Product Datasheet

LC3/MAP1LC3A Antibody
NB100-2331SS

Unit Size: 0.025 ml

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

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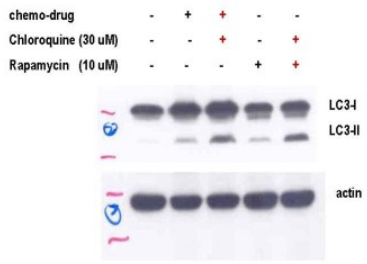
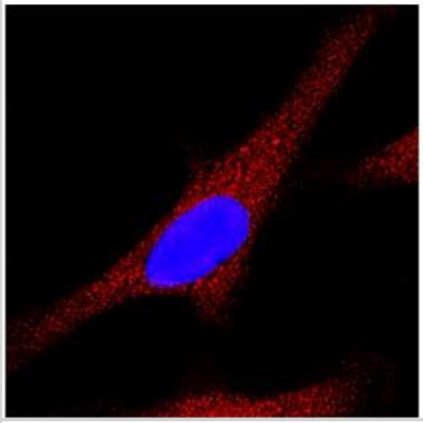
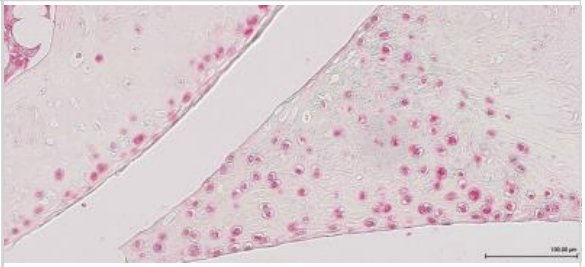
LC3/MAP1LC3A Antibody

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

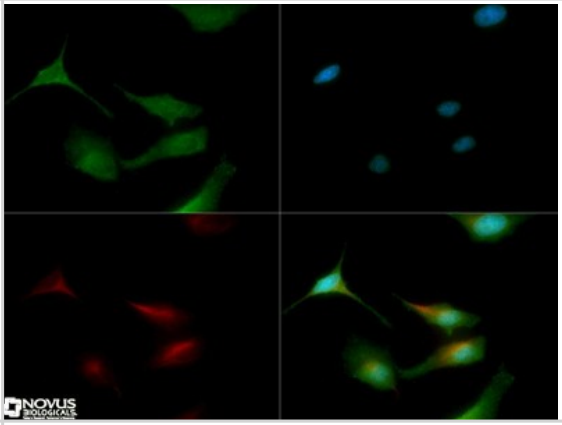
Product Description	
Host	Rabbit
Gene ID	84557
Gene Symbol	MAP1LC3A
Species	Human, Mouse, Rat, Fish, Zebrafish
Species Reactivity	Human, mouse, rat and Zebrafish.Fish reactivity reported in scientific literature (PMID: 25522711) Predicted to react with Xenopus and bovine based on 100% sequence homology.
Marker	Autophagosomes Marker
Specificity/Sensitivity	This antibody detects both LC3A and LC3B.
Immunogen	A synthetic peptide made to an internal portion of the human LC3 protein sequence (between residues 25-121). [UniProt# Q9GZQ8].

Product Application Details	
Applications	Western Blot, Simple Western, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Immunocytochemistry/Immunofluorescence 1:100-1:300, Immunohistochemistry 1:200-1:400, Immunohistochemistry-Paraffin 1:200-1:400, Immunoprecipitation 20 ug / 500 ug of lysate, Western Blot 2 ug/ml, Immunohistochemistry-Frozen, Flow Cytometry, Simple Western 1:50
Application Notes	This LC3 antibody is useful for Western blot, Immunocytochemistry/Immunofluorescence (PMID 21545732), Immunoprecipitation and Immunohistochemistry paraffin embedded sections. By Western blot bands are seen at ~19 kDa, representing LC3-I, and ~17 kDa, representing LC3-II. In ICC/IF, cytoplasmic staining was observed in HeLa cells.Use in FLOW cytometry reported in scientific literature (PMID 24419333)In Simple Western only 10-15 uL of the recommended dilution is used per data point.

