Product Datasheet

Calnexin Antibody - BSA Free
NB100-1974

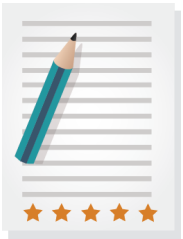
Unit Size: 0.1 ml

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

Reviews: 1 Publications: 11

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
www.novusbio.com/NB100-1974

Updated 12/20/2023 v.20.1



NB100-1974

Calnexin Antibody - BSA Free

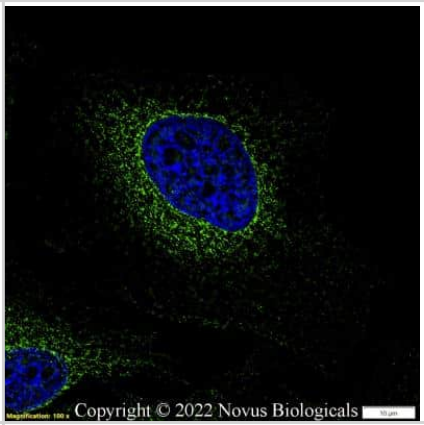
Product Information	
Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	97 kDa

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]

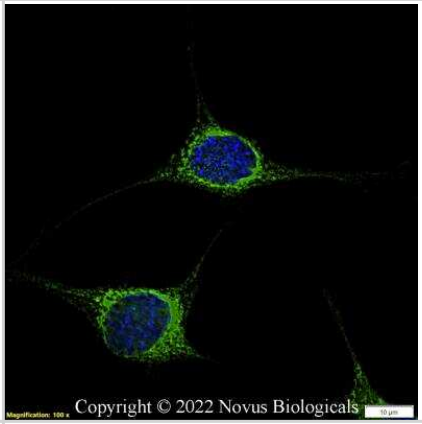
Product Application Details	
Applications	Western Blot, Simple Western, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Western Blot 1:1000, Simple Western 1:50, Flow Cytometry, Immunohistochemistry 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. Separated by Size-Wes, Sally Sue/Peggy Sue.

Images

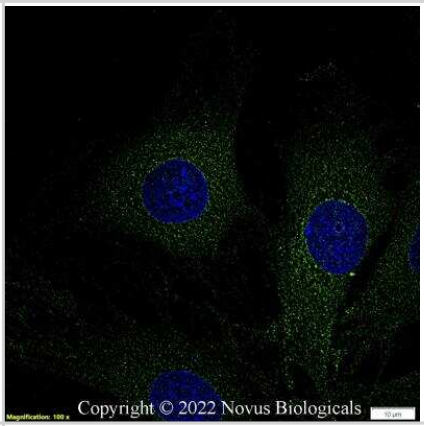
Immunocytochemistry/Immunofluorescence: Calnexin Antibody [NB100-1974] - HeLa cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with (NB100-1974) at 1 ug/ml overnight at 4C and detected with an anti-rabbit DyLight 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.



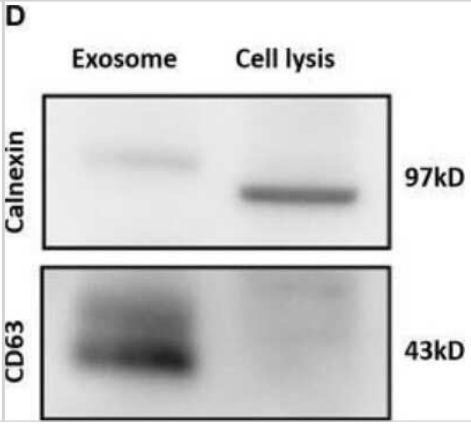
Immunocytochemistry/Immunofluorescence: Calnexin Antibody [NB100-1974] - NIH3T3 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with (NB100-1974) at 1 ug/ml overnight at 4C and detected with an anti-rabbit DyLight 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.



