

POLYCLONAL ANTIBODY

Anti-Derlin-1

Code No.	Quantity	Form
PM018	100 µL	Purified IgG

BACKGROUND: Many proteins of eukaryotic cells undergo folding and modification in the endoplasmic reticulum (ER). Properly folded polypeptides leave the ER along the secretory pathway, whereas misfolded proteins or unassembled protein complexes are retained. These proteins are eventually degraded by proteasomes and must therefore be transported back into the cytosol by a multi-step process called retro-translocation, dislocation, or ERAD (ER-associated protein degradation). The *S. cerevisiae* Der1 was identified in a genetic screen for components required for the degradation of misfolded luminal ER proteins and the Der1 homologue Derlin-1 is found in every eukaryotic organism. All species contain at least one other related protein, called Derlin-2, that belongs to a distinct group. Mammalian Derlin-1 is predicted to have four transmembrane segments with both the amino and carboxy termini in the cytosol.

SOURCE: This antibody was purified from rabbit serum using an ion exchange chromatography. The rabbit was immunized with KLH-conjugated Derlin-1 C-terminal peptides (239-251 aa).

FORMULATION: 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

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

REACTIVITY: This antibody reacts with Derlin-1 (28 kDa) on Western blotting.

APPLICATIONS:

Western blotting; 1:1,000 for chemiluminescence detection system

Immunoprecipitation; 1 µL/250 µL of cell extract from 5x10⁶ cells

Immunohistochemistry; Not tested

Immunocytochemistry; 1:50

Flow cytometry; Not tested

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

INTENDED USE:

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Jurkat, HUVEC,U937, KG-1	RAW, Ba/F3	Not Tested
Reactivity on WB	+	+	

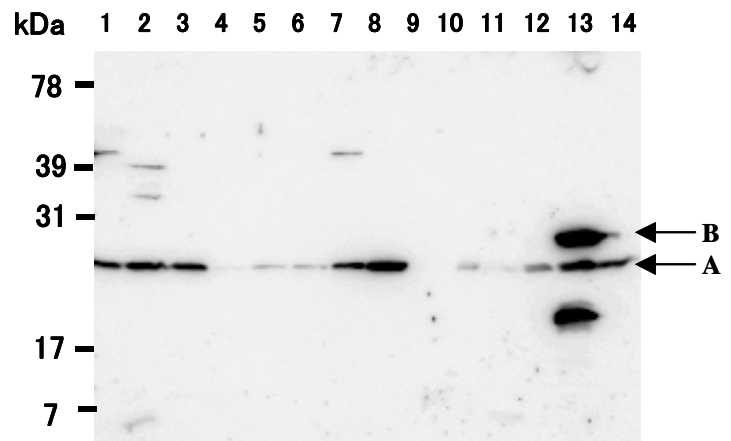
REFERENCES:

- 1) Oda, Y., *et al.*, *J. Cell Biol.* **172**, 383-393 (2006)
- 2) Brendan, N.L., *et al.*, *Nature* **429**, 834-840 (2004)
- 3) Yihong, Y., *et al.*, *Nature* **429**, 841-847 (2004)

This antibody is used in reference number 1).

RELATED PRODUCT:

PM019 Anti-Derlin-2 (polyclonal)



PROTOCOLS:

SDS-PAGE & Western Blotting

- 1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (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. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.
- 3) Mix the sample with equal volume of Laemmli's sample buffer.
- 4) Boil the samples for 3 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
- 5) 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% MeOH). See the manufacture's manual for precise transfer procedure.
- 6) 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.
- 7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 5% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
- 8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
- 9) Incubate the membrane with the 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.
- 10) Wash the membrane with PBS-T (10 minutes x 3 times).
- 11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
- 12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 13) Expose to an X-ray film in a dark room for 3 minutes.
- 14) Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; HUVEC, U937, PM-1, RAW, Jurkat, HeLa, KG-1, Ba/F3, 293T)

