

Product Datasheet

LC3B Antibody (1251A) NBP2-46892SS

Unit Size: 0.025 mg

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

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NBP2-46892SS

LC3B Antibody (1251A)

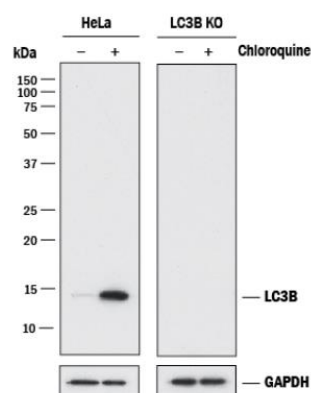
Product Information	
Unit Size	0.025 mg
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	1251A
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Protein G purified
Buffer	PBS

Product Description	
Host	Rabbit
Gene ID	81631
Gene Symbol	MAP1LC3B
Species	Human, Mouse, Rat
Marker	Autophagosomes
Specificity/Sensitivity	Specific for LC3B.
Immunogen	A synthetic peptide made to an N-terminal portion of the human LC3 protein sequence (between residues 1-100). [UniProt# Q9GZQ8].

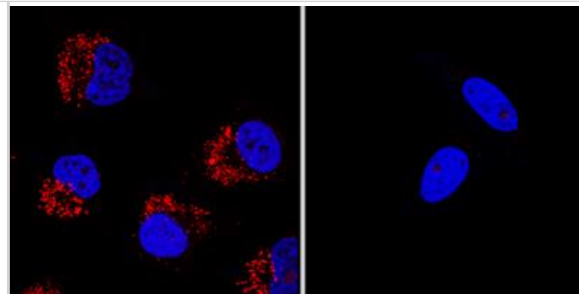
Product Application Details	
Applications	Western Blot, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, Knockout Validated
Recommended Dilutions	Western Blot, Flow Cytometry, Immunohistochemistry 1:100-1:500, Immunocytochemistry/Immunofluorescence 10-20 ug/ml, Immunoprecipitation 2-10 ug, Immunohistochemistry-Paraffin 1:100-1:500, Flow (Intracellular) 1 - 2.5 ug/mL, Knockout Validated
Application Notes	In Western blot this LC3B recombinant monoclonal antibody detects both LC3I and LC3II with Chloroquine treatment. With ICC autophagosome staining was observed after treatment with Chloroquine.

Images

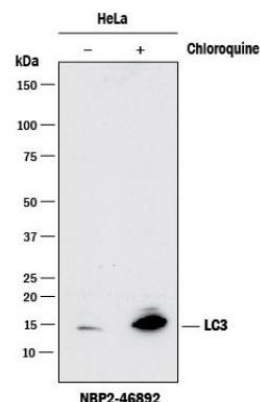
Knockout Validated: LC3B Antibody (1251A) [NBP2-46892] - Western blot shows lysates of HeLa human cervical epithelial carcinoma parental cell line and LC3B knockout HeLa cell line (KO) untreated (-) or treated (+) with 50 uM Chloroquine for 18 hours. PVDF membrane was probed with 2.0 ug/mL of Rabbit Anti-Human/Mouse/Rat LC3B Monoclonal Antibody (Catalog # NB100-2220) 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 is shown as a loading control. This experiment was conducted under reducing conditions.



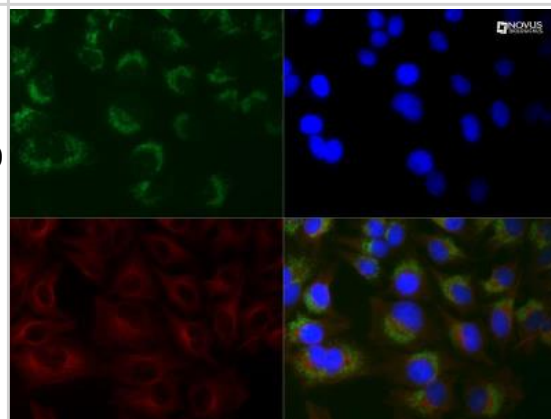
Knockout Validated: LC3B Antibody (1251A) [NBP2-46892] - LC3 was detected in immersion fixed Chloroquine treated HeLa cells (left) but was not detected in LC3 knockout HeLa cells (right) using rabbit anti-human LC3 monoclonal antibody (Catalog #NBP2-46892) at 0.3 ug/mL for 3 hours at room temperature. 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.



