Product Datasheet

Calnexin Antibody - BSA Free
NB100-1974-0.025ml

Unit Size: 0.025 ml

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

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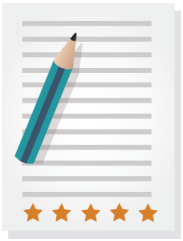
Updated 4/13/2025 v.20.1



NB100-1974-0.025ml

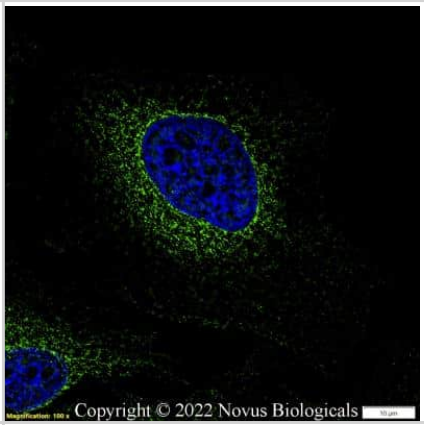
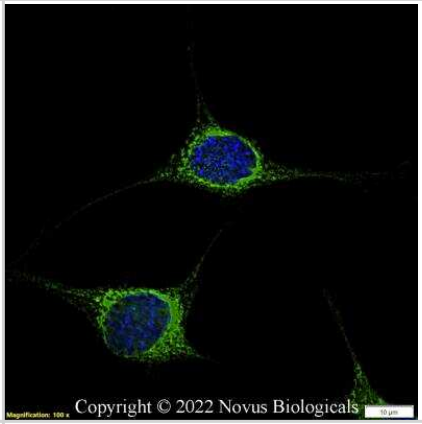
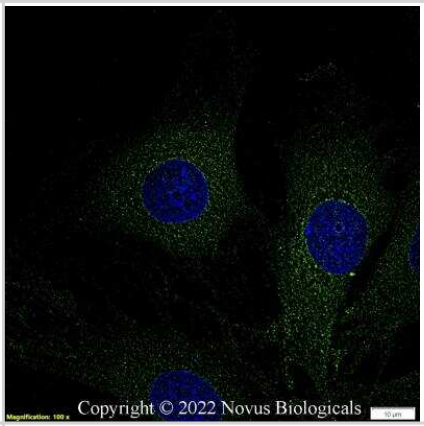
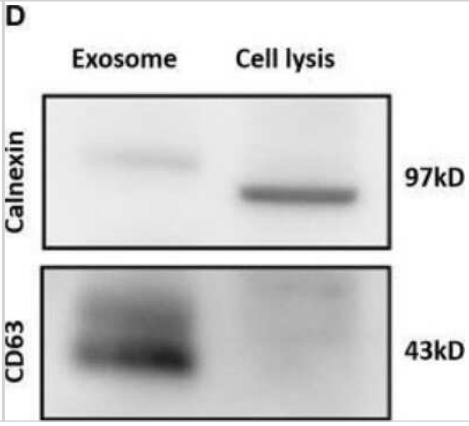
Calnexin Antibody - BSA Free

Product Information	
Unit Size	0.025 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	97 kDa

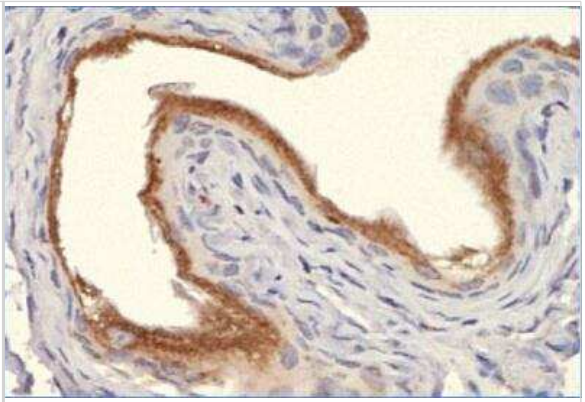
Product Description	
Host	Rabbit
Gene ID	821
Gene Symbol	CANX
Species	Human, Mouse, Rat, Hamster, Zebrafish
Reactivity Notes	Hamster reactivity reported in the scientific literature (PMID: 23760268). Use in Zebrafish reported in scientific literature (PMID:23049555).
Marker	Endoplasmic Reticulum Membrane Marker
Immunogen	A synthetic peptide made to a C-terminal portion of the rat Calnexin protein (between residues 550-591) [Uniprot: P35565]

