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ER Marker

Anti-KDEL mAb

Code No.	Clone	Subclass	Quantity	Concentration
M181-3MS	1D5	Mouse IgG2a κ	20 µL	1 mg/mL

BACKGROUND: The KDEL (lys-asp-glu-leu) sequence is most common endoplasmic reticulum (ER) retention signal. The ER resident proteins, which should be located in the ER, have this retention motif, and this retention is mediated by KDEL receptor.

SOURCE: This antibody was purified from hybridoma (clone 1D5) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with C3H mouse lymphocyte immunized with KLH conjugated synthetic peptide CTGEEDTSEKDEL corresponding to the amino acid residues 644-655 of mouse GRP78.

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 proteins containing KDEL sequence on Western blotting and Immunocytochemistry. The reactivity to mouse, rat and hamster proteins containing KDEL sequence was confirmed by Western blotting.

APPLICATIONS:

Western blotting; 0.1 µg/mL
Bands may be detected at 48, 60, 78, 94 kDa.
Immunoprecipitation; Reference 4)
Immunohistochemistry; Reference 2) and 5)
Immunocytochemistry; 0.5 µg/mL
Flow cytometry; Not tested

Detailed procedure is provided in the following **PROTOCOLS**.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster	Others*
Cells	HeLa, 293T	NIH/3T3	Rat1	CHO	
Reactivity on WB	+	+	+	+	

*Reactivity of this clone to Drosophila Txnip/VDUP1 is described in the reference number 1).

INTENDED USE:

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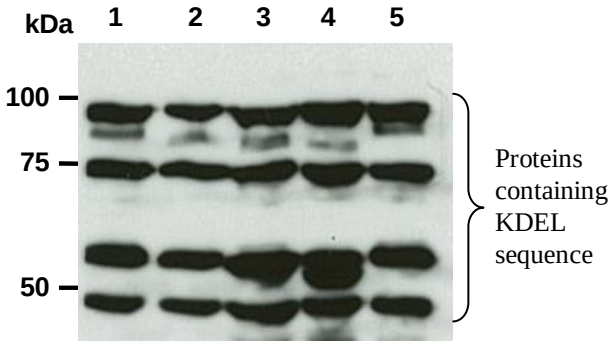
REFERENCES:

1) van de Hoef, D. L., *et al.*, *Development* **140**, 810-819 (2013) [IC]
2) Furuichi, T., *et al.*, *Mamm Genome.* **22**, 318-28 (2011) [IHC]
3) Zhang, B., *et al.*, *Blood* **118**, 3384-91 (2011) [IC]
4) Xiang, Y., *et al.*, *PLoS One* **5**, e13820 (2010) [WB, IP, IC]
5) Hino, S., *et al.*, *J. Bone Miner. Metab.* **28**, 131-138 (2010) [IHC]
6) Pelham, H. R., *EMBO J.* **7**, 913-918 (1988)
7) Munro, S., *et al.*, *Cell* **13**, 899-907 (1987)



Western blot analysis of proteins containing KDEL sequence in HeLa (1), 293T (2), NIH/3T3 (3), Rat1 (4) and CHO (5) using M181-3.

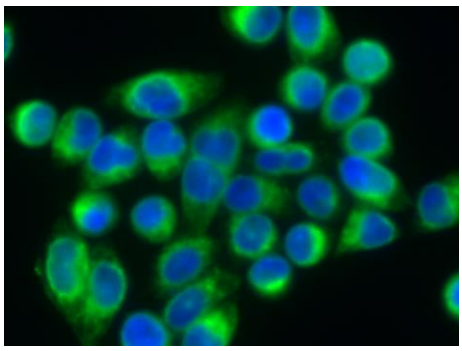
PROTOCOLS:

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 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/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 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 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 30 seconds. Develop the film under usual settings. The conditions for exposure and development may vary.

(Positive controls for Western blotting; HeLa, 293T, NIH/3T3, Rat1, CHO)



Immunocytochemical detection of proteins containing KDEL sequence in HeLa using M181-3.
Green: anti-KDEL
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 of HeLa cells for one slide, then incubate in a CO_2 incubator for one night.)
2) Wash the cells 3 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 twice with PBS.
7) Add the primary antibody diluted with 2% FCS/PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 60 minutes 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 twice 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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