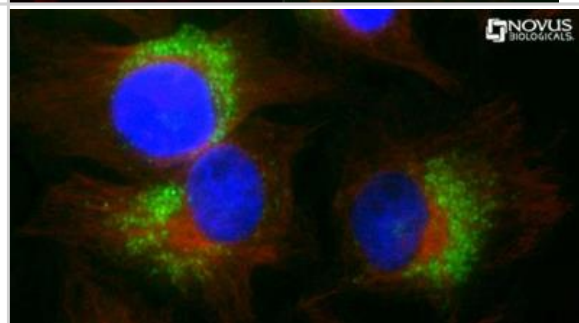
Western Blot: LC3B Antibody (1251A) [NBP2-46892] - Analysis using HeLa cells



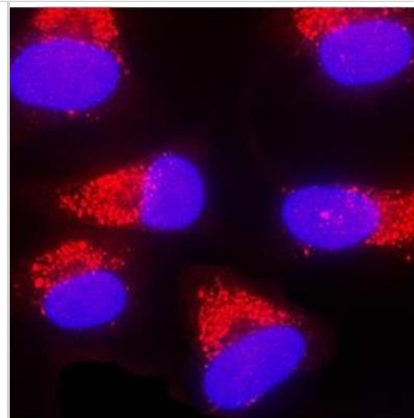
Immunocytochemistry/Immunofluorescence: LC3B Antibody (1251A) [NBP2-46892] - LC3B/MAP1 [NBP2-46892] - HeLa cells were treated with 50 uM Chloroquine for 24 hour prior to fixation in 10% buffered formalin for 10 min. Cells were permeabilized in 0.1% Triton X-100 and incubated with 20 ug/ml anti-LC3B (1251A) and 1:500 anti-tubulin NB100-690) for 1 h at room temperature. LC3 reactivity (green) was detected with ant-rabbit Dylight 488 and tubulin (red) with anti-mouse Dylight 550. Nuclei were counterstained with DAPI (blue).



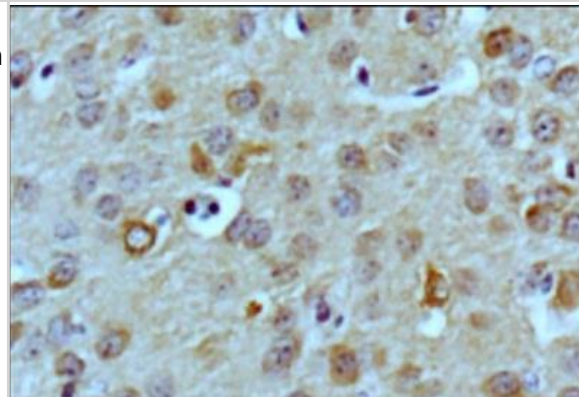
Immunocytochemistry/Immunofluorescence: LC3B Antibody (1251A) [NBP2-46892] - LC3B/MAP1 [NBP2-46892] - HeLa cells were treated with Chloroquine for 24 hours prior to fixation, permeabilization and incubation with anti-LC3B (1251A) and anti tubulin (NB100-690) antibodies. Image enlargement shows the accumulation of LC3 (green) on autophagosomes in response to chloroquine treatment. Tubulin staining is shown in red and DNA is counterstained with DAPI (blue).



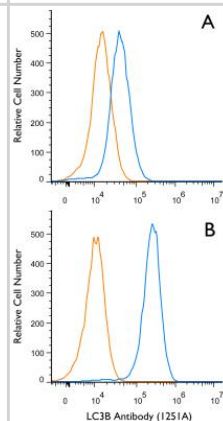
Immunocytochemistry/Immunofluorescence: LC3B Antibody (1251A) [NBP2-46892] - LC3B was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line treated with Chloroquine using 1 ug/mL rabbit anti-LC3B monoclonal (NBP2-46892, Novus Biologicals). Cells were stained using donkey anti-rabbit IgG and counterstained with DAPI (blue).



Immunohistochemistry-Paraffin: LC3B Antibody (1251A) [NBP2-46892] - LC3B/MAP1 [NBP2-46892] - IHC analysis of a formalin fixed and paraffin embedded tissue section of normal mouse brain using rabbit monoclonal LC3B (1251A) antibody 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.



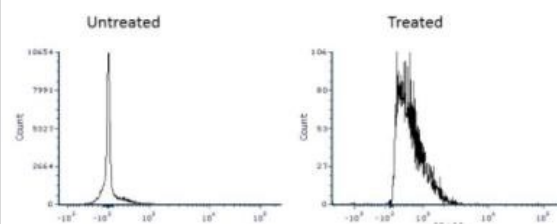
Flow (Intracellular): LC3B Antibody (1251A) [NBP2-46892] - Jurkat cells were either untreated (A) or treated with 50uM chloroquine for 24 hours (B). An intracellular stain was performed with LC3B (1251A) antibody NBP2-46892 (blue) and a matched isotype control MAB1050 (orange). Cells were fixed with 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).



