Product Datasheet

LC3B Antibody (1251D) - BSA Free
NBP2-59800

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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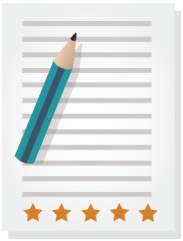


NBP2-59800

LC3B Antibody (1251D) - BSA Free

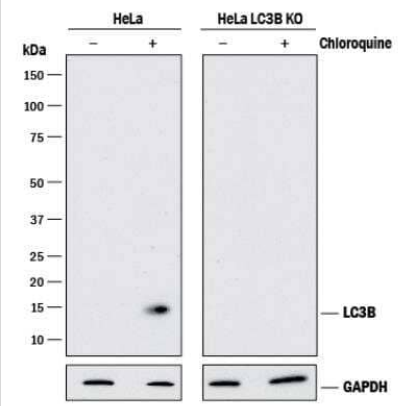
Product Information	
Unit Size	0.1 mg
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	1251D
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Protein A or G purified
Buffer	PBS
Target Molecular Weight	14.688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

Product Description	
Description	Novus Biologicals Knockout (KO) Validated Rabbit LC3B Antibody (1251D) - BSA Free (NBP2-59800) is a recombinant monoclonal antibody validated for use in IHC, WB, Flow, ICC/IF and Simple Western. Anti-LC3B Antibody: Cited in 1 publication. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	81631
Gene Symbol	MAP1LC3B
Species	Human, Mouse, Rat
Immunogen	Recombinant monoclonal LC3B Antibody (1251D) was made to a synthetic peptide made to an N-terminal portion of the human LC3B protein sequence (between residues 1-100). [Uniprot: Q9GZQ8].

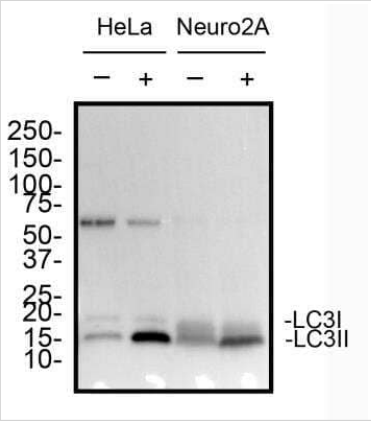
Product Application Details	
Applications	Western Blot, Simple Western, Immunohistochemistry-Paraffin, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Knockout Validated
Recommended Dilutions	Western Blot 1 - 2 ug/ml, Simple Western 5 ug/ml, Immunohistochemistry 2 - 5ug/ml, Immunocytochemistry/ Immunofluorescence 5 ug/ml, Immunohistochemistry-Paraffin 2 - 5ug/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

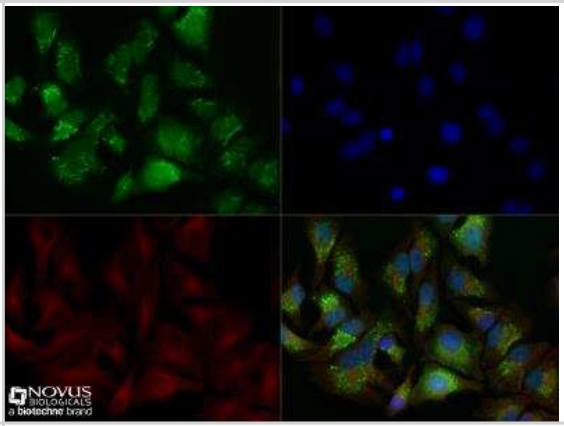
Western Blot: LC3B Antibody (1251D) [NBP2-59800] - HeLa human cervical epithelial carcinoma parental cell line and LC3B knockout HeLa cell line (KO) untreated (-) or treated (+) with 50 uM Chloroquine for 18 hrs. PVDF membrane was probed with 0.1 ug/mL of Rabbit Anti-LC3B Monoclonal Antibody (1251D) [Catalog# NBP2-59800] 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. This experiment was conducted under reducing conditions.



