

# Product Datasheet

## LC3B Antibody (1251A) - BSA Free NBP2-46892

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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**NBP2-46892**

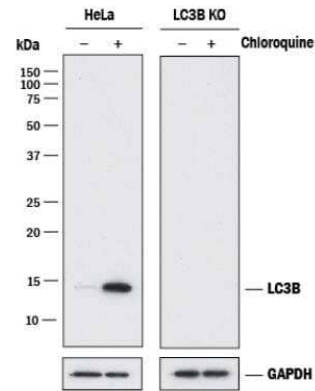
LC3B Antibody (1251A) - BSA Free

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	1251A
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Protein G purified
Buffer	PBS
Target Molecular Weight	14.688 kDa
Product Description	
Host	Rabbit
Gene ID	81631
Gene Symbol	MAP1LC3B
Species	Human, Mouse, Rat
Reactivity Notes	Mouse and Rat reactivity reported from verified customer reviews.
Marker	Autophagosomes
Immunogen	Recombinant monoclonal LC3B Antibody (1251A) 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, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Knockout Validated
Recommended Dilutions	Western Blot, Flow Cytometry, Immunohistochemistry 1:100-1:500, Immunocytochemistry/ Immunofluorescence 10-20 ug/ml, Immunoprecipitation 2-10 ug, Immunohistochemistry-Paraffin 1:100-1:500, Immunohistochemistry-Frozen reported in scientific literature (PMID 32278100), Flow (Intracellular) 1 - 2.5 ug/mL, Knockout Validated
Application Notes	In WB this LC3B recombinant monoclonal antibody detects both LC3B I and LC3B II with chloroquine treatment. With ICC autophagosome staining was observed after treatment with chloroquine.

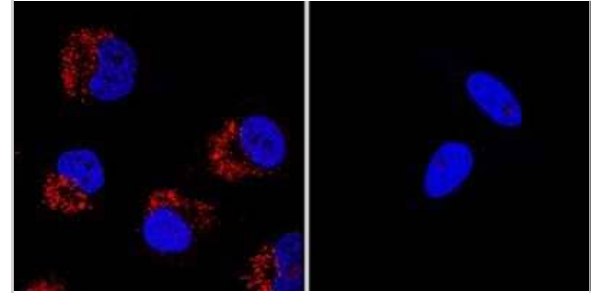


## Images

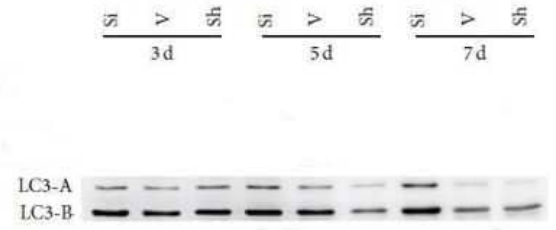
Knockout Validated: LC3B Antibody (1251A) [NBP2-46892] - Western Blot analysis shows lysates of HeLa human cervical epithelial carcinoma parental cell line and LC3B knockout HeLa cell line (KO) untreated (-) or treated (+) with 50  $\mu$ M Chloroquine for 18 hours. PVDF (Polyvinylidene difluoride) membrane was probed with 2.0  $\mu$ g/mL of Rabbit Anti-Human/Mouse/Rat LC3B Monoclonal Antibody (1251A) [Catalog # NBP2-46892] 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.



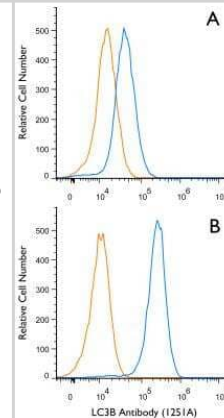
Knockout Validated: LC3B Antibody (1251A) [NBP2-46892] - LC3B was detected in immersion fixed Chloroquine treated Hela cells (left) but was not detected in LC3B knockout Hela cells (right) using rabbit anti-human LC3B monoclonal antibody (1251A) [Catalog #NBP2-46892] at 0.3  $\mu$ g/mL for 3 hours at 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.



