Product Datasheet

LC3A Antibody - BSA Free
NBP1-19167

Unit Size: 0.1 ml

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

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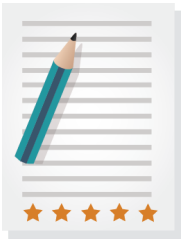
Updated 2/21/2025 v.20.1



NBP1-19167

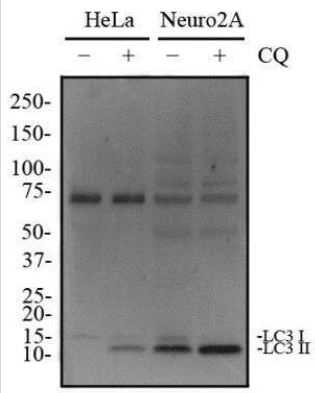
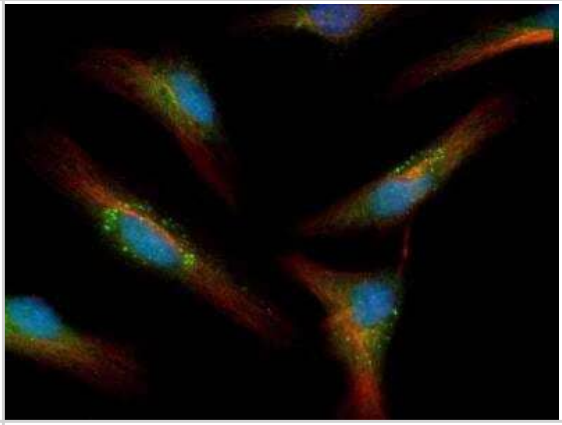
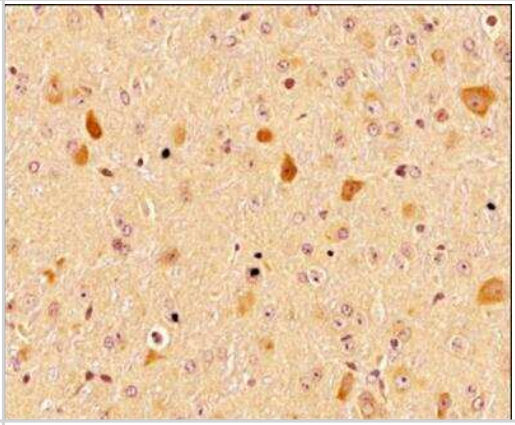
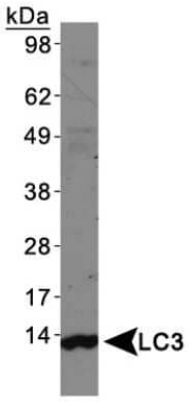
LC3A Antibody - BSA Free

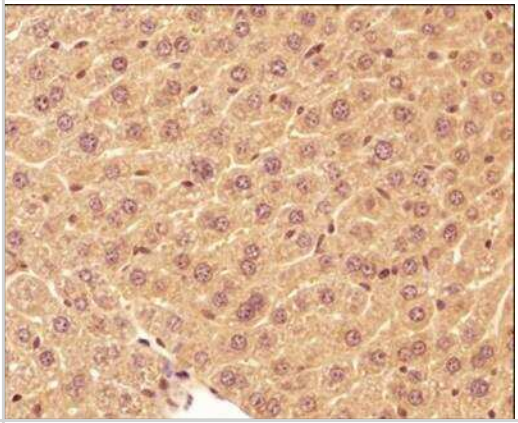
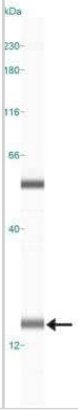
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	14 kDa

Product Description	
Host	Rabbit
Gene ID	84557
Gene Symbol	MAP1LC3A
Species	Human, Mouse, Rat, Bovine, Zebrafish
Marker	Autophagosome Marker
Specificity/Sensitivity	Although specificity between this LC3A Antibody 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	Partial recombinant protein 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	Western Blot 1:2500, Simple Western 1:40, Flow Cytometry 1:100, Immunohistochemistry 1:100 - 1:400, Immunocytochemistry/ Immunofluorescence 1:100, Immunohistochemistry-Paraffin 1:100 - 1:400, Immunohistochemistry-Frozen reported in scientific literature (PMID 23936035)
Application Notes	In WB, bands are seen at approx. 14-17 kDa. In ICC/IF, autophagosome formation has been seen in HeLa cells after treatment with 50 uM chloroquine. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. See Simple Western Antibody Database for Simple Western validation: Tested in Neuro2A lysate 0.5 mg/mL, separated by Size, antibody dilution of 1:40, apparent MW was 17 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.