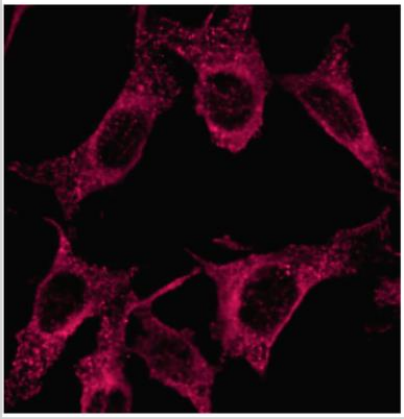
Images

Western Blot: LC3 Antibody [NB100-2331] - WB analysis of LC3 in human lysates.	
Immunocytochemistry/Immunofluorescence: LC3/MAP1LC3A Antibody [NB100-2331] - analysis of LC3 in HeLa cells using anti-LC3 antibody (red). Nuclei were counterstained with DAPI (blue).	
Immunohistochemistry-Paraffin: LC3 Antibody [NB100-2331] - Mouse meniscus and cartilage stained with anti-LC3 antibody. Image from verified customer review.	
Western Blot: LC3 Antibody [NB100-2331] - WB analysis of LC3 in human brain lyaste.	

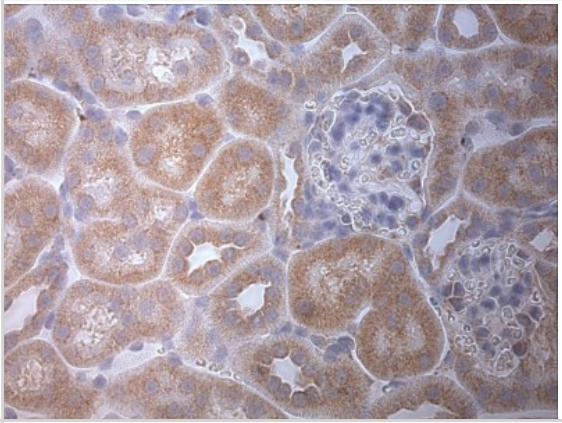
Immunocytochemistry/Immunofluorescence: LC3 Antibody [NB100-2331] - LC3 antibody was tested in HeLa cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).



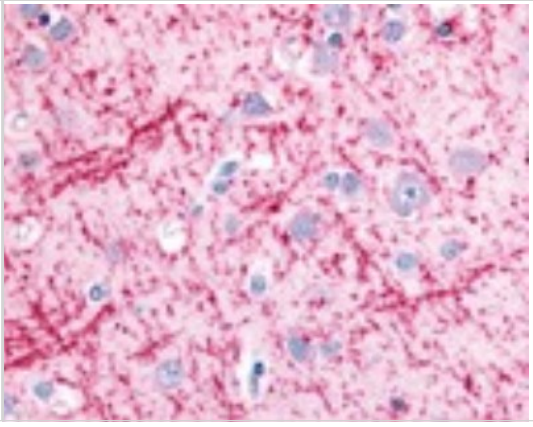
Immunocytochemistry/Immunofluorescence: LC3/MAP1LC3A Antibody [NB100-2331] - analysis of LC3 in PFA fixed NIH/3T3 cells using anti-LC3 antibody. Image from verified customer review.



Immunohistochemistry-Paraffin: LC3 Antibody [NB100-2331] - Analysis of LC3 in mouse renal tissue. Image courtesy of an anonymous customer review.



Immunohistochemistry: LC3 Antibody [NB100-2331] - Staining of human brain, cerebral cortex, cell crocesses in gray matter.



