

POLYCLONAL ANTIBODY

Anti-Atg14 pAb

Code No.
PD026

Quantity
100 µL

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 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 for chemiluminescence detection system

Immunoprecipitation; 5 µL/300 µL of cell extract from 3 x 10⁶ 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:

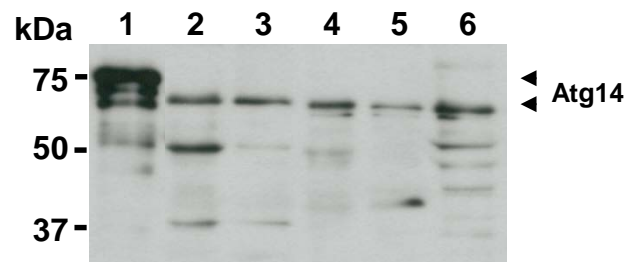
Species	Human	Mouse	Rat	Hamster
Cells	HeLa, A549	NIH/3T3, MEF	PC12	CHO
Reactivity on WB	+	+	+	-

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)



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

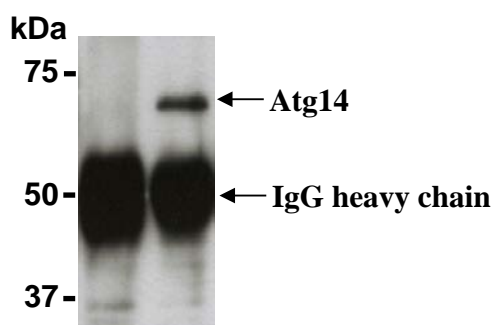
PROTOCOLS:

SDS-PAGE & Western Blotting

- 1) Wash cells (approximately 1 x 10⁷ 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/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 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) 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:

PM036	Anti-LC3 pAb (polyclonal) [WB, IP, IC, IHC, FCM]
M152-3	Anti-LC3 mAb (4E12) [WB, IP, IC, FCM, EM]
M186-3	Anti-LC3 mAb (8E10) [WB]
M186-7	Anti-LC3 mAb-HRP-Direct (8E10)
PD014	Anti-LC3 pAb (polyclonal) [WB]
PM037	Anti-GABARAP pAb (polyclonal)
M135-3	Anti-GABARAP mAb (1F4)
PM038	Anti-GATE-16 pAb (polyclonal)
PD041	Anti-Atg2A pAb (polyclonal)
PM034	Anti-Atg3 pAb (polyclonal)
M133-3	Anti-Atg3 mAb (3E8)
M134-3	Anti-Atg4B mAb (9H5)
PM050	Anti-Atg5 pAb (polyclonal)
M153-3	Anti-Atg5 mAb (4D3)
PM039	Anti-Atg7 (Human) pAb
PD042	Anti-Atg9A pAb (polyclonal)
M151-3	Anti-Atg10 (Human) mAb (5A7)
M154-3	Anti-Atg12 (Human) mAb (6E5)
PD036	Anti-Atg13 (Human) pAb (polyclonal)
M183-3	Anti-Atg13 mAb (5G4)
PD026	Anti-Atg14 pAb (polyclonal)
M184-3	Anti-Atg14 (Human) mAb (4H8)
PM040	Anti-Atg16L pAb (polyclonal)
M150-3	Anti-Atg16L mAb (1F12)
M160-3	Anti-UVRAG mAb (1H4)
PD017	Anti-Becclin 1 pAb (polyclonal)
PD027	Anti-Rubicon (Human) pAb (polyclonal)
M170-3	Anti-Rubicon (Human) mAb (1H6)
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)
PM045	Anti-p62 (SQSTM1) pAb (polyclonal)
PM066	Anti-p62 C-terminal pAb (polyclonal)
PM066-7	Anti-p62 C-terminal pAb-HRP-Direct (polyclonal)
M217-3	Anti-Phospho-p62 (SQSTM1) (Ser351) mAb (5D5)
PM074	Anti-Phospho-p62 (SQSTM1) (Ser351) pAb
D343-3	Anti-Phospho-p62 (SQSTM1) (Ser403) mAb (4F6)
D344-3	Anti-Phospho-p62 (SQSTM1) (Ser403) mAb (4C8)
PM035	Normal Rabbit IgG
8485	Autophagy Ab Sampler Set
8486	Autophagy Watch
PM036-PN	Positive control for anti-LC3 antibody y
8455	MaxBlot Solution 1 & 2

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