Product Datasheet

LC3B/MAP1LC3B Antibody
NB100-2220SS

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

NB100-2220SS

LC3B/MAP1LC3B Antibody

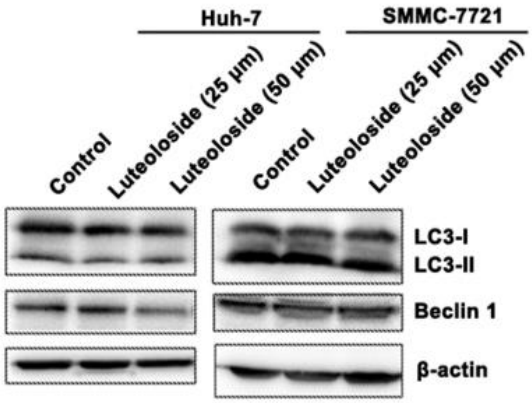
Product Information	
Unit Size	0.025 ml
Concentration	1.0 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Purity	Immunogen affinity purified
Buffer	PBS

Product Description	
Host	Rabbit
Gene ID	81631
Gene Symbol	MAP1LC3B
Species	Human, Mouse, Rat, Bacteria, Bovine, Primate, Porcine, Golden Syrian Hamster, Zebrafish
Species Reactivity	Human, rat, mouse, zebrafish, bacteria, hamster, and porcine. Bovine reactivity reported in scientific literature (PMID: 24895572) Primate reactivity reported in scientific literature (PMID: 25142602) The mouse detection has been reported to be weaker than the human. Immunogen sequence has 84% homology to Xenopus.
Marker	Autophagosomes Marker
Immunogen	A synthetic peptide made to an N-terminal portion of the human LC3 protein sequence (between residues 1-100). [UniProt# Q9GZQ8]

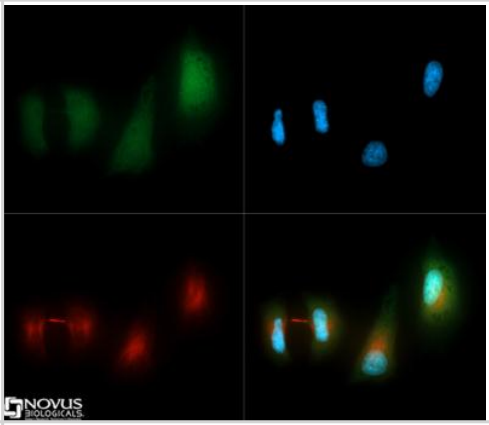
Product Application Details	
Applications	Western Blot, Simple Western, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Immunocytochemistry/Immunofluorescence 1:200, Immunohistochemistry 1:200-1:400, Immunohistochemistry-Paraffin 1:200-1:400, Immunoprecipitation 20ug/500ug of protein, Immunohistochemistry-Frozen, Western Blot 0.5-2 ug/ml, Simple Western 1:50
Application Notes	This LC3 antibody is useful for Immunocytochemistry/Immunofluorescence, Immunohistochemistry-paraffin embedded sections, Immunoprecipitation and Western Blot. Use in Immunohistochemistry-Frozen sections reported in scientific literature (PMID: 20008275) In Western blot bands are seen ~17 and 19 kDa corresponding to LC3-II and LC3-I. In some cases a non-specific band is seen at ~21 kDa in mouse protein. In ICC/IF, cytoplasmic staining was observed in HeLa cells.In Simple Western only 10-15 uL of the recommended dilution is used per data point.

Images

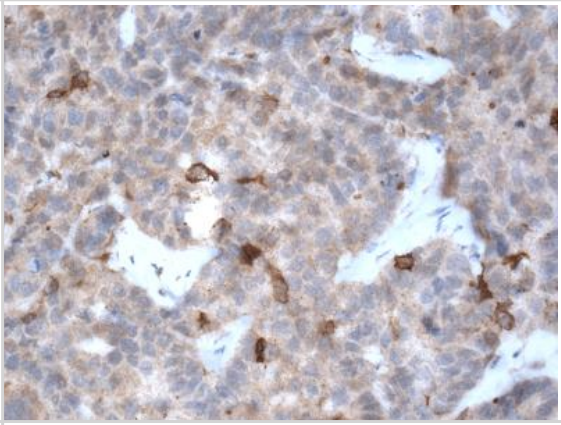
Western Blot: LC3B/MAP1LC3B Antibody [NB100-2220] - analysis of LC3 in Huh-7 and SMMC-7721 cells using anti-LC3B antibody. Image from verified customer review.