Simple Western: LC3/MAP1LC3A Antibody [NB100-2331] - 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

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

Nuschke A, Rodrigues M, Stolz DB et al. Human mesenchymal stem cells/multipotent stromal cells consume accumulated autophagosomes early in differentiation. Stem Cell Res Ther. 2014 Dec 17 [PMID: 25523618] (WB, Human)

Wilson WN, Baumgarner BL, Watanabe WO et al. Effects of resveratrol on growth and skeletal muscle physiology of juvenile southern flounder. Comp. Biochem. Physiol., Part A Mol. Integr. Physiol. 2014 Dec 16 [PMID: 25522711] (WB, Fish)

Rohailla S, Clarizia N, Sourour M et al. Acute, Delayed and Chronic Remote Ischemic Conditioning Is Associated with Downregulation of mTOR and Enhanced Autophagy Signaling. PLoS OnE. 2014 Oct 28 [PMID: 25347774] (WB, Mouse)

Gan M, Moussaud S, Jiang P, Mclean Pj. Extracellular ATP induces intracellular alpha-synuclein accumulation via P2X1 receptor-mediated lysosomal dysfunction Neurobiology of Aging et al. 2014 Nov 05 [PMID: 25480524] (WB, Human)

Details:
LC3/MAP1LC3A antibody used for WB on lysates of S1S2 cells (derived from founder H4/Tetoff cells) and differentiated Lund human mesencephalic/Luhmes cells treated or not with 3 mM ATP or 200 nM bafilomycin A1 for 48 hours (Fig. 6B and Fig. 6E).

Carlisi D, Lauricella M, D'Anneo A et al. The synergistic effect of SAHA and Parthenolide in MDA-MB231 breast cancer cells J. Cell. Physiol. 2014 Nov 05 [PMID: 25370819] (WB, Human)

Details:
LC3 antibody used for WB on MDA-MB231 breast cancer cells treated or not with suberoylanilide hydroxamic acid (SAHA, an histone deacetylase inhibitor), sesquiterpene lactone Parthenolide (Pn) and bafilomycin A1 (BafA1, a powerful inhibitor of the autophagic flux). WB data is shown in Figure 3b.

Lim YM, Lim H, Hur KY et al. Systemic autophagy insufficiency compromises adaptation to metabolic stress and facilitates progression from obesity to diabetes. Nat Commun. 2014 Sep 26 [PMID: 25255859]

Oliverio S, Corazzari M, Sestito C et al. The spermidine analogue GC7 (N1-guanyl-1,7-diamineoheptane) induces autophagy through a mechanism not involving the hypusination of eIF5A. Amino Acids. 2014 Sep 14 [PMID: 25218134]

Xiong R, Siegel D, Ross D. Quinone-induced protein handling changes: Implications for major protein handling systems in quinone-mediated toxicity. Toxicol. Appl. Pharmacol. 2014 Aug 22 [PMID: 25151970] (WB, ICC/IF, Rat)

Duarte-Silva S, Neves-Carvalho A, Soares-Cunha C et al. Lithium Chloride Therapy Fails to Improve Motor Function in a Transgenic Mouse Model of Machado-Joseph Disease. Cerebellum. 2014 Aug 12 [PMID: 25112410] (WB, Mouse)

Li J, Rohailla S, Gelber N et al. MicroRNA-144 is a circulating effector of remote ischemic preconditioning. Basic Res. Cardiol. 2014 Sep 01 [PMID: 25060662] (WB, Mouse)

Details:
LC3 antibody used for WB in hearts from mice injected with PBS, miR-Co or miR-144 (Figure 2b).

Barmada SJ, Serio A, Arjun A et al. Autophagy induction enhances TDP43 turnover and survival in neuronal ALS models. Nat. Chem. Biol. 2014 Jun 29 [PMID: 24974230] (ICC/IF, Rat)

More publications at http://www.novusbio.com/NB100-2331

Procedures

Western Blot protocol specific for LC3 Antibody (NB100-2331)
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 100-2331) 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 manufacturers 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: Add 3 ml of 30% Hydrogen Peroxide to 200 ml of Methanol.
**Use within 4 hours of preparation
A.Place slides in peroxidase quenching solution: 15-30 minutes.

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.
S. Lay slides on a flat surface to dry prior to viewing under microscope.

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.