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

POLYCLONAL ANTIBODY

Anti-Atg7

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:

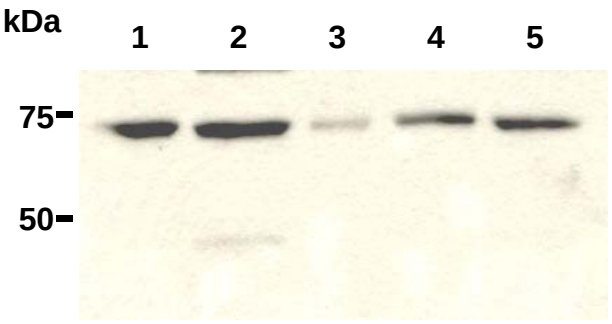
For Research Use Only. Not for use in diagnostic procedures.

SPECIES CROSS REACTIVITY:

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

REFERENCES:

1) Komatsu, M., *et al., J. Cell Biol.* **169**, 425-434 (2005)
2) 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:

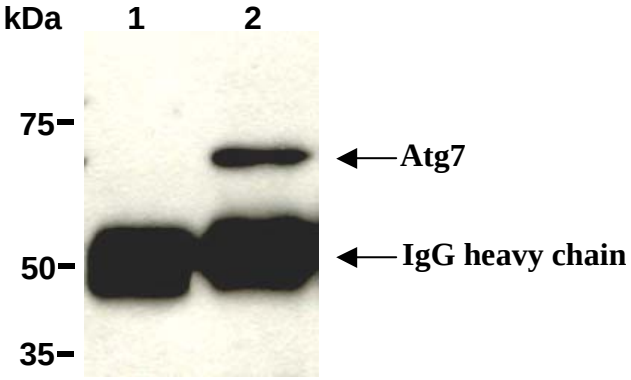
SDS-PAGE & Western Blotting

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 the 1:10,000 HRP-conjugated anti-rabbit IgG (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, 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:

PD014	anti-LC3 (polyclonal)	(for WB)
PD015	anti-LC3 (polyclonal)	(for IC)
PM036	anti-LC3 (polyclonal)	(for WB, IP, IC, IHC)
PM046	anti-LC3 (polyclonal)	(for WB, IC)
M115-3	anti-LC3 (51-11)	(for WB)
M152-3	anti-LC3 (4E12)	(for WB, IP, IC)
M135-3	anti-GABARAP (1F4)	
PM037	anti-GABARAP (polyclonal)	
PM038	anti-GATE-16 (polyclonal)	
PM034	anti-Atg3 (polyclonal)	
M133-3	anti-Atg3 (3E8)	
M134-3	anti-Atg4B (9H5)	
M153-3	anti-Atg5 (4D3)	
PM050	anti-Atg5 (polyclonal)	
PM039	anti-Atg7 (polyclonal)	
M151-3	anti-Atg10 (5A7)	
M154-3	anti-Atg12 (6E5)	
PM040	anti-Atg16L (polyclonal)	
M150-3	anti-Atg16L (1F12)	
M160-3	anti-UVRAG (1H4)	
M162-3	anti-p62/SQSTM1 (5F2)	
PM045	anti-p62/SQSTM1 (polyclonal)	
PM036-P	Positive control for anti-LC3 antibody	

WB: Western blotting
IP: Immunoprecipitation
IC: Immunocytochemistry
IHC: Immunohistochemistry