

POLYCLONAL ANTIBODY

Anti-Atg14 pAb

Code No.
PD026

Quantity
100 μ L

Form
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: 100 μ L 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 μ L/300 μ L of cell extract from 3×10^6 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	+	+	+	-

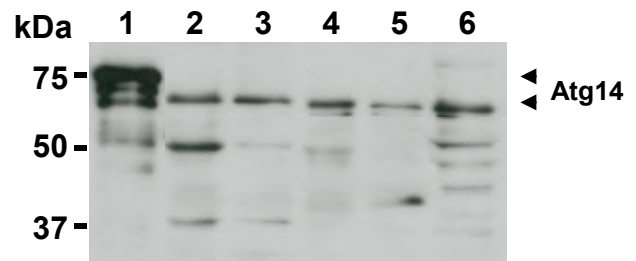
INTENDED USE:

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

REFERENCES:

- 1) Nemazanyy, I., *et al.*, *Nat. Commun.* **6**, 8283 (2015) [IP]
- 2) Zhong, Y., *et al.*, *J. Biol. Chem.* **289**, 26021-26037 (2014) [IP]
- 3) Bejarano, E., *et al.*, *Nat. Cell Biol.* **16**, 401-414 (2014) [WB, IC]
- 4) Kim, C. and Bergelson, J. M., *J. Virol.* **88**, 434-443 (2014) [WB]
- 5) Maejima, Y., *et al.*, *Nat. Med.* **19**, 1478-1488 (2013) [WB]
- 6) Hamasaki, M., *et al.*, *Nature* **495**, 389-393 (2013) [WB]
- 7) Kim, J., *et al.*, *Cell* **152**, 290-303 (2013) [IP]
- 8) Matsunaga, K., *et al.*, *Nat. Cell Biol.* **11**, 385-396 (2009)
- 9) Zhong, Y., *et al.*, *Nat. Cell Biol.* **11**, 468-476 (2009)
- 10) Itakura, E., *et al.*, *Mol. Biol. Cell* **19**, 5360-5372 (2008)
- 11) Sun, Q., *et al.*, *PNAS* **105**, 19211-19216 (2008)

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



Western blot 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:

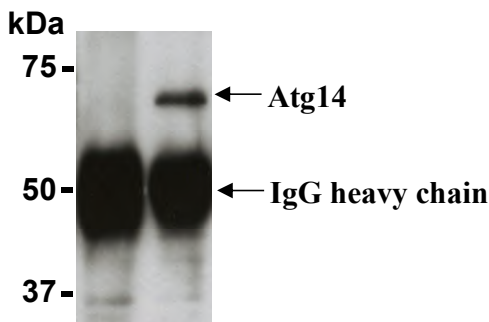
SDS-PAGE & Western Blotting

- 1) Wash cells (approximately 1×10^7 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 μ L 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/cm² 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 MaxBlot Solution 1 (MBL; code no. 8455) 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 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 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 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 1×10^7 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 μ L of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C.
- 4) Add 20 μ L 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 μ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 20 μ L/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting**.)

RELATED PRODUCTS:

Other related antibodies and kits are also available.

Please visit our website at <https://ruo.mbl.co.jp/>.