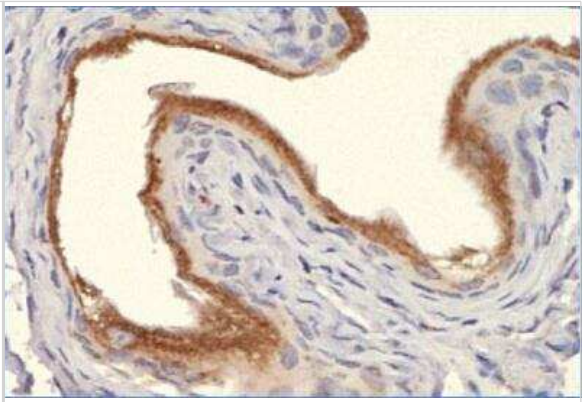
Immunocytochemistry/Immunofluorescence: Calnexin Antibody [NB100-1974] - Rat FR cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with (NB100-1974) at 1 ug/ml overnight at 4C and detected with an anti-rabbit DyLight 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.



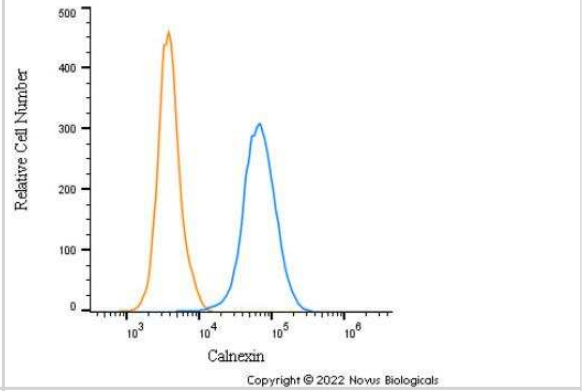
Western Blot: Calnexin Antibody [NB100-1974] - Identification of plasma exosomes and differentially expressed exosomal miRNAs. Confirmation of the exosomes markers with Western blotting indicated the presence of CD63 but the absence of calnexin in exosomes. Image collected and cropped by CiteAb from 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.

