

Product Datasheet

Niemann-Pick type C1 Like-1 Antibody - BSA Free NB400-128

Unit Size: 0.1 ml

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

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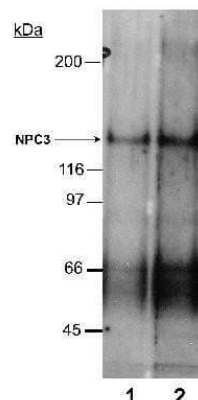
NB400-128

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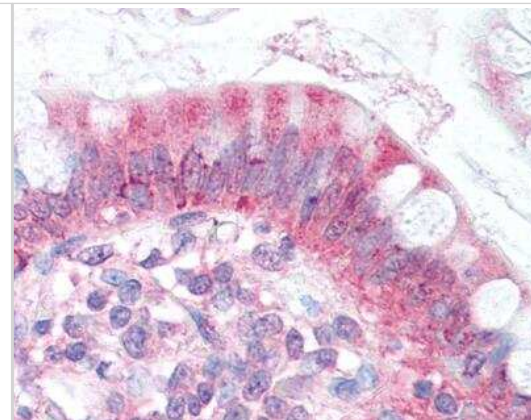
Product Information	
Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	140 kDa
Product Description	
Host	Rabbit
Gene ID	29881
Gene Symbol	NPC1L1
Species	Human, Mouse, Rat
Immunogen	A synthetic peptide made to an internal region of rat Niemann-Pick type C1 Like-1 (between residues 500-600). [Uniprot: Q6T3U3]
Product Application Details	
Applications	Western Blot, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Western Blot 1:500, Immunohistochemistry 1:50-1:100, Immunoprecipitation, Immunohistochemistry-Paraffin 1:50-1:100
Application Notes	In Western blot a band is observed at ~140 kDa representing NPC1L1, and a non-specific band may be seen ~60 kDa.

Images

Western Blot: Niemann-Pick type C1 Like-1 Antibody [NB400-128] - Detection of NPC3 in rat small intestine membrane preparations (20 ug). (1) 2 ug/ml and (2) 4 ug/ml. ECL: 30 minute exposure.



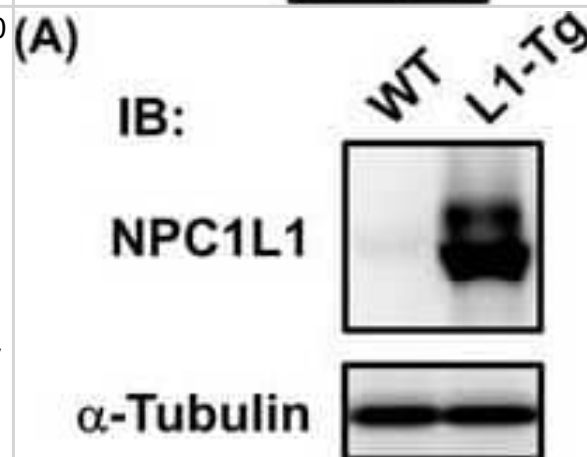
Immunohistochemistry-Paraffin: Niemann-Pick type C1 Like-1 Antibody [NB400-128] - Staining of human small intestine, enterocytes.



Western Blot: Niemann-Pick type C1 Like-1 Antibody [NB400-128] - Decrease in the biliary cholesterol secretion in L1-Tg mice. Immunoblot detection of hepatic NPC1L1 protein in L1-Tg mice using an anti-NPC1L1 (Niemann-Pick type C1 Like-1) antibody. WT, wild-type. Image collected and cropped by CiteAb from the following publication (<https://lipidworld.biomedcentral.com/articles/10.1186/s12944-019-1179-0>), licensed under a CC-BY license.



Western Blot: Niemann-Pick type C1 Like-1 Antibody - BSA Free [NB400-128] - Hepatic NPC1L1-mediated steatosis in the liver of L1-Tg mice. A, Hepatic expression of the human NPC1L1 protein in L1-Tg mice demonstrated by immunoblotting using an anti-NPC1L1 antibody. α -Tubulin, a loading control. B, Decrease of biliary cholesterol levels in L1-Tg mice. Data are expressed as the mean \pm SEM $n = 6$ (WT) & 7 (L1-Tg). C, Photographic images of the livers of WT & L1-Tg mice fed a control fat diet (CFD) or a high-fat diet (HFD) for 2 weeks. The coin diameter was 1 cm. D, Hematoxylin & eosin (H&E) & Oil Red O staining of the livers of WT & L1-Tg mice fed a HFD for 2 weeks. E & F, Hepatic cholesterol levels (E, left), hepatic triglyceride (TG) levels (E, right), body weight (BW) (F, left), & the ratios of liver weight to BW (L/B ratio) (F, right) in each group of mice fed a CFD or HFD for 2 weeks. Data are expressed as the mean \pm SEM $n = 11$ (WT-CFD) & 7 (the other groups). Statistical analyses for significant differences were performed using Bartlett's test, followed by a parametric Tukey-Kramer multiple-comparison test (E, right; F) or a non-parametric Steel-Dwass test (E, left) (### $P < 0.01$ vs CFD controls; ** $P < 0.01$ vs. the other groups; * $P < 0.05$ among two groups; NS, not significantly different among groups) as well as a two-sided t test ($\dagger\dagger P < 0.01$). Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/32123832>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Zhang Z, Wen H, Peng B, et al. High-fat diet-induced TRAF6 Upregulation Promotes Liver Cholesterol Accumulation and Fatty Liver Development Through EZH2-mediated miR-429/PPAR alpha axis Mol Ther Nucleic Acids 2021-05-17 [PMID: 33996254]

