

 My select sampler set

# Anti-VMP1 pAb

<b>CODE No.</b>	PM072MS
<b>CLONALITY</b>	Polyclonal
<b>ISOTYPE</b>	Rabbit Ig, affinity purified
<b>QUANTITY</b>	20 µL
<b>SOURCE</b>	Purified Ig from rabbit serum
<b>IMMUNOGEN</b>	Human VMP1, 131-217 aa (recombinant)
<b>FORMULATION</b>	PBS containing 50% Glycerol (pH 7.2). No preservative is contained.
<b>STORAGE</b>	This antibody solution is stable for one year from the date of purchase when stored at -20°C.

## APPLICATIONS-CONFIRMED

<u>Western blotting</u>	1:500 for chemiluminescence detection system
<u>Immunoprecipitation</u>	5 µL/2 x 10 <sup>6</sup> cells/sample

## SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Cell	293T, A431, transfectant	NIH/3T3, MEF	NRK, PC12	CHO
Reactivity	+	+	+	+

**Entrez Gene ID** 81671 (Human), 75909 (Mouse), 192129 (Rat)

**REFERENCES**  
1) Itakura, E., *et al.*, *Autophagy*. **6**, 764-76 (2010)  
2) Itakura, E., *et al.*, *J. Cell Biol.* **192**, 17-27 (2011)

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## RELATED PRODUCTS

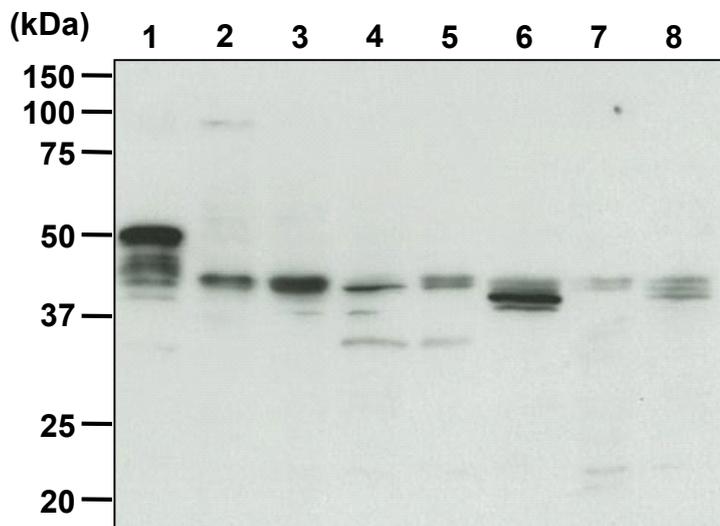
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The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

### **SDS-PAGE & Western blotting**

- 1) Wash  $1 \times 10^7$  cells 3 times with PBS and suspend them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 20 sec.).
- 2) Boil the samples for 3 min. and centrifuge. Load 10  $\mu$ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
- 3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm<sup>2</sup> for 1 hr. 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 overnight at 4°C.
- 5) Wash the membrane with PBS-T (0.05% Tween-20 in PBS) [5 min. x 3 times].
- 6) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
- 7) Wash the membrane with PBS-T (5 min. x 3 times).
- 8) Incubate the membrane with the 1:10,000 of anti-IgG (Rabbit)-HRP (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 9) Wash the membrane with PBS-T (5 min. x 3 times).
- 10) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 11) Expose to an X-ray film in a dark room for 3 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; 293T transfectant, 293T, A431, NIH/3T3, MEF, NRK, PC12, CHO)



#### ***Western blot analysis of VMP1***

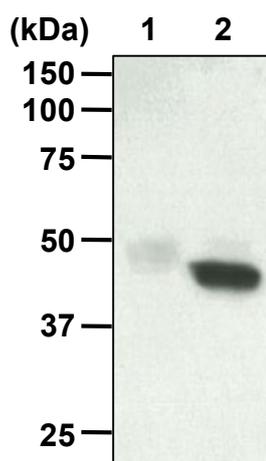
- Lane 1: 293T transfectant
- Lane 2: 293T
- Lane 3: A431
- Lane 4: NIH/3T3
- Lane 5: MEF
- Lane 6: NRK
- Lane 7: PC12
- Lane 8: CHO

Immunoblotted with Anti-VMP1 pAb (PM072)

### Immunoprecipitation

- 1) Wash  $5 \times 10^6$  cells 2 times with PBS and resuspend them with 1 mL of ice-cold Extraction buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40) containing appropriate protease inhibitors, then sonicate briefly (up to 20 sec.).
- 2) Incubate on ice for 5min.
- 3) Centrifuge the tube at  $12,000 \times g$  for 10 min. at  $4^\circ\text{C}$  and transfer the supernatant to another tube.
- 4) Mix 20  $\mu\text{L}$  of 50% protein A agarose beads slurry resuspended in 400  $\mu\text{L}$  of IP buffer (10 mM Tris-HCl (pH 8.0), 500 mM NaCl, 0.1% NP-40) with primary antibody as suggested in the **APPLICATIONS**. Incubate with gentle agitation for 1 hr. at room temperature.
- 5) Wash the beads 3 times with 1 mL of IP buffer.
- 6) Add 400  $\mu\text{L}$  of cell lysate, then incubate with gentle agitation for 1 hr. at room temperature.
- 7) Wash the beads 5 times with 1 mL of Extraction buffer.
- 8) Resuspend the beads in 20  $\mu\text{L}$  of Laemmli's sample buffer, boil for 3 min. and centrifuge.
- 9) Load 20  $\mu\text{L}$  of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
- 10) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at  $1 \text{ mA}/\text{cm}^2$  for 1 hr. 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.
- 11) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for overnight at  $4^\circ\text{C}$ .
- 12) Wash the membrane with PBS-T (0.05% Tween-20 in PBS) [5 min. x 3 times].
- 13) Incubate the membrane with 1:500 of anti-VMP1 pAb (MBL; code no. PM072) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
- 14) Wash the membrane with PBS-T (5 min. x 3 times).
- 15) Incubate the membrane with the 1:1,000 of Rabbit TrueBlot<sup>®</sup> anti-Rabbit IgG-HRP (eBioscience; code no. 18-8816-33) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 16) Wash the membrane with PBS-T (5 min. x 3 times).
- 17) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min.
- 18) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 19) Expose to an X-ray film in a dark room for 1 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; 293T)



#### ***Immunoprecipitation of VMP1 from 293T***

Lane 1: Normal rabbit IgG (PM035)

Lane 2: Anti-VMP1 pAb (PM072)

Immunoblotted with Anti-VMP1 pAb (PM072)