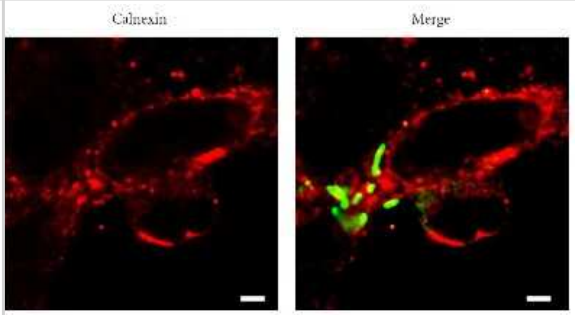


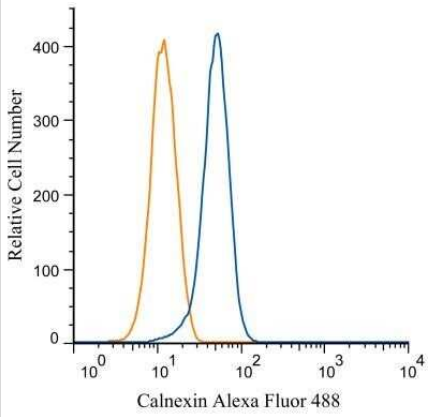
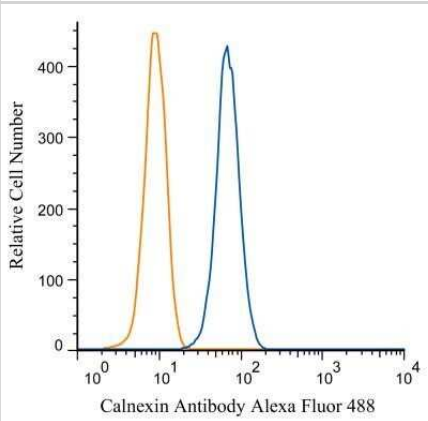
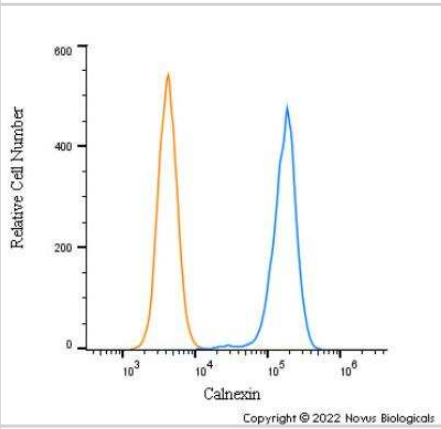
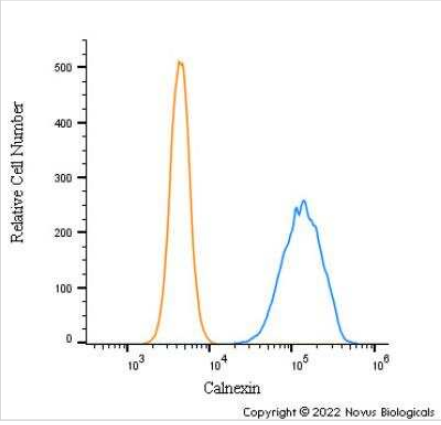
Flow Cytometry: Calnexin Antibody [NB100-1974] - An intracellular stain was performed on rat FR cells with Calnexin Antibody NB100-1974 (blue) and a matched isotype control NBP2-24891 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody 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).

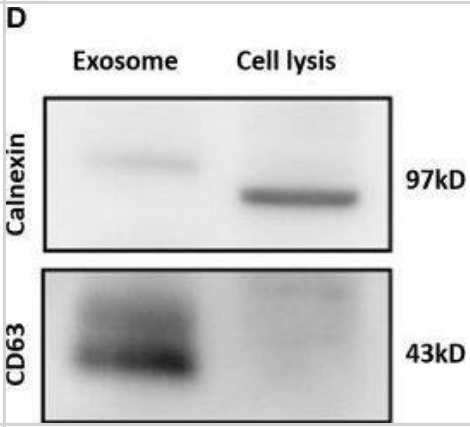
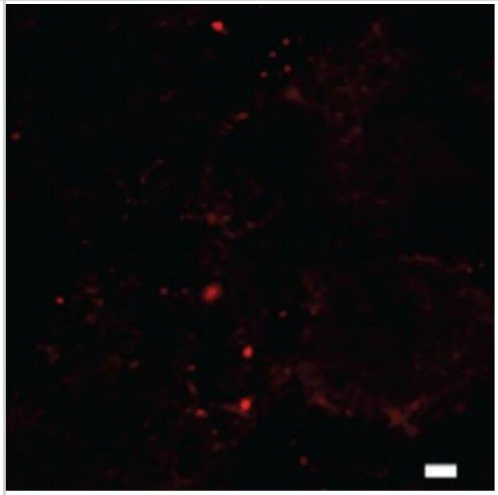
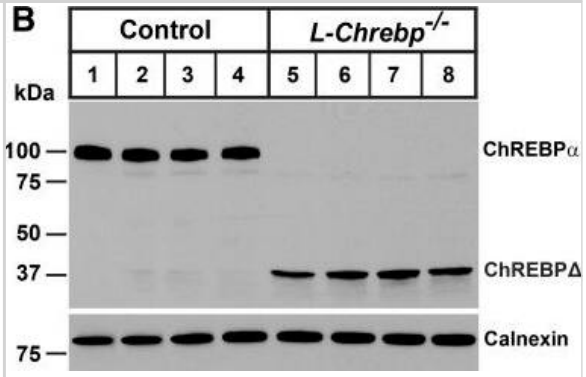


Western Blot: Calnexin Antibody [NB100-1974] - Analysis of Calnexin in HeLa whole cell lysate.

