Product Datasheet

Calnexin Antibody
NB100-1965SS

Unit Size: 0.025 ml

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

Publications: 4

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Updated 6/15/2014 v.20.1

NB100-1965SS

Calnexin Antibody

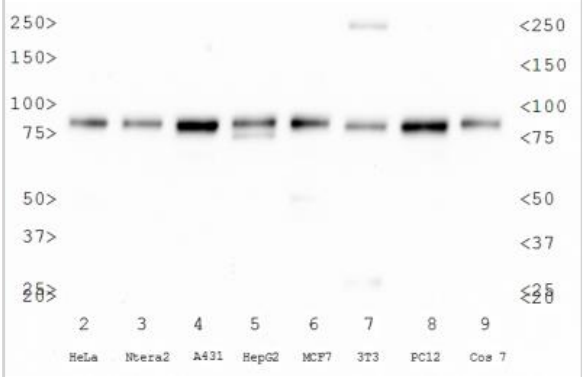
Product Information	
Unit Size	0.025 ml
Concentration	1 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.5% Sodium Azide
Purity	Immunogen affinity purified
Buffer	Tris-glycine, 150 mM NaCl
Target Molecular Weight	97 kDa

Product Description	
Host	Rabbit
Gene ID	821
Gene Symbol	CANX
Species	Human, Mouse, Rat, Avian, Bovine, Chicken, Drosophila, Guinea Pig, Porcine, Rabbit, Sheep, Xenopus, Zebrafish
Species Reactivity	Human, mouse, rat, Xenopus laevis, bovine, chicken, Drosophilia melanogaster, guinea pig, pig, quail, rabbit, zebrafish and sheep. 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]

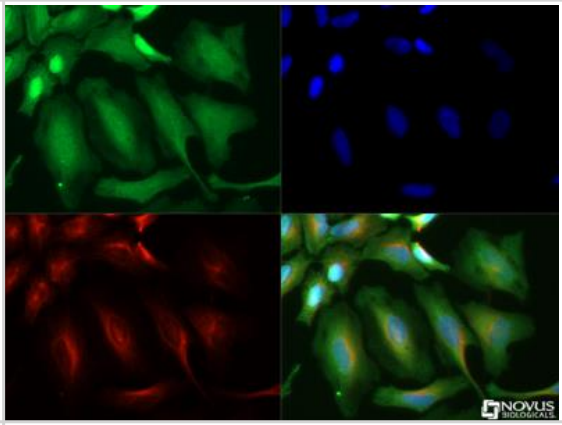
Product Application Details	
Applications	Western Blot, Simple Western, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Immunohistochemistry 1:40, Immunohistochemistry-Paraffin 1:40, Immunoprecipitation 1:100, Western Blot 2 ug/ml, Immunocytochemistry/Immunofluorescence 1:50, Simple Western 1:25
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 ~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.

Images

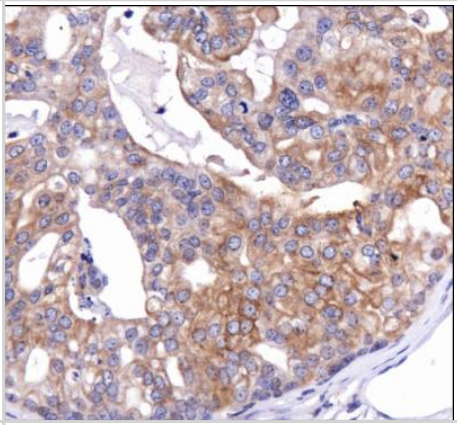
Western Blot: Calnexin Antibody [NB100-1965] - WB analysis of CANX in cell lysates as noted.



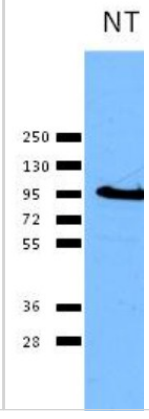
Immunocytochemistry/Immunofluorescence: Calnexin Antibody [NB100-1965] - Calnexin antibody (1:50) was tested in HeLa cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).



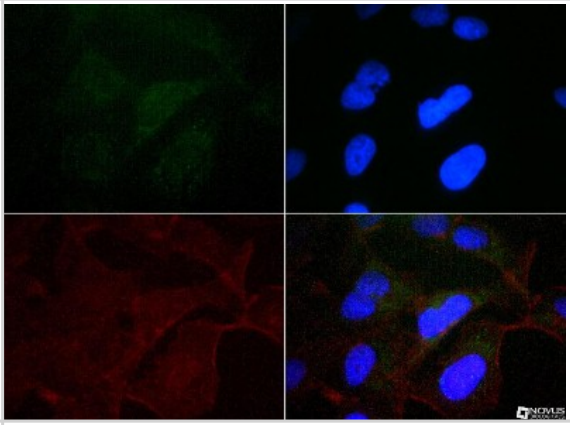
Immunohistochemistry: Calnexin Antibody [NB100-1965] - IHC analysis of CANX in mouse prostate using DAB with hematoxylin counterstain.



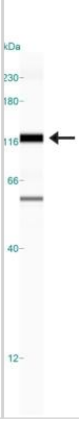
Western Blot: Calnexin Antibody [NB100-1965] - A dilution of 1/2000 used in TBS with 0.05% Tween-20. Bands visualized by ECL using goat anti-rabbit HRP at 1:2000.



Immunocytochemistry/Immunofluorescence: Calnexin Antibody [NB100-1965] - Antibody was tested at 1:50 in HeLa cells with FITC (green). Nuclei and actin were counterstained with DAPI (blue) and Phalloidin (red).



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.



Publications

Riemersma M, Sandrock J, Boltje TJ et al. Disease mutations in CMP-sialic acid transporter SLC35A1 result in abnormal alpha-dystroglycan O-mannosylation, independent from sialic acid. Hum. Mol. Genet. 2014 Dec 30 [PMID: 25552652] (WB, Human)

Saeki T, Sato K, Ito S et al. Importance of uncharged polar residues and proline in the proximal two-thirds (Pro107-Ser128) of the highly conserved region of mouse ileal Na+-dependent bile acid transporter, Slc10a2, in transport activity and cellular expression. BMC Physiol 2013 Feb 4 [PMID: 23374508] (WB, Porcine)

Yuen MY, Webb SE, Chan CM et al. Characterization of Ca(2+) signaling in the external yolk syncytial layer during the late blastula and early gastrula periods of zebrafish development Biochim Biophys Acta 2012 Nov 8 [PMID: 23142640] (IHC, ICC/IF, Zebrafish)

Tian E, Hoffman MP, Ten Hagen KG. O-glycosylation modulates integrin and FGF signalling by influencing the secretion of basement membrane components. Nat Commun 3:869. 2012 May 29. [PMID: 22643896] (ICC/IF, Mouse)

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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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 guarantee, please visit www.novusbio.com/guarantee.