

Product Datasheet

LC3B/MAP1LC3B Antibody

NB600-1384SS

Unit Size: 0.025 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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Updated 6/15/2014 v.20.1

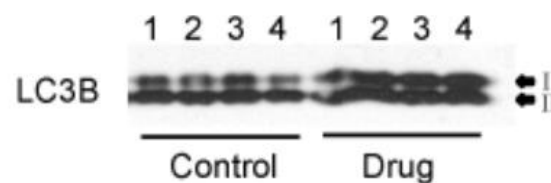
NB600-1384SS

LC3B/MAP1LC3B Antibody

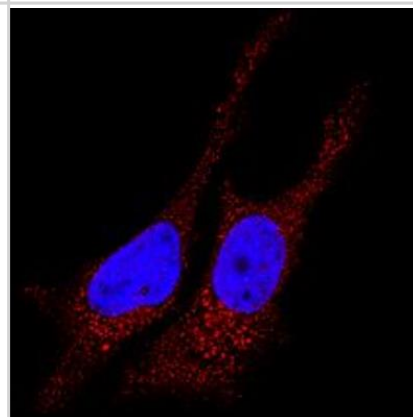
Product Information	
Unit Size	0.025 ml
Concentration	1.0 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Purity	Immunogen affinity purified
Buffer	Tris-glycine, 150 mM NaCl
Product Description	
Host	Rabbit
Gene ID	81631
Gene Symbol	MAP1LC3B
Species	Human, Mouse, Rat, Bovine, Canine, Primate, Porcine, Zebrafish
Species Reactivity	Human, mouse, rat and bovine. 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) Other species have not been tested.
Marker	Autophagosomes Marker
Immunogen	A synthetic peptide made to the N-terminal region of the human LC3B protein. [UniProt# Q9GZQ8]
Product Application Details	
Applications	Western Blot, Simple Western, Electron Microscopy, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin
Recommended Dilutions	Flow Cytometry 1:200, Immunohistochemistry-Frozen 1:400, Western Blot 1:1000, Immunohistochemistry, Electron Microscopy, Immunohistochemistry-Paraffin 1:200-1:400, Immunocytochemistry/Immunofluorescence 0.1-2 ug/ml, Simple Western 1:100
Application Notes	This LC3 antibody is useful for Immunocytochemistry/Immunofluorescence, Immunohistochemistry-frozen sections, Flow Cytometry and Western Blot where bands are seen ~17 and 19 kDa corresponding to LC3-II and LC3-I. Electron Microscopy was reported in scientific literature. In Simple Western only 10-15 uL of the recommended dilution is used per data point.

Images

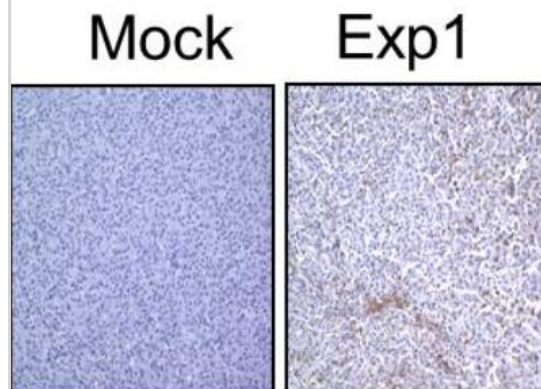
Western Blot: LC3B/MAP1LC3B Antibody [NB600-1384] - analysis of LC3B in IB3-1 whole cell lysate using anti-LC3B antibody. Image from verified customer review.



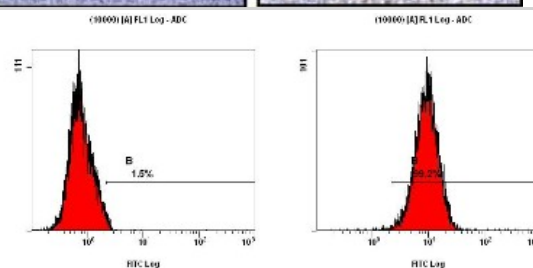
Immunocytochemistry/Immunofluorescence: LC3B/MAP1LC3B Antibody [NB600-1384] - analysis of LC3B in HeLa cells using anti-LC3B antibody (red). Nuclei were counterstained with DAPI (blue).