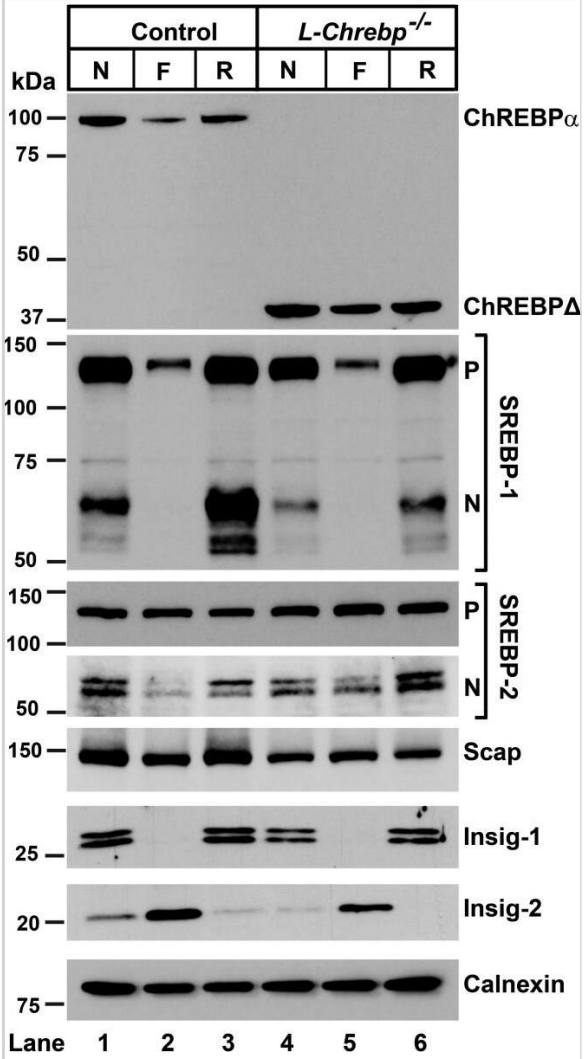
Immunocytochemistry/Immunofluorescence: Calnexin Antibody [NB100-1974] - Zebrafish Vwf forms pseudo-Weibel-Palade bodies (pseudo-WPBs) in mammalian cell culture. pzVwf/Myc-HIS (zebrafish Vwf) plasmids were transfected into HEK293T cells. Anti-Myc antibody conjugated to Alexa Fluor 488 (green channel) 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.



