Product Datasheet

LC3A Antibody (2312D) - BSA Free
NBP2-75924

Unit Size: 0.1 mg

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

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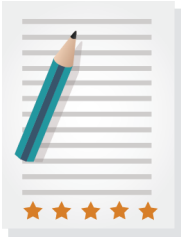


NBP2-75924

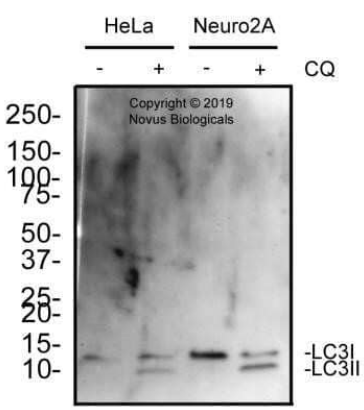
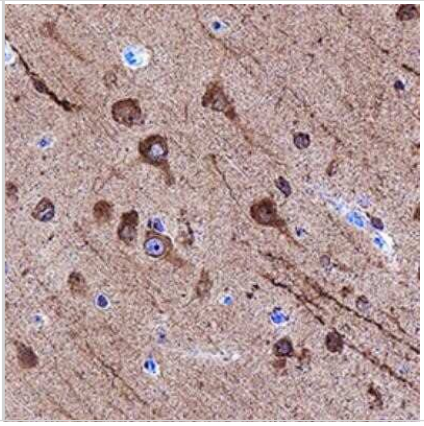
LC3A Antibody (2312D) - BSA Free

Product Information	
Unit Size	0.1 mg
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	2312D
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Protein A or G purified from cell culture supernatant
Buffer	PBS

Product Description	
Description	Novus Biologicals Rabbit LC3A Antibody (2312D) - BSA Free (NBP2-75924) is a recombinant monoclonal antibody validated for use in IHC and WB. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	84557
Gene Symbol	MAP1LC3A
Species	Human, Mouse
Immunogen	This LC3A Antibody (2312D) was immunized with a partial synthetic peptide from the C-terminal of human LC3A [UniProt Q9H492]

Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, Immunohistochemistry
Recommended Dilutions	Western Blot 1 ug/mL, Immunohistochemistry 3-25 ug/mL, Immunohistochemistry-Paraffin 3-25 ug/mL

Images

Western Blot: LC3A Antibody (2312D) [NBP2-75924] - Total protein from human HeLa and mouse Neuro2A cells treated with and without 50 uM chloroquine for 24 hours was separated on a 4-20% 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 blocking buffer and detected with an anti-rabbit HRP secondary antibody using West Pico PLUS chemiluminescence detection reagent.	
Immunohistochemistry-Paraffin: LC3A Antibody (2312D) [NBP2-75924] - This LC3A antibody was detected in immersion fixed paraffin-embedded sections of human brain (frontal cortex) using Rabbit Anti-Human LC3A Monoclonal Antibody (Catalog # NBP2-75924) at 3 ug/mL for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC003). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to nuclei.	

Procedures

Immunohistochemistry protocol for LC3A Antibody (NBP2-75924)
LC3A Antibody (2312D):
Recommended Protocol for IHC and ICC Staining

1. Deparaffinize paraffin-embedded sections in Xylene and hydrate in a series of graded alcohol to water. Perform Heat Induced Epitope Retrieval (HIER) if required. Rinse with deionized water. For frozen tissue sections deparafinization is not required. Cells can be fixed in dishes they are cultured in, but don't require heat-induced epitope retrieval: for example, cells can be fixed with freshly made 2-4% formaldehyde solution for 20 minutes at room temperature and then rinsed 3 x 10 minutes with PBS. Do not let tissue sections or cells dry from this point on.
2. Block endogenous peroxidase by incubating slides with tissue sections with 3% H2O2/Methanol solution for 15 minutes at room temperature.
3. Rinse the sample 3 times with PBS containing 0.05% Tween 20.
4. Block with normal serum of choice, by incubating slides for 15 minutes at room temperature.
5. Drain or blot off solution, but do not rinse.
6. Apply primary antibody and incubate for 60 minutes at room temperature. It is the responsibility of the investigator to optimize working dilution and incubation time for primary antibodies.
7. Repeat step 3.
8. Apply the VisUCyte(TM) HRP Polymer, covering the entire area of the tissue sections, and incubate for 30 to 60 minutes at room temperature.
9. Repeat step 3.
10. Add HRP-sensitive substrate solution (which produces insoluble precipitate) to tissue sections and incubate using conditions recommended by the substrate's supplier. For example, HRP-sensitive substrate solution can be either, 3' 3'-Diaminobenzidine (known as DAB) 3-Amino-9-ethylcarbazol (known as AEC).
11. Rinse with distilled tap water.
12. Counterstain tissue sections if required using hematoxylin or other counterstaining dyes as needed for 15 seconds to 2 minutes depending on the tissue.
13. Rinse slides with tap water (if hematoxylin was used) or other solution as required for the particular counterstaining dye and mount tissue sections under coverslips with either aqueous or non-aqueous (e.g. xylene-based) mounting media. Note: Unlike DAB, AEC is soluble in alcohols and xylene. Tissue sections subjected to an HRP-AEC protocol should be coverslipped using only aqueous mounting media.
14. Let slides dry and visualize staining under a microscope.

Precautions
Avoid using preservatives in solutions, such as Sodium Azide which is a strong inhibitor of HRP enzyme.

Western Blot protocol for LC3A Antibody (NBP2-75924)
LC3A Antibody (2312D):
Protocol: Inhibition of Autophagy and LC3 Antibody (NBP2-75924) 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 (NB2-75924) 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 (NBP2-75924) (~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.

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Products Related to NBP2-75924

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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