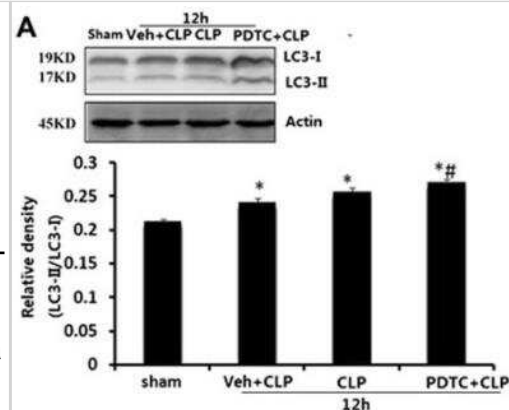
Western Blot: LC3B Antibody (1251A) [NBP2-46892] - The expression of LC3B in rat tissue. This image was submitted via customer Review.



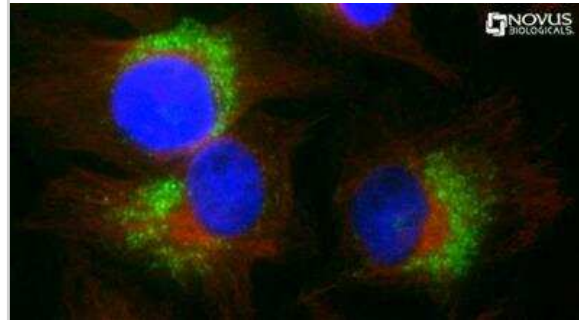
Flow (Intracellular): LC3B Antibody (1251A) [NBP2-46892] - Jurkat cells were either untreated (A) or treated with 50 $\mu$ M chloroquine for 24 hours (B). An intracellular stain was performed with anti-LC3B (1251A) antibody [Catalog # NBP2-46892] (blue) and a matched isotype control [Catalog # MAB1050] (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  $\mu$ g/mL for 30 minutes at room temperature, followed by rabbit IgG APC-conjugated secondary antibody (F0111, R&D Systems).



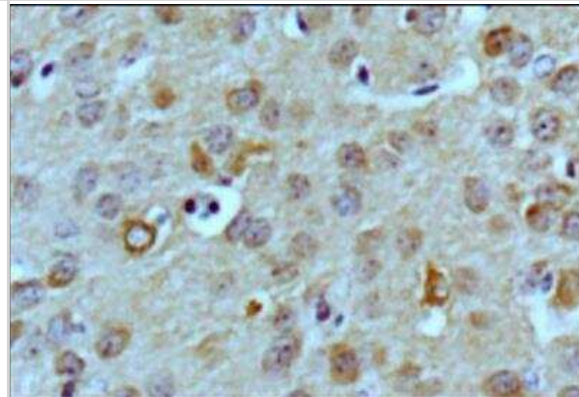
**Western Blot: LC3B Antibody (1251A) [NBP2-46892]** - Effects of PDTC on LC3 on the hippocampus of rats harvested 12 h after CLP. Values of protein data are normalized to actin level and given in relative arbitrary units. LC3-II increased in CLP rats receiving vehicle or PDTC compared with those in sham-operated rats. PDTC + CLP rats showed an increase in LC3-II compared with vehicle-treated rats. Data were expressed as mean  $\pm$  SEM, n = 6/group. \*P < 0.05 vs sham-operated group. # P < 0.05 vs 12 h or Veh + CLP group. PDTC pyrrolidine dithiocarbamate, LC3 microtubule-associated protein light chain-3, CLP cecal ligation and puncture. Image collected and cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/26067996>) licensed under a CC-BY license.



