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POLYCLONAL ANTIBODY

Anti-Rubicon (Human) pAb

Code No.	Quantity	Form
PD027	100 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: 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 human Rubicon on Western blotting and Immunoprecipitation.

APPLICATIONS:

Western blotting; 1:1,000
Immunoprecipitation; 5 µL/300 µL of cell extract from 3 x 10^6 cells
Immunohistochemistry; Can be used
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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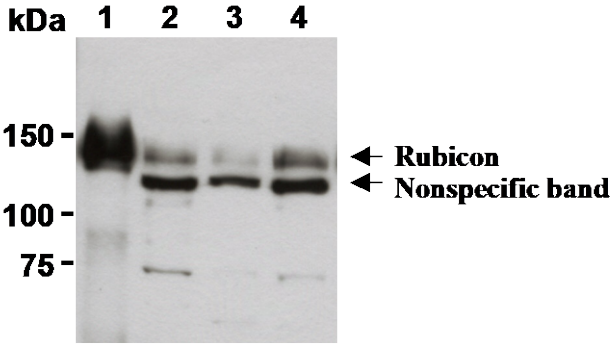
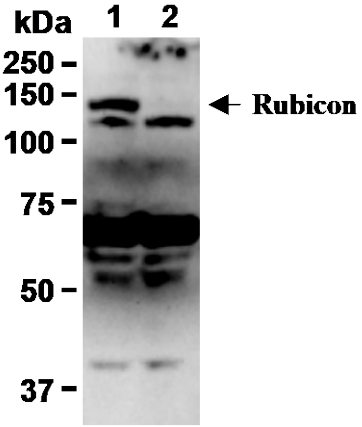
REFERENCES:

1) Bejarano, E., *et al., Nat. Cell Biol.* **16**, 401-414 (2014) [WB]
2) Maejima, Y., *et al., Nat. Med.* **19**, 1478-14 88 (2013) [WB]
3) Matsunaga, K., *et al., Nat. Cell Biol.* **11**, 385-396 (2009)
4) Zhong, Y., *et al., Nat. Cell Biol.* **11**, 468-476 (2009)

This antibody is used in the reference number 1)-3).



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)

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 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 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) 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 and 293T)

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**.)

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