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

POLYCLONAL ANTIBODY **ER Marker**

Anti-KDEL pAb

Code No.	Quantity	Form
PM059	100 µL	Affinity Purified

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. This antibody can specifically detect GRP78 (78 kDa protein) and GRP94 (94 kDa protein) including a KDEL sequence by WB.

SOURCE: This antibody was purified from rabbit serum using affinity column. The rabbit was immunized with the KLH conjugated synthetic peptide CTGEEDTSEKDEL corresponding to amino acid residues 644-654 from mouse GRP78.

FORMULATION: 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 GRP78 and GRP94 including KDEL sequence for Western blotting.

APPLICATIONS:
Western blotting; 1:500
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested*
*It is reported that this antibody can be used in Immunohistochemistry in the reference number 2-4).
Immunocytochemistry; 1:1,000
Flow cytometry; Not tested

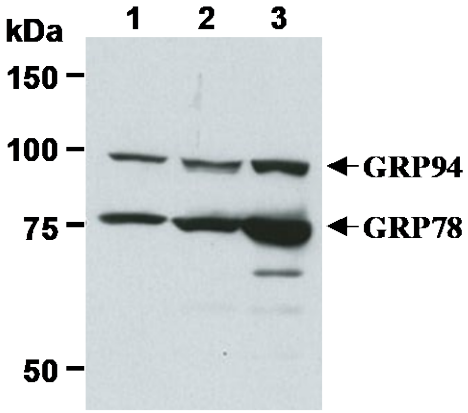
Detailed procedures are provided in the following **PROTOCOLS**.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	HeLa, 293T	NIH/3T3	Not tested
Reactivity on WB	+	+	

INTENDED USE:
For Research Use Only. Not for use in diagnostic procedures.

REFERENCES:
1) Ishii, S., *et al.*, *Biochem. Biophys. Res. Commun.* **486**, 767-773 (2017) [IC]
2) Yamanaka, T., *et al.*, *Sci. Rep.* **6**, 34575 (2016) [IHC]
3) Yamanaka, T., *et al.*, *Nat. Commun.* **5**, 3354 (2014) [IHC]
4) Saito, A., *et al.*, *Nat. Cell Biol.* **11**, 1197-1204 (2009) [IHC]
5) Pelham, H. R., *EMBO J.* **7**, 913-918 (1988)
6) Munro, S., and Pelham, H. R., *Cell* **48**, 899-907 (1987)



Western blot analysis of GRP78 and GRP94 expression on HeLa (1), 293T (2), and NIH/3T3 (3) using PM059.

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

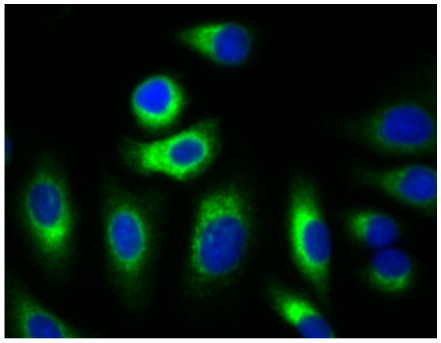
PROTOCOLS:

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 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/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). 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 times).
7) Incubate the membrane with 1:10,000 Anti-IgG (Rabbit) pAb-HRP (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 times).
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 and NIH/3T3)



Immunocytochemical detection of GRP78 and GRP94 in A549 with PM059.
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 for one slide, then incubate in a CO_2 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 is recommended if necessary).
8) Wash the glass slide 2 times with PBS.
9) Add 100 µL of 1:500 Alexa Fluor® 488 conjugated anti-rabbit IgG (Invitrogen; 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)

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