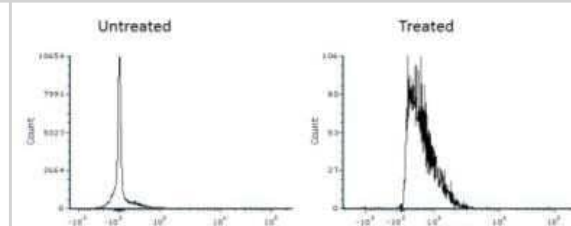
**Immunocytochemistry/Immunofluorescence: LC3B Antibody (1251A) [NBP2-46892]** - HeLa cells were treated with Chloroquine for 24 hours prior to fixation, permeabilization and incubation with anti- [Catalog # NBP2-46892] and anti tubulin (NB100-690) antibodies. Image enlargement shows the accumulation of LC3 (green) on autophagosomes in response to Chloroquine treatment. Tubulin staining is shown in red and DNA is counterstained with DAPI (blue).



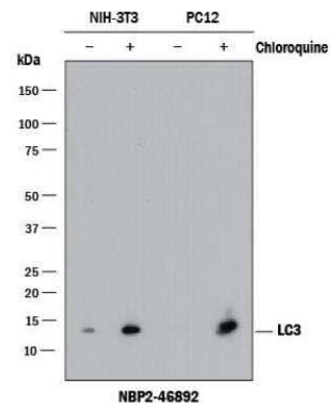
**Immunohistochemistry-Paraffin: LC3B Antibody (1251A) [NBP2-46892]** - IHC (Immunohistochemical) analysis of a formalin fixed and paraffin embedded tissue section of normal mouse brain using rabbit monoclonal LC3B (1251A) antibody [Catalog # NBP2-46892] at 1:100 dilution with HRP-DAB detection. The antibody generated a weak diffused cytoplasmic staining in most of the cells but some cells, especially within empty areas on the section, showed punctate signal also which signifies the presence of autophagy in those areas.



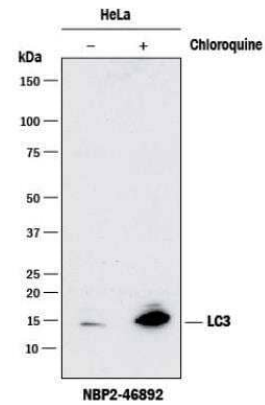
**Flow Cytometry: LC3B Antibody (1251A) [NBP2-46892]** - HeLa cells untreated or treated with 50  $\mu$ M chloroquine for 24h. Image from the Alexa Fluor 405 version of this antibody.



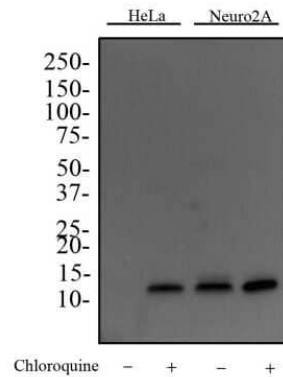
Western Blot: LC3B Antibody (1251A) [NBP2-46892] - Analysis shows lysates of HeLa human cervical epithelial carcinoma cell line untreated (-) or treated (+) with Chloroquine. PVDF membrane was probed with 0.2 ug/mL rabbit anti-LC3B monoclonal Antibody (1251A) (Catalog # NBP2-46892, Novus Biologicals), followed by 1:2000 dilution of goat anti-rabbit IgG secondary antibody. A specific band was detected for LC3B at a molecular weight of approximately 15 kDa in CQ treated NIH-3T3 and PC12 cell lines.



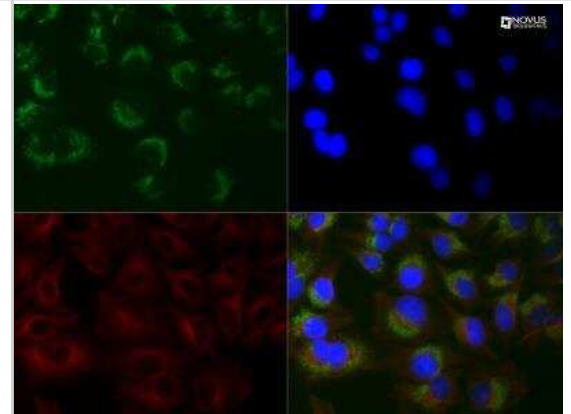
Western Blot: LC3B Antibody (1251A) [NBP2-46892] - Western Blot analysis using HeLa cells treated with (+) or without (-) Chloroquine (CQ). A specific band was detected for LC3B at a molecular weight of approximately 15 kDa.



