

Product Datasheet

LC3A Antibody - BSA Free NBP1-78964

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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NBP1-78964

LC3A Antibody - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	1.09 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
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS and 30% Glycerol

Product Description	
Description	Novus Biologicals Rabbit LC3A Antibody - BSA Free (NBP1-78964) is a polyclonal antibody validated for use in IHC, WB and ICC/IF. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	84557
Gene Symbol	MAP1LC3A
Species	Human, Mouse, Rat, Porcine, Bovine, Primate
Specificity/Sensitivity	This LC3I antibody specifically detects the cytosolic form of LC3 before it gets converted into LC3II during autophagy.
Immunogen	This LC3A Antibody was prepared from a synthetic peptide made to a C-terminal region of the human LC3 protein (within residues 50-120). [Swiss-Prot Q9H492].

Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, Immunocytochemistry/Immunofluorescence, Immunohistochemistry
Recommended Dilutions	Western Blot 1:1000, Immunohistochemistry 1:400, Immunocytochemistry/Immunofluorescence 1:75, Immunohistochemistry-Paraffin 1:400
Application Notes	This LC3I antibody is useful for Western Blot, Immunocytochemistry/Immunofluorescence, and IHC-paraffin embedded sections. In Western Blot, a band is seen ~15kDa representing LC3I. In ICC/IF, observed staining showed inactivated LC3 throughout the cytoplasm of Neuro2a cells. In IHC-P, staining was observed in the cytoplasm of mouse testes tissue. Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended.

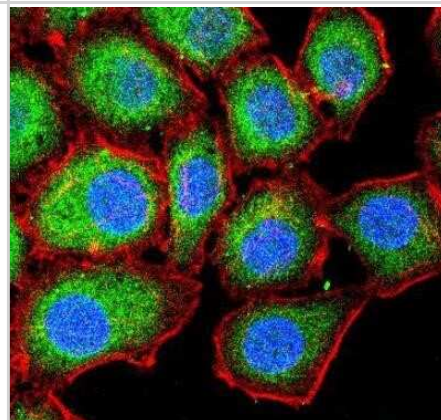


Images

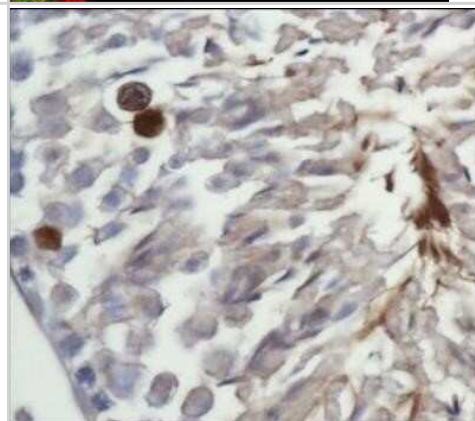
Western Blot: LC3A Antibody [NBP1-78964] - Analysis of LC3I in Neuro2A cell lysate.

<250
<150
<100
<75
<50
<37
<25
<20
<15
<10

Immunocytochemistry/Immunofluorescence: LC3A Antibody [NBP1-78964] - IF Confocal analysis of A549 cells using LC3I antibody (NBP1-78964, 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: LC3A Antibody [NBP1-78964] - Analysis of LC3I in mouse testis using DAB with hematoxylin counterstain.



Procedures

Western Blot protocol for LC3A Antibody (NBP1-78964)

LC3A Antibody:

Protocol: Inhibition of Autophagy and LC3 Antibody (NBP1-78964) 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 (NBP1-78964) 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 (NBP1-78964) (~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 its 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.



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Products Related to NBP1-78964

NB820-59177	Human Brain Whole Tissue Lysate (Adult Whole Normal)
NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
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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