# Product Datasheet

## Calnexin Antibody - BSA Free
## NB100-1965SS

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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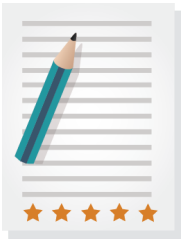
Updated 4/13/2025 v.20.1





# NB100-1965SS

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, 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.<br><br>In Simple Western only 10 - 15 uL of the recommended dilution is used per data point.<br>See Simple Western Antibody Database for Simple Western validation: Tested in HOK-16B, NOK-SI, UM-SCC-104, UM-SCC-105, UM-SCC-118, UM-SCC-17A, UM-SCC-38, UM-SCC-47, UM-SCC-92, UPCI-SCC-152; separated by Size; apparent MW was 115 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue. |

## Images

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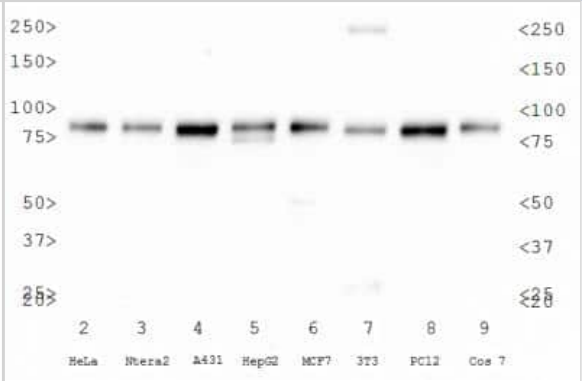
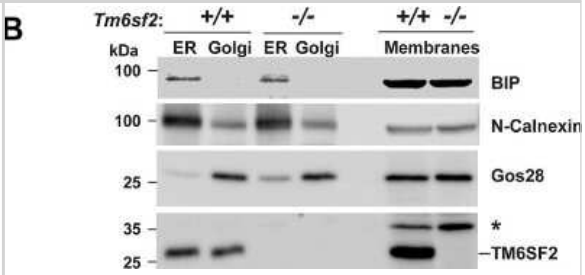
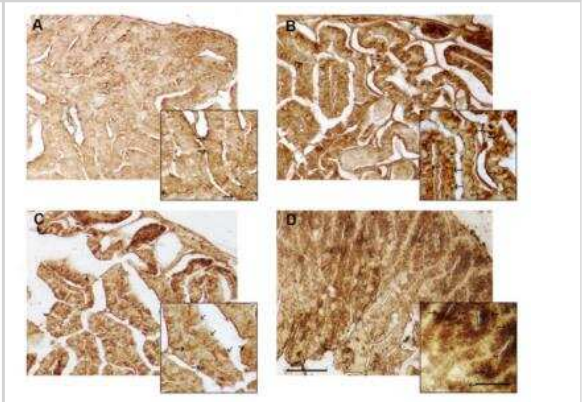
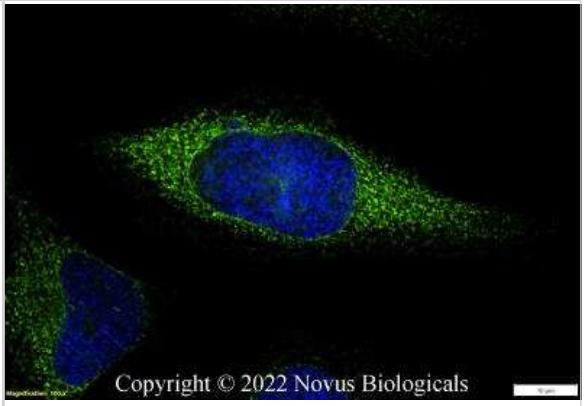
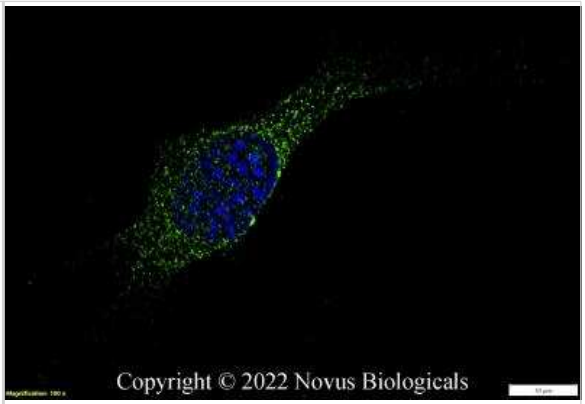
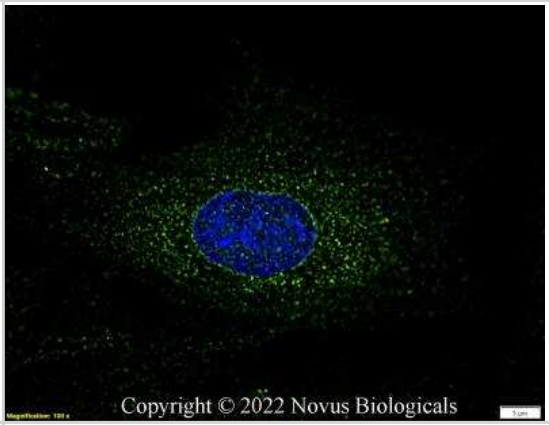
|---|---|
| Western Blot: Calnexin Antibody [NB100-1965] - WB analysis of CANX in cell lysates as noted. |   |
| Western Blot: Calnexin Antibody [NB100-1965] - Subcellular localization of TM6SF2.Immunoaffinity isolation of ER and Golgi complex from mouse liver. ER and Golgi fractions were prepared from 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. |   |