Product Application Details	
Applications	Western Blot, Simple Western, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Western Blot 1:1000, Simple Western 1:50, Flow Cytometry, Immunohistochemistry 1:100, Immunocytochemistry/ Immunofluorescence 1:100, Immunoprecipitation 1:100, Immunohistochemistry-Paraffin 1:100
Application Notes	In ICC/IF, endoplasmic reticulum staining was observed in HeLa cells. In Western Blot, a band is seen at ~ 90 kDa representing Calnexin. In IHC-P, staining was observed in the endoplasmic reticulum of mouse bladder tissue. Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. See Simple Western Antibody Database for Simple Western validation: Tested in HeLa lysate 0.1 mg/mL, separated by Size, antibody dilution of 1:50, apparent MW was 117 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue.

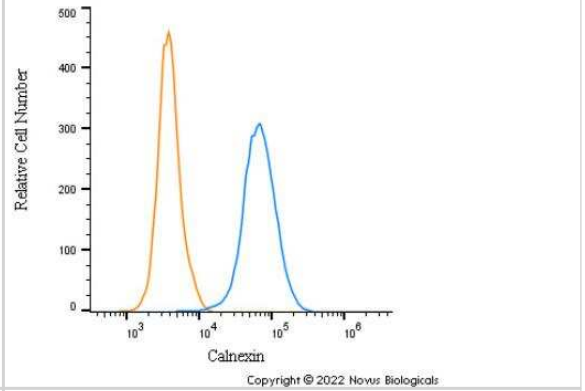
Images

Immunocytochemistry/Immunofluorescence: Calnexin Antibody [NB100-1974] - HeLa cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with (NB100-1974) at 1 ug/ml overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.	
Immunocytochemistry/Immunofluorescence: Calnexin Antibody [NB100-1974] - NIH3T3 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with (NB100-1974) at 1 ug/ml overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.	
Immunocytochemistry/Immunofluorescence: Calnexin Antibody [NB100-1974] - Rat FR cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with (NB100-1974) at 1 ug/ml overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.	
Western Blot: Calnexin Antibody [NB100-1974] - Identification of plasma exosomes and differentially expressed exosomal miRNAs. Confirmation of the exosomes markers with Western blotting indicated the presence of CD63 but the absence of calnexin in exosomes. Image collected and cropped by CiteAb from the following publication (https://www.frontiersin.org/article/10.3389/fonc.2019.00459/full), licensed under a CC-BY license.	

Immunohistochemistry: Calnexin Antibody [NB100-1974] - Analysis of Calnexin in mouse bladder using DAB with hematoxylin counterstain.

