Product Datasheet

Calnexin Antibody (IE2.1C12) - BSA Free
NBP2-36571

Unit Size: 0.1 mg

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

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Publications: 2

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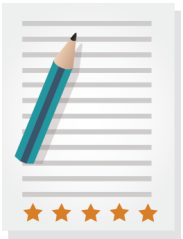
Updated 10/23/2024 v.20.1



NBP2-36571

Calnexin Antibody (IE2.1C12) - BSA Free

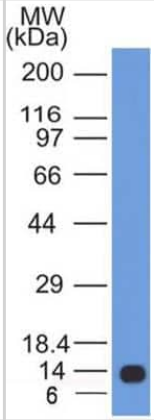
Product Information	
Unit Size	0.1 mg
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	IE2.1C12
Preservative	0.05% Sodium Azide
Isotype	IgG2b Kappa
Purity	Protein G purified
Buffer	PBS

Product Description	
Host	Mouse
Gene ID	821
Gene Symbol	CANX
Species	Human
Marker	Endoplasmic Reticulum Membrane Marker
Immunogen	Partial recombinant human Calnexin protein (between amino acids 1-300) [UniProt# P27824]

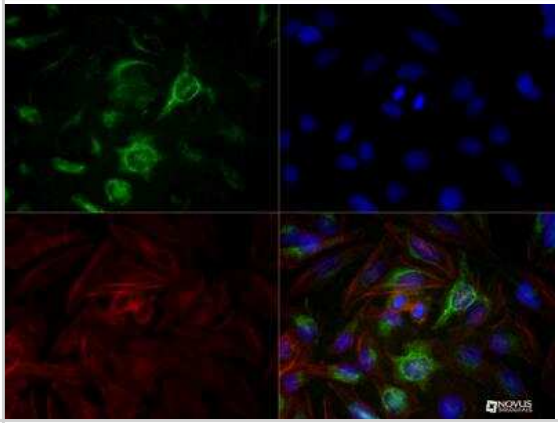
Product Application Details	
Applications	Western Blot, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot, Flow Cytometry, Immunohistochemistry 7 ug/ml, Immunocytochemistry/ Immunofluorescence 10 ug/ml, Immunohistochemistry-Paraffin 7 ug/ml, Flow (Intracellular) 5 ug/million cells

Images

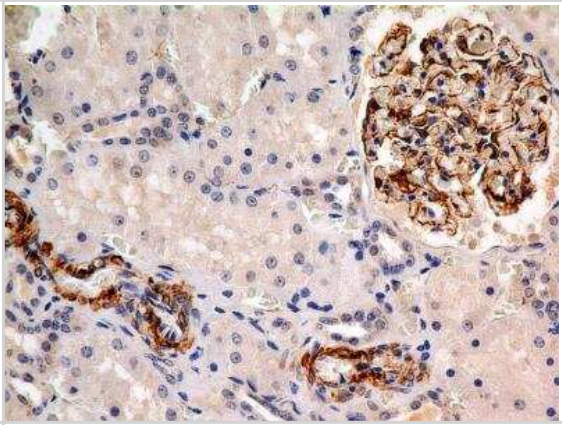
Western Blot: Calnexin Antibody (IE2.1C12) [NBP2-36571] - Analysis of 11 kDa Partial Recombinant Human Calnexin protein with Calnexin antibody (clone IE2.1C12) at 0.5 ug/ml concentration.



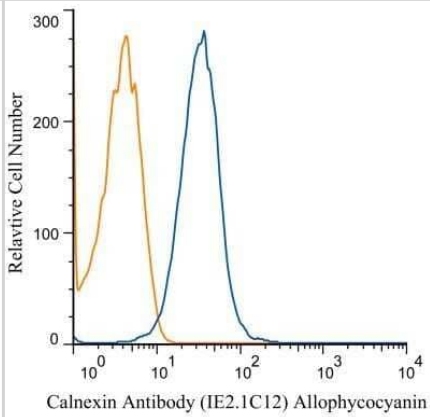
Immunocytochemistry/Immunofluorescence: Calnexin Antibody (IE2.1C12) [NBP2-36571] - Calnexin (1E2.1C12) antibody was tested in Hela cells with DyLight488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and DyLight 550 (red).



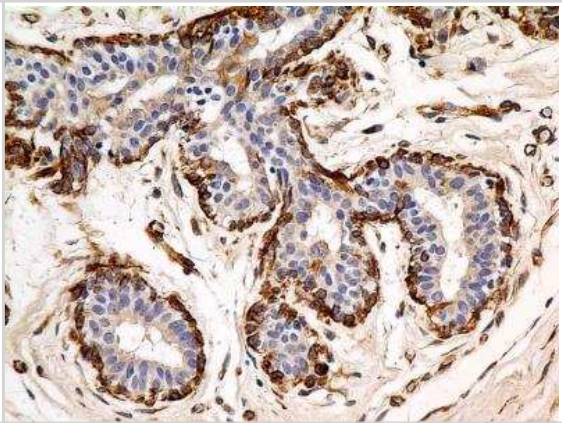
Immunohistochemistry-Paraffin: Calnexin Antibody (IE2.1C12) [NBP2-36571] - Analysis of FFPE tissue section of normal human kidney using mouse monoclonal Calnexin antibody (clone IE2.1C12) at 7 ug/ml concentration. The cells of Glomeruli developed strong cytoplasmic staining.

