Product Datasheet

Calnexin Antibody - BSA Free
NB100-1965

Unit Size: 0.1 ml

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

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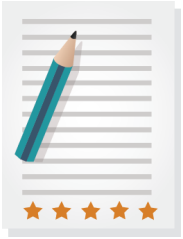
Updated 12/20/2023 v.20.1



NB100-1965

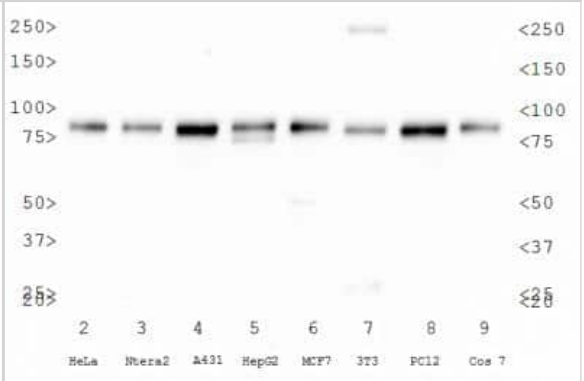
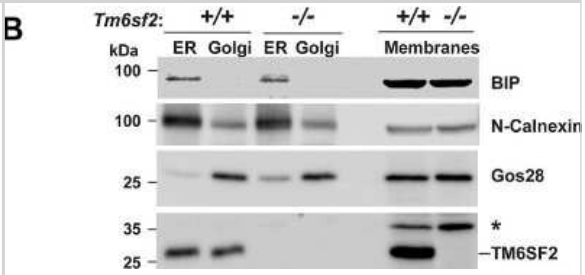
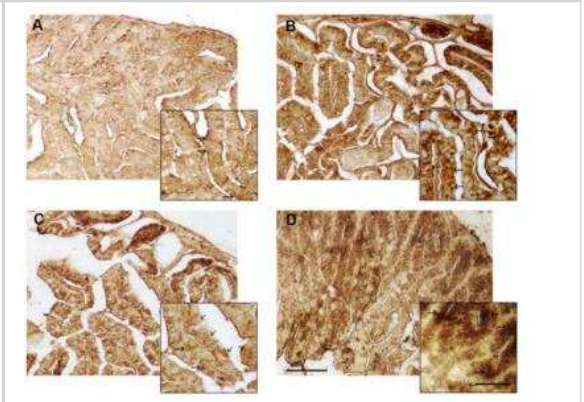
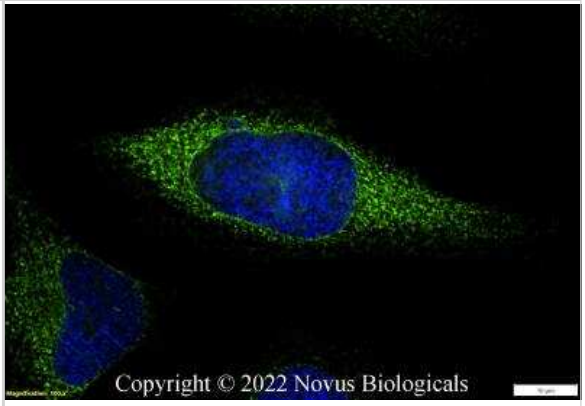
Calnexin Antibody - BSA Free

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

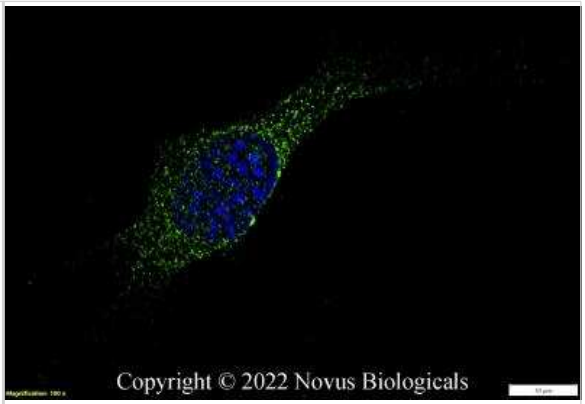
Product Description	
Host	Rabbit
Gene ID	821
Gene Symbol	CANX
Species	Human, Mouse, Rat, Porcine, Avian, Bovine, Chicken, Drosophila, Guinea Pig, Rabbit, Sheep, Xenopus, Zebrafish
Reactivity Notes	Quail. Predicted to react with dog based on 100% sequence homology.
Marker	Endoplasmic Reticulum Membrane Marker
Immunogen	A synthetic peptide made to an internal region of the canine Calnexin protein (within residues 25-100). [Swiss-Prot P24643]

