Product Datasheet

Beclin 1/ATG6 Antibody
NB500-249SS

Unit Size: 0.025 ml

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

Reviews: 4 Publications: 38

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Beclin 1/ATG6 Antibody

Product Information

Unit Size	0.025 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Purity	Immunogen affinity purified
Buffer	PBS

Product Description

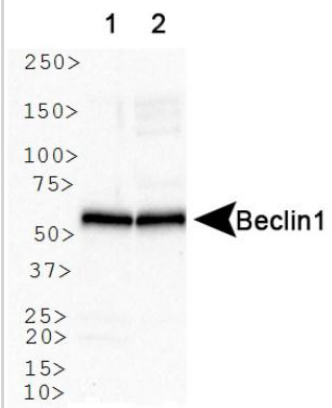
Host	Rabbit
Gene ID	8678
Gene Symbol	BECN1
Species	Human, Mouse, Rat
Species Reactivity	Human, mouse and rat.
Immunogen	Internal synthetic peptide to human Beclin 1, within residues 1-100 [UniProt# Q14457].

Product Application Details

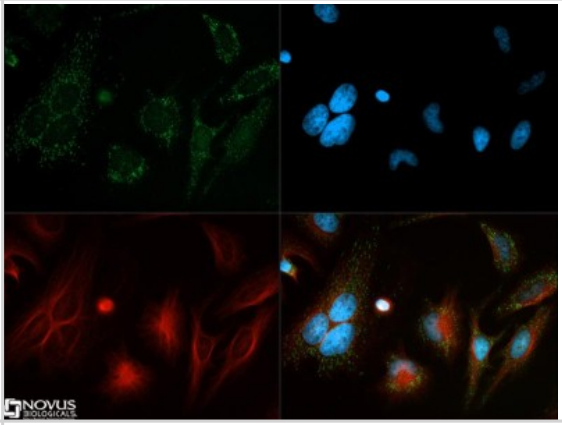
Applications	Western Blot, Simple Western, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Immunocytochemistry/Immunofluorescence 1:50-1:200, Immunohistochemistry 1:400, Immunohistochemistry-Paraffin 1:400, Immunoprecipitation 1:80, Western Blot 1:500-1:2000, Simple Western 1:50
Application Notes	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.

Images

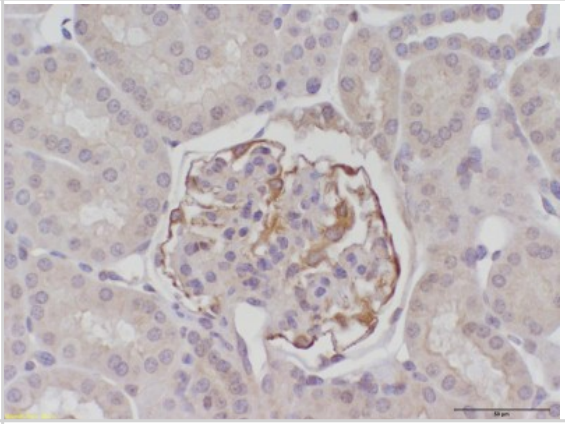
Western Blot: Beclin 1 Antibody [NB500-249] - WB analysis of Belclin1. Lane 1 human brain and Lane 2 mouse brain.



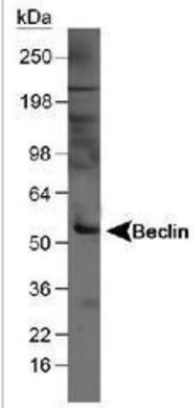
Immunocytochemistry/Immunofluorescence: Beclin 1 Antibody [NB500-249] - Beclin 1 antibody was tested in HeLa cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).



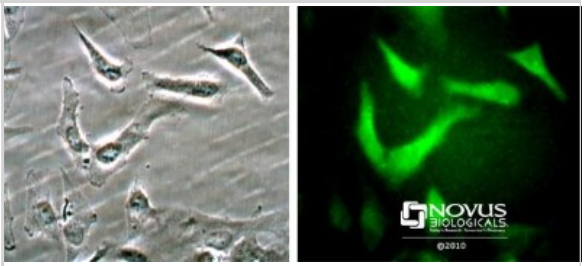
Immunohistochemistry-Paraffin: Beclin 1 Antibody [NB500-249] - Analysis of Beclin1 in mouse kidney. Image courtsey of product review submitted by Kelly Hudkins.

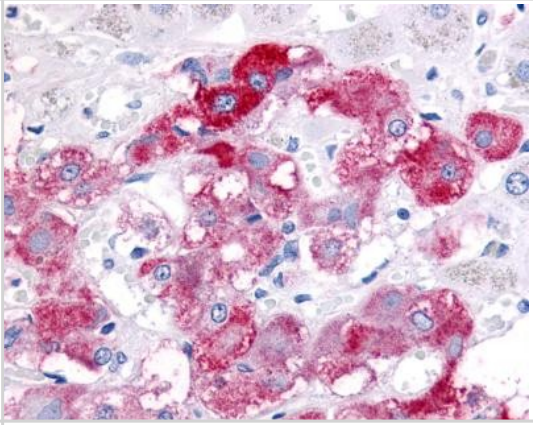
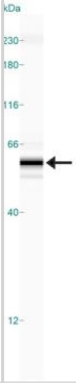


Western Blot: Beclin 1 Antibody [NB500-249] - Detection of Beclin 1 in mouse liver tissue lysate (50 ug) using NB500-249. ECL detection at 1 minute.