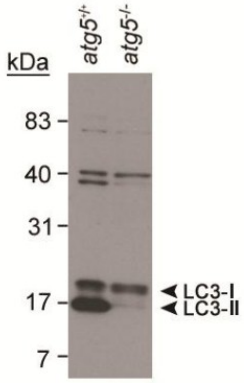
Immunocytochemistry/Immunofluorescence: LC3 Antibody [NB100-2220] - LC3 antibody was tested in HeLa cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).

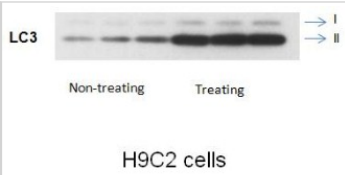
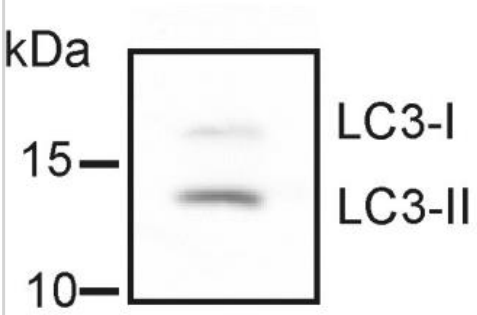
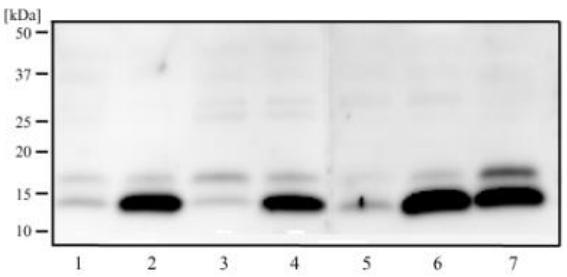
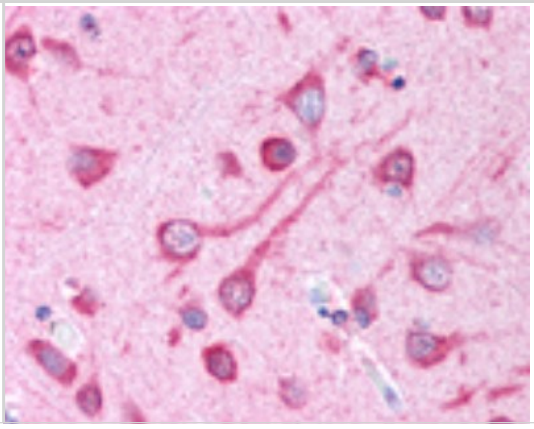


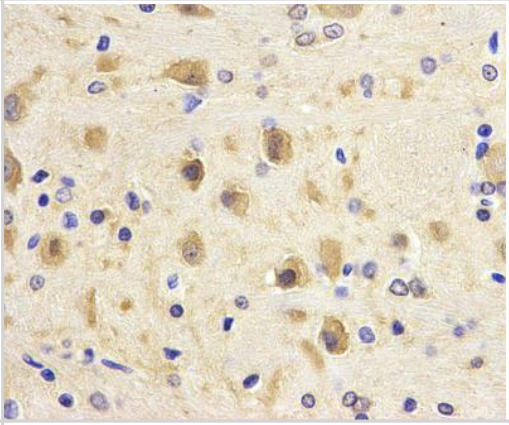
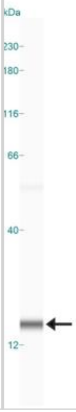
Immunohistochemistry-Paraffin: LC3 Antibody [NB100-2220] - Human ovarian Cancer tissue stained using heat mediated antigen retrieval in pH 6.0 citrate buffer at 1:200 dilution. Image provided by verified customer review.



Western Blot: LC3 Antibody [NB100-2220] - Detection of LC3 in mouse ES cell lysates. Atg5-/- ES cells from Dr. Noboru Mizushima [Mizushima, N. et al. J. Cell Biol. 152 (2001)] Photo courtesy of Dr. Beth Levine, UT SW Medical Center.