Product Application Details	
Applications	Western Blot, Simple Western, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Western Blot 2 ug/ml, Simple Western 1:25, Flow Cytometry 1-5 ug/ml, Immunohistochemistry 1:40, Immunocytochemistry/ Immunofluorescence 1-5 ug/ml, Immunoprecipitation 1:100, Immunohistochemistry-Paraffin 1:40
Application Notes	This Calnexin antibody is useful for Immunocytochemistry/Immunofluorescence, Immunohistochemistry-paraffin embedded sections, Immunoprecipitation and Western Blot. In Western blot a band is observed approx. 97 kDa. 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.

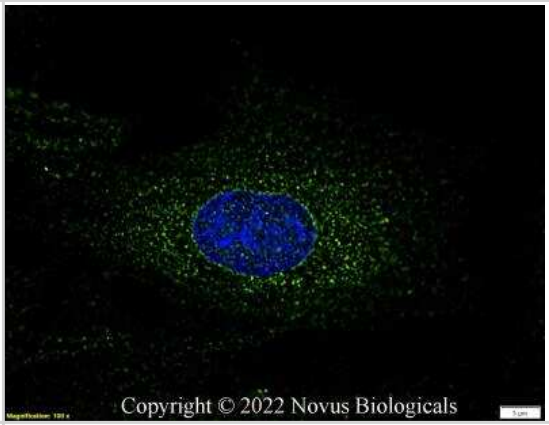
Images

Western Blot: Calnexin Antibody [NB100-1965] - WB analysis of CANX in cell lysates as noted.	
Western Blot: Calnexin Antibody [NB100-1965] - Subcellular localization of TM6SF2.Immunoaffinity isolation of ER and Golgi complex from mouse liver. ER and Golgi fractions were prepared from mouse liver microsomes by immunoaffinity chromatography as described under Experimental Procedures. Microsome membranes were dissolved in RIPA buffer, and equal volumes were separated on 10% SDS-PAGE and immunoblotting as described under Experimental Procedures. BiP, binding immunoglobulin protein; Gos28, Golgi SNAP receptor complex member 1; *, nonspecific band. Image collected and cropped by Citeab from the following publication (Inactivation of Tm6sf2, a Gene Defective in Fatty Liver Disease, Impairs Lipidation but Not Secretion of Very Low Density Lipoproteins. *J Biol Chem* (2016) licensed under a CC-BY license.	
Immunohistochemistry: Calnexin Antibody [NB100-1965] - Calnexin immunostaining in the anterior (AI) and mid (MI) intestine of ctrl (control zebrafish), D.I.O. (diet-induced obesity zebrafish), D.I.O. flw 3,5-T2 (D.I.O. zebrafish followed by 3,5-T2), D.I.O. with 3,5-T2 (D.I.O. zebrafish treated with 3,5-T2). (A) AI of ctrl zebrafish (B) AI of D.I.O. (C) AI of D.I.O. flw 3,5-T2. (D) AI of D.I.O. with 3,5-T2. The arrows indicate calnexin immunoexpression in the enteroendocrine and goblet cells. Image collected and cropped by CiteAb from the following publication (https://www.mdpi.com/2076-2615/10/7/1131/htm#) licensed under a CC-BY license.	
Immunocytochemistry/Immunofluorescence: Calnexin Antibody [NB100-1965] - 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 (NB100-1965) 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.	