Immunocytochemistry/Immunofluorescence: Beclin 1 Antibody [NB500-249] - Beclin 1 staining in Hela cells detected with a Dylight 488 labeled secondary antibody using NB500-249.



Immunohistochemistry-Paraffin: Beclin 1 Antibody [NB500-249] - Pheochromocytes of the Adrenal Medulla 40x.	
Simple Western: 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.	

Publications

Wakabayashi K, Mori F, Kakita A et al. Analysis of microRNA from archived formalin-fixed paraffin-embedded specimens of amyotrophic lateral sclerosis. Acta Neuropathol Commun. 2014 Dec 14 [PMID: 25497327] (IHC-P, Human)

Lim SW, Doh KC, Jin L et al. Ginseng Treatment Attenuates Autophagic Cell Death in Chronic Cyclosporine Nephropathy. Nephrology (Carlton). 2014 May 05 [PMID: 24796922] (WB, Mouse)

Stellrecht CM, Vangapandu HV, Le XF et al. ATP directed agent, 8-chloro-adenosine, induces AMP activated protein kinase activity, leading to autophagic cell death in breast cancer cells. J Hematol Oncol 3/21/2014 [PMID: 24628795] (WB, Human)

Dai DF, Karunadharma PP, Chiao YA et al. Altered proteome turnover and remodeling by short-term caloric restriction or rapamycin rejuvenate the aging heart. Aging Cell 2/25/2014 [PMID: 24612461] (WB, Mouse)

Lee CH, Lin ST, Liu JJ et al. Salmonella induce autophagy in melanoma by the downregulation of AKT/mTOR pathway. Gene Ther. 2014 Mar 6 [PMID: 24451116] (WB, Mouse)

Casadei N, Pohler AM, Tomas-Zapico C et al. Overexpression of synphilin-1 promotes clearance of soluble and misfolded alpha-synuclein without restoring the motor phenotype in aged A30P transgenic mice. Hum. Mol. Genet. 2014 Feb 1 [PMID: 24064336] (WB, Mouse)

Murphy KE, Gysbers AM, Abbott SK et al. Reduced glucocerebrosidase is associated with increased alpha-synuclein in sporadic Parkinson's disease. Brain 2014 Feb 19 [PMID: 24477431] (WB, Human)

Joshi-Barr S, Bett C, Chiang WC et al. De novo prion aggregates trigger autophagy in skeletal muscle. J Virol 2013 Dec 4 [PMID: 24307586] (WB, Mouse)

Romao S, Gasser N, Becker AC et al. Autophagy proteins stabilize pathogen-containing phagosomes for prolonged MHC II antigen processing. J Cell Biol 2013 Dec 9 [PMID: 24322427] (ICC/IF, Human)

Shin JN, Abdel Fattah E, Bhattacharya A et al. Inflammasome activation by altered proteostasis. J Biol Chem. 2013 Oct 31 [PMID: 24178293] (WB, Mouse)

Roy K, Raychaudhuri M, Chakrabarti O, Mukhopadhyay D. Growth Factor Receptor-Bound Protein 2 Promotes Autophagic Removal of Amyloid-beta Protein Precursor Intracellular Domain Overload in Neuronal Cells. J Alzheimers Dis. 2013 Oct 7 [PMID: 24100123]

Pampliega O, Orhon I, Patel B et al. Functional interaction between autophagy and ciliogenesis. Nature. 2013 Oct 10 [PMID: 24089209] (ICC/IF, Mouse)

More publications at 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. 0.1 ml 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. A SDS-PAGE gel is run and the proteins are transferred to a membrane.
12. The efficiency of IP is determined by using a monoclonal anti-beclin antibody.

Western Blot Protocol (NB500-249)
Western Blot Protocol

1. Perform SDS-PAGE (3-8%) on samples to be analyzed, loading 50ug of total protein per lane.
2. Transfer proteins to nitrocellulose 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 proteins onto the nitrocellulose membrane. 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 TBS for approximately 5 minutes.
5. Block the membrane using 5% non-fat dry milk + 0.5% BSA in TBS for 1 hour.
6. Dilute the rabbit anti-Beclin primary antibody (NB 500-249) in blocking buffer and incubate for 2 hours at room temperature.
7. Wash the membrane in water for 5 minutes and apply the diluted rabbit-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) and incubate 1 hour at room temperature.
8. Wash the blot in TBS containing 0.05-0.1% Tween-20 for 10-20 minutes.
9. Wash the blot in type I water for an additional 10-20 minutes (this step can be repeated as required to reduce background).
10. Apply the detection reagent of choice in accordance with the manufacturer's instructions (Amersham ECL is the standard reagent used at Novus Biologicals).

**Note: Tween-20 can be added to the blocking buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.

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).

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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 guarantee, please visit www.novusbio.com/guarantee.