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ER Marker

Anti-Calnexin mAb

Code No.	Clone	Subclass	Quantity	Concentration
M178-3MS	4F10	Mouse IgG2a κ	20 μ L	1 mg/mL

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 hybridoma (clone 4F10) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with C3H mouse lymphocyte immunized with synthetic peptide corresponding to N-terminus of human Calnexin.

FORMULATION: 20 μ g IgG in 20 μ L volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

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: 0.1 μ g/mL

Immunoprecipitation: 1 μ g/300 μ L of cell extract from 3×10^6 cells

Immunohistochemistry: Not tested

Immunocytochemistry: 1 μ g/mL

Flow cytometry: Not tested

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

SPECIES CROSS REACTIVITY:

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

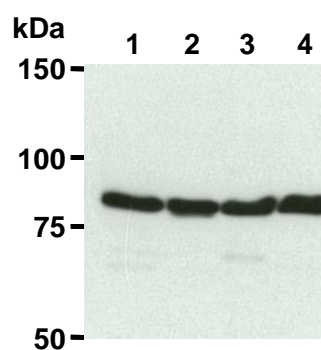
*Reactivity of clone 4F10 to monkey is not confirmed in our laboratory. However, it is reported that this clone reacts with COS-7 cells²⁾.

REFERENCES:

- 1) Matsuzaka, Y., *et al.*, *PLoS One* **11**, e0167811 (2016) [WB]
- 2) Oh-hashi, K., *et al.*, *FEBS Lett.* **585**, 2481-2487 (2011) [IC]
- 3) Kleizen, B. and Braakman, I., *Curr. Opin. Cell Biol.* **16**, 343-349 (2004)
- 4) David, V., *et al.*, *J. Biol. Chem.* **268**, 9585-9592 (1993)

INTENDED USE:

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



Western blot analysis of Calnexin in HeLa (1), 293T (2), A549 (3) and Jurkat (4) using M178-3.

PROTOCOLS:

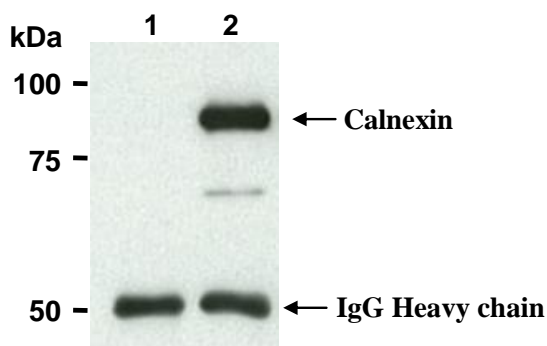
SDS-PAGE & Western Blotting

- 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 3 minutes and centrifuge. Load 20 μ L 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 5% 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 PBS (pH 7.2) containing 1% skimmed milk 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 Anti-IgG (Mouse)

pAb-HRP (MBL, code no. 330) 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 1 minute. Develop the film under usual settings. The conditions for exposure and development may vary.

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

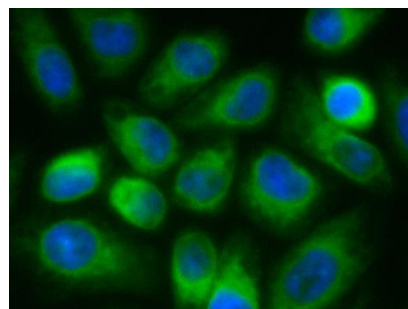


Immunoprecipitation of Calnexin from HeLa with mouse IgG2a isotype control, M076-3 (1) or M178-3 (2). After immunoprecipitated with the antibody, immunocomplexes were resolved on SDS-PAGE and immunoblotted with M178-3.

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 fresh tube.
- 3) Add primary antibody as suggested in the **APPLICATIONS** into 300 µL of the supernatant. Mix well and incubate with gentle agitation for 60-120 minutes at 4°C. Add 20 µL of 50% protein A agarose beads resuspended in the cold IP buffer (10 mM Tris-HCl pH 8.0, 500 mM NaCl, 0.1% NP-40). 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 discard the supernatant.
- 5) Resuspend the agarose with the cold Lysis buffer.
- 6) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
- 7) Repeat steps 5)-6) 2-4 times
- 8) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 20 µL/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation; HeLa)



Immunocytochemical detection of Calnexin in HeLa using M178-3.

Green: anti-Calnexin
Blue: DAPI

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 twice 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 twice with PBS.
- 7) Add the primary antibody diluted with PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 1 hour at room temperature (Optimization of antibody concentration or incubation condition is recommended if necessary).
- 8) Wash the glass slide twice with PBS.
- 9) Add 100 µL of 1:500 Alexa Fluor® 488 conjugated anti-mouse IgG (Thermo Fisher Scientific, code no. A-11001) 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 twice 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; HeLa)

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