# Product Datasheet

## Niemann-Pick C1 Antibody - BSA Free
## NB400-148

Unit Size: 0.1 ml

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

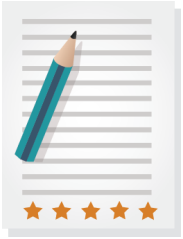


**Reviews: 6 Publications: 54**

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

Updated 10/23/2024 v.20.1





# NB400-148

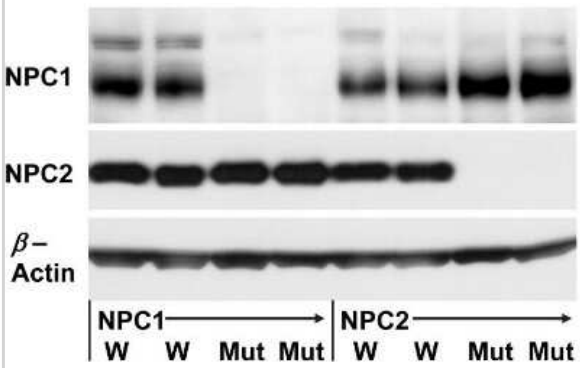
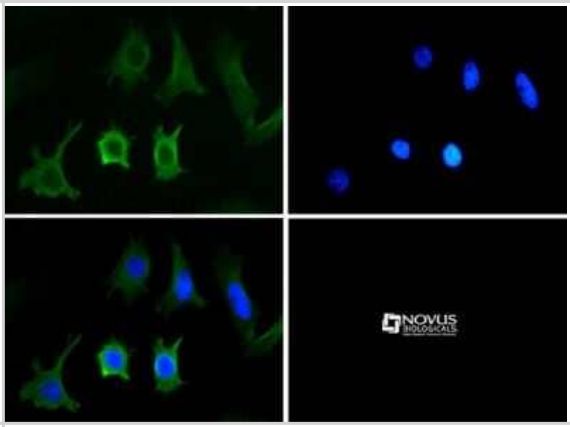
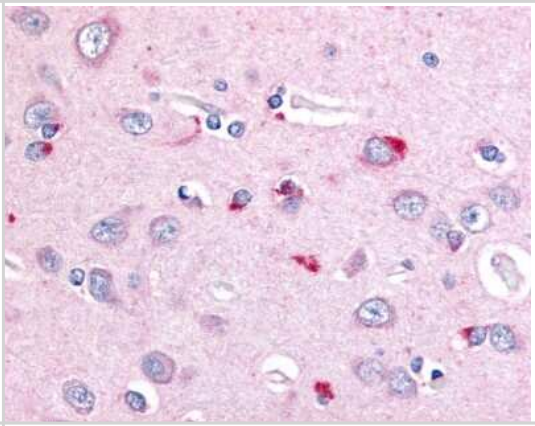
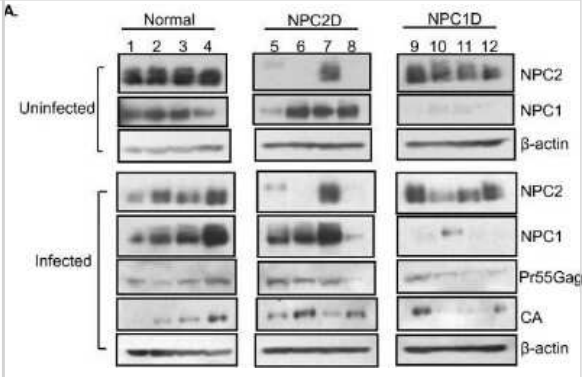
Niemann-Pick C1 Antibody - BSA Free

