Product Datasheet

LC3A Antibody
NB100-2331

Unit Size: 0.1 ml
Aliquot and store at -20°C or -80°C. Avoid freeze-thaw cycles.

Reviews: 18  Publications: 201

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Updated 12/17/2017 v.20.1

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### Product Information

<table>
<thead>
<tr>
<th>Feature</th>
<th>Specification</th>
</tr>
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<tbody>
<tr>
<td>Unit Size</td>
<td>0.1 ml</td>
</tr>
<tr>
<td>Concentration</td>
<td>1.0 mg/ml</td>
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<tr>
<td>Storage</td>
<td>Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.</td>
</tr>
<tr>
<td>Clonality</td>
<td>Polyclonal</td>
</tr>
<tr>
<td>Preservative</td>
<td>0.02% Sodium Azide</td>
</tr>
<tr>
<td>Isotype</td>
<td>IgG</td>
</tr>
<tr>
<td>Purity</td>
<td>Immunogen affinity purified</td>
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<tr>
<td>Buffer</td>
<td>PBS</td>
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### Product Description

**Host**  
Rabbit

**Gene ID**  
84557

**Gene Symbol**  
MAP1LC3A

**Species**  
Human, Mouse, Rat, Canine, Fish, Plant, Zebrafish

**Reactivity Notes**  
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. Canine reactivity reported in scientific literature (PMID: 26179070). Plant reactivity reported in scientific literature (PMID: 27861739).

**Marker**  
Autophagosome 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# Q9H492].

### Product Application Details

**Applications**  
Western Blot, Simple Western, ELISA, Flow Cytometry, Immunoblotting, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Southern Blot

**Recommended Dilutions**  

**Application Notes**  
This LC3 antibody is useful for Western blot, Immunocytochemistry (PMID 21545732), Immunoprecipitation and Immunohistochemistry in paraffin embedded sections. By Western blot bands are seen at ~19 kDa, representing LC3-I, and ~17 kDa, representing LC3-II. In ICC, cytoplasmic staining was observed in HeLa cells. Use in FLOW cytometry reported in scientific literature (PMID 24419333). Use in ELISA reported in scientific literature (PMID 20930550). Use in southern blot reported in scientific literature (PMID 21262964). Use in immunoblotting reported in scientific literature (PMID 28253371). In Simple Western only 10 - 15 uL of the recommended dilution is used per data point.
Western Blot: LC3A Antibody [NB100-2331] - WB analysis of heart tissue lysates from mice which were subjected or not to 48 hours of starvation. The signal was developed using ECL method and this LC3 antibody was found to detect both forms of LC3, i.e. LC3-I and LC3-II. As expected, the levels of LC3-II form were higher in the heart tissue lysates from starved mice. The bottom image is of the coomassie blue staining.

Western Blot: LC3A Antibody [NB100-2331] - Detection of HRP conjugated autophagic LC3 in mouse ES cell lysate. The atg5-/- lane (ES cells, cultured to form embryonic bodies, that are deficient in conversion of LC3-1 to LC3-11) demonstrates the specificity of NB 100-2331, as there is no detection of LC3-11. Photo courtesy of Dr. Beth Levine, UT Southwestern Medical Center.

Western Blot: LC3A Antibody [NB100-2331] - Analysis in human lysates.

Immunocytochemistry/Immunofluorescence: LC3A Antibody [NB100-2331] - Analysis in HeLa cells using anti-LC3 antibody (red). Nuclei were counterstained with DAPI (blue).
Western Blot: LC3A Antibody [NB100-2331] - LC3A expression in Mouse macrophage cell line RAW 264.7. This image was submitted via customer Review. *(Mouse WB)*

Immunohistochemistry-Paraffin: LC3A Antibody [NB100-2331] - Staining in mouse meniscus and cartilage. Image from verified customer review.

Western Blot: LC3A Antibody [NB100-2331] - Analysis in human brain lysate.

Immunocytochemistry/Immunofluorescence: LC3A Antibody [NB100-2331] - Tested in HeLa cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).
Immunocytochemistry/Immunofluorescence: LC3A Antibody [NB100-2331] - Analysis in PFA fixed NIH/3T3 cells using anti-LC3A antibody. Image from verified customer review.

Immunohistochemistry: LC3A Antibody [NB100-2331] - Staining of human brain, cerebral cortex, cell processes in gray matter.

Immunohistochemistry-Paraffin: LC3A Antibody [NB100-2331] - Analysis in mouse renal tissue. Image courtesy of an anonymous customer review.

Simple Western: LC3A 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.
<table>
<thead>
<tr>
<th>Publications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solanas G, Peixoto FO, Perdiguerio E et al. Aged Stem Cells Reprogram Their Daily Rhythmic Functions to Adapt to Stress Cell 2017 Aug 10 [PMID: 28802040] (Mouse)</td>
</tr>
<tr>
<td>Nazim UM, Moon JH, Lee YJ et al. PPARy activation by troglitazone enhances human lung cancer cells to TRAIL-induced apoptosis via autophagy flux. Oncotarget Apr 18 2017 12:00AM [PMID: 28460464] (WB, Human)</td>
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More publications at [http://www.novusbio.com/NB100-2331](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 µg 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 Peroxidase: 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).
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 100% guarantee, please visit www.novusbio.com/guarantee

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