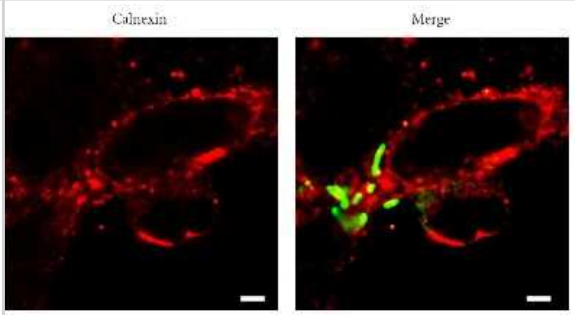


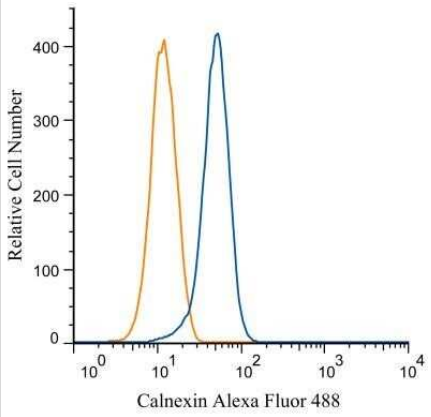
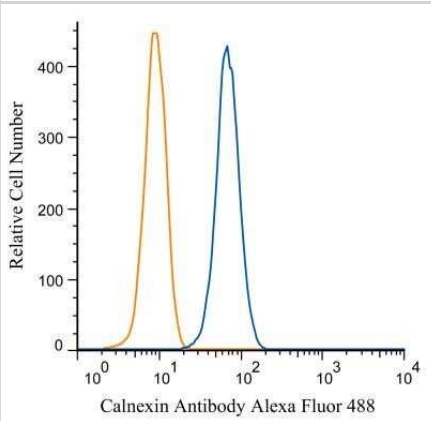
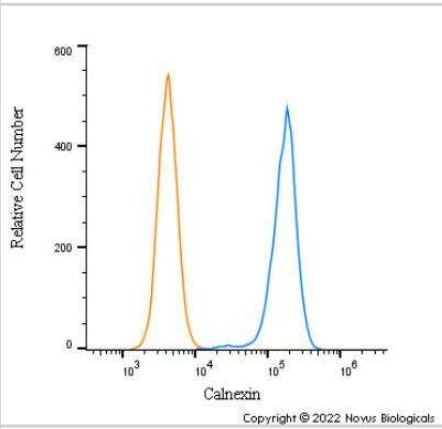
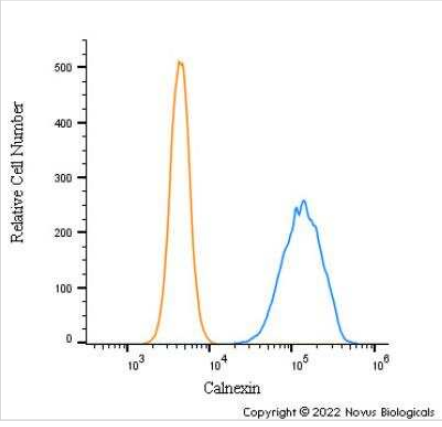
Flow Cytometry: Calnexin Antibody [NB100-1974] - An intracellular stain was performed on rat FR cells with Calnexin Antibody NB100-1974 (blue) and a matched isotype control NBP2-24891 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1.0 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (SA5-10033, Thermo Fisher).

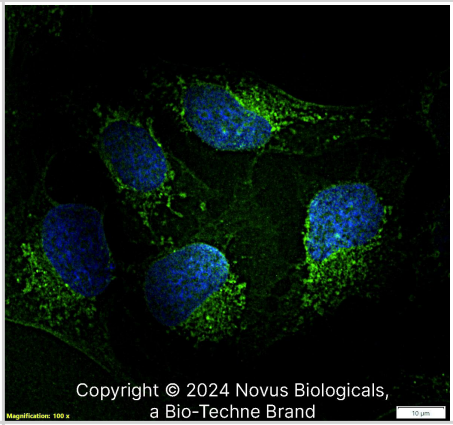
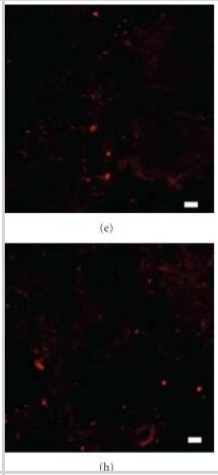
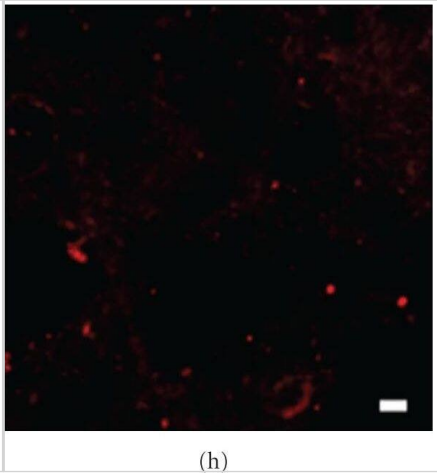


Western Blot: Calnexin Antibody [NB100-1974] - Analysis of Calnexin in HeLa whole cell lysate.

