Product Datasheet

LC3B Antibody
NB600-1384

Unit Size: 0.1 ml

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

Reviews: 18  Publications: 276

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Updated 5/6/2020 v.20.1

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## Product Information

<table>
<thead>
<tr>
<th>Feature</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unit Size</td>
<td>0.1 ml</td>
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<tr>
<td>Concentration</td>
<td>1.0 mg/ml</td>
</tr>
<tr>
<td>Storage</td>
<td>Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.</td>
</tr>
<tr>
<td>Clonality</td>
<td>Polyclonal</td>
</tr>
<tr>
<td>Preservative</td>
<td>0.02% Sodium Azide</td>
</tr>
<tr>
<td>Isotype</td>
<td>IgG</td>
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<tr>
<td>Purity</td>
<td>Immunogen affinity purified</td>
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<tr>
<td>Buffer</td>
<td>PBS</td>
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<tr>
<td>Target Molecular Weight</td>
<td>14.688 kDa</td>
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## Product Description

<table>
<thead>
<tr>
<th>Feature</th>
<th>Details</th>
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<tbody>
<tr>
<td>Host</td>
<td>Rabbit</td>
</tr>
<tr>
<td>Gene ID</td>
<td>81631</td>
</tr>
<tr>
<td>Gene Symbol</td>
<td>MAP1LC3B</td>
</tr>
<tr>
<td>Species</td>
<td>Human, Mouse, Rat, Porcine, Bacteria, Bovine, Canine, Primate, Zebrafish</td>
</tr>
<tr>
<td>Reactivity Notes</td>
<td>Zebrafish reactivity reported in scientific literature (PMID: 23724125). Canine and primate reactivity reported in scientific literature (PMID: 24027311) Porcine reactivity reported in scientific literature (PMID: 25378587). Rat reactivity reported in scientific literature (30067379). Bacteria reactivity reported in scientific literature (31110360). Bovine reactivity reported in scientific literature (21868124). Other species have not been tested.</td>
</tr>
<tr>
<td>Marker</td>
<td>Autophagosome Marker</td>
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## Product Application Details

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<tr>
<th>Feature</th>
<th>Details</th>
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Application Notes

In Western Blot, bands are seen at ~17 and 19 kDa corresponding to LC3-II and LC3-I. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors. Electron Microscopy was reported in scientific literature (PMID: 21885071). Use in Immunohistochemistry-free floating reported in scientific literature (PMID: 24928515). Use in Immunohistochemistry on both paraffin-embedded and frozen sections reported in scientific literature (PMID: 18259115). Use in immunoprecipitation reported in scientific literature (PMID: 26098573). Use in immunoblotting reported in scientific literature (PMID: 25383539). Use in flow reported in scientific literature (PMID: 27622036). Use in ELISA reported in scientific literature (PMID: 21885071). In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. Separated by Size-Wes, Sally Sue/Peggy Sue.

Images

Western Blot: LC3B Antibody [NB600-1384] - Analysis of LC3B in treated U87-MG (human glioblastoma astrocytoma) lysates using anti- [Catalog # NB600-1384].

Immunohistochemistry: LC3B Antibody [NB600-1384] - Staining of treated U373-MG (human glioblastoma) cells using anti- [Catalog # NB600-1384].

Immunocytochemistry/Immunofluorescence: LC3B Antibody [NB600-1384] - Immunocytochemical/Immunofluorescent staining of treated U373-MG cells using the HRP conjugate of anti- (Catalog # NB600-1384). The nuclei were stained with DAPI.
Western Blot: LC3B Antibody [NB600-1384] - Depletion of RARalpha prevents ATRA-induced autophagic flux in SKBR3 cells. LC3B western blotting and quantification of the autophagic activity in control and RARalpha-knockdown SKBR3 cells upon treatment with 1 μM ATRA for 2 days in the presence or absence of BafA for 2 h at 200 nM. LC3B-II expression was normalized to GAPDH and to vehicle-treated control cells. Standard deviations from at least five independent experiments are shown. Image collected and cropped by CiteAb from the following publication (http://www.nature.com/articles/cddis2015236), licensed under a CC-BY licence.