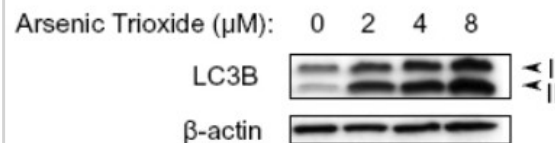
Immunohistochemistry-Paraffin: LC3B/MAP1LC3B Antibody [NB600-1384] - analysis of U87MG glioma xenografts using anti-LC3B antibody. Image from verified customer review.



Flow Cytometry: LC3B Antibody [NB600-1384] - Staining of NTERA-2 cells using NB600-1384 at a 1:200 dilution detected using Dylight-488 conjugated goat anti-rabbit IgG secondary.



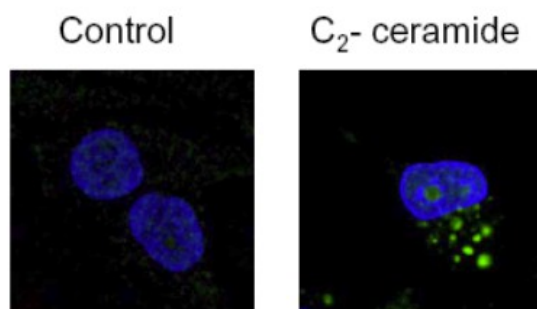
Western Blot: LC3B Antibody [NB600-1384] - Detection of LC3B in treated U87-MG (human glioblastoma astrocytoma) lysates.



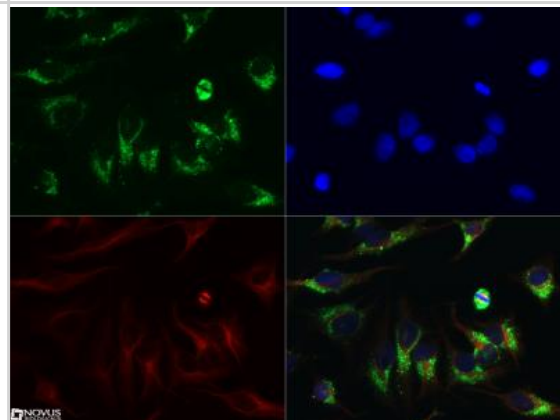
Western Blot: LC3B/MAP1LC3B Antibody [NB600-1384] - analysis of LC3B in mouse heart tissue lysates using anti-LC3B antibody. Image from verified customer review.



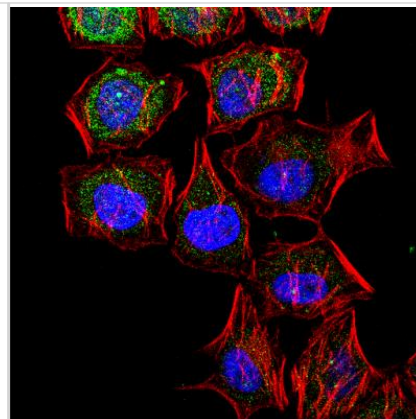
Immunocytochemistry/Immunofluorescence: LC3B Antibody [NB600-1384] - Immunofluorescent staining of treated U373-MG cells using NB600-1384. The nuclei were stained with DAPI.



Immunocytochemistry/Immunofluorescence: LC3B Antibody [NB600-1384] - LC3 antibody was tested in 50 μM 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 $\mu\text{g/ml}$ was used. Image objective 40x.



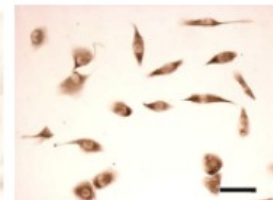
Immunocytochemistry/Immunofluorescence: LC3B Antibody [NB600-1384] - IF Confocal analysis of HeLa cells using LC3B antibody (NB600-1384, 1:5). An Alexa Fluor 488-conjugated Goat to rabbit IgG was used as secondary antibody (green). Actin filaments were labeled with Alexa Fluor 568 phalloidin (red). DAPI was used to stain the cell nuclei (blue).



Immunohistochemistry: LC3B Antibody [NB600-1384] - Staining of treated U373-MG (human glioblastoma).

Control

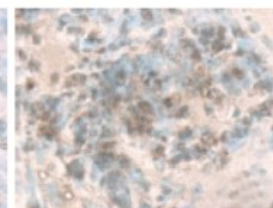
Temozolomide



Immunohistochemistry: LC3B Antibody [NB600-1384] - LC3B staining in treated U87-MG cultured & subcutaneous tumors.

