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My select sampler set

Anti-Atg14 pAb

Code No.	Quantity	Form
PD026MS	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. Mammalian homologues of Atg14/Atg14L/BARKOR localizes on the isolation membrane and autophagosome, and it necessary for autophagosome formation. Atg14 binds to the PI3K complex (Beclin-1, Vps34, Vps15) and promotes the autophagosome formation.

SOURCE: This antibody was purified from rabbit serum using affinity column. The rabbit was immunized with recombinant human Atg14 (167-404 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, mouse and rat Atg14 on Western blotting and Immunoprecipitation.

APPLICATIONS:

Western blotting; 1:500
Immunoprecipitation; 5 µL/300 µL of cell extract from 3 x 10^6 cells
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested*
*It is reported that this antibody can be used in this application in the reference number 3).
Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster
Cells	HeLa, A549	NIH/3T3, MEF	PC12	CHO
Reactivity on WB	+	+	+	-

INTENDED USE:

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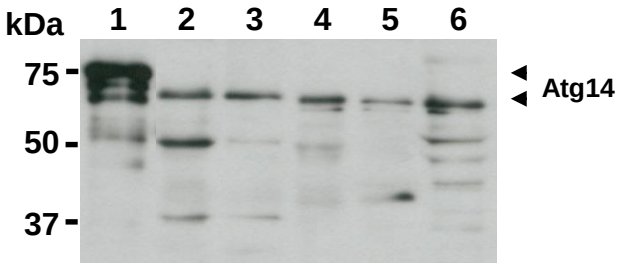


Western blotting analysis of Atg14
Lane 1: Flag-tagged Atg14 transfectant
Lane 2: HeLa
Lane 3: A549
Lane 4: NIH/3T3
Lane 5: MEF
Lane 6: PC12
Immunoblotted with PD026

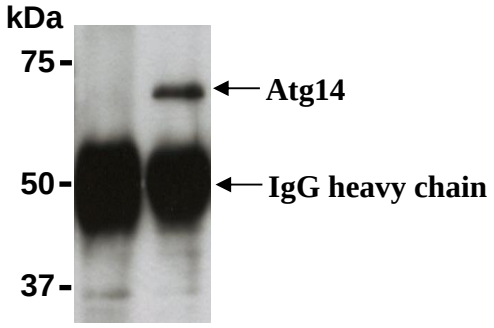
PROTOCOLS:

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 2 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% methanol). 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 1% skimmed milk (in PBS, pH 7.2) 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).
7) Incubate the membrane with 1:10,000 of 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).
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 2 minutes. Develop the film under usual settings. The conditions for exposure and development may vary.

(Positive controls for Western blotting; transfectant, HeLa, A549, NIH/3T3, MEF and PC12)



Immunoprecipitation of Atg14 from HeLa
Lane 1: IP with normal rabbit IgG (PM035)
Lane 2: IP with PD026
Immunoblotted with PD026

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 30-120 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) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
6) Resuspend the agarose with cold Lysis buffer.
7) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
8) Repeat steps 6)-7) 2-4 times.
9) 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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