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

My select sampler set

Anti-Rubicon (Human) pAb

Code No.	Quantity	Form
PD027MS	20 µL	Affinity Purified

BACKGROUND: Autophagy is a process of intracellular bulk degradation in which cytoplasmic components including organelles are sequestered within double-membrane vesicles that deliver the contents to the lysosome /vacuole for degradation. Rubicon was identified as Beclin1 interacting protein. Three distinct Beclin1 complexes exist in cells, one of the complexes including Rubicon (Beclin1, hVps34, hVps15, UVRAG, Rubicon) down regulates the process of autophagosome maturation and endocytosis.

SOURCE: This antibody was purified from rabbit serum using affinity column. The rabbit was immunized with recombinant C terminus of human Rubicon (722-972 aa).

FORMULATION: 20 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 Rubicon on Western blotting and Immunoprecipitation.

APPLICATIONS:

Western blotting; 1:1,000 for chemiluminescence detection system
Immunoprecipitation; 5 µL/300 µL of cell extract from 3 x 10^6 cells
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster
Cells	HeLa, A549, 293T	NIH/3T3, MEF	Not Tested	Not Tested
Reactivity on WB	+	-		

INTENDED USE:
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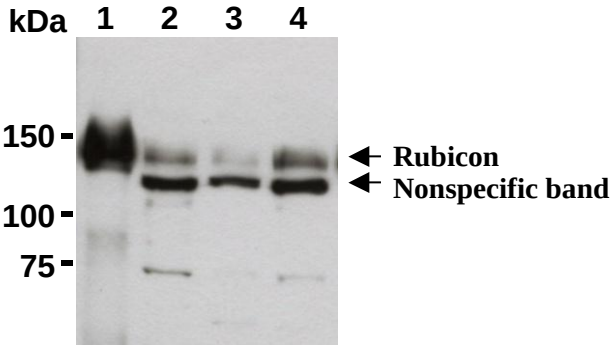
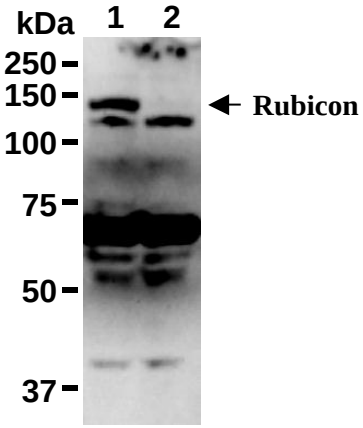
REFERENCES:

1) Matsunaga, K., *et al.*, *Nat. Cell Biol.* **11**, 385-396 (2009)
2) Zhong, Y., *et al.*, *Nat. Cell Biol.* **11**, 468-476 (2009)

This antibody is used in the reference number 1).



Western blot analysis of Rubicon in Flag tagged Rubicon transfectant (1), 293T (2), A549 (3) and HeLa (4) using PD027.



Western blot analysis of Rubicon in control shRNA transfected A549 (1) and Rubicon shRNA transfected A549 (2) using PD027.

This data was kindly provided from Dr. Kohichi, Matsunaga, Ph. D. and Professor Dr. Tamotsu Yoshimori, Ph. D. (The Department of cellular Regulation Research Institute for Microbial Diseases, Osaka University)

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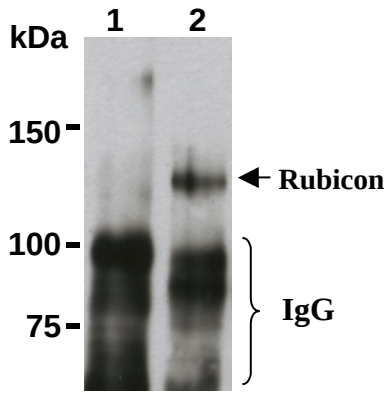
PROTOCOLS:

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% 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) 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 times).
7) Incubate the membrane with 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.
8) Wash the membrane with PBS-T (5 minutes x 3 times).
9) Drain excess buffer on 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; transfectant, HeLa, A549, 293T)



Immunoprecipitation of Rubicon from HeLa with normal rabbit IgG (1) or PD027 (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with PD027.*

Immunoprecipitation

1) Wash cells (approximately 1 x 10^7 cells) 3 times with PBS and resuspend them in 1 mL of cold Lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.05% NP-40) containing protease inhibitors at appropriate concentrations. Incubate it at 4°C with rotating for 30 minutes; thereafter, briefly sonicate the mixture (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 fresh tube.
3) Add primary antibody as suggested in the **APPLICATIONS** into 300 μL of the supernatant. Mix well and incubate with gentle agitation for 60-120 minutes at 4°C.
4) 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.
5) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
6) Resuspend the beads in 20 μL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 20 μL/lane for the SDS-PAGE analysis.
   (See **SDS-PAGE & Western blotting**.)

RELATED PRODUCTS

Antibodies

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 mAb (4D3)	
PM039	Anti-Atg7 (Human) 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	
PM072	Anti-VMP1 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 web site at http://ruo.mbl.co.jp