Vehicle alone

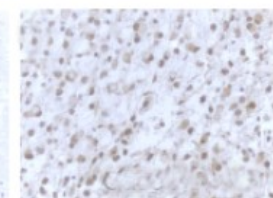
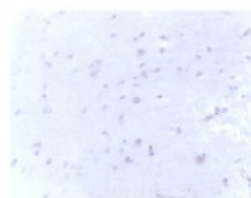
Temozolomide



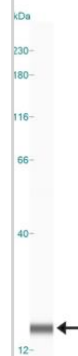
Immunohistochemistry: LC3B Antibody [NB600-1384] - LC3B staining in glioblastoma multiforme tissue.

normal brain

glioblastoma multiforme



Simple Western: LC3B/MAP1LC3B Antibody [NB600-1384] - Simple Western lane view shows a specific band for LC3B in 0.5 mg/ml of Neuro2A lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



Publications

Bernard M, DieudeM, Yang B et al. Autophagy fosters myofibroblast differentiation through MTORC2 activation and downstream upregulation of CTGF. *Autophagy*. 2014 Dec 12 [PMID: 25495560] (WB)

Yang Q, Stevenson HL, Scott MJ, Ismail N. Type I Interferon Contributes to Noncanonical Inflammasome Activation, Mediates Immunopathology, and Impairs Protective Immunity during Fatal Infection with Lipopolysaccharide-Negative *Ehrlichiae*. *Am. J. Pathol.* 2014 Dec 04 [PMID: 25481711] (WB, Mouse)

Details:
LC3B antibody used for WB at dilution 1:2500 on liver lysates of naive, Nlrp3^{-/-}, Casp1^{-/-}, Ifnar1^{-/-} and WT mice (Figure 10A).

Lee Jm, Wagner M, Xiao R et al. Nutrient-sensing nuclear receptors coordinate autophagy. *Nature*. 2014 Nov 12 [PMID: 25383539]

Wang J, Gu S, Huang J et al. Inhibition of autophagy potentiates the efficacy of Gli inhibitor GANT-61 in MYCn-amplified neuroblastoma cells. *BMC Cancer*. 2014 Oct 23 [PMID: 25323222] (WB, Human)

Toops Ka, Tan Lx, Jiang Z et al. Cholesterol-mediated activation of acid sphingomyelinase disrupts autophagy in the retinal pigment epithelium *Mol. Biol. Cell et al.* 2014 Nov 05 [PMID: 25378587] (WB, Porcine)

Details:
LC3B/MAP1LC3B antibody used for WB on porcine primary retinal pigment epithelium/RPE cells.

Mathew TS, Ferris RK, Downs RM et al. Caffeine promotes autophagy in skeletal muscle cells by increasing the calcium-dependent activation of AMP-activated protein kinase. *Biochem. Biophys. Res. Commun.* 2014 Sep 28 [PMID: 25268764] (WB, ICC/IF, Mouse)

Details:
LC3B antibody used for WB and ICC-IF in experiments involving C2C12 cells (mouse myoblast cell line).

Juarez E, Carranza C, Hernandez-Sanchez F et al. Nucleotide-oligomerizing domain-1 (NOD1) receptor activation induces pro-inflammatory responses and autophagy in human alveolar macrophages. *BMC Pulm Med.* 2014 Sep 25 [PMID: 25253572] (WB, EM, Human)

Li Y, Chen C, Yao F et al. AMPK inhibits cardiac hypertrophy by promoting autophagy via mTORC1. *Arch. Biochem. Biophys.* 2014 Jul 04 [PMID: 25009141] (WB, Rat, Mouse)

Details:
LC3B antibody used for WB on hearts of male C57BL/6J mice subjected or not (sham) to cardiac hypertrophy induced by pressure overload performed by descending aortic banding/AB (Fig. 1A). Antibody also used for WB on sham or AB groups treated or not with AMPK activator AICAR or metformin. LC3B WB also performed on cultured Rat's cardiomyocytes transfected with Ad-CA-AMPK or Ad-GFP - treated or not with phenylephrine/PE and/or Rapamycin (Fig. 4A, 4C; Fig. 6C).