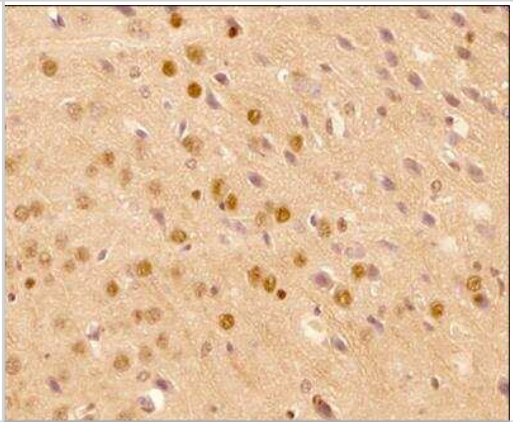
Western Blot: LC3B Antibody (1251D) [NBP2-59800] - Total protein from HeLa and Neuro2A cells treated with or without 50 uM chloroquine for 24 hrs was separated on a 4-15% gel by SDS-PAGE, transferred to 0.2 um PVDF (Polyvinylidene difluoride) membrane and blocked in 5% non-fat milk in TBST (Tris-buffered saline, 0.1% Tween 20). The membrane was probed with 2.0 ug/ml anti-LC3B Antibody (1251d) [Catalog # NBP2-59800] in blocking buffer and detected with an anti-rabbit HRP secondary antibody using chemiluminescence. Note the detection of LC3I and LC3II at a molecular weight of approximately 15 kDa.



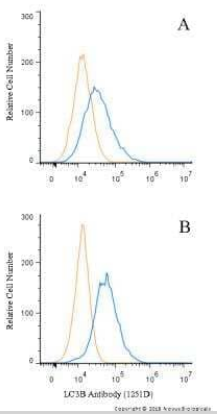
Immunocytochemistry/Immunofluorescence: LC3B Antibody (1251D) [NBP2-59800] - HeLa cells were treated with 50uM CQ (Chloroquine) overnight, fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton X-100. The cells were incubated with anti-LC3B (1251d) [Catalog # NBP2-59800] at 5 ug/ml overnight at 4C and detected with an anti-mouse DyLight 488 (Green) at a 1:500 dilution. Actin was detected with Phalloidin 568 (Red) at a 1:200 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



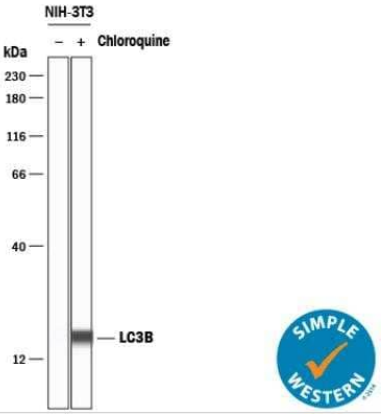
Immunohistochemistry-Paraffin: LC3B Antibody (1251D) [NBP2-59800] - Analysis of a FFPE (Formalin-Fixed Paraffin-Embedded) tissue section of mouse brain using anti-LC3B antibody (clone 1251D) [Catalog # NBP2-59800] at 5ug/ml concentration (1:200 dilution). The primary antibody binding to LC3 in cells 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.



Flow (Intracellular): LC3B Antibody (1251D) [NBP2-59800] - HeLa cells were either (A) untreated or (B) treated with 50uM chloroquine for 24 hrs. An intracellular stain was performed with anti-LC3B (1251D) antibody [Catalog # NBP2-59800] (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).



Simple Western: LC3B Antibody (1251D) [NBP2-59800] - Lane view shows lysates of NIH-3T3 mouse embryonic fibroblast cell line untreated (-) or treated (+) with 50 uM Chloroquine for 18 hrs, 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 LC3B Monoclonal Antibody (1251D) [Catalog# NBP2-59800]. This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.



Publications

Klionsky DJ, Abdel-Aziz AK, Abdelfatah S et al. Guidelines for the use and interpretation of assays for monitoring autophagy (4th edition)1 Autophagy 2021-01-01 [PMID: 33634751] (WB)

Procedures

Western Blot protocol for LC3B Antibody (NBP2-59800)
LC3B Antibody (1251D):
Protocol: Inhibition of Autophagy and LC3 Antibody (NBP2-59800) 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 (NBP2-59800) 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 (NBP2-59800) (~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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HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control
NB100-2220PEP	LC3B Antibody Blocking Peptide

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