Product Datasheet

LC3A Antibody
NBP1-19167

Unit Size: 0.1 ml

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

Reviews: 2  Publications: 16

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Updated 12/11/2017 v.20.1

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### NBP1-19167
#### LC3A Antibody

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<tr>
<th><strong>Product Information</strong></th>
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</tr>
</thead>
<tbody>
<tr>
<td><strong>Unit Size</strong></td>
<td>0.1 ml</td>
</tr>
<tr>
<td><strong>Concentration</strong></td>
<td>0.2 mg/ml</td>
</tr>
<tr>
<td><strong>Storage</strong></td>
<td>Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.</td>
</tr>
<tr>
<td><strong>Clonality</strong></td>
<td>Polyclonal</td>
</tr>
<tr>
<td><strong>Preservative</strong></td>
<td>0.05% Sodium Azide</td>
</tr>
<tr>
<td><strong>Isotype</strong></td>
<td>IgG</td>
</tr>
<tr>
<td><strong>Purity</strong></td>
<td>Immunogen affinity purified</td>
</tr>
<tr>
<td><strong>Buffer</strong></td>
<td>PBS, 0.1% BSA, and 50% Glycerol</td>
</tr>
<tr>
<td><strong>Target Molecular Weight</strong></td>
<td>14 kDa</td>
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### Product Description

#### Host
Rabbit

#### Gene ID
84557

#### Gene Symbol
MAP1LC3A

#### Species
Human, Mouse, Rat, Bovine, Zebrafish

#### Reactivity Notes
Human, mouse, bovine, rat and zebrafish.

#### Marker
Autophagosome 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
- 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

#### Application Notes
- In WB, bands are seen at approx. 14-17 kDa position. 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. 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.
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 50uM chloroquine to induce autophagosome formation. Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).

Immunohistochemistry-Paraffin: LC3A Antibody [NBP1-19167] - LC3/MAP1 [NBP1-19167] - IHC analysis of a formalin fixed and paraffin embedded 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.

**Immunohistochemistry-Paraffin: LC3/MAP1LC3A Antibody [NBP1-19167]** - IHC analysis of a formalin fixed and paraffin embedded 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: 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.
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Procedures

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

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 and then maintain at a sub-boiling temperature for approximately 5 minutes.
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 (1X 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 for 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 1:25,000 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.
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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