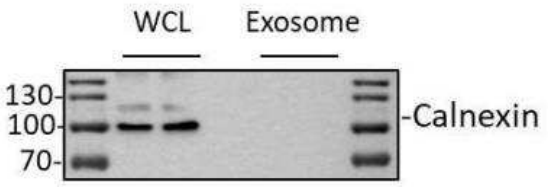
Immunocytochemistry/Immunofluorescence: Calnexin Antibody [NB100-1965] - NIH3T3 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with (NB100-1965) at 1 ug/ml overnight at 4C and detected with an anti-rabbit DyLight 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.



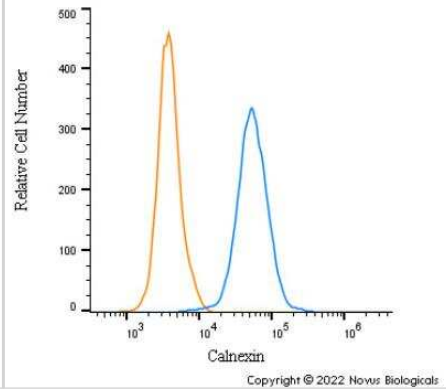
Immunocytochemistry/Immunofluorescence: Calnexin Antibody [NB100-1965] - Rat FR cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with Calnexin Antibody (NB100-1965) at 1 ug/ml overnight at 4C and detected with 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.

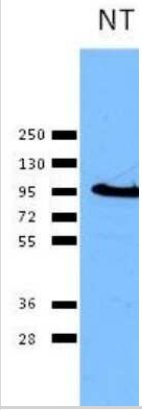
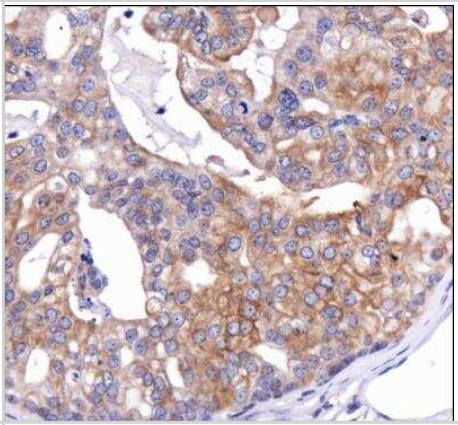
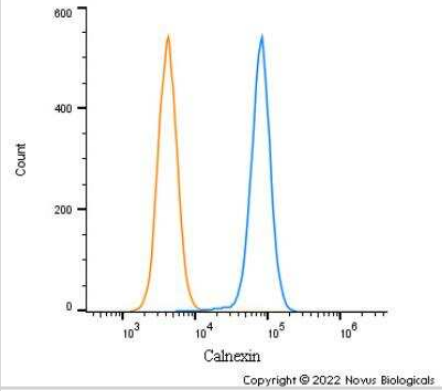
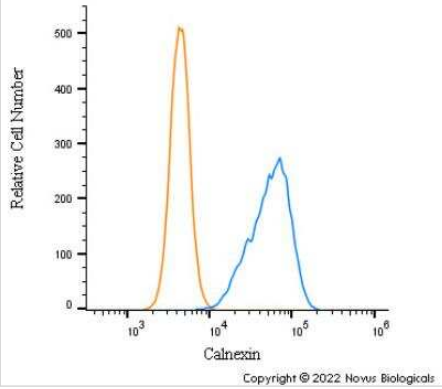


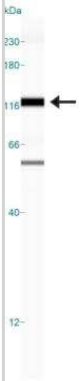
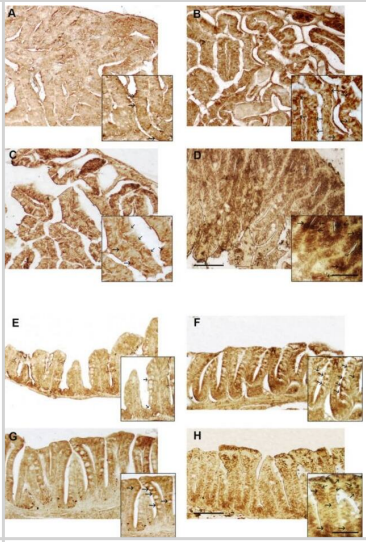
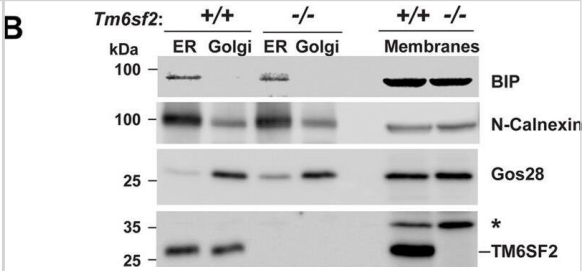
Western Blot: Calnexin Antibody - BSA Free [NB100-1965] - Whole cell lysate or exosome from MDA-MD-231 cells was loaded with 20 ug/lane. 10% SDS-PAGE. Calnexin Antibody (NB100-1965) was used for primary antibody: 1:3000, 4C, overnight. Image from verified customer review.