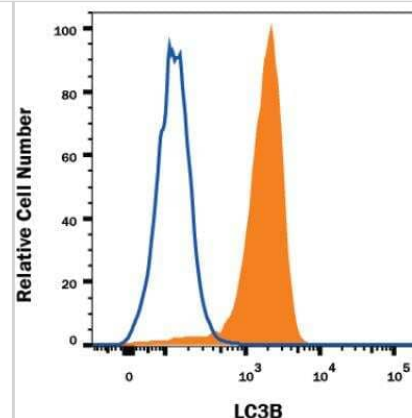
Western Blot: LC3B Antibody (1251A) [NBP2-46892] - Western Blot image of monoclonal anti-LC3B Antibody (Clone 1251D) [Catalog # NBP2-46892]. HeLa and Neuro2A cells were treated with or without 50 uM chloroquine for 24 hours as indicated. Whole cell protein was then separated on a 4-15% gel by SDS-PAGE, transferred to 0.2 um PVDF membrane for 30 min and blocked in 5% non-fat milk in TBST (Tris-buffered saline, 0.1% Tween 20). The membrane was probed with 2 ug/ml anti-LC3B Antibody in 1% milk, and detected with an anti-rabbit HRP secondary antibody using chemiluminescence. Note the accumulation of LC3B II upon chloroquine treatment. Bands for LC3 were detected at a molecular weight of approximately 15 kDa in treated HeLa cells, and both treated and untreated Neuro2A cells.



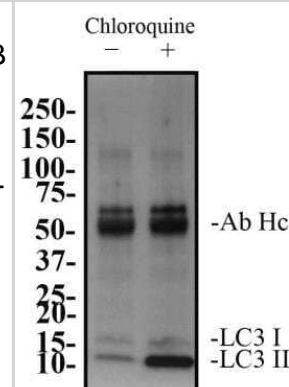
Immunocytochemistry/Immunofluorescence: LC3B Antibody (1251A) [NBP2-46892] - HeLa cells were treated with 50 uM Chloroquine for 24 hour prior to fixation in 10% buffered formalin for 10 min. Cells were permeabilized in 0.1% Triton X-100 and incubated with 20 ug/ml anti-[Catalog # NBP2-46892] and 1:500 anti-tubulin [Catalog # NB100-690] for 1 h at room temperature. LC3B reactivity (green) was detected with anti-rabbit Dylight 488 and tubulin (red) with anti-mouse Dylight 550. Nuclei were counterstained with DAPI (blue).



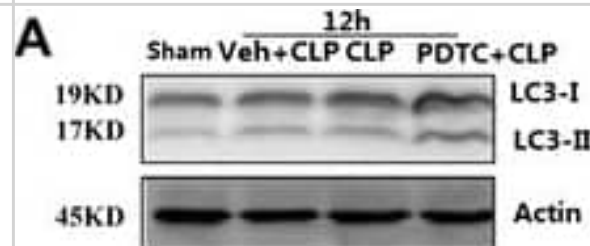
Flow (Intracellular): LC3B Antibody (1251A) [NBP2-46892] - HeLa human cervical epithelial carcinoma cell line either treated with 50  $\mu$ M chloroquine for 24 hours (filled histogram) or untreated (open histogram) was stained with Rabbit anti-Human LC3B Alexa Fluor 647 conjugated monoclonal antibody (1251A) [Catalog # NBP2-46892AF647]. To facilitate intracellular staining, cells were fixed and permeabilized with FlowX FoxP3 Fixation and Permeabilization Buffer Kit (FC012-NOV).