Western Blot: LC3 Antibody [NB100-2220] - Analysis of LC3 in H9C2 cells.	
Western Blot: LC3 Antibody [NB100-2220] - Detection of LC3 in mouse HL-1 cell lysate. Photo courtesy of product review by verified customer.	
Western Blot: LC3 Antibody [NB100-2220] - Western blot analysis of HeLa (1), HeLa + CQ (2), SHSY5Y (3), SHSY5Y +CQ (4), A431 (5), A431 +CQ (6) and Ntera2 (7) using LC3 antibody at 2 ug/ml.	
Immunohistochemistry: LC3 Antibody [NB100-2220] - Staining of human brain, cerebral cortex, neurons with cell processes using NB100-2220.	

Immunohistochemistry: LC3 Antibody [NB100-2220] - IHC of rat brain from confirmed customer review.	
Simple Western: LC3B/MAP1LC3B Antibody [NB100-2220] - Simple Western lane view shows a specific band for LC3 in 0.5 mg/ml of Neuro2A lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	

Publications

Yang M, Liu L, Xie M et al. Poly-ADP-ribosylation of HMGB1 regulates TNFSF10/TRAIL resistance through autophagy Autophagy. 2015 Jan 21 [PMID: 25607248]

Yan Y, Jiang W, Liu L et al. Dopamine Controls Systemic Inflammation through Inhibition of NLRP3 Inflammasome Cell. 2015 Jan 15 [PMID: 25594175] (WB, Mouse)

Xu Y, Tian C, Sun J et al. FBXW7-Induced MTOR Degradation Forces Autophagy to Counteract Persistent Prion Infection Mol. Neurobiol. 2015 Jan 13 [PMID: 25579381] (WB, Mouse)

Details:
LC3B/MAP1LC3B antibody used for WB on SMB-PS and SMB-S15 cell lines (murine cell line, persistent prion infection/scrapie-infected) - data is shown in Fig 1a and higher levels of LC3II were observed in SMB-S15 cells compared to SMB-P.

Sampaolo S, Esposito T, Gianfrancesco F et al. A novel GBE1 mutation and features of polyglucosan bodies autophagy in adult polyglucosan body disease. Neuromuscular Disorders 2014 Nov 18 [PMID: 25544507]

Seguin SJ, Morelli,FF, Vinet J et al. Inhibition of autophagy, lysosome and VCP function impairs stress granule assembly. Cell Death Differ. [PMID: 25034784]

Varga M, Fodor E, Vellai T. Autophagy in zebrafish. Methods. 2014 Dec 09 [PMID: 25498006] (WB, Zebrafish)

Sykora P, Misiak M, Wang Y et al. DNA polymerase b deficiency leads to neurodegeneration and exacerbates Alzheimer disease phenotypes. Nucleic Acids Res. 2014 Dec 30 [PMID: 25552414] (WB, Mouse)

Cossette SM, Gastonguay AJ, Bao X et al. Sucrose non-fermenting related kinase enzyme is essential for cardiac metabolism. Biol Open. 2014 Dec 12 [PMID: 25505152] (WB, Mouse)

Bueno M, Lai Y, Romero Y et al. PINK1 deficiency impairs mitochondrial homeostasis and promotes lung fibrosis. J. Clin. Invest. 2014 Dec 22 [PMID: 25562319] (IHC, Mouse)

Krishnaswamy S, Lin Y, Rajamohamedsait WJ et al. Antibody-derived in vivo imaging of tau pathology. J. Neurosci. 2014 Dec 10 [PMID: 25505335] (IHC, Mouse)

Campbell GR, Bruckman RS, Chu Y, Spector SA. Autophagy Induction by Histone Deacetylase Inhibitors Inhibits HIV Type 1. J. Biol. Chem. 2014 Dec 24 [PMID: 25540204] (WB, Human)

Johnson CE, Hunt DK, Wiltshire M et al. Endoplasmic reticulum stress and cell death in mTORC1-overactive cells is induced by nelfinavir and enhanced by chloroquine. Mol Oncol. 2014 Nov 22 [PMID: 25498902] (WB, Human)

More publications at http://www.novusbio.com/NB100-2220

Procedures

Western Blot Protocol protocol specific for LC3 Antibody (NB100-2220)
Western Blot Protocol

1. Perform SDS-PAGE (4-12%) on samples to be analyzed, loading 40 ug of total protein per lane.
2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.
3. Rinse membrane with dH2O and then stain the blot using ponceau S for 1-2 minutes to access the transfer of proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations and locations of molecular weight markers using a pencil.
4. Rinse the blot in TBS for approximately 5 minutes.
5. Block the membrane using 5% non-fat dry milk + 1% BSA in TBS for 1 hour at room temperature (RT).
6. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
7. Dilute the rabbit anti-LC3 primary antibody (NB 100-2220) in blocking buffer and incubate 1 hour at RT.
8. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
9. Apply the diluted rabbit-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer [TBS + 0.1% Tween] 3 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 manufacturer's instructions (we used BioFX Super Plus ECL).

