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Product data sheet

Calnexin Polyclonal Antibody

Catalog Number PA5-19169

| Details | | Species Reactivity | |
|--------------------|---|--|--------------------------|
| Size | 100 µg | Species reactivity | Human |
| Host/Isotope | Goat / IgG | Published species | Human, Not Applicable |
| Class | Polyclonal | Tested Applications | Dilution * |
| Туре | Antibody | Immunohistochemistry (Paraffin) (IHC (P)) | 6 μg/mL |
| | Synthetic peptide sequence (SKTPELNLDQFHDKT) | Western Blot (WB) | 0.1-0.5 μg/mL |
| Immunogen | corresponding to the internal amino acids of CANX | Immunocytochemistry (ICC/IF) | 10 µg/ml |
| Conjugate | Unconjugated | Published Applications | |
| Form | Liquid | Western Blot (WB) | See 1 publications below |
| Concentration | 0.5 mg/mL | * Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls. | |
| Purification | Ammonium sulfate precipitation | | |
| Storage buffer | TBS, pH 7.3, with 0.5% BSA | | |
| Contains | 0.02% sodium azide | | |
| Storage Conditions | -20° C, Avoid Freeze/Thaw Cycles | | |

Product specific information

This antibody is predicted to react with bovine, canine, porcine and rat based on sequence homology. This antibody is tested in Peptide ELISA: antibody detection limit dilution 128,000.

Background/Target Information

Calnexin, also referred to as IP90, p88 and p90, is an approximately 90 kDa integral membrane protein of the endoplasmic reticulum (ER). Many resident ER proteins act as molecular chaperones and participate in the proper folding of polypeptides and their assembly into multisubunit proteins. Studies indicate that calnexin associates with the major histocompatibility complex (MHC) class I heavy chains, partial complexes of the T cell receptor and B cell membrane immunoglobulin, but not with completed receptor complexes. It has been shown that calnexin is a chaperone that retains incompletely or improperly folded proteins in the ER. The sequence Lys-Asp-Glu-Leu (KDEL) or a closely related sequence, is present at the carboxy-terminus of soluble ER resident proteins such as GRP78 and GRP94 and protein disulfide isomerase. Integral membrane ER resident proteins, like calnexin, often lack this KDEL sequence but contain positively charged cytosolic residues that ensure ER retention. Calnexin contains a large ER luminal domain (461 amino acids), a transmembrane segment (22 amino acids), and a cytoplasmic tail (89 amino acids).

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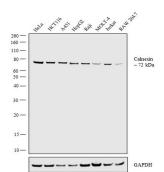
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Product Images For Calnexin Polyclonal Antibody



Calnexin Antibody (PA5-19169) in WB

Western blot analysis was performed on whole cell extracts (30 µg lysate) of HeLa (Lane 1), HCT116 (Lane 2), A431 (Lane 3), HepG2 (Lane 4), Raji (Lane 5), MOLT-4 (Lane 6), Jurkat (Lane 7) and RAW 264.7 (Lane 8). The blot was probed with anti-Calnexin Polyclonal Antibody (Product # PA5-19169, 1:3000 dilution) and detected by chemiluminescence using Rabbit anti-Goat IgG Heavy Chain Superclonal[™] Secondary Antibody, HRP conjugate (Product # A27014, 0.25 µg/mL, 1:4000 dilution). A 72 kDa band corresponding to Calnexin was observed across all the cell lines tested.

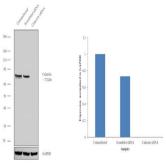
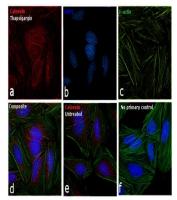


Fig. i

Calnexin Antibody (PA5-19169)

Antibody specificity was demonstrated by siRNA mediated knockdown of target protein. HeLa cells were transfected with Calnexin siRNA and reduction of signal was observed in Western Blot using Calnexin Polyclonal Antibody (Product # PA5-19169). {KD}



Calnexin Antibody (PA5-19169)

Altered expression of target protein upon cell treatment demonstrates antibody specificity. Immunofluorescence analysis of Calnexin using Anti-calnexin Rabbit Polyclonal Antibody (Product # PA5-19169) shows increased expression of proteins in HeLa cell line upon treatment with Thapsigargin. {TM}



Calnexin Antibody (PA5-19169) in WB

Western blot analysis of Calnexin using Calnexin Polyclonal Antibody (Product # PA5-19169) (0.1 µg/mL) in staining of CaCo-2 (A) and (1 µg/mL) NIH3T3 (B) cell lysate (35 µg protein in RIPA buffer). Detected by chemiluminescence.