Flow Cytometry: Calnexin Antibody [NB100-1965] - An intracellular stain was performed on rat FR cells with Calnexin Antibody NB100-1965 (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 [NB100-1965] - A dilution of 1/2000 used in TBS with 0.05% Tween-20. Bands visualized by ECL using goat anti-rabbit HRP at 1:2000.	
Immunohistochemistry: Calnexin Antibody [NB100-1965] - IHC analysis of CANX in mouse prostate using DAB with hematoxylin counterstain.	
Flow Cytometry: Calnexin Antibody [NB100-1965] - An intracellular stain was performed on Daudi cells with Calnexin Antibody NB100-1965 (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 [NB100-1965] - An intracellular stain was performed on NIH3T3 cells with Calnexin Antibody NB100-1965 (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 [NB100-1965] - Simple Western lane view shows a specific band for Calnexin in 1.0 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	
Calnexin immunostaining in the anterior (AI) and mid (MI) intestine of ctrl (control zebrafish), D.I.O. (diet-induced obesity zebrafish), D.I.O. flw 3,5-T2 (D.I.O. zebrafish followed by 3,5-T2), D.I.O. with 3,5-T2 (D.I.O. zebrafish treated with 3,5-T2). (A) AI of ctrl zebrafish (B) AI of D.I.O. (C) AI of D.I.O. flw 3,5-T2. (D) AI of D.I.O. with 3,5-T2. (E) MI of ctrl zebrafish. (F) MI of D.I.O. (G) MI of D.I.O. flw 3,5-T2. and (H) MI of D.I.O. with 3,5-T2. The arrows indicate calnexin immunoexpression in the enteroendocrine and goblet cells. (I,J) Bar graphs showing calnexin optical density (O.D.) in the (I) AI and (J) MI of ctrl, D.I.O., D.I.O. flw 3,5-T2 and D.I.O. with 3,5-T2. Data are expressed as mean +/- SE. ** $p < 0.001$, * $p < 0.05$ compared to the control group. $p < 0.05$ compared to D.I.O. Scale bar: 100 um in the low magnification and 50 um in the higher magnification in the boxes. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32635261), licensed under a CC-BY licence.	
(a1) Q-PCR assay of TLR4 mRNA expression levels after transfecting JJN3 cell line with TLR4 RNAi oligonucleotides or a non-targeting pool (siCtrl) for 48 h. (a2) Representative immunoblotting analyses of protein samples probed with an antibody against TLR4 after TLR4 RNAi for 48 h. (b1) % cell viability and (b2) % proliferation of JJN3 cells after TLR4 RNAi for 48 h. (c,d1) Q-PCR expression analyses of TLR4 mRNA expression levels after transfecting H929 (c) and U266 (d1) cell lines with the pCMV6-TLR4 construct or a pCMV6 empty vector for 48 h. (d2) Immunobloting analyses of U266 cells transfected with pCMV6-TLR4 or pCMV6 vector for 48 h; protein samples were probed with an antibody against TLR4. (e1) % cell viability and (e2) % proliferation of H929 and U266 cells after transfection with the pCMV6-TLR4 construct or with the empty pCMV6 vector for 48 h. β-ACTIN probing and β-ACTIN mRNA expression were used as reference for total protein and mRNA input, respectively. Cells transfected with the pCMV6-TLR4 construct are labelled as TLR4 OE (TLR4 overexpression), whereas cells transfected with the pCMV6 empty vector are labelled as Con (control). Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/30824741), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Publications