Vodicka P, Lim J, Williams DT et al. Assessment of Chloroquine Treatment for Modulating Autophagy Flux in Brain of WT and HD Mice. *J Huntingtons Dis* 2014 Jul 02 [PMID: 25062859] (WB, Mouse)

Details:

LC3B antibody used for WB in lysates of brain and muscle tissues of WT and HD (Q175/Q175) mice that received or not the intraperitoneal injections of chloroquine.

Brigger D, Proikas-Cezanne T, Tschan MP. WIPI-dependent autophagy during neutrophil differentiation of NB4 acute promyelocytic leukemia cells. *Cell Death Dis* 2014 Jul 04 [PMID: 24991767] (WB, ICC/IF, Human)

Details:

LC3B antibody used for WB for measuring autophagic flux in SHC002 and shWIPI-1 or shWIPI-2 expressing NB4 cells (human acute promyelocytic leukemic/APL cell lines NB4) upon 1 uM ATRA treatment at day 4 in the presence or absence of bafilomycin A1 (Figure 4a). Antibody also used for ICC-IF application.

Sheng R, Zhang TT, Felice VD et al. Preconditioning stimuli induce autophagy via sphingosine kinase 2 in mouse cortical neurons. *J. Biol. Chem.* 2014 Jun 13 [PMID: 24928515] (ICC/IF, IHC-FrFI, Mouse)

Details:

Used for ICC-IF on murine cortical neurons and IHC-FrFI on mouse brain free floating sections. ICC-IF - Neurons grown on coverglasses fixed for 15 min with 4% PFA and permeablized with PBS/0.1% Triton X-100 for 30 min, blocked with 2% BSA for 1 h at RT, incubated with LC3 (1:800; Novus; NB600-1384) or SPK2 (1:500) at 4°C for 24 h followed by Cy3-conjugated anti-rabbit IgG for 2 h (FIGURE 2A). IHC-FrFI - 24h past isoflurane, C57 mice perfused with PBS/4% PFA-PBS, brains postfixed in 4% PFA/ON, 40 micron-thick coronal sections cut and blocked in 5% normal goat serum, 1% BSA, 0.2% Triton X-100, 0.03% H2O2 in PBS/1 h RT, incubated with 1:800 dilution of LC3-antibody for 48 h at 4C followed by biotinylated anti-rabbit secondary antibody/2 h RT and ABC -DAB detection (FIGURE 6C).

Zhou Y, Sun K, Ma Y et al. Autophagy inhibits chemotherapy-induced apoptosis through downregulating Bad and Bim in hepatocellular carcinoma cells *Sci Rep.* 2014 Jun 20 [PMID: 24947039] (WB, Human)

Details:

LC3B antibody used for WB in human hepatocellular carcinoma cells/HCC cell line SMMC-7721 and HepG2 (transiently transfected with GFP-LC3 plasmids) cultured under low glucose and hypoxia (LH) conditions in the presence or absence of CQ or 3-MA for 8 h (Figure 1C).

More publications at <http://www.novusbio.com/NB600-1384>

Procedures

Protocol specific for LC3B Antibody (NB600-1384)

Fluorescent Staining

1. After washing sample(s) in PBS, fix for 20 minutes at room temperature.
 2. Wash sample(s) in PBS 3x 3 minutes, each.
 3. Block sample(s) with 5% NGS (keep Triton X-100 at 0.1%) for 60 minutes at room temperature
 4. Prepare primary anti-LC3B (NB 600-1384) solution during this time at a 1:5,000 dilution with 1%NGS (keep Triton X-100 at 0.1%).
 5. Incubate sample(s) in the primary Ab overnight at 4C in a humidified container
- Note: If thick tissue samples, Ab must be prepared in 0.5% Triton X-100/PBS

Day 2

1. Wash sample(s) in PBS 3x 5 minutes with wells on. Prepare Alexa-fluor antibodies (Goat anti-Rabbit IgG H+L) as secondary Ab with 1% NGS (keep Triton X-100 at 0.1%).
2. Incubate sample(s) in the appropriate secondary Ab for 1 hour in a dark container.
3. Rinse the sample(s) in PBS 4x 10 minutes, each.
4. Place one drop of Vectashield onto the slide and cover with a coverslip





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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 guarantee, please visit www.novusbio.com/guarantee.