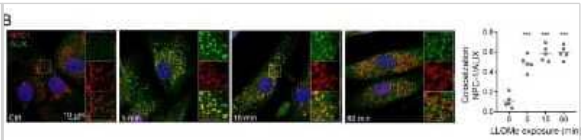
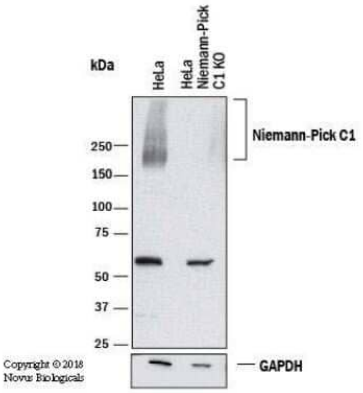
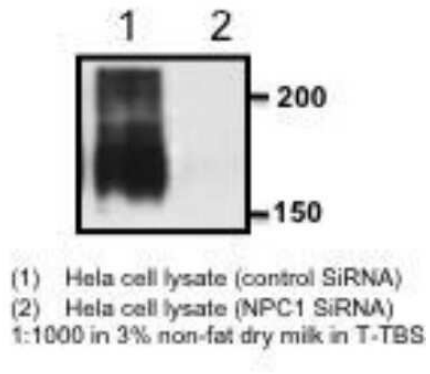
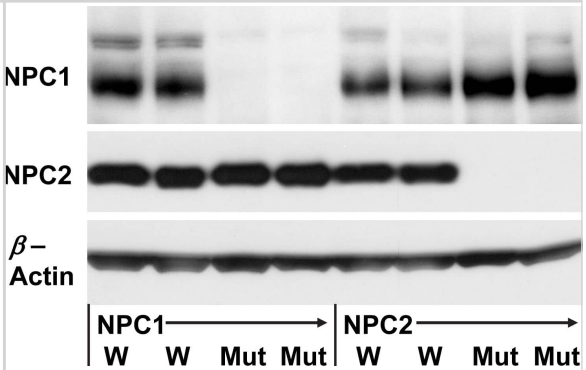
| Product Information | |
|---|---|
| **Unit Size** | 0.1 ml |
| **Concentration** | 1.0 mg/ml |
| **Storage** | Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles. |
| **Clonality** | Polyclonal |
| **Preservative** | 0.1% Sodium Azide |
| **Isotype** | IgG |
| **Purity** | Immunogen affinity purified |
| **Buffer** | PBS |

| Product Description | |
|---|---|
| **Host** | Rabbit |
| **Gene ID** | 4864 |
| **Gene Symbol** | NPC1 |
| **Species** | Human, Mouse, Rat, Porcine, Chinese Hamster, 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. Use in Hamster reported in scientific literature (PMID:21878321). |
| **Specificity/Sensitivity** | |
| **Immunogen** | A synthetic peptide made to the C-terminal region of human Niemann-Pick C. [UniProt# O15118] |

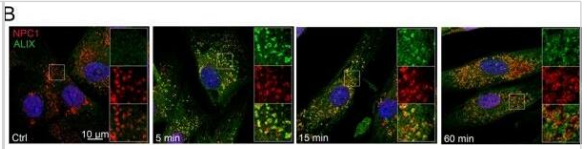
| Product Application Details | |
|---|---|
| **Applications** | Western Blot, Electron Microscopy, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, Knockdown Validated, Knockout Validated |
| **Recommended Dilutions** | Western Blot 1:1000-1:3000. Use reported in scientific literature (PMID 24209575), Immunohistochemistry 5-10 ug/ml, Immunocytochemistry/ Immunofluorescence 1:250. Use reported in scientific literature (PMID 24209575), Immunoprecipitation 1:10-1:500. Use reported in scientific literature (PMID 18216017), Immunohistochemistry-Paraffin 5-10 ug/ml, Electron Microscopy reported in scientific literature (PMID 21051527), 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). |

## Images

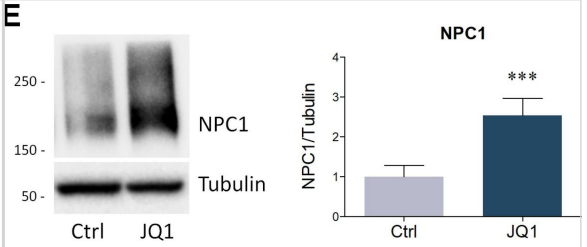
| | |
|---|---|
| Western Blot: Niemann-Pick C1 Antibody - BSA Free [NB400-148] - NPC proteins in mouse lungs. Western blot of wild type (W) littermates, NPC1 (Mut) or NPC2 (Mut) mutant mouse lungs using anti-NPC1 or -NPC2 antibody. beta-actin used as a loading control. 30 ug protein/lane. Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0067084), licensed under a CC-BY license. |  |
| Immunocytochemistry/Immunofluorescence: Niemann-Pick C1 Antibody - BSA Free [NB400-148] - NPC1 antibody was tested in HeLa cells with DyLight 488 (green). Nuclei were counterstained with DAPI (blue). |  |
| Immunohistochemistry-Paraffin: Niemann-Pick C1 Antibody - BSA Free [NB400-148] - Staining of human brain, cortex, neurons and astrocytes. |  |
| Western Blot: Niemann-Pick C1 Antibody - BSA Free [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 (https://virologyj.biomedcentral.com/articles/10.1186/1743-422X-9-31), licensed under a CC-BY license. |  |

| | |
|---|---|
| Immunocytochemistry/Immunofluorescence: Niemann-Pick C1 Antibody - BSA Free [NB400-148] - Immunocytochemical staining with corresponding colocalization analysis of ALIX (green) and NPC-1 (red) in fibroblasts exposed to LLOMe for 15 min. Nuclei are stained with DAPI (blue) and merged images show colocalization in yellow (n = 5). Image collected and cropped by CiteAb from the following publication (//pubmed.ncbi.nlm.nih.gov/32409651/) licensed under a CC-BY license. |  |
| Knockout Validated: Niemann-Pick C1 Antibody - BSA Free [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. |  |
| Knockdown Validated: Niemann-Pick C1 Antibody - BSA Free [NB400-148] - HeLa lysate, BHK lysate, A431 lysate tested at 1:1000. Image from verified customer review. |  |
| 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. β-actin used as a loading control. 30 µg protein/lane. |  |