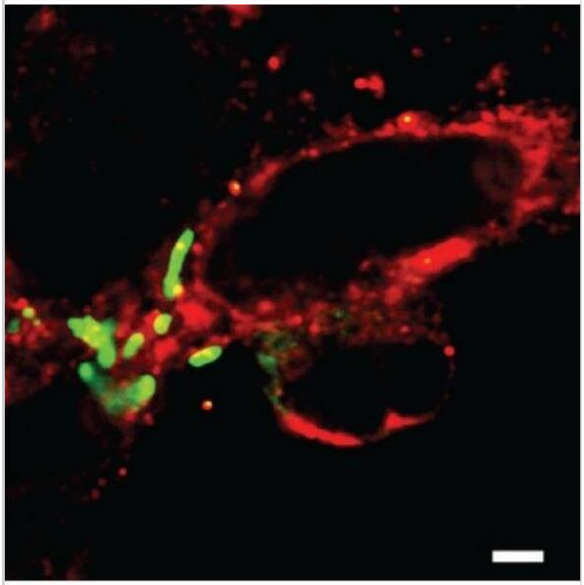
Flow Cytometry: Calnexin Antibody [NB100-1974] - Analysis of Alexa Fluor (R) 488 conjugate of NB100-1974. An intracellular stain was performed on Jurkat cells with Calnexin antibody NB100-1974AF488 (blue) and a matched isotype control NBP2-24893AF488 (orange).	
Flow Cytometry: Calnexin Antibody [NB100-1974] - 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.	
Identification of plasma exosomes and differentially expressed exosomal miRNAs. (D) 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://pubmed.ncbi.nlm.nih.gov/31249805), licensed under a CC-BY licence.	
EMPA treatment attenuated cardiomyocyte overproduction of reactive oxygen species (ROS) induced by TAC. (A) Left, Mitosox staining of cardiomyocytes in sham, sham + EMPA, TAC and TAC + EMPA groups and Right, quantitative results. (B) Representative blots of NRF-2 and HO-1, and quantitative results. (C) The relative mRNA levels of genes related to endogenous antioxidants. Results are expressed as mean ± SEM, n = 5–7, *p < 0.05 vs. corresponding sham group, †p < 0.05 vs. corresponding TAC vehicle group. One-way ANOVA and Tukey post hoc test. EMPA, empagliflozin; SEM, standard error of the mean; TAC, transverse aortic constriction; NRF-2, the nuclear factor erythroid 2–related factor 2; HO-1, heme oxygenase-1; GCLM, glutamate-cysteine ligase modifier subunit; GAPDH, glyceraldehyde 3-phosphate dehydrogenase. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/35647080), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	 (e)
HIF□2α depletion counteracts tumour growth and non□canonical glutamine metabolism in vivo. (A) Representative images of tumour□bearing mice and tumours removed from the mice. (B) Tumour volumes were measured on the indicated days. (C) Tumour weights were shown as the means ± S.D. when the tumours were harvested. (D) Representative images (×200) of H&E and IHC staining of the tumours. Ki67 was used as proliferation index. (E and F) qRT□PCR and Western blot analysed the expression of HIF□2α and GOT1 in tumour tissues from sh□HIF□2α Panc□1 group compared with control group, *P < 0.05, **P < 0.01. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/28544376), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Bmal1 regulates tumor-associated macrophage polarization.(A) Bmal1 gene expression (left panel) and protein levels (right panel) in WT macrophages treated with control medium or increasing doses of B16-F10 tumor-conditioned medium (T-CM, diluted 1:3 or 1:1 with control medium) for 8 hr. N = 3 biological replicates for qPCR, statistical analysis was performed using Student's T test. (B) Measurement of mROS using MitoSox Red (mean fluorescent intensity, MFI) in mitochondria from macrophages treated with control medium or T-CM diluted 1:1 with control medium for 1 hr. N = 3 biological replicates, statistical analysis was performed using Student's T test. (C) Hif-1α protein levels in WT and M-BKO macrophages treated with control medium, T-CM diluted 1:1 with control medium, or undiluted T-CM for 4 hr. (D) Glycolytic stress test in macrophages pretreated with control medium or T-CM diluted 1:1 with control medium for 4 hr. N = 5 biological replicates. Statistical analysis was performed using two-way ANOVA comparing T-CM-treated M-BKO with WT cells across the time course. (E) Relative expression of genes involved in amino-acid metabolism and oxidative stress response in macrophages treated with control medium or T-CM diluted 1:3 with control medium for 8 hr, as determined by qPCR. N = 3 biological replicates, statistical analysis was performed using Student's T test. (F) Tumor volume in male (left) and female (right) WT and M-BKO mice. 300,000 B16-F10 cells were injected subcutaneously into the right flank. N = 18 (male) and 8 (female) mice, statistical analysis was performed using two-way ANOVA for WT vs M-BKO mice across the time course. (G) Gene expression for F4/80+ cells isolated from B16-F10 tumors or spleens of female mice 14 days after injection. Tissues from six mice per genotype were pooled into three groups for leukocyte isolation. Statistical analysis was performed using Student's T test. Data are presented as mean ± S.E.M. *, p<0.05. Experiments were repeated at least twice.Increased mROS levels and glucose uptake in M-BKO TAMs.(A) Tumor volumes of B16-F10 subcutaneous allografts in 3-month-old female WT and M-BKO mice 14 days after tumor cell injection. Each mouse was injected with 500,000 B16-F10 cells in the right and left flanks, and tumors from each mouse were pooled for immune cell isolation and flow cytometry. N = 4 mice, with two tumors per mouse. (B, C) mROS production and glucose uptake by tumor-associated CD45+F4/80+ cells determined by flow cytometry (mean fluorescent intensities) of Mitosox Red and 2-NBDG staining, respectively. N = 4. Statistical analysis was performed using Student's T test. Data are presented as mean ± S.E.M. *, p<0.05. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/32396064), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

