Product Datasheet

Niemann-Pick C1 Antibody
NB400-148

Unit Size: 0.1 ml

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

Reviews: 5  Publications: 35

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
www.novusbio.com/NB400-148

Updated 5/11/2020 v.20.1
# Product Information

<table>
<thead>
<tr>
<th><strong>Unit Size</strong></th>
<th>0.1 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Concentration</strong></td>
<td>1.0 mg/ml</td>
</tr>
<tr>
<td><strong>Storage</strong></td>
<td>Store at 4°C short term. Aliquot and store at -20°C 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>0.1% 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</td>
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</tbody>
</table>

## Product Description

**Host** | Rabbit
---|---
**Gene ID** | 4864
**Gene Symbol** | NPC1

**Species** | Human, Mouse, Rat, Porcine, Chinese Hamster, Primate

**Reactivity Notes**

- Human, mouse, rat and Chinese hamster and primate (PMID 22212234).
- Porcine reactivity reported in scientific literature (PMID: 21051527).
- Results with mouse in Western blot have been mixed.

**Specificity/Sensitivity**

**Immunogen**

A synthetic peptide made to the C-terminal region of human Niemann-Pick C. [UniProt# O15118]

## Product Application Details

### Applications

Western Blot, Electron Microscopy, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, Knockdown Validated, Knockout Validated

### Recommended Dilutions

- **Western Blot** 1:1000-1:3000, Immunohistochemistry 5-10 ug/ml, Immunocytochemistry/Immunofluorescence 1:250, Immunoprecipitation 1:10-1:500, Immunohistochemistry-Paraffin 5-10 ug/ml, Electron Microscopy, Knockout Validated, Knockdown Validated

### Application Notes

In Western blot the antibody detects heterogeneously glycosylated NPC1 protein with prominent bands at 170 and 220 kDa. Results with mouse in Western blot have been mixed. It has also been tested for immuno-EM (on human protein only). Use in Electron Microscopy reported in scientific literature (PMID: 21051527). Use in Immunocytochemistry/immunofluorescence and Western blot reported in scientific literature (PMID:24209575). Use in immunoprecipitation reported in scientific literature (PMID: 18216017).
Knockout Validated: Niemann-Pick C1 Antibody [NB400-148] - Western blot shows lysates of HeLa human cervical epithelial carcinoma parental cell line and NPC1 knockout (KO) HeLa cell line. PVDF membrane was probed with 1:1000 of Rabbit Anti-Human NPC1 Polyclonal Antibody (Catalog # NB400-148) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). Specific band was detected for NPC1 at approximately 240-260 kDa (as indicated) in the parental HeLa cell line, but is not detectable in the knockout HeLa cell line. This experiment was conducted under reducing conditions.


Western Blot: Niemann-Pick C1 Antibody [NB400-148] - NPC proteins in mouse lungs. Western blot of wild type (W) littermates, NPC1 (Mut) or NPC2 (Mut) mutant mouse lungs using anti-NPC1 or -NPC2 antibody. beta-actin used as a loading control. 30 ug protein/lane. Image collected and cropped by CiteAb from the following publication (http://dx.plos.org/10.1371/journal.pone.0067084), licensed under a CC-BY licence.

Immunocytochemistry/Immunofluorescence: Niemann-Pick C1 Antibody [NB400-148] - NPC1 antibody was tested in HeLa cells with DyLight 488 (green). Nuclei were counterstained with DAPI (blue).
Western Blot: Niemann-Pick C1 Antibody [NB400-148] - Protein expression analysis of normal and NPC-deficient cells after HIV-1 infection. Cells were uninfected or infected with VSVG-HIV-1 and harvested 96 h post-infection. NPC2, NPC1, and beta-actin protein expression was detected via Western blotting in uninfected and infected cells. Gag expression was also detected in the infected cells. Image collected and cropped by CiteAb from the following publication [http://virologyj.biomedcentral.com/articles/10.1186/1743-422X-9-31], licensed under a CC-BY licence.

| Publications |
|-----------------
| Mitroi DN, Pereyra-Gomez G, Soto-Huelin B et al. NPC1 enables cholesterol mobilization during long-term potentiation that can be restored in Niemann-Pick disease type C by CYP46A1 activation EMBO Rep. Sep 18 2019 12:00AM [PMID: 31535451] (WB, ICC/IF, Mouse) |
| Kanerva K, Uronen RL, Blom T et al. LDL Cholesterol Recycles to the Plasma Membrane via a Rab8a-Myosin5b-Actin-Dependent Membrane Transport Route. Dev Cell. 2013 Nov 11 [PMID: 24209575] (IP, Human) |

Procedures

Protocol specific for Niemann Pick C1 Antibody (NB400-148)

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).
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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