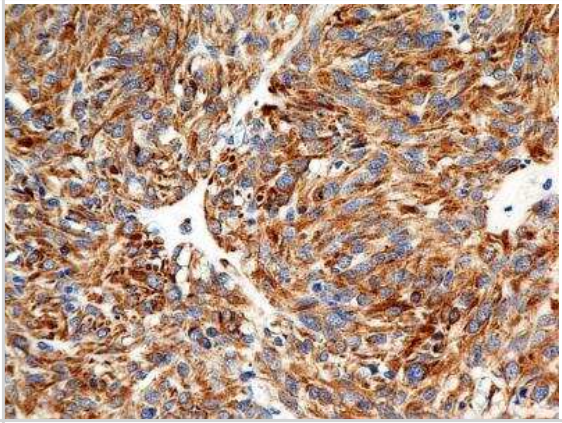
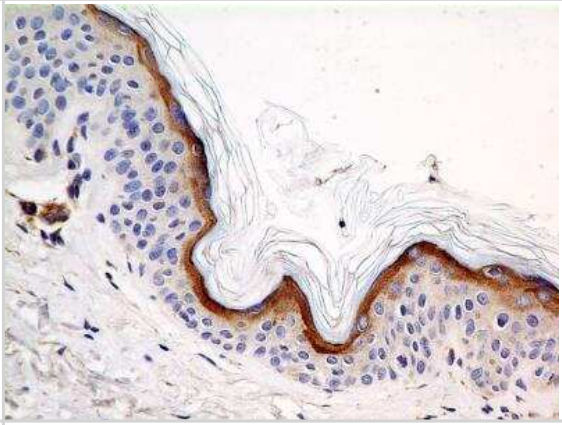
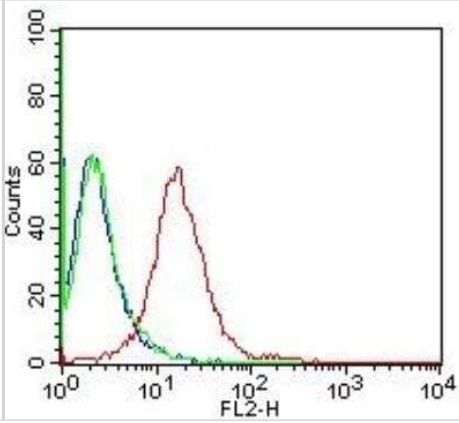


Flow Cytometry: Calnexin Antibody (IE2.1C12) [NBP2-36571] - Analysis of Allophycocyanin conjugate of NBP2-36571. An intracellular stain was performed on Jurkat cells with Calnexin antibody (IE2.1C12) NBP2-36571APC (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeablized with 0.1% saponin.

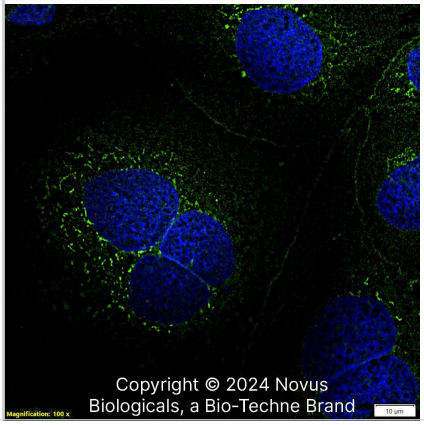


Immunohistochemistry-Paraffin: Calnexin Antibody (IE2.1C12) [NBP2-36571] - Analysis of FFPE tissue section of normal human breast using mouse monoclonal Calnexin antibody (clone IE2.1C12) at 7 ug/ml concentration. The myoepithelial cells around the lobules depicted a very strong cytoplasmic staining.



Immunohistochemistry-Paraffin: Calnexin Antibody (IE2.1C12) [NBP2-36571] - Analysis of FFPE tissue section of malignant stromal tumor of the human small bowel using mouse monoclonal Calnexin antibody (clone IE2.1C12) at 7 ug/ml concentration. The cancer cells showed a very strong cytoplasmic reactivity for Calnexin.	
Immunohistochemistry-Paraffin: Calnexin Antibody (IE2.1C12) [NBP2-36571] - Analysis of FFPE tissue section of human placenta using mouse monoclonal Calnexin antibody (clone IE2.1C12) at 7 ug/ml concentration.	
Immunohistochemistry-Paraffin: Calnexin Antibody (IE2.1C12) [NBP2-36571] - Analysis of FFPE tissue section of human skin using mouse monoclonal Calnexin antibody (clone IE2.1C12) at 7 ug/ml concentration. The outermost keratinocytes layer of the epidermis showed cytoplasmic positivity for Calnexin protein.	
Flow (Intracellular): Calnexin Antibody (IE2.1C12) [NBP2-36571] - Intracellular staining of human Calnexin in Flow cytometry using 5.0 ug of antibody per 1 million cells. Isotype control was mouse IgG2b kappa.	