Immunocytochemistry/Immunofluorescence: LC3B Antibody [NB600-1384] - Confocal analysis of HeLa cells using Rabbit anti-LC3B antibody (Catalog # NB600-1384, 1:5). An Alexa Fluor 488-conjugated Goat to rabbit IgG was used as secondary antibody (green). Actin filaments were labeled with Alexa Fluor 568 phalloidin (red). DAPI was used to stain the cell nuclei (blue).


Electron Microscopy: LC3B Antibody [NB600-1384] - NOD1-dependent induction of autophagy contributes to IRGM and LC3 recruitment to Mtb-containing vesicles. The subcellular localization of autophagy proteins was observed in untreated (A, C) and Tri-DAP treated cells (B, D) using anti-IRGM and anti-LC3 antibodies, which were detected using a secondary antibody coupled to 5 nm gold particles (indicated with arrowheads; TEM X 62,000). Gold particles colocalizing with bacteria were manually counted in 10 macrophages of each condition (E, F) and the differences between the treatments are indicated: ***p < 0.01 and *p < 0.05 vs. Mtb, ++p < 0.01 vs. Tri-DAP, Wilcoxon Rank test. Box plots indicate median and quartiles. Image collected and cropped by CiteAb from the following publication (http://bmcpeuimmed.biomedcentral.com/articles/10.1186/1471-2466-14-152), licensed under a CC-BY licence.
Immunohistochemistry: LC3B Antibody [NB600-1384] - LC3B staining in glioblastoma multiform tissue.

Knockdown Validated: LC3B Antibody [NB600-1384] - LC3A and LC3B siRNAs specifically block the expression of the LC3A and LC3B proteins, respectively, in A549 cell line (E1,2,3). In E4 the reactivity of and of the LC3B (5F10 antibody) is shown, following silencing of the LC3A or of the LC3B genes, in the A549 cell line. Image collected and cropped by CiteAb from the following publication (http://dx.plos.org/10.1371/journal.pone.0137675), licensed under a CC-BY licence.

Immunocytochemistry/Immunofluorescence: LC3B Antibody [NB600-1384] - Analysis of LC3B in HeLa cells using anti-LC3B antibody (red) [Catalog # NB600-1384]. Nuclei were counterstained with DAPI (blue).

Simple Western: LC3B Antibody [NB600-1384] - Lane view shows a specific band for LC3B in 0.5 mg/ml of Neuro2A lysate at a molecular weight of approximately 15 kDa. This experiment was performed under reducing conditions using the 12-230 kDa separation system.

Publications

Bernard M, Yang B, Migneault F et al. Autophagy drives fibroblast senescence through MTORC2 regulation
Autophagy Jan 13 2020 12:00AM [PMID: 31931659] (WB, Human)


Zhou W, Fang C, Zhang L et al. Thioredoxin domain-containing protein 9 (TXNDC9) contributes to oxaliplatin resistance through regulation of autophagy-apoptosis in colorectal adenocarcinoma Biochemical and Biophysical Research Communications Feb 1 2020 12:00AM [PMID: 32029274]

Dubois-Deruy E, Gelinas R, Beauloye C, Esfahani H Beta-3 Adrenoreceptors protect from hypertrophic remodelling through AMP-Activated Protein Kinase and Autophagy. bioRxiv Jan 1 2020 12:00AM (ICC/IF, Mouse)


Omary Z Understanding the role of LRBA in patients with inflammatory bowel disease Thesis

Shao Q, Yang M, Liang C, et al. C9orf72 and smcr8 mutant mice reveal MTORC1 activation due to impaired lysosomal degradation and exocytosis Autophagy Dec 26 2019 12:00AM [PMID: 31847700] (ICC/IF, Mouse)


Details:
Citation used the Alexa Fluor 488 format of this antibody.


Yu Y, Xiang N, Lin M et al. miR- 26a Sensitizes Melanoma Cells To Dabrafenib Via Targeting HMGB1-Dependent Autophagy Pathways Drug Des Devel Ther Oct 29 2019 12:00AM [PMID: 31754297] (WB, Human)

More publications at http://www.novusbio.com/NB600-1384
Procedures

Western Blot Protocol for LC3B Antibody (NB600-1384)
Protocol: Inhibition of Autophagy and LC3B Antibody (NB600-1384) 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 (NB600-1384) (~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 16-18 kDa.

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.

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**Immunohistochemistry-Paraffin Protocol for LC3B Antibody (NB600-1384)**

**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.

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