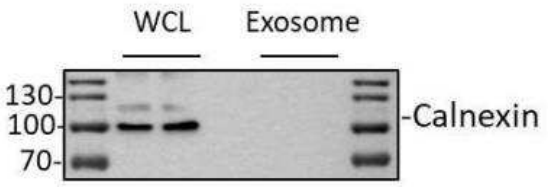
Immunocytochemistry/Immunofluorescence: Calnexin Antibody [NB100-1965] - NIH3T3 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with (NB100-1965) at 1 ug/ml overnight at 4C and detected with an anti-rabbit DyLight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



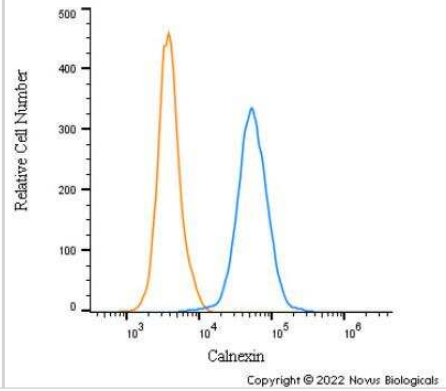

Immunocytochemistry/Immunofluorescence: Calnexin Antibody [NB100-1965] - Rat FR cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with Calnexin Antibody (NB100-1965) at 1 ug/ml overnight at 4C and detected with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



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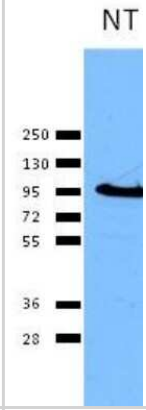
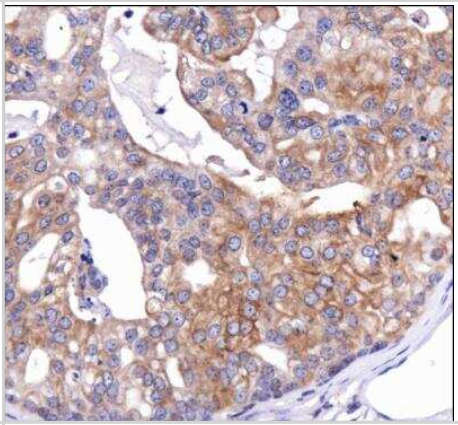
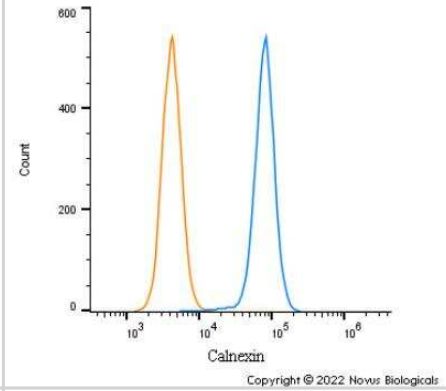
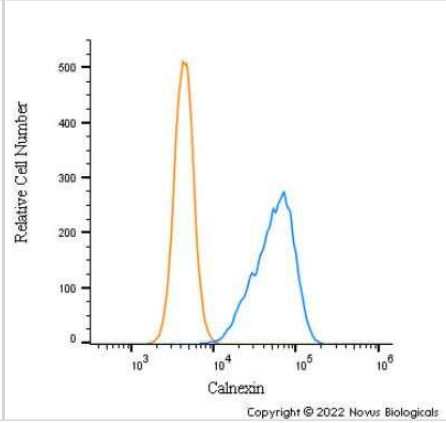
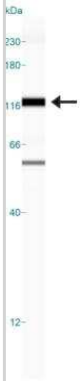
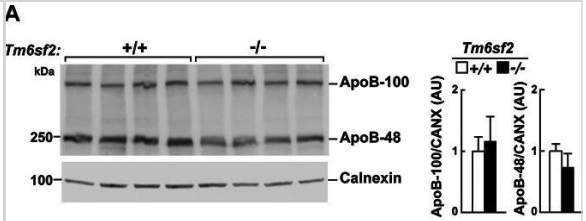
| Western Blot: Calnexin Antibody [NB100-1965] - A dilution of 1/2000 used in TBS with 0.05% Tween-20. Bands visualized by ECL using goat anti-rabbit HRP at 1:2000. |   |
| Immunohistochemistry: Calnexin Antibody [NB100-1965] - IHC analysis of CANX in mouse prostate using DAB with hematoxylin counterstain. |  |
| 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. |   |
| Western Blot: Calnexin Antibody - BSA Free [NB100-1965] - Hepatic apoB levels (A), liver function tests (B), & relative hepatic levels of selected transcripts encoding proteins involved in activation of fibrosis & ER stress (C) in male WT & Tm6sf2−/− mice described in Fig. 3A.A, immunoblotting analysis was performed on liver protein (50 µg) using a rabbit anti-mouse apoB polyclonal antibody (1:1,000; Abcam) & ECL (SuperSignal West Pico Kit, Thermo Scientific). The ECL signal was visualized using a LI-COR imager (Odyssey Fc imager) & analyzed using LI-COR Image Studio software. B, plasma levels of aspartate aminotransferase (AST) & alanine aminotransferase (ALT) in chow-fed male mice. C, RNA levels were detected using quantitative real-time PCR (qRT-PCR), normalized to levels of 36B4 & expressed relative to the levels in the WT animals (n = 5). COL1A1, collagen alpha-1(I) chain; ACTA2, actin, aortic smooth muscle; XBP1s/u, X-box-binding protein 1 spliced/unspliced; ATF4, activating transcription factor 4; EDEM, ER degradation enhancer, mannosidase α-like 1; CHOP(DDIT3), C/EBP-homologous protein. Values are means ± S.E. (error bars). *, p < 0.05. AU, arbitrary units. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/27013658), licensed under a CC-BY license. Not internally tested by Novus Biologicals. |   |