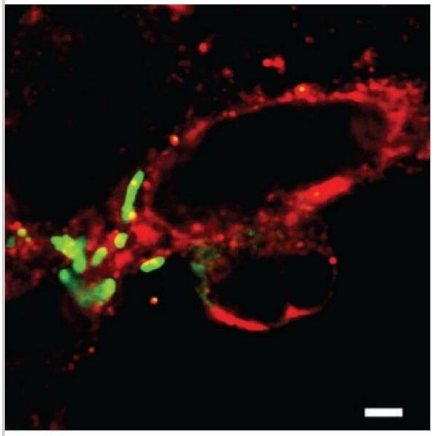
Immunocytochemistry/Immunofluorescence: Calnexin Antibody [NB100-1974] - Zebrafish Vwf forms pseudo-Weibel-Palade bodies (pseudo-WPBs) in mammalian cell culture. pzVwf/Myc-HIS (zebrafish Vwf) plasmids were transfected into HEK293T cells. Anti-Myc antibody conjugated to Alexa Fluor 488 (green channel) was used for detection and anti-calnexin antibody conjugated to Alexa Fluor 594 (red channel) labeled endoplasmic reticulum (ER). Both constructs demonstrate formation of elongated Myc positive and ER negative structures (absence of yellow signal in the merged panels) characteristic of pseudo-WPBs. Scale bars, 2.5 um. Image collected and cropped by CiteAb from the following publication (https://www.hindawi.com/journals/ah/2012/214209/), licensed under a CC-BY license.



Flow Cytometry: Calnexin Antibody [NB100-1974] - Analysis of Alexa Fluor (R) 488 conjugate of NB100-1974. An intracellular stain was performed on Jurkat cells with Calnexin antibody NB100-1974AF488 (blue) and a matched isotype control NBP2-24893AF488 (orange).	
Flow Cytometry: Calnexin Antibody [NB100-1974] - An intracellular stain was performed on HeLa cells with Calnexin antibody NB100-1974AF488 (blue) and a matched isotype control NBP2-24893AF488 (orange). Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 488. Image using the Alexa Fluor 488 form of this antibody.	
Flow Cytometry: Calnexin Antibody [NB100-1974] - An intracellular stain was performed on Daudi cells with Calnexin Antibody NB100-1974 (blue) and a matched isotype control NBP2-24891 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1.0 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (SA5-10033, Thermo Fisher).	
Flow Cytometry: Calnexin Antibody [NB100-1974] - An intracellular stain was performed on NIH3T3 cells with Calnexin Antibody NB100-1974 (blue) and a matched isotype control NBP2-24891 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1.0 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (SA5-10033, Thermo Fisher).	

