Product Datasheet

LC3B Antibody (1251B) - BSA Free
NBP2-60735

Unit Size: 0.1 mg

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

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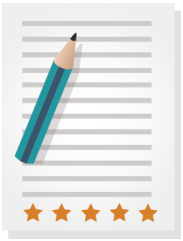


NBP2-60735

LC3B Antibody (1251B) - BSA Free

Product Information

Unit Size	0.1 mg
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	1251B
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Protein A or G purified
Buffer	PBS
Target Molecular Weight	14.688 kDa

Product Description

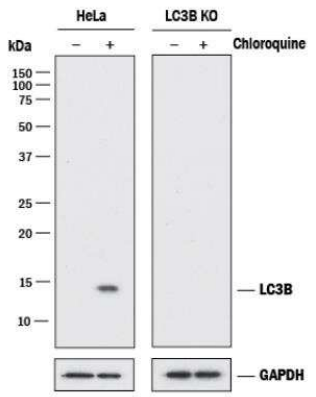
Host	Rabbit
Gene ID	81631
Gene Symbol	MAP1LC3B
Species	Human, Mouse, Rat
Immunogen	Recombinant monoclonal LC3B Antibody (1251B) was made to a synthetic peptide made to an N-terminal portion of the human LC3B protein sequence (between residues 1-100). [UniProt# Q9GZQ8].

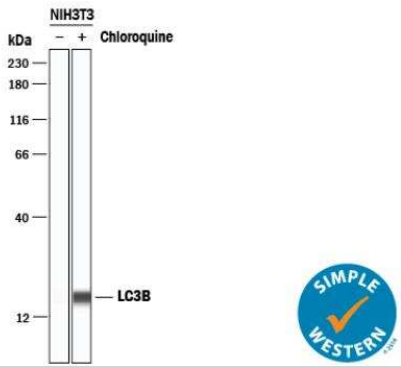
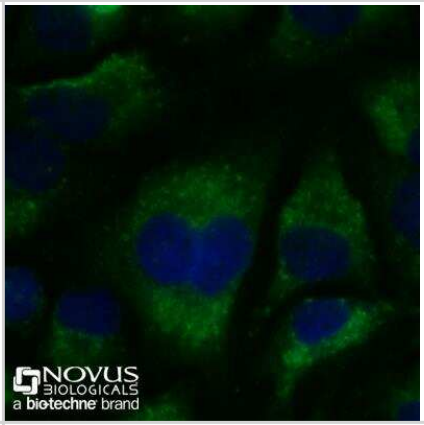
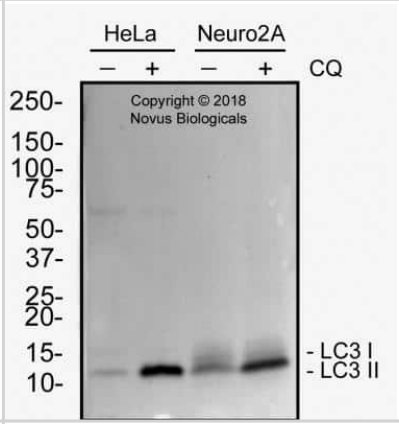
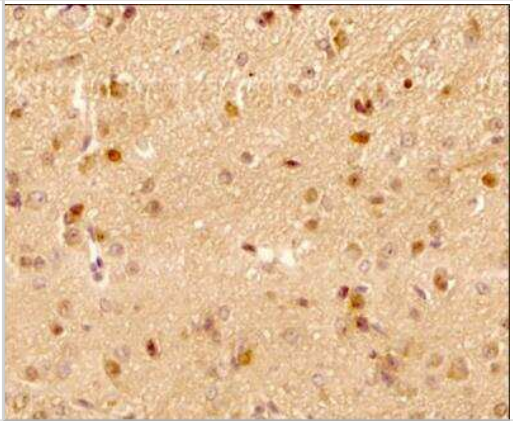
Product Application Details

Applications	Western Blot, Simple Western, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Knockout Validated
Recommended Dilutions	Western Blot 0.1 ug/ml, Simple Western 5 ug/ml, Immunohistochemistry 2 - 5 ug/ml, Immunocytochemistry/ Immunofluorescence 1 - 25 ug/ml, Immunohistochemistry-Paraffin 2 - 5 ug/ml, Flow (Intracellular) 1 ug/ml, Knockout Validated
Application Notes	Western blot bands are seen at ~19 kDa, representing LC3-I, and ~17 kDa, representing LC3-II.