Images

Western Blot: LC3A Antibody [NBP1-19167] - Total protein from HeLa and Neuro2A cells treated with or without 50 uM chloroquine for 24 hours was separated on a 4-15% gel by SDS-PAGE, transferred to 0.2 um PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 2.0 ug/mL anti-LC3A in 1% non-fat milk in TBST and detected with an anti-rabbit HRP secondary antibody using chemiluminescence. Note the detection LC3 II upon chloroquine treatment.	
Immunocytochemistry/Immunofluorescence: LC3A Antibody [NBP1-19167] - LC3/MAP1 [NBP1-19167] - LC3 antibody was tested in HeLa cells with Dylight 488 (green). Cells were treated overnight with 50 uM chloroquine to induce autophagosome formation. Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).	
Immunohistochemistry-Paraffin: LC3A Antibody [NBP1-19167] - Analysis of a FFPE tissue section of mouse brain using LC3 antibody at 1:300 dilution. The signal was developed using HRP-labelled secondary antibody and DAB reagent, and the sections/nuclei were further counterstained with hematoxylin. Note the diffused cytoplasmic staining of LC3 in all of the cells with highest positivity in various neurons.	
Western Blot: LC3/MAP1LC3A Antibody [NBP1-19167] - Human brain lysate.	

Immunohistochemistry-Paraffin: LC3A Antibody [NBP1-19167] - Analysis of a FFPE tissue section of mouse liver using LC3 antibody at 1:300 dilution. The signal was developed using HRP-labelled secondary antibody and DAB reagent, and the sections/nuclei were further counterstained with hematoxylin. Note the diffused cytoplasmic staining of LC3 in all of the hepatocytes and other liver cells.	
Simple Western: LC3A Antibody [NBP1-19167] - Image 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

Jiang Z, Bo L, et al. Overexpression of homeodomain-interacting protein kinase 2 (HIPK2) attenuates sepsis-mediated liver injury by restoring autophagy. Cell Death Dis 2018-08-28 [PMID: 30154452] (WB, Mouse)

Yang A, Herter-Sprie G, Zhang H, et al. Autophagy Sustains Pancreatic Cancer Growth through Both Cell-Autonomous and Nonautonomous Mechanisms. Cancer Discov. 2018-01-09 [PMID: 29317452] (IF/IHC, Mouse)

Korski KI, Kubli DA, Wang BJ et al. Hypoxia Prevents Mitochondrial Dysfunction and Senescence in Human c-Kit+ Cardiac Progenitor Cells. Stem Cells 2019-01-10 [PMID: 30629785] (ICC/IF, Human)

Kohler LJ, Reed SR, Sarraf SA et al. Effector Protein Cig2 Decreases Host Tolerance of Infection by Directing Constitutive Fusion of Autophagosomes with the Coxiella-Containing Vacuole MBio 2016 Jul 20 [PMID: 27435465] (WB). Elife. 2016-07-20 [PMID: 27435465] (WB)

Details:
This citation used the PE version of this antibody.

Rao S, Tortola L, Perlot T et al. A dual role for autophagy in a murine model of lung cancer. Nat Commun. 2014-01-01 [PMID: 24445999] (IHC-P, Mouse)

Saito T, Asai K, Sato S et al. Autophagic vacuoles in cardiomyocytes of dilated cardiomyopathy with initially decompensated heart failure predict improved prognosis Autophagy 2016-02-18 [PMID: 26890610] (IHC-P, Human)

Zois CE, Giatromanolaki A, Sivridis E et al. Autophagic flux in normal mouse tissues: focus on endogenous LC3A processing. Autophagy 2011-11-01 [PMID: 21997374]

Yang Annan, Rajeshkumar N V, Wang Xiaoxu et al. Autophagy is critical for pancreatic tumor growth and progression in tumors with p53 alterations. Cancer Discov. 2014-05-29 [PMID: 24875860]

Campos T, Ziehe J, Palma M et al. Rheb promotes cancer cell survival through p27Kip1-dependent activation of autophagy Mol. Carcinog. 2015-01-15 [PMID: 25594310] (WB, Human)

Giovannini M, Bonne NX, Vitte J et al. mTORC1 inhibition delays growth of neurofibromatosis type 2 schwannoma. Neuro-oncology 2014-01-10 [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. 2013-11-20 [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 2013-07-30 [PMID: 23936035] (ICC/IF, IHC-Fr, Mouse)

