Product Datasheet

LC3B Antibody
NB100-2220

Unit Size: 0.1 ml
Store at -20C.

Reviews: 44  Publications: 1004

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Updated 2/20/2020 v.20.1

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NB100-2220
LC3B Antibody

**Product Information**

<table>
<thead>
<tr>
<th><strong>Unit Size</strong></th>
<th>0.1 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Concentration</strong></td>
<td>1.0 mg/ml</td>
</tr>
<tr>
<td><strong>Storage</strong></td>
<td>Store at -20C.</td>
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<tr>
<td><strong>Clonality</strong></td>
<td>Polyclonal</td>
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<tr>
<td><strong>Preservative</strong></td>
<td>0.02% Sodium Azide</td>
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<tr>
<td><strong>Isotype</strong></td>
<td>IgG</td>
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<tr>
<td><strong>Purity</strong></td>
<td>Immunogen affinity purified</td>
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<tr>
<td><strong>Buffer</strong></td>
<td>PBS</td>
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<td><strong>Target Molecular Weight</strong></td>
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**Product Description**

<table>
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<tr>
<th><strong>Host</strong></th>
<th>Rabbit</th>
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<tr>
<td><strong>Gene ID</strong></td>
<td>81631</td>
</tr>
<tr>
<td><strong>Gene Symbol</strong></td>
<td>MAP1LC3B</td>
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<tr>
<td><strong>Species</strong></td>
<td>Human, Mouse, Rat, Porcine, Avian, Bovine, Canine, Chicken, Guinea Pig, Hamster, Invertebrate, Monkey, Primate, Rabbit, Golden Syrian Hamster, Zebrafish</td>
</tr>
</tbody>
</table>

**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). Hamster reactivity reported in scientific literature (PMID: 26423766). Rabbit reactivity reported in scientific literature (PMID: 26497211). 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. Rat reactivity reported in scientific literature (PMID: 30744518). Bacteria reactivity reported in scientific literature (PMID: 28783414). Chicken reactivity reported in scientific literature (PMID: 30649814). Porcine reactivity reported in scientific literature (PMID: 30789643). Golden Syrian hamster reactivity reported in scientific literature (PMID: 23180219). Zebrafish reactivity reported in scientific literature (PMID: 29185873).

**Marker**

Autophagosome Marker

**Immunogen**

Polyclonal LC3B Antibody was made to 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), Knockdown Validated, Knockout Validated |

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Recommended Dilutions

Western Blot 0.5 - 2.0 ug/ml, 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, Knockdown Validated

Application Notes

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 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 Proximity Ligation Assay reported in scientific literature (PMID 27219062). Use in FLOW reported in scientific literature (PMID: 27980456). Use in IHC on paraffin-embedded sections reported in scientific literature (PMID: 31071489). Use in Immunoprecipitation reported in scientific literature (PMID: 31209065). In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. Knockdown validation (PMID: 31754102).

Images

Western Blot: LC3B Antibody [NB100-2220] - GNA triggers the formation of autophagic markers in A549 and HeLa cell. Effects of GNA on LC3 protein. A549 cells were treated with various concentrations of GNA for 24 hours or 3 uM GNA for the indicated periods of time, then analyzed by western blotting using anti-LC3 antibodies. GAPDH protein was used as the loading control. The bar graph shows the band intensities of LC3-II relative to those of GAPDH. Mean+/− SEM, n = 3, *means p<0.05, **p<0.01, one-way ANOVA. Image collected and cropped by CiteAb from the following publication (http://dx.plos.org/10.1371/journal.pone.0083604), licensed under a CC-BY licence.

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 (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.
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] - Autophagy Investigation. (A) Representative WBs (Western Blots) 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

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.

Immunohistochemistry: LC3B Antibody [NB100-2220] - Localization of LC3 by immunohistochemical method. The immunohistochemical staining shows that LC3, CRF, and HIF-1alpha have an overlapping localization in villous and extravillous trophoblast. d: decidua; v: villi; DeVe: decidual vessel. Original magnification 40x. Image collected and cropped by CiteAb from the following publication (http://www.hindawi.com/journals/bmri/2013/689768/) licensed under a CC-BY licence.

Photo courtesy of Dr. Beth Levine, UT SW Medical Center.

Western Blot: LC3B Antibody [NB100-2220] - Autophagy induced by ceramide in CNE2 and SUNE1 cells. Ceramide dose and time-dependently induced the formation of LC3-II, a marker for autophagy. SUNE1 cells were treated with ceramide in the indicated concentrations for 24 h or treated with 20 uM ceramide for the indicated times. Lysates were analyzed by immunoblotting with LC3 antibody. Image collected and cropped by CiteAb from the following publication (http://translational-medicine.biomedcentral.com/articles/10.1186/1479-5876-9-161) licensed under a CC-BY licence.