Elsabrouty R, Jo Y, Hwang S et al. Type 1 polyisoprenoid diphosphate phosphatase modulates geranylgeranyl-mediated control of HMG CoA reductase and UBIAD1 eLife 2021-11-29 [PMID: 34842525]

Sung SE, Seo MS, Kang KK et al. Isolation and Characterization of Extracellular Vesicle from Mesenchymal Stem Cells of the Epidural Fat of the Spine Asian Spine Journal 2022-04-30 [PMID: 34461688] (B/N, WB)

Xu Y, Zhu Y, Hu S et al. Hepatocyte Nuclear Factor 4? Prevents the Steatosis-to-NASH Progression by Regulating p53 and Bile Acid Signaling (in mice) Hepatology 2021-06-01 [PMID: 33098092] (WB)

Zar M, Campodonico J, Cosentino N et al. Plasma Exosome Profile in ST-Elevation Myocardial Infarction Patients with and without Out-of-Hospital Cardiac Arrest International Journal of Molecular Sciences 2021-07-28 [PMID: 34360827] (WB)

Zhu Y, Hu S, Pan X et al. Hepatocyte Sirtuin 6 Protects against Atherosclerosis and Steatohepatitis by Regulating Lipid Homeostasis Cells 2023-08-05 [PMID: 37566087] (WB)

Johnson B, Jun D, DeBose-Boyd R Membrane Topology of UbiA Prenyltransferase Domain-Containing Protein-1 (UBIAD1), a Novel Regulator of Cholesterol Homeostasis bioRxiv 2023-03-06 (WB)

Adamo G, Santonicola P, Picciotto S et al. Microalgae as a novel biofactory for biocompatible and bioactive extracellular vesicles bioRxiv 2023-04-04 (ICC/IF, Human)

Turner N, Abeysinghe P, Sadowski P, Mitchell M Cross-species proteomic and microRNA comparison of extracellular vesicles in human milk, cow's milk, and infant formula products: moving towards next generation infant formula products bioRxiv 2023-02-24 (WB, Human, Bovine)

Goudsmit, C, da Veiga Leprevost, F, et al. Differences in Extracellular Vesicle Protein Cargo Are Dependent on Head and Neck Squamous Cell Carcinoma Cell of Origin and Human Papillomavirus Status. Cancers (Basel) [PMID: 34359613] (Simple Western, Human)

Chen H, Qi X, Faulkner RA et al. Regulated degradation of HMG CoA reductase requires conformational changes in sterol-sensing domain Nature communications 2022-07-25 [PMID: 35879350] (WB, Chinese Hamster)

Sandona M, Consalvi S, et al. HDAC inhibitors tune miRNAs in extracellular vesicles of dystrophic muscle-resident mesenchymal cells. EMBO Rep 2020-09-03 [PMID: 32754983] (WB, Mouse)

Xu Y, Hu S, Jadhav K et al. Hepatocytic Activating Transcription Factor 3 Protects Against Steatohepatitis Via Hepatocyte Nuclear Factor 4 alpha Diabetes 2021-09-02 [PMID: 34475098]

More publications at http://www.novusbio.com/NB100-1965

Procedures

Western Blot Protocol Specific for CANX antibody (NB100-1965)

1. Perform SDS-PAGE on samples to be analyzed, loading 40 ug of total protein per lane.
2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot.
5. Block the membrane using standard blocking buffer for at least 1 hour.
6. Wash the membrane in wash buffer three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate 1 hour at room temperature.
8. Wash the membrane in wash buffer three times for 10 minutes each.
9. Apply the diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer 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 manufacturers instructions.

Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

Immunohistochemistry-Paraffin protocol for Calnexin Antibody (NB100-1965)

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.

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

Immunocytochemistry/Immunofluorescence protocol for Calnexin Antibody (NB100-1965)

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

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

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Products Related to NB100-1965

HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control
NB100-1965B	Calnexin Antibody [Biotin]

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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