

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

 **My select** sampler set

Anti-VMP1 pAb

CODE No. PM072MS

CLONALITY Polyclonal
ISOTYPE Rabbit Ig, affinity purified
QUANTITY 20 µL

SOURCE Purified Ig from rabbit serum
IMMUNOGEN Human VMP1, 131-217 aa (recombinant)
FORMURATION 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.

APPLICATIONS-CONFIRMED

Western blotting 1:500 for chemiluminescence detection system
Immunoprecipitation 5 µL/2 x 10⁶ 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)

For more information, please visit our web site <http://ruo.mbl.co.jp/>

RELATED PRODUCTSAntibodies

PM072	Anti-VMP1 pAb	
PM036	Anti-LC3 pAb	[WB, IP, IC, IHC, FCM]
M152-3	Anti-LC3 mAb (4E12)	[WB, IP, IC, FCM, EM]
M186-3	Anti-LC3 mAb (8E10)	[WB]
PD014	Anti-LC3 pAb	[WB]
PD015	Anti-LC3 pAb	[IC]
PM046	Anti-LC3 pAb	[WB, IC]
M115-3	Anti-LC3 mAb (51-11)	[WB]
PM045	Anti-p62/SQSTM1 pAb	
M162-3	Anti-p62/SQSTM1 (Human) mAb (5F2)	
M162-A48	Anti-p62/SQSTM1 (Human) mAb -Alexa Fluor [®] 488 (5F2)	
M162-A59	Anti-p62/SQSTM1 (Human) mAb -Alexa Fluor [®] 594 (5F2)	
M162-A64	Anti-p62/SQSTM1 (Human) mAb -Alexa Fluor [®] 647 (5F2)	
PM066	Anti-p62 C-terminal pAb	
PD017	Anti-Beclin 1 pAb	
PM037	Anti-GABARAP pAb	
M135-3	Anti-GABARAP mAb (1F4)	
PM038	Anti-GATE-16 pAb	
PD041	Anti-Atg2A pAb	
PM034	Anti-Atg3 pAb	
M133-3	Anti-Atg3 mAb (3E8)	
M134-3	Anti-Atg4B mAb (9H5)	
PM050	Anti-Atg5 pAb	
M153-3	Anti-Atg5 (4D3)	
PM039	Anti-Atg7 pAb	
PD042	Anti-Atg9A pAb	
M151-3	Anti-Atg10 (Human) mAb (5A7)	
M154-3	Anti-Atg12 (Human) mAb (6E5)	
PD036	Anti-Atg13 (Human) pAb	
M183-3	Anti-Atg13 mAb (5G4)	
PD026	Anti-Atg14 pAb	
M184-3	Anti-Atg14 (Human) mAb (4H8)	
PM040	Anti-Atg16L pAb	
M150-3	Anti-Atg16L mAb (1F12)	
M160-3	Anti-UVRAG mAb (1H4)	
PD027	Anti-Rubicon (Human) pAb	
M170-3	Anti-Rubicon (Human) mAb (1H6)	
PM069	Anti-NRF2 pAb	
M200-3	Anti-NRF2 mAb (1F2)	
PD037	Anti-Tel2 pAb	

Kits

8485	Autophagy Ab Sampler Set
PM036-PN	Positive control for anti-LC3 antibody

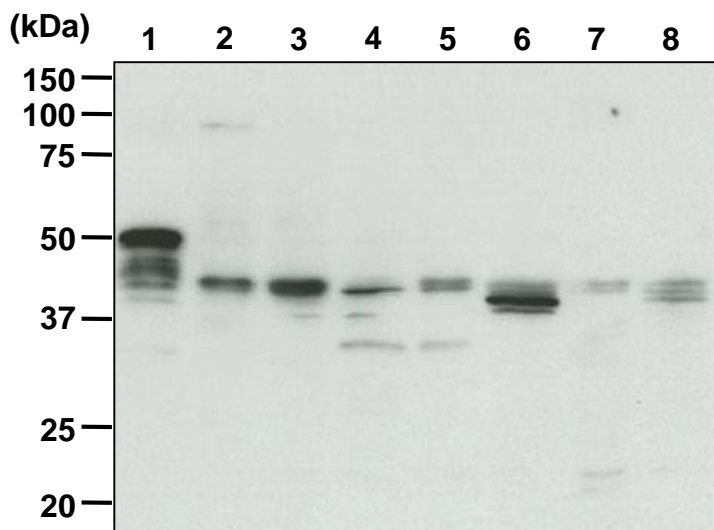
WB:	Western blotting
IP:	Immunoprecipitation
IC:	Immunocytochemistry
IHC:	Immunohistochemistry
FCM:	Flow cytometry
EM:	Immuno-electron microscopy

Other related antibodies and kits are also available.
Please visit our website at <http://ruo.mbl.co.jp/>

SDS-PAGE & Western blotting

- 1) Wash 1×10^7 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 μ 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/cm² 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

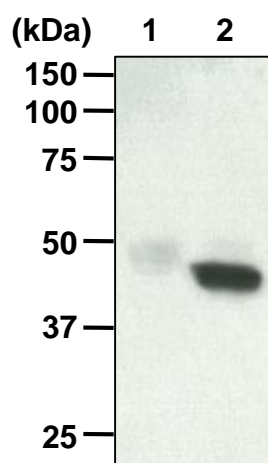
- Lane 1: 293T transfectant
- Lane 2: 293T
- Lane 3: A431
- Lane 4: NIH/3T3
- Lane 5: MEF
- Lane 6: NRK
- Lane 7: PC12
- Lane 8: CHO

Immunoblotted with Anti-VMP1 pAb (PM072)

Immunoprecipitation

- 1) Wash 5×10^6 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 x g for 10 min. at 4°C and transfer the supernatant to another tube.
- 4) Mix 20 μ L of 50% protein A agarose beads slurry resuspended in 400 μ 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 μ 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 μ L of Laemmli's sample buffer, boil for 3 min. and centrifuge.
- 9) Load 20 μ 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 mA/cm² 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°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[®] 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)