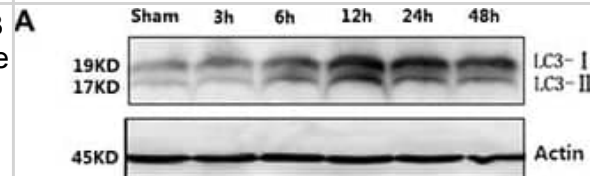
Immunoprecipitation: LC3B Antibody (1251A) [NBP2-46892] - Western blot analysis of LC3 immunoprecipitation. 10  $\mu$ g of monoclonal anti-LC3B antibody (clone 1251A) [Catalog # NBP2-46892] was used to immunoprecipitate LC3 from 200  $\mu$ g of total protein from HeLa cells treated with or without 50  $\mu$ M chloroquine for 24 h. Antibody-protein was captured by magnetic protein A/G beads, washed and subjected to SDS-PAGE on a 4-15% gel. Protein was transferred to 0.2  $\mu$ m PVDF membrane and probed with 2  $\mu$ g/ml anti-LC3B (NB100-2220) and detected with an anti-rabbit HRP conjugated secondary antibody using chemiluminescence. The detection of LC3 at a molecular weight of approximately 12 kDa and the antibody heavy chain (Ab Hc) at a molecular weight of approximately 50 kDa is indicated.



Western Blot: LC3B Antibody (1251A) - BSA Free [NBP2-46892] - Effects of PDTC on LC3 (a), Beclin1 (b), LAMP-1 (c), & Rab7 (d) on the hippocampus of rats harvested 12 h after CLP. Values of protein data are normalized to actin level & given in relative arbitrary units. LC3-II increased while Beclin1, Rab7, & LAMP-1 declined in CLP rats receiving vehicle or PDTC compared with those in sham-operated rats. PDTC + CLP rats showed an increase in LC3-II, Beclin1, Rab7, & LAMP-1 compared with vehicle-treated rats. Data were expressed as mean  $\pm$  SEM, n = 6/group. \*P < 0.05 vs sham-operated group. #P < 0.05 vs 12 h or Veh + CLP group. PDTC pyrrolidine dithiocarbamate, LC-3 microtubule-associated protein light chain-3, CLP cecal ligation & puncture Image collected & cropped by CiteAb from the following publication (<https://jneuroinflammation.biomedcentral.com/articles/10.1186/s12974-015-0336-2>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: LC3B Antibody (1251A) - BSA Free [NBP2-46892] - a LC3 in brains harvested 3, 6, 12, 24, & 48 h after CLP. b Immunofluorescence for LC3 in neurons after CLP injury. Values of protein data were related to signals obtained from actin protein & given as relative arbitrary units. LC3-II significantly increased in CLP rats at 6, 12, 24, & 48 h after surgery compared with sham-operated rats. Three-color staining for anti-LC3 antibody (green), NeuN (red), & DAPI (blue) showed that a staining pattern changes from largely diffuse to predominantly punctate & cytoplasmic in hippocampal neurons after CLP. Data were expressed as mean  $\pm$  SEM, n = 6/group. \*P < 0.05 vs sham-operated group. LC-3 microtubule-associated protein light chain-3, CLP cecal ligation & puncture, DAPI 4,6-diamidino-2-phenylindole Image collected & cropped by CiteAb from the following publication (<https://jneuroinflammation.biomedcentral.com/articles/10.1186/s12974-015-0336-2>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

Anna N Ligezka, Rohit Budhraj, Yurika Nishiyama, Fabienne C Fiesel, Graeme Preston, Andrew Edmondson, Wasantha Ranatunga, Johan L K Van Hove, Jens O Watzlawik, Wolfdieter Springer, Akhilesh Pandey, Eva Morava, Tamas Kozicz Interplay of Impaired Cellular Bioenergetics and Autophagy in PMM2-CDG. *Genes* 2023-08-28 [PMID: 37628636]