More publications at http://www.novusbio.com/NBP1-19167

Procedures

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

Protocol: Inhibition of Autophagy and LC3 Antibody (NBP1-19167) Western Blot

Materials

Chloroquine diphosphate (CQ) (10 mM) in dH2O
1X PBS
Sample buffer, 2X Laemmli buffer: 4% SDS, 5% 2-mercaptoethanol (BME), 20% glycerol, 0.004% bromophenol blue, 0.125 M Tris HCl, pH 6.8
RIPA buffer: 150 mM NaCl, 1% NP-40 or Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris-HCl, pH 8.0, 20 mM Tris-HCl, pH 7.5
1X Running Buffer: 25 mM Tris-base, 192 mM glycine, 0.1% SDS. Adjust to pH 8.3
1X Transfer buffer (wet): 25 mM Tris-base, 192 mM glycine, 20% methanol, Adjust to pH 8.3
TBS
TBST, TBS and 0.1% Tween
Blocking solution: TBST, 5% non-fat dry milk
rabbit anti-LC3 primary antibody (NBP1-19167) in blocking buffer (~2 ug/mL)

Methods

Tip: For more information on Western Blotting, see our Western Blot handbook.

1. Grow cells (e.g. HeLa or Neuro2A) in vitro to semi-confluency (70-75%).

2. Add CQ to culture dishes to a final concentration of 50 uM and incubate overnight (16 hours). Remember to include an untreated sample as a negative control.
Note: Validated autophagy inducers should be included as positive controls.

3. Rinse cells with ice-cold 1X PBS and lyse cells with sample buffer.
Note: LC3-I and LC3-II are sensitive to degradation, although LC3-I is more labile. These proteins are sensitive to freeze-thaw cycles and SDS sample buffers. Fresh samples should be analyzed quickly to prevent protein degradation.

4. Sonicate and incubate cells for 5 minutes at 95oC.
Tip: Cells are lysed directly in sample buffer or may be lysed in RIPA buffer.

5. Load samples of Chloroquine-treated and -untreated cell lysates 40 ug/lane on a 4-20% polyacrylamide gradient gel (SDS-PAGE).
Tip: For detection of LC3 it is particularly important to monitor the progress of the gel as this protein is relatively small (~14kDa).

Tip: Alternatively, for non-gradient gels, use a 20% polyacrylamide gel.

6. Transfer proteins to a 0.2 um PVDF membrane for 30 minutes at 100V.

7. After transfer, rinse the membrane with dH2O and stain with Ponceau S for 1-2 minutes to confirm efficiency of protein transfer.

8. Rinse the membrane in dH2O to remove excess stain and mark the loaded lanes and molecular weight markers using a pencil.

9. Block the membrane using blocking buffer solution (5% non-fat dry milk in TBST) for 1 hour at room temperature.

10.Rinse the membrane with TBST for 5 minutes.

11.Dilute the rabbit anti-LC3 primary antibody (NBP1-19167) (~2 ug/mL) in blocking buffer and incubate the

membrane for 1 hour at room temperature.

12.Rinse the membrane with dH2O.

13.Rinse the membrane with TBST, 3 times for 10 minutes each.

14.Incubate the membrane with diluted secondary antibody, according with product's specifications, (e.g. anti-rabbit-IgG HRP-conjugated) in blocking buffer for 1 hour at room temperature.
Note: Tween-20 may 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.

15.Rinse the membrane with TBST, 3 times for 10 minutes each.

16.Apply the detection reagent of choice (e.g. BioFX Super Plus ECL) in accordance with the manufacturer's instructions.

17.Image the blot.
Tip: LC3-I and it's lipidated form LC3-II have different electrophoretic mobility properties, with the lipidated form moving faster in an SDS-PAGE gel, albeit its larger molecular weight. LC3-II runs at 14-16 kDa while LC3-I runs at 16 -18kDa.

Note: This assay measures the difference in the LC3-II signal in the presence and absence of inhibitors (e.g., lysosomotropic agents). When autophagic flux is present or induced in a system an increase in the LC3-II signal should be observed with the inhibitor.

Immunohistochemistry-Paraffin protocol for LC3A Antibody (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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Products Related to NBP1-19167

NB820-59177	Human Brain Whole Tissue Lysate (Adult Whole Normal)
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NBP2-24891	Rabbit IgG Isotype Control

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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