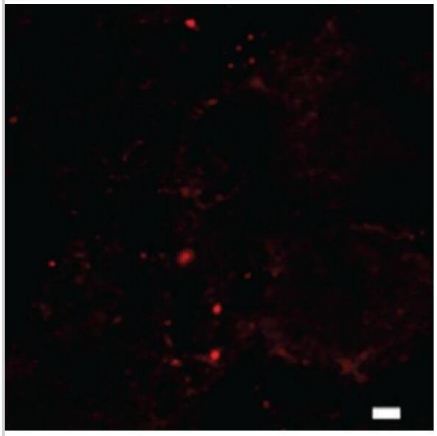
Simple Western: Calnexin Antibody [NB100-1974] - Image shows a specific band for Calnexin in 0.1 mg/mL of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	
Calnexin was detected in immersion fixed U-2 OS human osteosarcoma cell line using Rabbit anti-Calnexin Affinity Purified Polyclonal Antibody conjugated to FITC (Catalog # NB100-1974F) (green) at 10 µg/mL overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.	
Immunocytochemistry/ Immunofluorescence: Calnexin Antibody - BSA Free [NB100-1974] - Zebrafish Vwf forms pseudo-Weibel-Palade bodies (pseudo-WPBs) in mammalian cell culture. pVWF/Myc-HIS (human VWF, (a–c)) or pzVwf/Myc-HIS (zebrafish Vwf, (d–i)) plasmids were transfected into HEK293T cells. Anti-Myc antibody conjugated to Alexa Fluor 488 (green channel, (a, d, g)) was used for detection & anti-calnexin antibody conjugated to Alexa Fluor 594 (red channel, (b, e, h)) labeled endoplasmic reticulum (ER). Both constructs demonstrate formation of elongated Myc positive & ER negative structures (absence of yellow signal in the merged panels, (c, f, i)) characteristic of pseudo-WPBs (examples are indicated in (a, d), & (g) by arrowheads). Scale bars, 2.5 µm. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/23049555), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Immunocytochemistry/ Immunofluorescence: Calnexin Antibody - BSA Free [NB100-1974] - Zebrafish Vwf forms pseudo-Weibel-Palade bodies (pseudo-WPBs) in mammalian cell culture. pVWF/Myc-HIS (human VWF, (a–c)) or pzVwf/Myc-HIS (zebrafish Vwf, (d–i)) plasmids were transfected into HEK293T cells. Anti-Myc antibody conjugated to Alexa Fluor 488 (green channel, (a, d, g)) was used for detection & anti-calnexin antibody conjugated to Alexa Fluor 594 (red channel, (b, e, h)) labeled endoplasmic reticulum (ER). Both constructs demonstrate formation of elongated Myc positive & ER negative structures (absence of yellow signal in the merged panels, (c, f, i)) characteristic of pseudo-WPBs (examples are indicated in (a, d), & (g) by arrowheads). Scale bars, 2.5 µm. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/23049555), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Immunocytochemistry/ Immunofluorescence: Calnexin Antibody - BSA Free [NB100-1974] - Zebrafish Vwf forms pseudo-Weibel-Palade bodies (pseudo-WPBs) in mammalian cell culture. pVWF/Myc-HIS (human VWF, (a–c)) or pzVwf/Myc-HIS (zebrafish Vwf, (d–i)) plasmids were transfected into HEK293T cells. Anti-Myc antibody conjugated to Alexa Fluor 488 (green channel, (a, d, g)) was used for detection & anti-calnexin antibody conjugated to Alexa Fluor 594 (red channel, (b, e, h)) labeled endoplasmic reticulum (ER). Both constructs demonstrate formation of elongated Myc positive & ER negative structures (absence of yellow signal in the merged panels, (c, f, i)) characteristic of pseudo-WPBs (examples are indicated in (a, d), & (g) by arrowheads). Scale bars, 2.5 µm. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/23049555), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



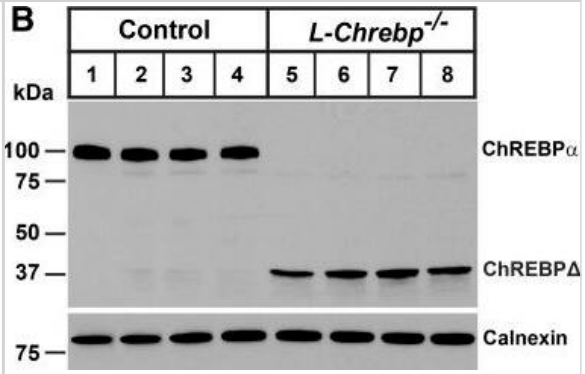
(c)

