abcam

Product datasheet

Anti-Niemann Pick C1 antibody ab108921

KO VALIDATED

6 References 4 Images

Overview

Product name	Anti-Niemann Pick C1 antibody
Description	Rabbit polyclonal to Niemann Pick C1
Host species	Rabbit
Specificity	From Jan 2024, QC testing of replenishment batches of this polyclonal changed. All tested and expected application and reactive species combinations are still covered by our Abcam product promise. However, we no longer test all applications. For more information on a specific batch, please contact our Scientific Support who will be happy to help.
Tested applications	**Suitable for:** IP, ICC/IF, WB
Species reactivity	**Reacts with:** Human **Predicted to work with:** Rabbit, Chimpanzee, Macaque monkey, Gorilla, Orangutan
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	This antibody gave a positive signal in Human Adrenal Gland tissue lysate as well as the following whole cell lysates: HepG2; WI38; HeLa; A431. It also gave a positive signal in HepG2 cell line.
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.

Purity Immunogen affinity purified

Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab108921 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IP		Use a concentration of 5 µg/ml.
ICC/IF		Use a concentration of 5 µg/ml.
WB		Use a concentration of 1 µg/ml. Detects a band of approximately 200 kDa (predicted molecular weight: 142 kDa).

Target

Function Involved in the intracellular trafficking of cholesterol. May play a role in vesicular trafficking in glia, a process that may be crucial for maintaining the structural and functional integrity of nerve terminals.

Involvement in disease Defects in NPC1 are the cause of Niemann-Pick disease type C1 (NPDC1) [MIM:257220]. A lysosomal storage disorder that affects the viscera and the central nervous system. It is due to defective intracellular processing and transport of low-density lipoprotein derived cholesterol. It causes accumulation of cholesterol in lysosomes, with delayed induction of cholesterol homeostatic reactions. Niemann-Pick disease type C1 has a highly variable clinical phenotype. Clinical features include variable hepatosplenomegaly and severe progressive neurological dysfunction such as ataxia, dystonia and dementia. The age of onset can vary from infancy to late adulthood. An allelic variant of Niemann-Pick disease type C1 is found in people with Nova Scotia ancestry. Patients with the Nova Scotian clinical variant are less severely affected.

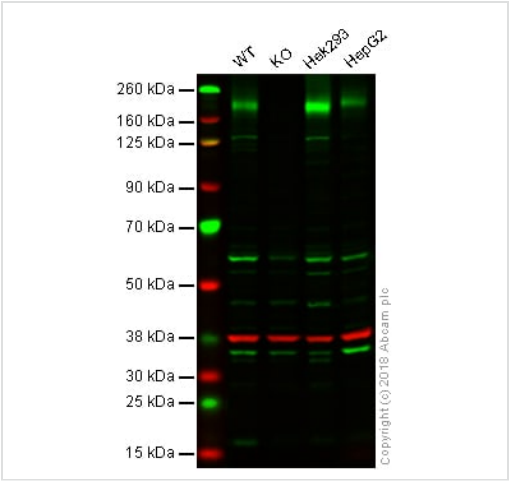
Sequence similarities Belongs to the patched family.
Contains 1 SSD (sterol-sensing) domain.

Domain A cysteine-rich N-terminal domain and a C-terminal domain containing a di-leucine motif necessary for lysosomal targeting are critical for mobilization of cholesterol from lysosomes.

Post-translational modifications Glycosylated.

Cellular localization Late endosome membrane. Lysosome membrane.

Images



Western blot - Anti-Niemann Pick C1 antibody (ab108921)

All lanes : Anti-Niemann Pick C1 antibody (ab108921) at 1 µg/ml

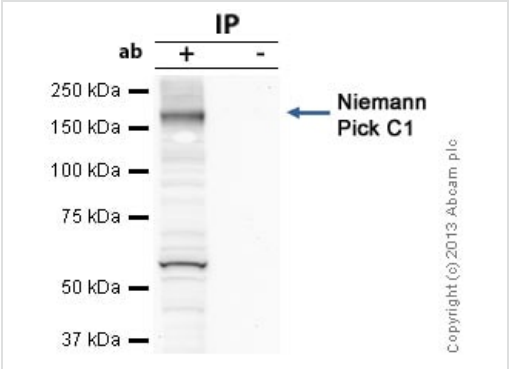
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : NPC1 (Niemann Pick C1) knockout HAP1 whole cell lysate
Lane 3 : HEK293 whole cell lysate
Lane 4 : HepG2 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 142 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab108921 observed at 200 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