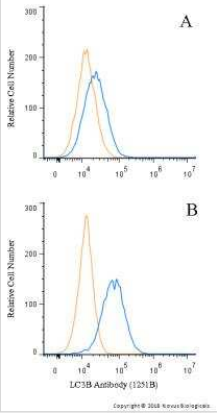
Images

Western Blot: LC3B Antibody (1251B) [NBP2-60735] - Western blot analysis of HeLa human cervical epithelial carcinoma parental cell line and LC3B knockout HeLa cell line (KO) untreated (-) or treated (+) with 50uM Chloroquine for 18 hours. PVDF (polyvinylidene difluoride) membrane was probed with 0.1 ug/mL of anti-LC3B monoclonal antibody (Catalog # NBP2-60735) 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 (Catalog # AF5718) is shown as a loading control.



Simple Western: LC3B Antibody (1251B) [NBP2-60735] - Lane view shows lysates of NIH-3T3 mouse embryonic fibroblast cell line untreated (-) or treated (+) with 50uM Chloroquine for 18 hours, loaded at 0.2 mg/mL. A specific band was detected for LC3B at approximately 17 kDa (as indicated) using 5 ug/mL of Rabbit Anti-Human/Mouse/Rat anti-LC3B Monoclonal Antibody (1251B) [Catalog # NBP2-60735]. This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.	
Immunocytochemistry/Immunofluorescence: LC3B Antibody (1251B) [NBP2-60735] - HeLa cells were treated with 50uM CQ (Chloroquine) 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- [Catalog # NBP2-60735] at 2 ug/ml overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.	
Western Blot: LC3B Antibody (1251B) [NBP2-60735] - Total protein from HeLa and Neuro2A cells treated with or without 50 uM chloroquine (CQ) for 24 hours was separated on a 4-15% gel by SDS-PAGE, transferred to 0.2 um PVDF (Polyvinylidene difluoride) membrane and blocked in 5% non-fat milk in TBST (Tris-buffered saline, 0.1% Tween 20). The membrane was probed with 12.0 ug/ml anti-LC3B Antibody (1251B) [Catalog # NBP2-60735] in 1% non-fat milk in TBST and detected with an anti-rabbit HRP secondary antibody using chemiluminescence.	
Immunohistochemistry-Paraffin: LC3B Antibody (1251B) [NBP2-60735] - Analysis of a FFPE (Formalin-Fixed Paraffin-Embedded) tissue section of mouse brain using anti-LC3B antibody (clone 1251B) [Catalog # NBP2-60735] at 5ug/ml concentration (1:200 dilution). The primary antibody binding to LC3 in cells was detected using HRP conjugated anti-Rabbit secondary antibody with DAB (3,3'Diaminobenzidine) reagent, and the sections were further counterstained with hematoxylin for labeling cellular nuclei. This LC3 antibody generated a diffused staining in all cell types (except the endothelial cells of blood vessels) and the signal was strongest in a subset of neuronal cells. A few cells depicted LC3 puncta formation/dotted staining in apparently autophagic cells.	

Flow (Intracellular): LC3B Antibody (1251B) [NBP2-60735] - HeLa cells were either (A) untreated or (B) treated with 50uM chloroquine for 24 hours. An intracellular stain was performed with anti-LC3B (1251B) antibody [Catalog # NBP2-60735] (blue) and a matched isotype control [Catalog # MAB1050] (orange). Cells were fixed with 4% paraformaldehyde, following fixation, cells were permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1 ug/mL for 30 minutes at room temperature, followed by rabbit IgG APC-conjugated secondary antibody (F0111, R&D Systems).



Procedures

Western Blot protocol for LC3B Antibody (NBP2-60735)
LC3B Antibody (1251B):
Protocol: Inhibition of Autophagy and LC3 Antibody (NBP2-60735) 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-LC3 primary antibody (NBP2-60735) 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: LC3-I and LC3-II are sensitive to degradation, although LC3-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 LC3 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-LC3 primary antibody (NBP2-60735) (~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: LC3-I and it's lipidated form LC3-II have different electrophoretic mobility properties, with the lipidated form moving faster in an SDS-PAGE gel, albeit its larger molecular weight. LC3-II runs at 14-16 kDa while LC3-I runs at 16 -18kDa.

Note: This assay measures the difference in the LC3-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 LC3-II signal should be observed with the inhibitor.

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NB100-2220PEP	LC3B Antibody Blocking Peptide

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