Immunocytochemistry/ Immunofluorescence: Calnexin Antibody - BSA Free [NB100-1974] - Zebrafish Vwf forms pseudo-Weibel-Palade bodies (pseudo-WPBs) in mammalian cell culture. pVWF/Myc-HIS (human VWF, (a–c)) or pzVwf/Myc-HIS (zebrafish Vwf, (d–i)) plasmids were transfected into HEK293T cells. Anti-Myc antibody conjugated to Alexa Fluor 488 (green channel, (a, d, g)) was used for detection & anti-calnexin antibody conjugated to Alexa Fluor 594 (red channel, (b, e, h)) labeled endoplasmic reticulum (ER). Both constructs demonstrate formation of elongated Myc positive & ER negative structures (absence of yellow signal in the merged panels, (c, f, i)) characteristic of pseudo-WPBs (examples are indicated in (a, d), & (g) by arrowheads). Scale bars, 2.5 µm. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/23049555), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

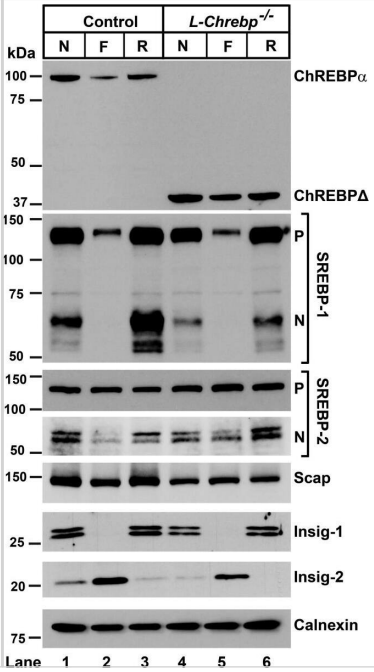


(e)

Western Blot: Calnexin Antibody - BSA Free [NB100-1974] - Liver-specific disruption of Chrebp in mice. A: Quantitative real-time PCR analysis of ChREBP mRNA. Total RNA was isolated from various tissues of control & liver-specific Chrebp knockout (L-Chrebp−/−) mice & subjected to real-time PCR analysis with 36B4 as the invariant control. Each value represents the mean ± SEM of four mice relative to that of controls, which was arbitrarily defined as 1.0. #P < 0.01 denotes the level of statistical significance (two-tailed Student's t-test) between control & L-Chrebp−/− mice. B: Immunoblot analysis of ChREBP in liver lysates of control & L-Chrebp−/− mice. Aliquots (60 µg of protein) of liver whole-cell lysates were subjected to SDS-PAGE & immunoblot analysis with anti-ChREBP & anti-calnexin antibodies. ChREBPΔ denotes a truncated aberrant ChREBP protein present only in lysates prepared from L-Chrebp−/− livers. The functional domains of ChREBP, including the glucose-sensing proline-rich bHLH-Zip DNA-binding & ZIP-like domains, are denoted. NLS, nuclear localization sequence. Image collected & cropped by CiteAb from the following publication (https://linkinghub.elsevier.com/retrieve/pii/S0022227520331369), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: Calnexin Antibody - BSA Free [NB100-1974] - Immunoblot analysis of livers from control & L-Chrebp−/− mice subjected to fasting & refeeding with a high-sucrose diet. Littermate control & L-Chrebp−/− mice (same as those described in supplemental Tables S2A & S2B) were subjected to fasting & refeeding. The nonfasted (N) groups were fed chow diet ad libitum. The fasted (F) group was fasted 12 h, & the refed (R) group was fasted for 12 h & then refed with 60% (w/w) high-sucrose diet for 12 h prior to study. Liver whole-cell lysates & membrane fractions were prepared individually, & equal amounts of protein from each mouse of the same group (four per group) were pooled. Aliquots (40 µg for whole-cell lysates & 30 µg for membrane fractions) of the pooled protein were subjected to SDS-PAGE & immunoblot analysis. Immunoblot analysis of Insig-1 & Insig-2 were carried out using membrane fractions. Whole-cell lysates were used to detect other proteins. The precursor & nuclear forms of SREBPs are denoted as P & N, respectively. Calnexin was used as loading control. Image collected & cropped by CiteAb from the following publication (https://linkinghub.elsevier.com/retrieve/pii/S0022227520331369), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Akoumianaki T, Vaporidi K, Diamantaki E et al. Uncoupling of IL-6 signaling and LC3-associated phagocytosis drives immunoparalysis during sepsis Cell host & microbe 2021-06-25 [PMID: 34214493]