Piyali Majumder, Chinmayee Shukla, Arjun Arya, Shubham Sharma, Bhaskar Datta G-quadruplexes in MTOR and induction of autophagy *Scientific Reports* 2024-01-30 [PMID: 38291093]

Su C, Cheng C, Rong Z et al. Repurposing fluphenazine as an autophagy modulator for treating liver cancer *Heliyon* 2023-12-01 [PMID: 38107270]

Gaffke L, Firyng N, Rintz E et al. Therapeutic potential of lithium chloride and valproic acid against neuronopathic types of mucopolysaccharidoses through induction of the autophagy process *Archives of biochemistry and biophysics* 2023-09-12 [PMID: 37708928] (WB, Human)

Ulferts R, Marcassa E, Timimi L Et al. Subtractive CRISPR screen identifies the ATG16L1/vacuolar ATPase axis as required for non-canonical LC3 lipidation *Cell reports* 2021-10-26 [PMID: 34706226]

Sun X, Zhu H, Cao R et al. BACH1 is transcriptionally inhibited by TET1 in hepatocellular carcinoma in a microRNA-34a-dependent manner to regulate autophagy and inflammation *Pharmacological research* 2021-04-18 [PMID: 33878446]

Su Y, Qu Y et al. Regulation of autophagy by the nuclear factor kappa B signaling pathway in the hippocampus of rats with sepsis. *J Neuroinflammation* 2015-12-06 [PMID: 26067996] (WB, Rat)

Vitry S, Bruyere J et al. Storage vesicles in neurons are related to Golgi complex alterations in mucopolysaccharidosis IIIB. *Am J Pathol* 2010-01-12 [PMID: 21037080] (WB, Mouse)

Wang H, Dou S, Zhu J et al. Ghrelin protects dopaminergic neurons against MPTP neurotoxicity through promoting autophagy and inhibiting endoplasmic reticulum mediated apoptosis *Brain Res* 2020-07-26 [PMID: 32710901] (ICC/IF, WB)

Butler M, Chotiwan N, Brewster CD et al. Cyclin-Dependent Kinases 8 and 19 Regulate Host Cell Metabolism during Dengue Virus Serotype 2 Infection *Viruses* 2020-06-17 [PMID: 32560467] (WB, Human)

Wang J, Lee CJ, Deci MB et al. MiR-101a loaded extracellular nanovesicles as bioactive carriers for cardiac repair *Nanomedicine* 2020-04-08 [PMID: 32278100] (IF/IHC, IHC-Fr, Mouse)

Martinez Legaspi S, Segatori L Aggregation Behavior of Nanoparticle-Peptide Systems Affects Autophagy *Endocrinology* 2019-07-03 [PMID: 31268689] (ICC/IF, Human)

More publications at <http://www.novusbio.com/NBP2-46892>



## Procedures

### Western Blot protocol for LC3B/MAP1LC3B Antibody (NBP2-46892)

Protocol: Inhibition of Autophagy and LC3 Antibody (NBP2-46892) Western Blot

#### Materials

Chloroquine diphosphate (CQ) (10 mM) in dH<sub>2</sub>O

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 (NBP2-46892) 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 dH<sub>2</sub>O and stain with Ponceau S for 1-2 minutes to confirm efficiency of protein transfer.
8. Rinse the membrane in dH<sub>2</sub>O 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 (NBP2-46892) (~2 ug/mL) in blocking buffer and incubate the



membrane for 1 hour at room temperature.

12. Rinse the membrane with dH<sub>2</sub>O.

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 its 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.

### **Immunocytochemistry/Immunofluorescence Protocol for LC3B/MAP1LC3B Antibody (NBP2-46892)**

LC3B Antibody (1251A):

Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

\*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

**Immunohistochemistry-Paraffin Protocol for LC3B/MAP1LC3B Antibody (NBP2-46892)**

LC3B Antibody (1251A):

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.





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### **Products Related to NBP2-46892**

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NBP2-49688	Neuro2a Chloroquine Treated / Untreated Cell Lysate
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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### **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](http://www.novusbio.com/guarantee)

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