Western Blot: LC3B Antibody [NB100-2220] - GNA triggers the formation of autophagic markers in A549 and HeLa cell. Effects of GNA on LC3 protein. A549 cells were treated with various concentrations of GNA for 24 hours or 3 uM GNA for the indicated periods of time, then analyzed by western blotting using anti-LC3 antibodies. GAPDH protein was used as the loading control. The bar graph shows the band intensities of LC3-II relative to those of GAPDH. Mean+-/ SEM, n=73, *means p<0.05, ** p<0.01, one-way ANOVA. Image collected and cropped by CiteAb from the following publication (http://dx.plos.org/10.1371/journal.pone.0083604), licensed under a CC-BY licence.

Immunocytochemistry/Immunofluorescence: LC3B Antibody [NB100-2220] - Autophagosomes accumulate in microglia and oligodendrocytes of the dorsal white matter adjacent to injury after SCI. Representative images of IHC staining for LC3 (green) and activated microglia marker CD11B (red) in dorsal white matter of sham and SCI animals. Increased co-localization between LC3 and CD11B is apparent at day 1 after SCI. Image collected and cropped by CiteAb from the following publication (http://www.nature.com/articles/cddis2014527) licensed under a CC-BY licence.
Immunohistochemistry: LC3B Antibody [NB100-2220] - SCI leads to lysosomal dysfunction. Representative high magnification images (x 60) of LC3 (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 licence.

Western Blot: LC3B Antibody [NB100-2220] - Lysates of mouse NIH3T3 and rat PC-12 cell lines untreated (-) or treated (+) with Chloroquine. PVDF (Polyvinylidene difluoride) membrane was probed with 0.5 ug/mL rabbit anti-LC3B polyclonal Antibody (Catalog # NB100-2220, Novus Biologicals), followed by 1:2000 dilution of goat anti-rabbit IgG secondary antibody. LC3 detected at a molecular weight of approximately 15 kDa in treated NIH3T3 and PC-12 cells.

Western Blot: LC3B Antibody [NB100-2220] - Analysis of LC3 in untreated and treated H9C2 cells using Rabbit anti-LC3B Antibody [Catalog # NB100-2220].

Western Blot: LC3B Antibody [NB100-2220] - Detection of LC3I and LC3II in mouse cochlea cell line SV-K1 using Rabbit anti-LC3B Antibody [Catalog # NB100-2220]. 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] - Expression of autophagic protein LC3 in BME-UV1 cells forming acinar structures on Matrigel. Representative images of Western blot analysis of LC3 in bovine MECs grown in 3D culture for 3, 6, 9, and 14 days in differentiation medium (control), enriched with 17beta-estradiol (E2, 1 nM), progesterone (P4, 5 ng/mL), or both (E + P); expression of beta-actin was used as a loading control; graphs below the images show the results of densitometric analysis, in which IOD of each band was measured, and the values were normalized to IOD of beta-actin; the IOD results are presented as means +/- SEM from at least three separate experiments. Image collected and cropped by CiteAb from the following publication [http://www.hindawi.com/journals/bmri/2014/382653/] licensed under a CC-BY licence.

Immunocytochemistry/Immunofluorescence: LC3B Antibody [NB100-2220] - Autophagosomes accumulate in neurons of the VH gray matter at day 1 after SCI. Representative images of IHC staining for LC3 (green) and neuronal marker NeuN (red) in VH of gray matter from sham and SCI animals. Stronger co-localization between NeuN and LC3 is apparent at day 1 after SCI. Scale bar is 20 um. Image collected and cropped by CiteAb from the following publication [http://www.nature.com/articles/cddis2014527] licensed under a CC-BY licence.


Immunohistochemistry-Paraffin: LC3B Antibody [NB100-2220] - FFPE (Formalin-Fixed Paraffin-Embedded) tissue section of mouse brain using 1:200 dilution of Rabbit anti-LC3B antibody [Catalog # NB100-2220]. The specific signal of LC3 was detected using HRP-conjugated secondary antibody with DAB (3, 3'-diaminobenzidine) 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.

Immunohistochemistry: LC3B Antibody [NB100-2220] - Analysis using the Biotin conjugate of Rabbit anti-LC3B Antibody [Catalog # NB100-2220]. Staining of brain, cerebral cortex, neurons with cell processes.

Simple Western: LC3B Antibody [NB100-2220] - Image shows a specific band for LC3B at a molecular weight of approximately 15 kDa in 0.5 mg/mL of Neuro2A lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.


Schaub T, Gürgen D, Maus D. mTORC1 and mTORC2 Differentially Regulate Cell Fate Programs to Coordinate Osteoblastic Differentiation in Mesenchymal Stromal Cells. Sci Rep Dec 27 2019 12:00AM [PMID: 31882658] (WB, ICC/IF, KD, Human, Mouse)


An HK, Chung KM, Park H, et al. CASP9 (caspase 9) is essential for autophagosome maturation through regulation of mitochondrial homeostasis. Autophagy Dec 10 2019 12:00AM [PMID: 31818185] (WB, Rat, 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 its 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)

**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.

**Staining:**
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in wash buffer for 5 minutes.
3. Block each section with 100-400 ul blocking solution 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 biotinylated diluted secondary antibody. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in deionized water.
12. Counterstain sections in hematoxylin.
13. Wash sections in deionized water two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.
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.

For more information on our 100% guarantee, please visit www.novusbio.com/guarantee

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