ab108921 was shown to recognize Niemann Pick C1 in wild-type HAP1 cells as signal was lost at the expected MW in NPC1 (Niemann Pick C1) knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and NPC1 (Niemann Pick C1) knockout samples were subjected to SDS-PAGE. Ab108921 and **ab9484** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1 µg/ml and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Immunoprecipitation - Anti-Niemann Pick C1 antibody (ab108921)

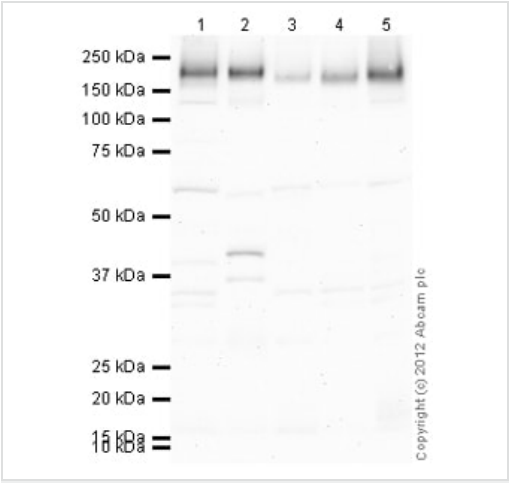
Niemann Pick C1 was immunoprecipitated using 0.5mg HeLa whole cell extract, 5µg of Rabbit polyclonal to Niemann Pick C1 and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

The antibody was incubated under agitation with Protein G beads for 10min, HeLa whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.

Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70°C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab108921.

Secondary: Mouse monoclonal [SB62a] Secondary Antibody to Rabbit IgG light chain (HRP) (**ab99697**).

Band: 142kDa; Niemann Pick C1, non specific bands - 55kDa: We are unsure as to the identity of this extra band.



Western blot - Anti-Niemann Pick C1 antibody (ab108921)

All lanes : Anti-Niemann Pick C1 antibody (ab108921) at 1 µg/ml (Milk Blocking - 3%)

Lane 1 : HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate
Lane 2 : Human adrenal normal tissue lysate - total protein (**ab29249**)
Lane 3 : WI-38 whole cell lysate (**ab3960**)
Lane 4 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 5 : A431 (Human epithelial carcinoma cell line) Whole Cell Lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (**ab97080**) at 1/5000 dilution

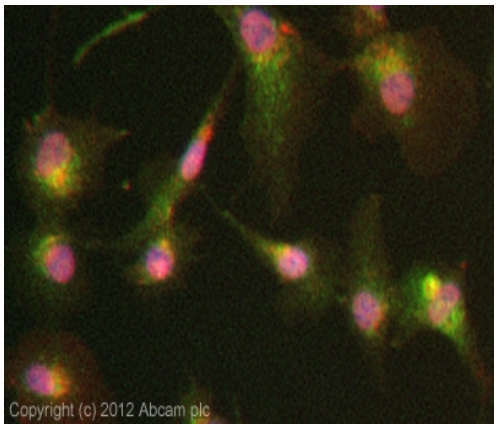
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 142 kDa
Observed band size: 200 kDa
Additional bands at: 42 kDa, 60 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 8 minutes

Niemann Pick C1 contains a number of potential glycosylation sites (SwissProt) which may explain its migration at a higher molecular weight than predicted.



Immunocytochemistry/ Immunofluorescence - Anti-Niemann Pick C1 antibody (ab108921)

ICC/IF image of ab108921 stained HepG2 cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab108921, 5µg/ml) overnight at +4°C. The secondary antibody (green) was **ab96899**, DyLight® 488 goat anti-rabbit IgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM. This antibody also gave a positive result in 4% formaldehyde fixed (10 min) HeLa cells at 5µg/ml, and in 100% methanol fixed (5 min) HepG2 cells at 5µg/ml.

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