Note: Tween-20 can 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.

I. Deparaffinization:

A. Treat slides with Xylene: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.
B. Treat slides with 100% Reagent Alcohol: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.

II. Quench Endogenous Peroxidase:

To Prepare 200 ml of Quenching Solution: Hydrogen Peroxide to 200 ml of Methanol.
**Use within 4 hours of preparation

A. Place slides in peroxidase quenching solution: 15-30 minutes.
B. Place slides in distilled water: 2 changes for 2 minutes each.

III. Retrieve Epitopes:

A. Preheat Citrate Buffer. Place 200 ml of Citrate Buffer Working Solution into container, cover and place into steamer. Heat to 90-96C.
B. Place rack of slides into hot Citrate Buffer for 20 minutes. Cover.
C. Carefully remove container with slides from steamer and cool on bench, uncovered, for 20 minutes.
D. Slowly add distilled water to further cool for 5 minutes.
E. Rinse slides with distilled water. 2 changes for 2 minutes each.

IV. Immunostaining Procedure:
A. Remove each slide from rack and circle tissue section with a hydrophobic barrier pen (e.g. Liquid Blocker-Super Pap Pen).
B. Flood slide with Wash Solution.

**Do not allow tissue sections to dry for the rest of the procedure.

C. Drain wash solution and apply 4 drops of Blocking Reagent to each slide and incubate for 15 minutes.
D. Drain Blocking Reagent (do not wash off the Blocking Reagent), apply 200 ul of Primary Antibody solution to each slide, and incubate for 1 hour.
E. Wash slides with Wash Solution: 3 changes for 5 minutes each.
F. Drain wash solution, apply 4 drops of Secondary antibody to each slide and incubate for 1 hour.
G. Wash slides with Wash Solution: 3 changes for 5 minutes each.
H. Drain wash solution, apply 4 drops of DAB Substrate to each slide and develop for 5-10 minutes. Check development with microscope.
I. Wash slides with Wash Solution: 3 changes for 5 minutes each.
J. Drain wash solution, apply 4 drops of Hematoxylin to each slide and stain for 1-3 minutes. Increase time if darker counterstaining is desired.
K. Wash slides with Wash Solution: 2-3 changes for 2 minutes each.
L. Drain wash solution and apply 4 drops of Bluing Solution to each slide for 1-2 minutes.
M. Rinse slides in distilled water.
N. Soak slides in 70% reagent alcohol: 3 minutes with intermittent agitation.
O. Soak slides in 95% reagent alcohol: 2 changes for 3 minutes each with intermittent agitation.
P. Soak slides in 100% reagent alcohol: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
Q. Soak slides in Xylene: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
R. Apply 2-3 drops of non-aqueous mounting media to each slide and mount coverslip.

Notes:
- Use treated slides (e.g. HistoBond) to assure adherence of FFPE sections to slide.
- Prior to deparaffinization, heat slides overnight in a 60C oven.
- All steps in which Xylene is used should be performed in a fume hood.
- For Epitope Retrieval, a microwave or pressure cooker may be substituted for the steamer method. Adjust times as necessary depending on conditions.
- For the initial IHC run with a new primary antibody, test tissues with and without Epitope Retrieval. In some instances, Epitope Retrieval may not be necessary.
- 200 ul is the recommended maximum volume to apply to a slide for full coverage. Using more than 200 ul may allow solutions to wick off the slide and create drying artifacts. - For small tissue sections less than 200 ul may be used.
- 5 minutes of development with DAB Substrate should be sufficient. Do not develop for more than 10 minutes. If 5 minutes of development causes background staining, further dilution of the primary antibody may be necessary.
- Hematoxylin should produce a light nuclear counterstain so as not to obscure the DAB staining. Counterstain for 1-1.5 minutes for nuclear antigens. Counterstain for 2-3 minutes for cytoplasmic and membranous antigens. If darker counterstaining is desired increase time (up to 10 minutes).

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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.