold 1X PBS and lyse cells with sample buffer.
Note: LC3B-I and LC3B-II are sensitive to degradation, although LC3B-I is more labile. These proteins are sensitive to freeze-thaw cycles and SDS sample buffers. Fresh samples should be analyzed quickly to prevent protein degradation.

4. Sonicate and incubate cells for 5 minutes at 95oC.
Tip: Cells are lysed directly in sample buffer or may be lysed in RIPA buffer.

5. Load samples of Chloroquine-treated and -untreated cell lysates 40 ug/lane on a 4-20% polyacrylamide gradient gel (SDS-PAGE).
Tip: For detection of LC3B it is particularly important to monitor the progress of the gel as this protein is relatively small (~14kDa).

Tip: Alternatively, for non-gradient gels, use a 20% polyacrylamide gel.

6. Transfer proteins to a 0.2 um PVDF membrane for 30 minutes at 100V.

7. After transfer, rinse the membrane with dH2O and stain with Ponceau S for 1-2 minutes to confirm efficiency of protein transfer.

8. Rinse the membrane in dH2O to remove excess stain and mark the loaded lanes and molecular weight markers using a pencil.

9. Block the membrane using blocking buffer solution (5% non-fat dry milk in TBST) for 1 hour at room temperature.

10.Rinse the membrane with TBST for 5 minutes.

11.Dilute the rabbit anti-LC3B primary antibody (NB100-2220) (~2 ug/mL) in blocking buffer and incubate the membrane for 1 hour at room temperature.

12.Rinse the membrane with dH2O.

13.Rinse the membrane with TBST, 3 times for 10 minutes each.

14.Incubate the membrane with diluted secondary antibody, according with product's specifications, (e.g. anti-rabbit-IgG HRP-conjugated) in blocking buffer for 1 hour at room temperature.
Note: Tween-20 may be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.

15.Rinse the membrane with TBST, 3 times for 10 minutes each.

16.Apply the detection reagent of choice (e.g. BioFX Super Plus ECL) in accordance with the manufacturer's instructions.

17.Image the blot.
Tip: LC3B-I and it's lipidated form LC3B-II have different electrophoretic mobility properties, with the lipidated form moving faster in an SDS-PAGE gel, albeit its larger molecular weight. LC3B-II runs at 14-16 kDa while LC3B-I runs at 16-18kDa.

Note: This assay measures the difference in the LC3B-II signal in the presence and absence of inhibitors (e.g., lysosomotropic agents). When autophagic flux is present or induced in a system an increase in the LC3B-II signal should be observed with the inhibitor.

Immunohistochemistry-Paraffin Protocol for LC3B/MAP1LC3B Antibody (NB100-2220)
I. Deparaffinization:

A. Treat slides with Xylene: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.
B. Treat slides with 100% Reagent Alcohol: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.

II. Quench Endogenous Peroxidase:

To Prepare 200 ml of Quenching Solution: Hydrogen Peroxide to 200 ml of Methanol.
**Use within 4 hours of preparation

A. Place slides in peroxidase quenching solution: 15-30 minutes.
B. Place slides in distilled water: 2 changes for 2 minutes each.

III. Retrieve Epitopes:

A. Preheat Citrate Buffer. Place 200 ml of Citrate Buffer Working Solution into container, cover and place into steamer. Heat to 90-96C.
B. Place rack of slides into hot Citrate Buffer for 20 minutes. Cover.
C. Carefully remove container with slides from steamer and cool on bench, uncovered, for 20 minutes.
D. Slowly add distilled water to further cool for 5 minutes.
E. Rinse slides with distilled water. 2 changes for 2 minutes each.

IV. Immunostaining Procedure:
A. Remove each slide from rack and circle tissue section with a hydrophobic barrier pen (e.g. Liquid Blocker-Super Pap Pen).
B. Flood slide with Wash Solution.

**Do not allow tissue sections to dry for the rest of the procedure.

C. Drain wash solution and apply 4 drops of Blocking Reagent to each slide and incubate for 15 minutes.
D. Drain Blocking Reagent (do not wash off the Blocking Reagent), apply 200 ul of Primary Antibody solution to each slide, and incubate for 1 hour.
E. Wash slides with Wash Solution: 3 changes for 5 minutes each.
F. Drain wash solution, apply 4 drops of Secondary antibody to each slide and incubate for 1 hour.

G. Wash slides with Wash Solution: 3 changes for 5 minutes each.
H. Drain wash solution, apply 4 drops of DAB Substrate to each slide and develop for 5-10 minutes. Check development with microscope.
I. Wash slides with Wash Solution: 3 changes for 5 minutes each.
J. Drain wash solution, apply 4 drops of Hematoxylin to each slide and stain for 1-3 minutes. Increase time if darker counterstaining is desired.
K. Wash slides with Wash Solution: 2-3 changes for 2 minutes each.
L. Drain wash solution and apply 4 drops of Bluing Solution to each slide for 1-2 minutes.
M. Rinse slides in distilled water.
N. Soak slides in 70% reagent alcohol: 3 minutes with intermittent agitation.
O. Soak slides in 95% reagent alcohol: 2 changes for 3 minutes each with intermittent agitation.
P. Soak slides in 100% reagent alcohol: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
Q. Soak slides in Xylene: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
R. Apply 2-3 drops of non-aqueous mounting media to each slide and mount coverslip.

Notes:
- Use treated slides (e.g. HistoBond) to assure adherence of FFPE sections to slide.
- Prior to deparaffinization, heat slides overnight in a 60C oven.
- All steps in which Xylene is used should be performed in a fume hood.
- For Epitope Retrieval, a microwave or pressure cooker may be substituted for the steamer method. Adjust times as necessary depending on conditions.
- For the initial IHC run with a new primary antibody, test tissues with and without Epitope Retrieval. In some instances, Epitope Retrieval may not be necessary.
- 200 ul is the recommended maximum volume to apply to a slide for full coverage. Using more than 200 ul may allow solutions to wick off the slide and create drying artifacts. - For small tissue sections less than 200 ul may be used.
- 5 minutes of development with DAB Substrate should be sufficient. Do not develop for more than 10 minutes. If 5 minutes of development causes background staining, further dilution of the primary antibody may be necessary.
- Hematoxylin should produce a light nuclear counterstain so as not to obscure the DAB staining. Counterstain for 1-1.5 minutes for nuclear antigens. Counterstain for 2-3 minutes for cytoplasmic and membranous antigens. If darker counterstaining is desired increase time (up to 10 minutes).

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