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

POLYCLONAL ANTIBODY

Anti-Atg7 (Human) pAb

Code No.	Quantity	Form
PM039	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. Microtubule-associated protein light chain 3 (LC3) is a homologue of yeast Atg8, an essential component of autophagy. Following synthesis, the C-terminus of LC3 is cleaved by a cysteine protease-Atg4, to produce LC3-I, which is located in cytosolic fraction. LC3-I is activated by the E1-like enzyme Atg7 and forms a Atg7-LC3-I thioester. Atg7-LC3-I is transferred to Atg3 to form Atg3-LC3-I thioester. Atg3 is an E2-like enzyme that catalyzes the conjugation of LC3-I and phosphatidylethanolamine (PE) to form LC3-II. The LC3-II-PE conjugate is essential for binding tightly to autophagosomal membrane.

SOURCE: This antibody was purified from rabbit serum using affinity column. The rabbit was immunized with the synthetic peptide at the C-terminus region of human Atg7.

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 Atg7 on Western blotting and Immunoprecipitation.

APPLICATIONS:

Western blotting; 1:1,000-1:2,000 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**.

INTENDED USE:

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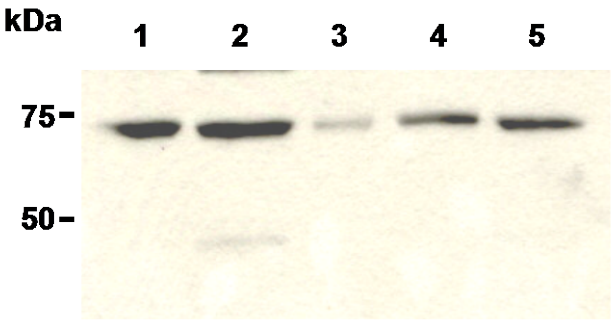
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster
Cells	293T, HeLa, Raji, HL-60, Jurkat	NIH/3T3	Rat1	CHO
Reactivity on WB	+	-	-	-

REFERENCES:

1) Dutta, P., *et al.*, *BMC Cancer* **16**, 33 (2016) [WB]
2) He, R., *et al.*, *Autophagy* **11**, 740-747 (2015) [WB]
3) Xu, D., *et al.*, *Autophagy* **11**, 617-628 (2015) [WB]
4) Horikawa, I., *et al.*, *Nat. Commun.* **5**, 4706 (2014) [WB]
5) Maejima, Y., *et al.*, *Nat. Med.* **19**, 1478-1488 (2013) [WB]
6) Komatsu, M., *et al.*, *J. Cell Biol.* **169**, 425-434 (2005)
7) Yu, L., *et al.*, *Science* **304**, 1500-1502 (2004)



Western blot analysis of Atg7 expression in 293T (1), HeLa (2), Raji (3), HL-60 (4) and Jurkat (5) using PM039.

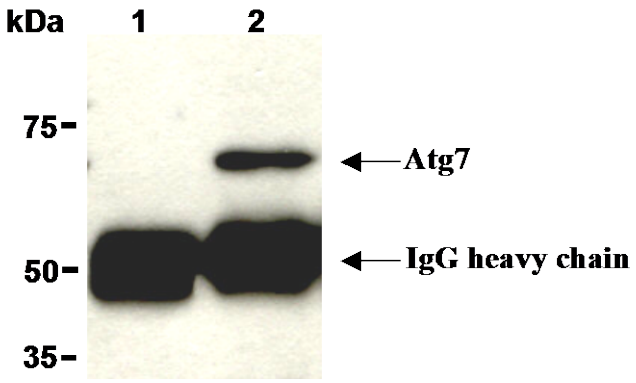
PROTOCOLS:

SDS-PAGE & Western Blotting

1) Wash the 1x10^7 cells 3 times with PBS and suspend with 1 mL of Laemmli's sample buffer.
2) Boil the samples for 2 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for 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 manufacture'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) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).

6) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
7) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
8) 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.
9) Wash the membrane with PBS-T (5 minutes x 3 times).
10) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
11) Expose to an X-ray film in a dark room for 3 minutes.
12) Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; 293T, HeLa, Raji, HL-60 and Jurkat)



Immunoprecipitation of HeLa with normal rabbit IgG (1) or PM039 (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with PM039.

Immunoprecipitation

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40] containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (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 tube.
3) Add primary antibody as suggest 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 10 µL/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation; HeLa)

RELATED PRODUCTS:

Antibodies

M152-3	Anti-LC3 mAb (4E12)	[WB, IP, IC, FCM, EM]
M186-3	Anti-LC3 mAb (8E10)	[WB]
PM036	Anti-LC3 pAb	[WB, IP, IC, IHC, FCM]
PD014	Anti-LC3 pAb	[WB]
M162-3	Anti-p62 (SQSTM1) (Human) mAb (5F2)	
PM045	Anti-p62 (SQSTM1) pAb	
PM066	Anti-p62 C-terminal pAb	
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)	
M230-3	Anti-Parkin mAb (Par6)	
M224-3	Anti-KEAP1 mAb (KP1)	
M200-3	Anti-NRF2 mAb (1F2)	
PM069	Anti-NRF2 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)	
PD037	Anti-Tel2 pAb	
PM072	Anti-VMP1 pAb	
PM076	Anti-Syntaxin-17 (Human) pAb	
M212-3	Anti-Syntaxin-17 (Human) mAb (2F8)	

Kits

8485	Autophagy Ab Sampler Set
8486	Autophagy Watch
PM036-PN	Positive control for anti-LC3 antibody

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