Pan X, Jiang X C et al. Impaired cholesterol metabolism and enhanced atherosclerosis in clock mutant mice. Circulation 2013-10-15 [PMID: 24014832] (WB, Mouse)

Toyoda Y, Takada T et al. Identification of hepatic NPC1L1 as an NAFLD risk factor evidenced by ezetimibe-mediated steatosis prevention and recovery. FASEB Bioadv 2019-01-05 [PMID: 32123832] (WB, Mouse)

Toyoda Y, Takada T, Yamanashi Y, Suzuki H Pathophysiological importance of bile cholesterol reabsorption: hepatic NPC1L1-exacerbated steatosis and decreasing VLDL-TG secretion in mice fed a high-fat diet Lipids Health Dis 2019-12-28 [PMID: 31883528] (WB, Mouse)

Ahn SB, Jun DW, Jang K et al. Duodenal Niemann-Pick C1-like 1 expression was negatively correlated with liver X receptor expression in nonalcoholic fatty liver disease Korean J. Intern. Med. 2019-07-01 [PMID: 29466845] (IHC-P, Human)

Koo M, Olevsky O, Ruchalski K, Song S Hepatic expression of Niemann-Pick C1-Like 1, a cholesterol re-absorber from bile, exacerbates western diet-induced atherosclerosis in LDL receptor mutant mice Mol. Pharmacol. 2019-05-07 [PMID: 31064810] (WB, Mouse)

Ontawong A, Duangjai A, Muanprasat C et al. Lipid-lowering effects of Coffea arabica pulp aqueous extract in Caco-2 cells and hypercholesterolemic rats. Phytomedicine 2018-06-01 [PMID: 30599898] (WB, Human, Rat)

Meng Z, Gwag T, Sui Y et al. The atypical antipsychotic quetiapine induces hyperlipidemia by activating intestinal PXR signaling JCI Insight 2019-02-07 [PMID: 30728326] (WB, Human)

Soayfane Z, Terce F, Cantiello M et al. Exposure to dietary lipid leads to rapid production of cytosolic lipid droplets near the brush border membrane. Nutr Metab (Lond) 2016-07-28 [PMID: 27478484] (WB)

Helsley RN. THE ROLE OF PXR AND IKKbeta SIGNALING IN CARDIOMETABOLIC DISEASE. Thesis. 2016-01-01 (WB, Mouse)

Kawase A, Hata S, Takagi M. Pravastatin Modulate Niemann-Pick C1-Like 1 and ATP-Binding Cassette G5 and G8 to Influence Intestinal Cholesterol Absorption. Journal of Pharmacy & Pharmaceutical Sciences. 2015-11-08 [PMID: 26670369] (WB, Human)

Levy E, Ouadda AB, Spahis S et al. PCSK9 plays a significant role in cholesterol homeostasis and lipid transport in intestinal epithelial cells. Atherosclerosis 2013-02-04 [PMID: 23422832] (WB, Human)

Details:

Using the HRP conjugated version of NB400-128, catalog number NB400-128H.

More publications at <http://www.novusbio.com/NB400-128>



Procedures

Serum protocol for Niemann-Pick type C1 Like-1 Antibody (NB400-128)

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 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 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 in TBS for 1 hour at room temperature.
6. Dilute the rabbit anti-NPC3 primary antibody (NB 400-128) in blocking buffer and incubate overnight at 4 degrees Celsius.
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's 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.

IHC-FFPE sections

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:

- A. Place slides in peroxidase quenching solution: 15-30 minutes. To Prepare 200 ml of Quenching Solution: Add 3 ml of 30% Hydrogen Peroxide to 200 ml of Methanol. Use within 4 hours of preparation
- B. Place slides in distilled water: 2 changes for 2 minutes each.

III. Retrieve Epitopes:

- A. Preheat Citrate Buffer. Place 200 ml of Citrate Buffer Working Solution into container, cover and place into steamer. Heat to 90-96 degrees Celsius.
- 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 60 degrees Celsius 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).





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Products Related to NB400-128

NB400-128PEP	Niemann-Pick type C1 Like-1 Antibody Blocking Peptide
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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