Product Datasheet

LC3B Antibody
NB600-1384

Unit Size: 0.1 ml
Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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## NB600-1384
### LC3B Antibody

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

### Product Description

- **Host**: Rabbit
- **Gene ID**: 81631
- **Gene Symbol**: MAP1LC3B
- **Species**: Human, Mouse, Rat, Porcine, Bacteria, Bovine, Canine, Primate, Zebrafish
- **Reactivity Notes**: 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.
- **Marker**: Autophagosome Marker
- **Immunogen**: Polyclonal LC3B Antibody was made to a synthetic peptide made to the N-terminal region of the human LC3B protein. [Uniprot: Q9GZQ8]

### Product Application Details

#### Applications

#### Recommended Dilutions
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). 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.
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).

Immunohistochemistry-Paraffin: LC3B Antibody [NB600-1384] - Analysis of U87MG glioma xenografts using anti- [Catalog # NB600-1384]. Image from verified customer review.

Immunohistochemistry: LC3B Antibody [NB600-1384] - LC3B staining in glioblastoma multiform tissue.

Immunocytochemistry/Immunofluorescence: LC3B Antibody [NB600-1384] - LC3B/MAP1 [NB600-1384] - Rabbit anti-LC3B antibody [Catalog # NB600-1384] was tested in 50uM Chloroquine treated HeLa cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red). An antibody concentration of 0.1 ug/ml was used. Image objective 40x.
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.

Hong JM, Kim JH, Kim H et al. SB365, Pulsatilla Saponin D Induces Caspase-Independent Cell Death and Augments the Anticancer Effect of Temozolomide in Glioblastoma Multiforme Cells Molecules Sep 5 2019 12:00AM [PMID: 31491945] (WB, Human, Mouse)


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.
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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