

POLYCLONAL ANTIBODY

ER Marker

Anti-Calnexin pAb

Code No.
PM060

Quantity
100 µL

Form
Affinity Purified

BACKGROUND: The endoplasmic reticulum (ER) is a eukaryotic organelle, which serves many general functions, including the facilitation of protein folding. Calnexin is a 90 kDa integral membrane protein of the ER. Calnexin is one of the chaperone proteins, which play a major role in the quality control of the ER by the retention of incorrectly folded proteins.

SOURCE: This antibody was purified from rabbit serum using affinity column. The rabbit was immunized with the synthetic peptide corresponding to N-terminus of human Calnexin.

FORMULATION: 100 µL volume of PBS containing 50% glycerol, pH 7.2. Contains no preservative.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with human Calnexin for Western blotting, Immunoprecipitation and Immunocytochemistry.

APPLICATIONS:

Western blotting; 1:1,000

Immunoprecipitation; 2 µL/300 µL of cell extract from 1×10^7 cells

Immunohistochemistry; Not tested

Immunocytochemistry; 1:1,000

Flow cytometry; Not tested

Detailed procedures are provided in the following **PROTOCOLS**.

SPECIES CROSS REACTIVITY:

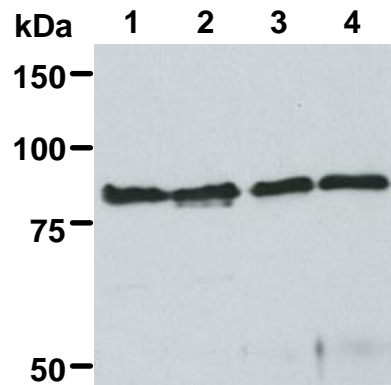
Species	Human	Mouse	Rat
Cells	HeLa, 293T, A549, Jurkat	NIH/3T3	Not Tested
Reactivity on WB	+	-	

REFERENCES:

- 1) Kleizen, B., and Braakman, I., *Curr. Opin. Cell Biol.* **16**, 343-349 (2004)
- 2) David, V., et al., *J. Biol. Chem.* **268**, 9585-9592 (1993)

INTENDED USE:

For Research Use Only. Not for use in diagnostic procedures.



Western blotting analysis of Calnexin expression on HeLa (1), 293T (2), A549 (3) and Jurkat (4) using PM060.

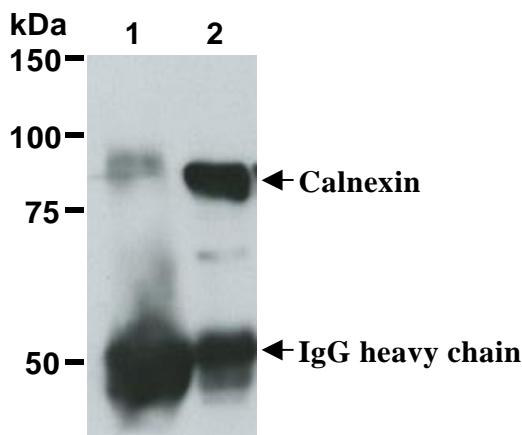
PROTOCOLS:

SDS-PAGE & Western blotting

- 1) Wash cells (approximately 1×10^7 cells) 3 times with PBS and resuspend them in 1 mL of Laemmli's sample buffer.
- 2) Boil the samples for 2 minutes and centrifuge. Load 10 µL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
- 3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). See the manufacturer's manual for precise transfer procedure.
- 4) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
- 5) Incubate the membrane for 1 hour at room temperature with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS**. (The concentration of antibody will depend on the conditions.)
- 6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
- 7) Incubate the membrane with 1:10,000 HRP-conjugated anti-rabbit IgG (MBL, code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 8) Wash the membrane with PBS-T (5 minutes x 3).
- 9) Wipe excess buffer off the membrane, and incubate membrane with an appropriate chemiluminescence reagent for 1 minute.

- 10) Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
- 11) Expose the membrane onto an X-ray film in a dark room for 3 minutes. Develop the film under usual settings. The conditions for exposure and development may vary.

(Positive controls for Western blotting; HeLa, 293T, A549, Jurkat)



Immunoprecipitation of Calnexin from HeLa with normal rabbit IgG (1) or PM060 (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with PM060.

Immunoprecipitation

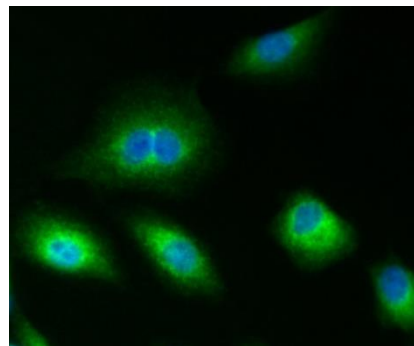
- 1) Wash cells (approximately 1×10^7 cells) 3 times with PBS and resuspend them in 1 mL of cold Lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40] containing protease inhibitors at appropriate concentrations. Incubate it at 4°C with rotating for 30 minutes; thereafter, briefly sonicate the mixture (up to 10 seconds).
- 2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
- 3) Add primary antibody as suggested in the **APPLICATIONS** into 300 μ L of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20 μ L of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
- 4) Centrifuge the tube at 2,500 x g for 10 seconds, and carefully discard the supernatant using a pipettor without disturbing the beads.
- 5) Resuspend the beads with cold Lysis buffer.
- 6) Centrifuge the tube at 2,500 x g for 10 seconds, and carefully discard the supernatant.
- 7) Repeat steps 5)-6) 3-5 times
- 8) Resuspend the beads in 20 μ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 μ L/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation; HeLa)

Immunocytochemistry

- 1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 1×10^4 cells for one slide, then incubate in a CO₂ incubator for one night.)
- 2) Wash the glass slide 2 times with PBS.
- 3) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde for 10 minutes at room temperature.
- 4) Wash the glass slide 3 times with PBS.
- 5) Immerse the slide in PBS containing 0.2% Triton X-100 for 10 minutes at room temperature.
- 6) Wash the glass slide 2 times with PBS.
- 7) Add the primary antibody diluted with 2% FCS/PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 1 hour at room temperature (Optimization of antibody concentration or incubation condition are recommended if necessary).
- 8) Wash the glass slide 2 times with PBS.
- 9) Add 100 μ L of 1:500 Alexa Fluor[®] 488 conjugated anti-rabbit IgG (Thermo Fisher Scientific, code no. A110374) diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
- 10) Wash the glass slide 3 times with PBS.
- 11) Counter stain with DAPI for 5 minutes at room temperature.
- 12) Wash the glass slide 2 times with PBS.
- 13) Wipe excess liquid off the slide but take care not to touch the cells. Never leave the cells to dry.
- 14) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; A549)



Immunocytochemical detection of Calnexin in A549 with PM060.

Green: Anti-Calnexin pAb (MBL, code no. PM060)
Blue: DAPI counter stain

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