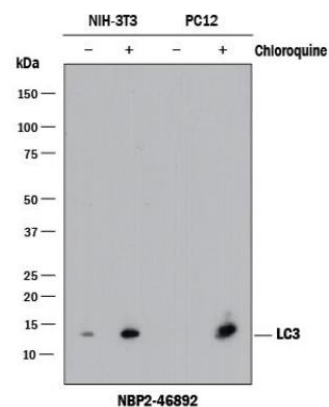
Western Blot: LC3B Antibody (1251A) [NBP2-46892] - The expression of LC3B in rat tissue. This image was submitted via customer Review.



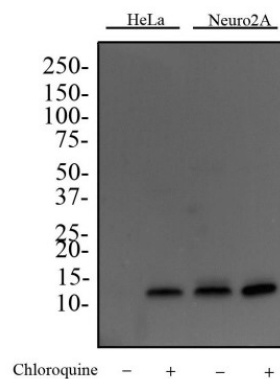
Flow Cytometry: LC3B Antibody (1251A) [NBP2-46892] - HeLa cells untreated or treated with 50 uM chloroquine for 24h. Image from the Alexa Fluor 405 version of this antibody.



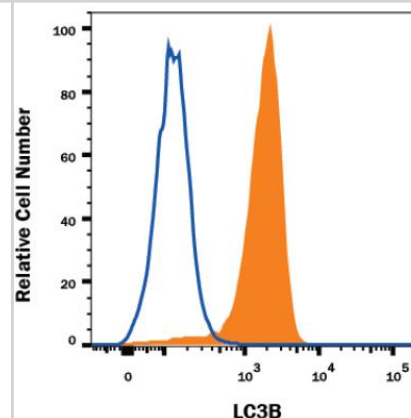
Western Blot: LC3B Antibody (1251A) [NBP2-46892] - analysis shows lysates of HeLa human cervical epithelial carcinoma cell line untreated (-) or treated (+) with Chloroquine. PVDF membrane was probed with 0.2 ug/mL rabbit anti-LC3B monoclonal (NBP2-46892, Novus Biologicals), followed by 1:2000 dilution of goat anti-rabbit IgG secondary antibody.



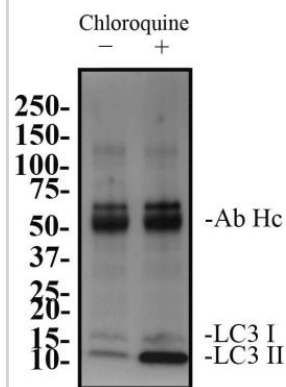
Western Blot: LC3B Antibody (1251A) [NBP2-46892] - Western Blot image of monoclonal anti-LC3B (Clone 1251D). HeLa and Neuro2A cells were treated with or without 50 uM chloroquine for 24 hours as indicated. Whole cell protein was then separated on a 4-15% gel by SDS-PAGE, transferred to 0.2 um PVDF membrane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



Flow (Intracellular): LC3B Antibody (1251A) [NBP2-46892] - HeLa human cervical epithelial carcinoma cell line either treated with 50 uM chloroquine for 24 hours (filled histogram) or untreated (open histogram) was stained with Rabbit anti-Human LC3B Alexa Fluor 647 conjugated monoclonal antibody (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] - LC3B/MAP1 [NBP2-46892] - Western blot analysis of LC3 immunoprecipitation. 10 ug of monoclonal antibody (clone 1251A) was used to immunoprecipitate LC3 from 200 ug of total protein from HeLa cells treated with or without 50 uM 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 um PVDF membrane and probed with 2 ug/ml anti-LC3 (NB100-2220) and detected with an anti-rabbit HRP conjugated secondary antibody using chemiluminescence. The detection of LC3 and the antibody heavy chain (Ab Hc) is indicated.



Publications

Verwey M, Nolte EM, Joubert AM, Theron AE. Autophagy induced by a sulphamoylated estrone analogue contributes to its cytotoxic effect on breast cancer cells. *Cancer Cell Int.* Dec 16 2016 12:00AM [PMID: 27980456] (FLOW, Human)

Wechman SL, Rao XM, Cheng PH et al. Development of an Oncolytic Adenovirus with Enhanced Spread Ability through Repeated UV Irradiation and Cancer Selection. *Viruses* Jun 18 2016 12:00AM [PMID: 27314377] (WB)

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

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
 2. Transfer protein to 0.2 um PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
 3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
 4. Rinse the blot.
 5. Block the membrane using standard blocking buffer for at least 1 hour.
 6. Dilute anti-LC3 primary antibody in blocking buffer and incubate 1 hour at room temperature.
 7. Wash the membrane in wash buffer three times for 10 minutes each.
 9. Apply the diluted HRP conjugated rabbit secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
 10. Wash the blot in wash buffer three times for 10 minutes each (this step can be repeated as required to reduce background).
 11. Apply the detection reagent of choice in accordance with the manufacturers instructions.
- Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

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

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)

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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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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