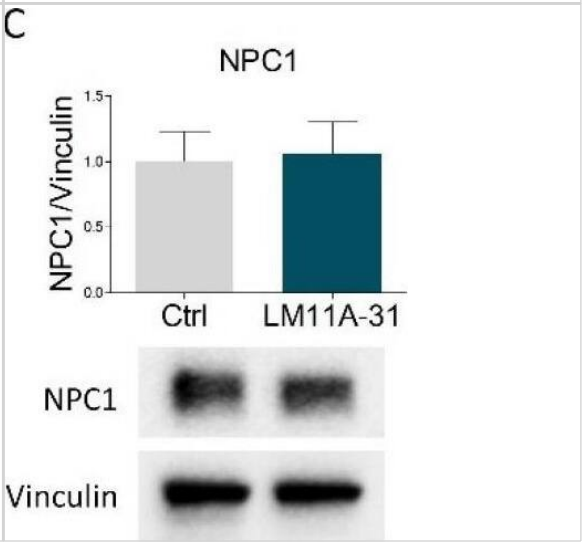
Immunocytochemistry/ Immunofluorescence: Niemann-Pick C1 Antibody - BSA Free [NB400-148] - ESCRT complex recruited to LLOMe-damaged lysosomes promotes cell survival.Human fibroblasts were exposed to 1 mM LLOMe & when indicated, the cell permeable calcium chelator BAPTA-AM (1 µM) was added 10 min prior to LLOMe exposure. Immunocytochemical staining with corresponding colocalization analysis of a CHMP4B (green) & LAMP2 (red), b ALIX (green) & NPC-1 (red), & c CHMP4B (green) & LAMP2 (red) in fibroblasts exposed to LLOMe for 15 min. Nuclei are stained with DAPI (blue) & merged images show colocalization in yellow (n = 5). d Caspase-3 like activity in cells exposed to LLOMe for 8 h (n = 3). e Quantification of apoptotic & necrotic cells using Annexin V/PI staining in fibroblasts exposed to LLOMe for 6 h (n = 3). f Immunoblot of LAMP2 in digitonin-extracted cytosolic fractions from cells exposed to LLOMe for 2 h (n = 8) with corresponding quantitative analysis. *p < 0.05, **p < 0.01 & ***p < 0.001 compared to control (a–b) or LLOMe only (c–f). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32409651), licensed under a CC-BY license. Not internally tested by Novus Biologicals.




Western Blot: Niemann-Pick C1 Antibody - BSA Free [NB400-148] - Expression of proteins involved in extracellular lipid uptake & intracellular cholesterol trafficking following JQ1 (0.4 µM) administration to HepG2 cells for 48 hours. (A) Representative Western blot (left panel) & densitometric analysis (right panel) of SR-B1. n = 6 independent experiments. (B) Immunofluorescence analysis of SR-B1 (green). Nuclei were counterstained with DAPI. n = 3 different experiments. Scale bar: 50 µm. (C) Representative Western blot (left panel) & densitometric analysis (right panel) of LDLr. n = 5 independent experiments. (D) LDLr immunofluorescence (green). Nuclei were counterstained with DAPI. n = 3 different experiments. (E–F) Representative Western blots & densitometric analysis of NPC1 & TMEM97. Tubulin was chosen as loading control. n = 6 independent experiments. Data represent means ± SD. Statistical analysis was performed by using unpaired Student's t test. ** p < 0.01; *** p < 0.001. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32075110), licensed under a CC-BY license. Not internally tested by Novus Biologicals.




Western Blot: Niemann-Pick C1 Antibody - BSA Free [NB400-148] - Effects of p75NTR modulation by LM11A-31 on cholesterol metabolism & secretion. (A–D) Representative Western blot & densitometric analysis of HMGCR, ABCA1, NPC1, & ApoE in U373 cells treated with vehicle (Ctrl) & LM11A-31 (0.1 µM) for 48 h. n = 4 different experiments. Vinculin served as a housekeeping protein to normalize protein loading. (E) Quantification of ApoE amount (µg/mL) by ELISA assay in culture medium from vehicle- & LM11A-31-treated U373 cells. n = 3 different experiments. (F) Cholesterol quantification (total cholesterol, free cholesterol, & cholesteryl esters) in the culture medium of U373 cells treated with vehicle (Ctrl) & LM11A-31 (0.1 µM) for 48 h. n = 3 different experiments. Data represent means ± SD. Statistical analysis was carried out by using unpaired Student's t test. *** p < 0.001. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35563230), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications


Ji Eon Kim, So□Young Park, Chulhwan Kwak, Yoonji Lee, Dae□Geun Song, Jae Woo Jung, Haesong Lee, Eun□Ae Shin, Yangie Pinanga, Kyung□hee Pyo, Eun Hae Lee, Wonsik Kim, Soyeon Kim, Chang□Duck Jun, Jeanho Yun, Sun Choi, Hyun□Woo Rhee, Kwang□Hyeon Liu, Jung Weon Lee Glucose□mediated mitochondrial reprogramming by cholesterol export at TM4SF5□enriched mitochondria□lysosome contact sites Cancer Communications 2023-12-22 [PMID: 38133457]

Skop□l A, Ujlaki G, Gerencs□r AT et al. Adenosine A(2A) Receptor Activation Regulates Niemann-Pick C1 Expression and Localization in Macrophages Current Issues in Molecular Biology 2023-06-07 [PMID: 37367064] (Western Blot)

Weng Y, Shepherd D, Liu Y et al. Inhibition of the Niemann-Pick C1 protein is a conserved feature of multiple strains of pathogenic mycobacteria Nature Communications 2022-09-09 [PMID: 36085278]

Cheng YS, Zhang T, Ma X et al. A proteome-wide map of 20(S)-hydroxycholesterol interactors in cell membranes Nature Chemical Biology 2021-12-01 [PMID: 34799735]

Guix FX, Capit□n AM, Casadom□-Perales □ et al. Increased exosome secretion in neurons aging in vitro by NPC1-mediated endosomal cholesterol buildup Life Science Alliance 2021-08-01 [PMID: 34183444]

N?ková H, Cortizo FG, Schwenker LS et al. Competition for cysteine acylation by C16:0 and C18:0 derived lipids is a global phenomenon in the proteome The Journal of biological chemistry 2023-07-24 [PMID: 37495107] (WB, Human)

Details:
1:1000 WB dilution

Martella N, Colardo M, Sergio W et al. Lavender Essential Oil Modulates Hepatic Cholesterol Metabolism in HepG2 Cells Current issues in molecular biology 2023-01-03 [PMID: 36661512] (WB, ICC/IF, Human)

Piggott V, Lloyd S, Matchynski J et al. Traumatic Stress, Chronic Ethanol Exposure, or the Combination, Alter Cannabinoid System Components in Reward and Limbic Regions of the Mouse Brain Molecules 2021-04-30 [PMID: 33917316]

Colardo M, Petraroia M, Lerza L et al. NGF Modulates Cholesterol Metabolism and Stimulates ApoE Secretion in Glial Cells Conferring Neuroprotection against Oxidative Stress International Journal of Molecular Sciences 2022-04-27 [PMID: 35563230] (WB)

Tonini C, Colardo M, et al. Inhibition of Bromodomain and Extraterminal Domain (BET) Proteins by JQ1 Unravels a Novel Epigenetic Modulation to Control Lipid Homeostasis. Int J Mol Sci 2020-02-14 [PMID: 32075110] (WB, Human)

Goedeke L, Canfran-Duque A, Rotllan N Et al. MMAB promotes negative feedback control of cholesterol homeostasis Nature communications 2021-11-08 [PMID: 34750386] (WB, Human)

Walch P, Selkrig J, Knodler La Et Al. Global mapping of Salmonella enterica-host protein-protein interactions during infection Cell host & microbe 2021-07-02 [PMID: 34237247]


More publications at http://www.novusbio.com/NB400-148

## 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).

**Novus Biologicals USA**
10730 E. Briarwood Avenue
Centennial, CO 80112
USA
Phone: 303.730.1950
Toll Free: 1.888.506.6887
Fax: 303.730.1966
nb-customerservice@bio-techne.com

**Bio-Techne Canada**
21 Canmotor Ave
Toronto, ON M8Z 4E6
Canada
Phone: 905.827.6400
Toll Free: 855.668.8722
Fax: 905.827.6402
canada.inquires@bio-techne.com

**Bio-Techne Ltd**
19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB, United Kingdom
Phone: (44) (0) 1235 529449
Free Phone: 0800 37 34 15
Fax: (44) (0) 1235 533420
info.EMEA@bio-techne.com

**General Contact Information**
www.novusbio.com
Technical Support: nb-technical@bio-techne.com
Orders: nb-customerservice@bio-techne.com
General: novus@novusbio.com

## Products Related to NB400-148

| | |
|---|---|
| NB400-148PEP | Niemann-Pick C1 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.

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

**Earn gift cards/discounts by submitting a review: www.novusbio.com/reviews/submit/NB400-148**

**Earn gift cards/discounts by submitting a publication using this product: www.novusbio.com/publications**