Xu S, Donnelly L, Kober DL et al. Development of a monoclonal antibody to study MARCH6, an E3 ligase that regulates proteins that control lipid homeostasis. Journal of lipid research 2024-09-19 [PMID: 39306038]

Castellani, G;Buccarelli, M;D'Alessandris, QG;Ilari, R;Cappannini, A;Pedini, F;Boe, A;Lulli, V;Parolini, I;Giannetti, S;Biffoni, M;Zappavigna, V;Marziali, G;Pallini, R;Ricci-Vitiani, L; Extracellular vesicles produced by irradiated endothelial or Glioblastoma stem cells promote tumor growth and vascularization modulating tumor microenvironment Cancer cell international 2024-02-12 [PMID: 38347567]

Hung YH, Kim Y, Mitchell SB et al. Absence of Slc39a14/Zip14 in mouse pancreatic beta cells results in hyperinsulinemia American journal of physiology. Endocrinology and metabolism 2023-11-29 [PMID: 38019082]

Galanopoulou O, Tachmatzidi E, Deligianni E et al. Endonucleosis mediates internalization of cytoplasm into the nucleus in senescent cells bioRxiv 2023-11-13 (ICC/IF)

Fernandez-Fuente G, Overmyer KA, Lawton AJ et al. The citrate transporters SLC13A5 and SLC25A1 elicit different metabolic responses and phenotypes in the mouse Commun Biol 2023-09-09 [PMID: 37689798] (Western Blot)

Chantziou A, Theodorakis K, Polioudaki H et al. Glycosylation Modulates Plasma Membrane Trafficking of CD24 in Breast Cancer Cells International Journal of Molecular Sciences 2021-07-29 [PMID: 34360932]

Sikorski K, Mehta A et al. A high-throughput pipeline for validation of antibodies. Nat Methods 2018-01-11 [PMID: 30377371] (Human)

Details:
Antibody validation based on denaturing gel electrophoresis of biotinylated cell lysates (PAGE) followed by mass spectrometry (MS) and antibody array analysis (MAP).

Ghosh A, Vo A, Twiss BK et al. Characterization of Zebrafish von Willebrand Factor Reveals Conservation of Domain Structure, Multimerization, and Intracellular Storage Adv Hematol 2012-09-24 [PMID: 23049555] (ICC/IF, Zebrafish)

Ma J, Xu M, Yin M, et al. Exosomal hsa-miR199a-3p Promotes Proliferation and Migration in Neuroblastoma Front Oncol 2019-06-12 [PMID: 31249805] (FLOW, WB, Human)

Linden AG, Li S, Choi HY et al. Interplay between ChREBP and SREBP-1c coordinates postprandial glycolysis and lipogenesis in livers of mice J Lipid Res 2018-01-01 [PMID: 29335275] (WB, Mouse)

Ye Z, Zhang J, Ancrum T et al. S-Glutathionylation of Endoplasmic Reticulum Proteins Impacts Unfolded Protein Response Sensitivity. Antioxid. Redox Signal. 2016-02-03 [PMID: 26838680]

More publications at http://www.novusbio.com/NB100-1974

Procedures

Western Blot protocol for Calnexin Antibody (NB100-1974)
Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturer's instructions.

Immunohistochemistry-Paraffin Protocol for Calnexin Antibody (NB100-1974)
Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer at all times).

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.

Immunocytochemistry/Immunofluorescence Protocol for Calnexin Antibody (NB100-1974)
Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeablization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPi if required.

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

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