Product Datasheet

Beclin 1 Antibody
NB500-249

Unit Size: 0.1 ml

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

Reviews: 8  Publications: 78

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Updated 8/22/2018 v.20.1

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# NB500-249
## Beclin 1 Antibody

### Product Information

<table>
<thead>
<tr>
<th>Feature</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unit Size</td>
<td>0.1 ml</td>
</tr>
<tr>
<td>Concentration</td>
<td>1.0 mg/ml</td>
</tr>
<tr>
<td>Storage</td>
<td>Store at 4°C short term. Aliquot and store at -20°C long term. 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>
</tr>
<tr>
<td>Buffer</td>
<td>PBS (pH 7.4)</td>
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</table>

### Product Description

<table>
<thead>
<tr>
<th>Feature</th>
<th>Details</th>
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<tbody>
<tr>
<td>Host</td>
<td>Rabbit</td>
</tr>
<tr>
<td>Gene ID</td>
<td>8678</td>
</tr>
<tr>
<td>Gene Symbol</td>
<td>BECN1</td>
</tr>
<tr>
<td>Species</td>
<td>Human, Mouse, Rat</td>
</tr>
<tr>
<td>Immunogen</td>
<td>Internal synthetic peptide to human Beclin 1, within residues 1-100 [UniProt# Q14457].</td>
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### Product Application Details

<table>
<thead>
<tr>
<th>Feature</th>
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<tr>
<td>Applications</td>
<td>Western Blot, Simple Western, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation</td>
</tr>
<tr>
<td>Application Notes</td>
<td>This Beclin 1 Antibody is useful for Western Blot, Immunoprecipitation, Immunocytochemistry and Immunohistochemistry paraffin-embedded sections. In Western Blot, a band is seen at ~52 kDa representing Beclin 1. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. Separated by Size-Wes, Sally Sue/Peggy Sue.</td>
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</tbody>
</table>

### Images

Western Blot: Beclin 1 Antibody [NB500-249] - Beclin 1/ATG6 Antibody [NB500-249] - WB analysis of Beclin1. Lane 1 human brain and Lane 2 mouse brain.
Simple Western: Beclin 1 Antibody [NB500-249] - Beclin 1/ATG6 Antibody [NB500-249] - Simple Western lane view shows a specific band for Beclin1 in 1.0 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.

Immunocytochemistry/Immunofluorescence: Beclin 1 Antibody [NB500-249] - Beclin 1/ATG6 Antibody [NB500-249] - Beclin 1 antibody was tested in HeLa cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).


Immunohistochemistry-Frozen: Beclin 1 Antibody [NB500-249] - Merged immunostaining of frozen section of Rat brain tissue. This image was submitted via customer review.
Western Blot: Beclin 1/ATG6 Antibody [NB500-249] - Detection of Beclin 1 in mouse liver tissue lysate (50 ug) using NB500-249. ECL detection at 1 minute.

Immunohistochemistry: Beclin 1/ATG6 Antibody [NB500-249] - ATG6 on pheochromocytes of the Adrenal Medulla 40x.
### Publications

<table>
<thead>
<tr>
<th>Title</th>
<th>Authors</th>
<th>Journal</th>
<th>Date</th>
<th>PMID</th>
<th>Species/Method</th>
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<tbody>
<tr>
<td>Critical Role of Beclin1 in HIV Tat and Morphine-Induced Inflammation and Calcium Release in Glial Cells from Autophagy Deficient Mouse</td>
<td>Lapierre J, Rodriguez M, Ojha CR, El-Hage N</td>
<td>J Neuroimmune Pharmacol</td>
<td>May 11 2018</td>
<td>29752681 (Mouse)</td>
<td></td>
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<tr>
<td>The role of autophagy in age-related macular degeneration.</td>
<td>Kivinen N</td>
<td>Acta Ophthalmol</td>
<td>Apr 1 2018</td>
<td>29633521 (IHC-P, Mouse)</td>
<td></td>
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<tr>
<td>Alteration of Interneuron Immunoreactivity and Autophagic Activity in Rat Hippocampus after Single High-Dose Whole-Brain Irradiation.</td>
<td>Ouyang YM, Ning S, Adler JR et al.</td>
<td>Cureus</td>
<td>Jun 30 2017</td>
<td>28861331 (ICC/IF, Rat)</td>
<td></td>
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<tr>
<td>Continued 26S proteasome dysfunction in mouse brain cortical neurons impairs autophagy and the Keap1-Nrf2 oxidative defence pathway.</td>
<td>Ugun-Klusek A, Tatham MH, Elkharaz J et al.</td>
<td>Cell Death Dis</td>
<td>Jan 5 2017</td>
<td>28055010 (IHC, Mouse)</td>
<td></td>
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More publications at [http://www.novusbio.com/NB500-249](http://www.novusbio.com/NB500-249)
Procedures

**Immunoprecipitation protocol specific for Beclin 1 Antibody (NB500-249)**

**Immunoprecipitation Protocol:**

1. Cells in 2x 75cm flasks (60% confluency) are scraped with 0.5ml of Tris lysis Buffer (50mM Tris, 150mM NaCl, 1mM EDTA, 100ug/ml PMSF, 1% triton).
2. Lyse 1h at 4C, with gentle agitation.
3. Centrifuge to clear the lysates.
4. 0.1ml of lysate is kept aside for Western Blot experiments.
5. IP : Add 5ul of polyclonal beclin antibody (NB 500-249) to 0.4ml of lysate (1:80 dilution).
6. Incubate overnight at 4C, with gentle agitation.
7. Next day, add 60ul of protein A sepharose beads to the lysate.
8. Incubate for one hour at 4C.
9. Wash beads 3X with Tris lysis buffer.
10. Beads are re-suspended with 15ul of Laemmli buffer and boiled.
11. The efficiency of IP is determined by using a monoclonal anti-beclin antibody.