## Publications


Alahari S, Ausman J, Porter T, Park C et Al. Fibronectin and JMJD6 Signature in Circulating Placental Extracellular Vesicles for the Detection of Preeclampsia Endocrinology 2023-01-23 [PMID: 36683415]

Weesner JA, van de Vlekkert D, Fremuth LE, d'Azzo A. et Al. Protocol for the isolation and purification of endoplasmic reticulum-plasma membrane junctions from the mouse brain STAR Protoc 2024-08-10 [PMID: 39126654]

Long T, Li D, Vale G, Jiang Y et Al. Molecular insights into human phosphatidylserine synthase 1 reveal its inhibition promotes LDL uptake Cell 2024-08-29 [PMID: 39208797]

Galli J, Almiñana C, Wiesendanger M, Schuler G et Al. Bovine placental extracellular vesicles carry the fusogenic syncytin BERV-K1 Theriogenology 2024-04-28 [PMID: 38678697]

Pan X, Hu S, Xu Y et Al. Krüppel-like factor 10 protects against metabolic dysfunction-associated steatohepatitis by regulating HNF4?-mediated metabolic pathways Metabolism 2024-06-07 [PMID: 38582490]

Wu X, Quan M, Hadisurya M et Al. Monitoring drug metabolic pathways through extracellular vesicles in mouse plasma PNAS Nexus 2024-02-01 [PMID: 38312223]

Oliveira Silva, R;Counil, H;Rabanel, JM;Haddad, M;Zaouter, C;Ben Khedher, MR;Patten, SA;Ramassamy, C; Donepezil-Loaded Nanocarriers for the Treatment of Alzheimer's Disease: Superior Efficacy of Extracellular Vesicles Over Polymeric Nanoparticles International journal of nanomedicine 2024-02-01 [PMID: 38317848]

Turner NP, Abeysinghe P, Sadowski P, Mitchell MD Omics Analysis of Extracellular Vesicles Recovered from Infant Formula Products and Milk: Towards Personalized Infant Nutrition Molecular nutrition & food research 2023-08-10 [PMID: 37562982] (Western Blot, Bovine, Human)

Jason A. Weesner, Ida Annunziata, Diantha van de Vlekkert, Camenzind G. Robinson, Yvan Campos, Ashutosh Mishra, Leigh E. Fremuth, Elida Gomero, Huimin Hu, Alessandra d'Azzo Altered GM1 catabolism affects NMDAR-mediated Ca 2+ signaling at ER-PM junctions and increases synaptic spine formation in a GM1-gangliosidosis model Cell reports 2024-06-20 [PMID: 38630590]

Miira M. Klemetti, Ante B. V. Pettersson, Aafaque Ahmad Khan, Leonardo Ermini, Tyler R. Porter, Michael L. Litvack, Sruthi Alahari, Stacy Zamudio, Nicholas P. Illsley, Hannes Röst, Martin Post, Isabella Caniggia Lipid profile of circulating placental extracellular vesicles during pregnancy identifies foetal growth restriction risk Journal of Extracellular Vesicles 2024-02-14 [PMID: 38353485]

Sabrina Garbo, Daniel D'Andrea, Alessio Colantoni, Francesco Fiorentino, Antonello Mai, Andres Ramos, Gian Gaetano Tartaglia, Andrea Tancredi, Marco Tripodi, Cecilia Battistelli m6A modification inhibits miRNAs' intracellular function, favoring their extracellular export for intercellular communication. Cell reports 2024-07-01 [PMID: 38878288]

Raja Gopoju, Jiayou Wang, Xiaoli Pan, Shuwei Hu, Li Lin, Alyssa Clark, Yanyong Xu, Liya Yin, Xinwen Wang, Yanqiao Zhang Hepatic FOXA3 overexpression prevents Western diet–induced obesity and MASH through TGR5 Journal of Lipid Research 2024-03-04 [PMID: 38447926]


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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