Product Datasheet

LC3/MAP1LC3A Antibody
NBP1-19167SS

Unit Size: 0.025 ml

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

Reviews: 1 Publications: 11

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NBP1-19167SS

LC3/MAP1LC3A Antibody

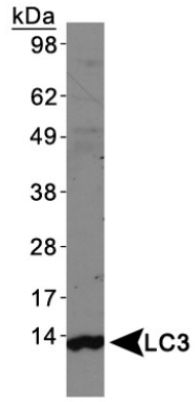
Product Information	
Unit Size	0.025 ml
Concentration	0.2 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Purity	Immunogen affinity purified
Buffer	PBS, 0.1% BSA, 50% glycerol
Target Molecular Weight	14 kDa

Product Description	
Host	Rabbit
Gene ID	84557
Gene Symbol	MAP1LC3A
Species	Human, Mouse, Rat, Bovine, Zebrafish
Species Reactivity	Human, mouse, bovine, rat and zebrafish.
Marker	Autophagosomes Marker
Specificity/Sensitivity	Although specificity between LC3A and LC3B has not been tested, this antibody was created to a peptide that has 100% identity to LC3A and 62% identity to LC3B.
Immunogen	Genomic sequence made to an N-terminal portion of the human LC3A protein [Swiss-Prot# Q9H492].

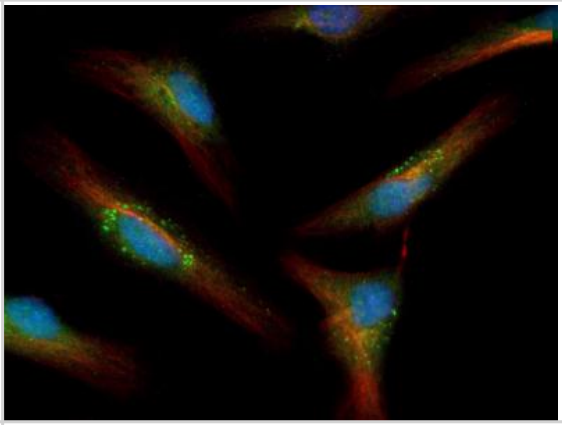
Product Application Details	
Applications	Western Blot, Simple Western, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin
Recommended Dilutions	Flow Cytometry 1:100, Immunocytochemistry/Immunofluorescence 1:100, Immunohistochemistry 1:100, Immunohistochemistry-Paraffin 1:100, Western Blot 1:2500, Immunohistochemistry-Frozen, Simple Western 1:40
Application Notes	This LC3 antibody is useful for Immunohistochemistry, Immunocytochemistry/Immunofluorescence, Flow Cytometry and Western blot. In WB a band is seen at ~14 kDa. IHC was done on formalin fixed paraffin embedded sections. In ICC/IF, autophagosome formation has been seen in HeLa cells after treatment with 50uM chloroquine. Use in Immunohistochemistry-Frozen reported in scientific literature (PMID: 23936035)In Simple Western only 10-15 uL of the recommended dilution is used per data point.

Images

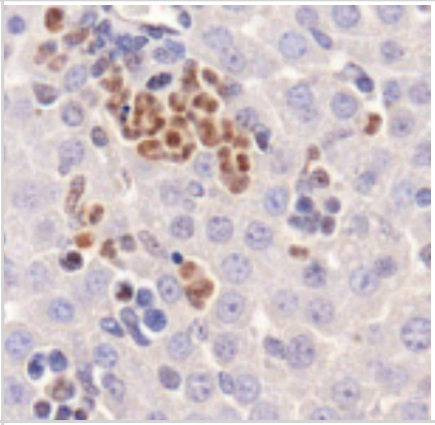
Western Blot: LC3 Antibody [NBP1-19167] - Human brain lysate.



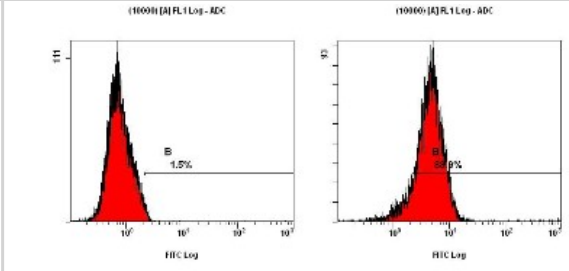
Immunocytochemistry/Immunofluorescence: LC3 Antibody [NBP1-19167] - LC3 antibody was tested in HeLa cells with Dylight 488 (green). Cells were treated overnight with 50uM chloroquine to induce autophagosome formation. Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).