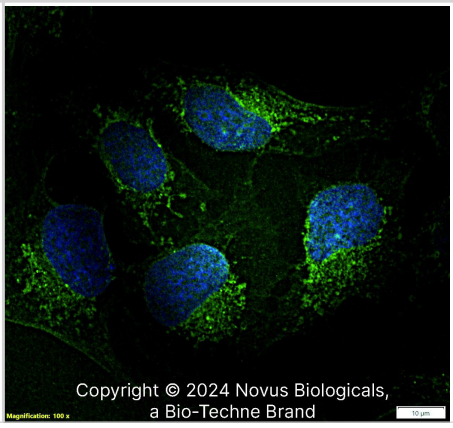


Tau clearance in vivo and autophagy function. Clearance kinetics of photoconverted Dendra-tau measured in neurons of WT-tau and AT152T-tau fish. Measurement of the intensity of the red Dendra-tau signal over time reflects the clearance or degradation of tau protein. (A) Representative images of photoconverted red Dendra-tau signal comparing a single neuron from WT-tau and A152T-tau fish at three different timepoints: immediately after photoconversion (0 h), 24 h and 48 h after photoconversion. (B) Quantification of red Dendra-tau intensity in photoconverted neurons in the spinal cord of WT-tau and A152T-Tau transgenic fish (representative images shown in Supplementary Fig. 8C). The percentage of residual photoconverted red Dendra-tau was measured over 48 h measured at 12-h intervals. Dendra-tagged A152T-tau clears at a significantly lower rate than WT-tau. (n = 30/group shown as mean ± SD; Student-Newman-Keuls one-way ANOVA, **P < 0.01 and ***P < 0.001 versus WT-tau). (C–F) Western blots for LC3-II, a well-characterized marker of autophagosome number, demonstrate that there are no differences in the levels of this protein between WT-tau and A152T-tau fish either at 24 hpf (pre-phenotype; C and D) or 72 hpf (post-phenotype; E and F). (E and F) Measurements of LC3-II levels in the presence or absence of ammonium chloride provides a method for measuring autophagic flux. No differences were observed between the two transgenic lines at 3 dpf, suggesting that autophagy functions normally in both WT-tau and A152T-tau fish (graph represents mean ± SD of four independent clutches per group for E and F and three for C and D; two-tailed t-test). (G and H) Clearance kinetics of Dendra-tau was measured in the presence or absence of ammonium chloride. Treatment with ammonium chloride blocks autophagic flux and delays the clearance of both WT-tau and A152T-tau to the same extent, indicating that flux occurs at the same rate in these two lines (mean ± SD, n = 62 neurons/group; Student-Newman-Keuls one-way ANOVA, **P < 0.01 and ***P < 0.001 versus untreated group). Note in G and H, the ‘WT-tau + NH4Cl’ (denoted by black squares and black dashed line) overlaps with the ‘A152T-tau’ line (denoted by grey triangles and grey solid line). The graphs in G and H are presented with a different line in the foreground and background to aid interpretation. Image collected and cropped by CiteAb from the following open publication (https://academic.oup.com/brain/article/140/4/1128/2980948), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



(c)

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.



Publications

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] (WB)

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]

Xu J, Xu M, Brown T et al. Stabilization of the m-Opioid Receptor by Truncated Single Transmembrane Splice Variants through a Chaperone-like Action. J Biol Chem 2013-07-19 [PMID: 23760268] (WB, Hamster)

Kang R, Tang D, Schapiro NE et al. The HMGB1/RAGE inflammatory pathway promotes pancreatic tumor growth by regulating mitochondrial bioenergetics. Oncogene 2013-01-14 [PMID: 23318458] (WB, Mouse)

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.

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