Calnexin (IE2.1C12) was detected in immersion fixed Caco-2 human colorectal adenocarcinoma cell line using Mouse anti-Calnexin (IE2.1C12) Protein G Purified Monoclonal Antibody conjugated to Alexa Fluor® 488 (Catalog # NBP2-36571AF488) (green) at 10 µg/mL overnight at 4C. Cells were counterstained with DAPI (dark blue). Cells were imaged using a 100X objective and digitally deconvolved.



Publications

Munson PB, Hall EM, Farina NH et al. Exosomal miR-16-5p as a target for malignant mesothelioma Sci Rep 2019-08-12 [PMID: 31406207] (WB, Human)

MacPherson Maximilian, Westbom Catherine, Kogan Helen, Shukla Arti Actin polymerization plays a significant role in asbestos-induced inflammasome activation in mesothelial cells in vitro. Histochem Cell Biol. 2016-12-24 [PMID: 28013367] (ICC/IF, Human)

Procedures

Western Blot protocol for Calnexin Antibody (NBP2-36571)

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 25 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 anti-Calnexin 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%.

Immunocytochemistry/Immunofluorescence protocol for Calnexin Antibody (NBP2-36571)

Immunocytochemistry Protocol

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.

Immunohistochemistry-Paraffin protocol for Calnexin Antibody (NBP2-36571)

1. Deparaffinize the tissue sections by immersing the slides in Xylene with two changes for 10 min each. Sections should not get dried at any stage from this point.
2. Rehydrate the tissue sections by immersing the slides in decreasing grades of ethanol as follows:
 a. Immerse in 100% ethanol with 2 changes for 5 minutes each
 b. Immerse in 95% ethanol with 2 changes for 5 minutes each
 c. Immerse in 90% ethanol for 5 minutes
 d. Immerse in 70% ethanol for 5 minutes
 e. Immerse in 50% ethanol for 5 minutes
 f. Immerse in distilled water for 5 minutes
3. Antigen Retrieval (Microwave Method):
 a. Immerse the slides in a microwave compatible tray containing 10 mM Sodium Citrate buffer (pH 6.0) with 0.05% Tween 20.
 b. Boil the slides and maintain the sub-boiling temperature for 5 minutes in the microwave. Thereafter, take out the tray very carefully and cool it at room temperature (RT) for about 30 minutes.
 c. Wash the slides 3 times, 3 minutes each by immersing them in TBST (Tris Buffered Saline having 0.05% Tween 20).
4. Quenching of Endogenous Peroxidase:
 a. Incubate the slides in 3% hydrogen peroxide prepared in methanol for 15 minutes (at RT, in dark conditions).
 b. Wash the slides in TBST 3 times, 3 minutes each.
5. Protein Blocking:
 a. Incubate the sections with background sniper solution at RT for 15 minutes (Biocare Medicals, USA).
 b. Wash the sections 3 times, 3 min each by immersing the slides in TBST.
6. Primary Antibody:
 a. Dilute the primary antibody at 5ug/ml concentration using PBS as a diluent.
 b. Incubate the sections with diluted primary antibody for 90 minutes at RT in a humidified chamber.
 c. Thereafter, wash the slides 4 times, 5 minutes each with TBST.
7. Probe (Secondary Reagent):
 a. Incubate with MACH 1 Mouse probe for 15 minutes at RT.
 b. Incubate for 30 min at room temperature with HRP-Polymer (Biocare Medical, USA).
 c. Wash the slides with TBST 4 times, 5 minutes each
8. Chromogen:
 a. Mix 32ul of DAB Chromogen with 1 ml of DAB substrate buffer (Biocare Medical, USA).
 b. Apply 200ul DAB mixture/section and incubate at RT in dark conditions (few seconds - 5 minutes).
 c. As soon as an appropriate color develops, rinse the slides with deionized water (2-3 brief rinses).
9. Counter stain:
 a. Counter stain with Hematoxylin for 30 seconds (Vector Labs, USA).
 b. Wash in deionized water for 1-2 minutes to clear the extra stain.
 c. Incubate the slides in bluing solution or Scott's water twice for 2 minutes each time.
10. Dehydrate the sections in increasing grades of alcohols:
 a. 50% alcohol for 1 minute
 b. 70% for 1 minute
 c. 90% for 1 minute
 d. 95% for 1 minute
 e. 100% for 1 minute
 f. Xylene with 2 changes for 2 minutes each
11. Mount with DPX mount and cover-slip glass (Fisher Scientific, USA), carefully not allowing any air bubbles to enter.

NOTE:- This protocol is provided as a reference tool only. Depending upon the type of tissues /tissue processing and reagents employed, the end user will need to optimize the final conditions for achieving an expected staining.

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Products Related to NBP2-36571

NBP1-30235	Recombinant Human Calnexin Protein
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-43317-0.5mg	Mouse IgG2b Kappa Light Chain Isotype Control (MG2b)

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