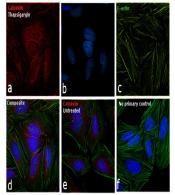
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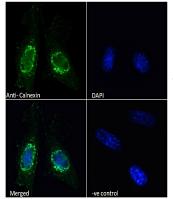
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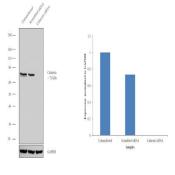
Calnexin Antibody (PA5-19169) in ICC/IF

Immunofluorescence analysis of Calnexin was performed using 70% confluent log phase HeLa cells treated with Thapsigargin (1uM for 24hrs). The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton[™] X-100 for 15 minutes, and blocked with 1% BSA for 1 hour at room temperature. The cells were labeled with Calnexin Polyclonal Antibody (Product # PA5-19169) at 1:200 dilution in 0.1% BSA, incubated at 4 degree celsius overnight and then labeled with Rabbit anti-Goat IgG (H+L) Superclonal[™] Secondary Antibody, Alexa Fluor 594 conjugate (Product # A27016) at a dilution of 1:2000 for 45 minutes at room temperature (Panel a: red). Nuclei (Panel b: blue) were stained with ProLong[™] Diamond Antifade Mountant with DAPI (Product # P36962). F-actin (Panel c: green) was stained with Alexa Fluor[™] 488 Phalloidin (Product # A12379, 1:300). Panel d represents the merged image showing Calnexin in the ER and cytoplasm. Panel e represents the untreated cells showing lower expression levels. Panel f represents control cells with no primary antibody to assess background. The images were captured at 60X magnification.



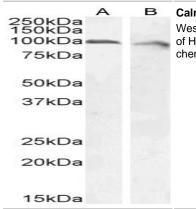
Calnexin Antibody (PA5-19169) in ICC/IF

Immunocytochemistry analysis of Calnexin using Calnexin Polyclonal Antibody (Product # PA5-19169) in paraformaldehyde fixed NIH3T3 cells, permeabilized with 0.15% Triton. Primary incubation 1hr (10 µg/mL) followed by Alexa Fluor 488 secondary antibody (2 µg/mL), showing endoplasmic reticulum staining. The nuclear stain is DAPI (blue). Negative control: Unimmunized goat IgG (10 µg/mL) followed by Alexa Fluor 488 secondary antibody (2 µg/mL).



Calnexin Antibody (PA5-19169) in WB

Knockdown of Calnexin was achieved by transfecting HeLa cells with Calnexin specific siRNAs (Silencer® select Product # s2376). Western blot analysis (Fig. a) was performed using whole cell extracts from the Calnexin knockdown cells (lane 3), non-specific scrambled siRNA transfected cells (lane 2) and untransfected cells (lane 1). The blot was probed with Calnexin Polyclonal Antibody (Product # PA5-19169, 1 µg/mL) and Goat anti-Rabbit IgG (Heavy Chain) Superclonal[™] Secondary Antibody, HRP conjugate (Product # A27036, 0.25 µg/mL, 1:4,000 dilution). Densitometric analysis of this western blot is shown in histogram (Fig. b). Decrease in signal upon siRNA mediated knock down confirms that antibody is specific to Calnexin.



Calnexin Antibody (PA5-19169) in WB

Western blot analysis of Calnexin using Calnexin Polyclonal Antibody (Product # PA5-19169) (0.1 µg/mL) in staining of Human Cerebellum (A) and Colorectal cancer (B) lysate (35 µg protein in RIPA buffer). Detected by chemiluminescence.

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| PubMed References For (| Calnexin Polyclonal Antibody |
|---------------------------|---|
| 1 Western Blot References | |
| Species / Dilution | Summary |
| | PA5-19169 was used in Western Blotting to investigate if targeting of G3BP1 weakened the stress granule-specific interaction network, resulting in granule disassembly. |
| Human / Not Cited | Science (New York, N.Y.) (2021; 372:) "Ubiquitination of G3BP1 mediates stress granule disassembly in a context-specific manner." Author(s):Gwon Y,Maxwell BA,Kolaitis RM,Zhang P,Kim HJ,Taylor JP PubMed Article URL:http://dx.doi.org/10.1126/science.abf6548 |

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