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MONOCLONAL ANTIBODY **ER Marker**

Anti-Calnexin mAb

Code No.	Clone	Subclass	Quantity	Concentration
M178-3	4F10	Mouse IgG2a κ	100 µL	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: 100 µg IgG in 100 µL 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 µg/mL
Immunoprecipitation; 1 µg/300 µL of cell extract from 3 x 10^6 cells
Immunohistochemistry; Not tested
Immunocytochemistry; 1 µg/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 cells[2].

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)

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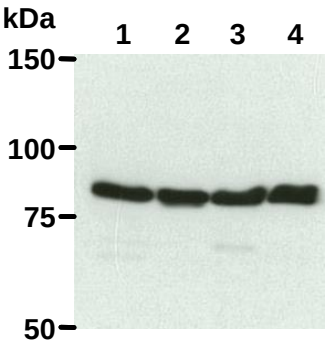


Western blot analysis of Calnexin in HeLa (1), 293T (2), A549 (3) and Jurkat (4) using M178-3.

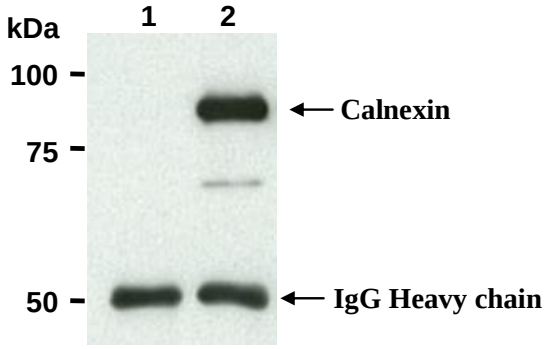
PROTOCOLS:

SDS-PAGE & Western blotting

1) Wash cells (approximately 1 x 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 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/cm^2 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 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 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)

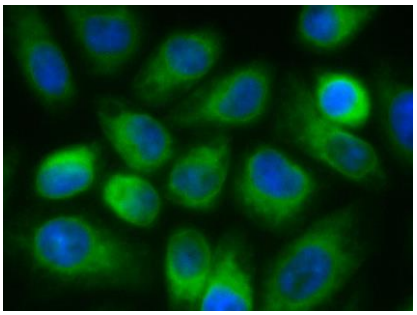


Immunoprecipitation of Calnexin from HeLa with mouse IgG2a isotype control, M076-3 (1) or M178-3 (2). *After immunoprecipitated with the antibody, immunocomplexes were resolved on SDS-PAGE and immunoblotted with M178-3.*

Immunoprecipitation

1) Wash cells (approximately 1 x 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 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 counter stain

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (For example, spread 1 x 10^4 cells for one slide, then incubate in a CO_2 incubator overnight.)
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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