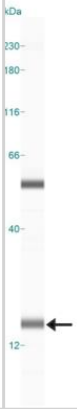
Immunohistochemistry: LC3 Antibody [NBP1-19167] - Staining of LC3 in mouse liver.



Flow Cytometry: LC3 Antibody [NBP1-19167] - Staining of NTERA-2 cells using NBP1-19167 at a 1:50 dilution detected using Dylight-488 conjugated goat anti-rabbit IgG secondary antibody.



Simple Western: LC3/MAP1LC3A Antibody [NBP1-19167] - 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

Campos T, Ziehe J, Palma M et al. Rheb promotes cancer cell survival through p27Kip1-dependent activation of autophagy Mol. Carcinog. 2015 Jan 15 [PMID: 25594310] (WB, Human)

Giovannini M, Bonne NX, Vitte J et al. mTORC1 inhibition delays growth of neurofibromatosis type 2 schwannoma. Neuro-oncology 2014 Jan 10 [PMID: 24414536] (IHC-P, Mouse)

Bruntz RC, Taylor HE, Lindsley CW, Brown HA. Phospholipase D2 mediates survival signaling through direct regulation of Akt in glioblastoma cells. J Biol Chem. 2013 Nov 20 [PMID: 24257753] (WB, Human)

Howell GM, Gomez H, Collage RD et al. Augmenting Autophagy to Treat Acute Kidney Injury during Endotoxemia in Mice. PLoS One 2013 Jul 30 [PMID: 23936035] (IHC-Fr, ICC/IF, Mouse)

Santoni M, Amantini C, Morelli MB et al. Pazopanib and sunitinib trigger autophagic and non-autophagic death of bladder tumour cells. Br J Cancer 2013 Jul 25 [PMID: 23887605] (WB, Human)

Kuo SH, Tang G, Ma K et al. Macroautophagy abnormality in essential tremor PLoS One 2012 [PMID: 23300858] (WB, Human)

Dehay B, Bove J, Rodriguez-Muela N et al. Pathogenic lysosomal depletion in Parkinson's disease J Neurosci 2010 Sep 15 [PMID: 20844148] (WB, Human, Mouse)

Jae-Kyo Jeong, Myung-Hee Moon et al. Autophagy induced by resveratrol prevents human prion protein-mediated neurotoxicity Neuroscience Research 2012 Mar 12 [PMID: 22465415] (WB, Human)

Schwarz L, Goldbaum O, Bergmann M et al. Involvement of Macroautophagy in Multiple System Atrophy and Protein Aggregate Formation in Oligodendrocytes J Mol Neurosci 2012 Mar 13 [PMID: 22411133] (IHC, Human)

Chang, C-F, Huang, H-J, Lee, H-C et al. Melatonin attenuates kainic acid-induced neurotoxicity in mouse hippocampus via inhibition of autophagy and ?-synuclein aggregation Journal of Pineal Research 2012 [PMID: 22212051] (WB, Mouse)

Herd HL, Malugin A, Ghandehari H. Silica nanoconstruct cellular toleration threshold in vitro. J Control Release;153 (1):40-8. 2011 Jul 15. [PMID: 21342660] (WB, Human, Mouse)

Procedures

Western Blot protocol specific for LC3 Antibody (NBP1-19167)
Western Blot Protocol

1. Perform SDS-PAGE (4-12% MEX) on samples to be analyzed, loading 30 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% NFDM + 1% BSA in TBS + Tween, 1 hour at 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 (NBP1-19167) in blocking buffer and incubate 1 hour at room temperature.
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 manufacturers 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 (Pierce 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.

Immunohistochemistry-paraffin embedded sections protocol (NBP1-19167)
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 dH2O three times for 5 minutes each.
2. Wash section in wash buffer (1X PBS/0.1% Tween-20 (1X PBST)) for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1X PBST, 5% goat serum) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul primary antibody diluted in 1X PBST, 5% goat serum to each section. Incubate overnight at 4C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul biotinylated secondary antibody, diluted in 1X PBST, 5% goat serum. 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 Striptavidin-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 dH2O.
12. Counterstain sections in hematoxylin.
13. Wash sections in dH2O two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.

Immunocytochemistry/Immunofluorescence Protocol for LC3 Antibody (NBP1-19167)
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 1:25,0000 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.

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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.