**Western Blot protocol for Beclin 1/ATG6 Antibody (NB500-249)**

**Western Blot Protocol**

1. Perform SDS-PAGE on samples to be analyzed, loading 20-30 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the transfer apparatus.
3. Stain the blot using ponceau S for 1-2 minutes to access the transfer of protein. Rinse the blot in water to remove excess stain and mark the lane and molecular weight markers location using a pencil.
4. Rinse the blot in TBST for approximately 5 minutes.
5. Block the membrane using 5% non-fat dry milk in TBST for 1 hour.
6. Dilute the rabbit anti-Beclin primary antibody (NB500-249) in 1% milk-TBST and incubate for 2 hours at room temperature or overnight.
7. Wash the membrane 3X for 10 min each in TBST and apply the diluted rabbit-IgG HRP-conjugated secondary antibody in 1% milk-TBST (as per manufacturer's instructions) and incubate for 1 hour at room temperature.
8. Wash the blot 3X for 10 min each in TBST.
9. Apply the detection reagent of choice in accordance with the manufacturer's instructions.
Immunohistochemistry Free-Floating Protocol for Beclin 1 Antibody (NB500-249)

IHC-FFPE sections

Deparaffinization:
1. Treat slides with Xylene: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.
2. Treat slides with 100% Reagent Alcohol: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes

Quench Endogenous Peroxidase:
1. Place slides in peroxidase quenching solution: 15-30 minutes. Add 3ml of 30% Hydrogen Peroxide to 200ml of Methanol.
2. Place slides in distilled water: 2 changes for 2 minutes each.

Retrieve Epitope:
1. Preheat citrate buffer. Place 200ml of citrate buffer working solution into container, cover and place into steamer. Heat to 90-96C.
2. Place rack of slides into hot citrate buffer for 20 minutes. Cover.
3. Carefully remove container with slides from steamer and cool on bench, uncovered, for 20 minutes.
4. Slowly add distilled water to further cool for 5 minutes.
5. Rinse slides with distilled water. 2 changes for 2 minutes each.

Immunostaining Procedure:
1. Remove each slide from rack and circle tissue section with a hydrophobic barrier pen (e.g. Liquid Blocker-Super Pap Pen).
2. Flood slide with Wash Solution. Do not allow tissue sections to dry for the rest of the procedure.
3. Drain the wash solution and apply 4 drops of blocking reagent to each slide and incubate for 15 minutes.
4. Drain blocking reagent (do not wash off the Blocking Reagent), apply 200ul of primary antibody solution to each slide, and incubate for 1 hour.
5. Wash slides with wash solution: 3 changes for 5 minutes each.
6. Drain wash solution, apply 4 drops of secondary antibody to each slide and incubate for 1 hour.
7. Wash slides with wash solution: 3 changes for 5 minutes each.
8. Drain wash solution, apply 4 drops of DAB substrate to each slide and develop for 5-10 minutes.
9. Wash slides with wash solution: 3 changes for 5 minutes each.
10. Drain wash solution, apply 4 drops of Hematoxylin to each slide and stain for 1-3 minutes.
11. Wash slides with wash solution: 2-3 changes for 2 minutes each.
12. Drain wash solution and apply 4 drops of Bluing Solution to each slide for 1-2 minutes.
13. Rinse slides in distilled water.
14. Soak slides in 70% reagent alcohol: 3 minutes with intermittent agitation.
15. Soak slides in 95% reagent alcohol: 2 changes for 3 minutes each with intermittent agitation.
16. Soak slides in 100% reagent alcohol: 3 changes for 3 minutes each with intermittent agitation.

Drain slides for 10 seconds between each change.
17. Soak slides in Xylene: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
18. Apply 2-3 drops of non-aqueous mounting media to each slide and mount coverslip.
19. Lay slides on a flat surface to dry prior to viewing under microscope.

NOTES:
-Use treated slides (e.g. HistoBond) to ensure 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.
-200ul is the recommended maximum volume to apply to a slide for full coverage. Using more than 200ul may allow solutions to wick off the slide and create drying artifacts. For small tissue sections less than 200ul 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 minute for nuclear antigens. Counterstain for 2-3 minutes for cytoplasmic and membranous antigens. If darker counterstaining is desired, increase the time (up to 10 minutes).
Immunocytochemistry/Immunofluorescence Protocol for Beclin 1/ATG6 Antibody (NB500-249)

Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates containing coverslips.

1. Remove the coverslips to a new dish and add 100% cold (-20°C) methanol to the dish. Fix at room temperature for 10 minutes.
2. Remove the coverslips and air dry for 5 minutes.
3. Rehydrate the cell by incubating the coverslips in PBS at room temperature for 5 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 to the coverslip and incubate at room temperature from 2 hours to overnight.
6. Remove primary antibody and wash with PBS. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution to the coverslip. Incubate for 1 hour at room temperature.
8. Remove antibody and wash with PBS. Wash three times for 10 minutes. Counterstain for DNA with DAPI.
9. Mount the coverslips in a fluorochrome anti-fade agent and image as required.

*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.

For more information on our 100% guarantee, please visit www.novusbio.com/guarantee

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