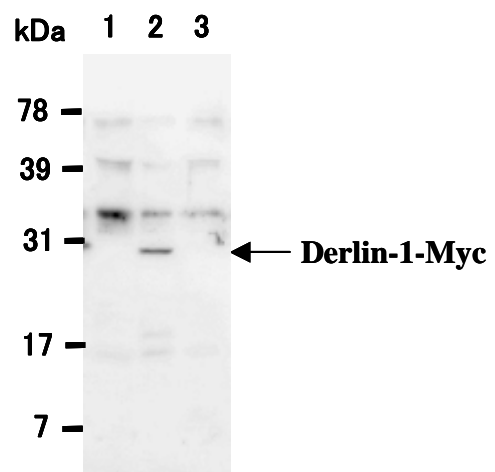
Immunoprecipitation

- 1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (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 suggest in the **APPLICATIONS** into 250 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20 µL 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) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
- 5) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
- 6) Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
- 7) 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% MeOH). See the manufacture's manual for precise transfer procedure.
- 8) 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.
- 9) Incubate the membrane with 1:500 of HRP conjugated Anti-Myc-Tag monoclonal antibody (MBL; code no. M047-7) as primary antibody diluted with PBS, pH 7.2 containing 5% skimmed milk for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
- 10) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
- 11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 12) Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The condition for exposure and development may vary.

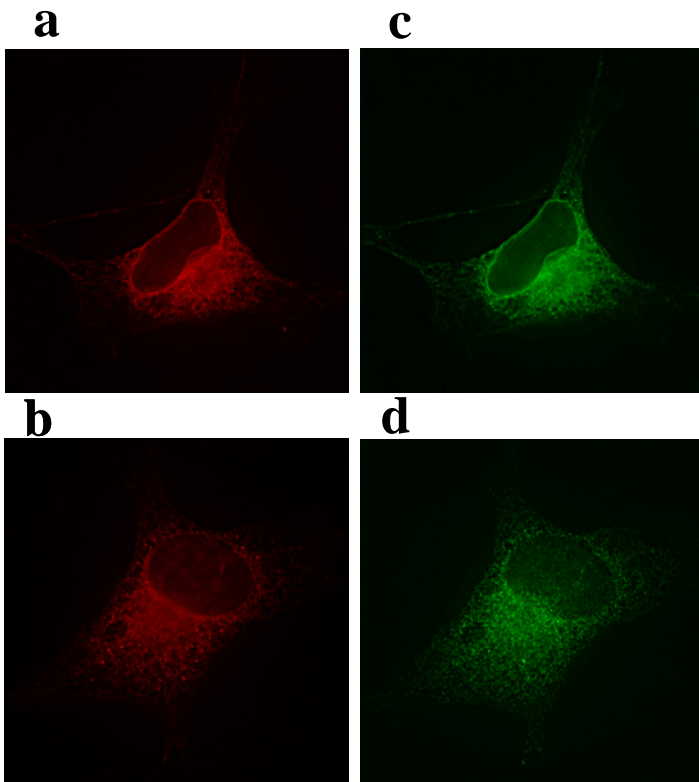
(Positive control for Immunoprecipitation; transfectant)



Immunoprecipitation of Derlin-1 from mock transfected 293T (1), Derlin-1-Myc transfected 293T (2) and Derlin-2-Myc transfected (3) with PM018. After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with M047-7 (HRP-conjugated anti-Myc-Tag antibody).

Immunocytochemistry

- 1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 1×10^4 cells of transfected cells for one slide, then incubate in a CO₂ incubator for one night.)
 - 2) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde for 10 minutes at room temperature.
 - 3) Immerse the slide in PBS containing 0.1% Triton X-100 for 10 minutes at room temperature.
 - 4) The glass slide was washed with PBS 3 times.
 - 5) Cover the slide with blocking buffer (PBS containing 5% BSA, 2% FCS) for 30 minutes at room temperature.
 - 6) Tip off the blocking buffer, add the primary antibody diluted with PBS as suggest 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.)
 - 7) The glass slide was washed with PBS 10 times.
 - 8) Add 100 μ L of 1:2,000 Alexa Fluor 546 conjugated anti-rabbit IgG (H+L) (Molecular Probes Inc.; code no. A11071) diluted with PBS onto the cells. Incubate for 1 hour at room temperature. Keep out light by aluminum foil.
 - 9) The glass slide was washed with PBS 10 times.
 - 10) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
 - 11) Promptly add mounting medium onto the slide, then put a cover slip on it.
- (Positive control for Immunocytochemistry; transfectant)



Immunocytochemical detection of Derlin-1 on 4% PFA fixed Derlin-1-Myc transfected cells with PM018. a,b, after the anti-Derlin-1 antibody (PM018) was added to the slides, Derlin-1 was detected Alexa Fluor 546 conjugated anti-rabbit IgG (H+L) (Molecular Probes Inc.). **c,d,** after the anti-Myc antibody (MBL code no. M047-3) (**c**) or the anti-KDEL antibody (Stressgen) (**d**) were added to the slides, Myc or KDEL were detected Alexa Fluor 488 conjugated anti-mouse IgG (H+L) (Molecular Probes Inc.).