

 **My select** sampler set

## Anti-Atg10 (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
M151-3MS	5A7	Mouse IgG1 $\kappa$	20 $\mu$ L	1 mg/mL

**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. Autophagy has two ubiquitin-like conjugation systems, the Atg12 and LC3-II systems. In the Atg12 conjugation system, Atg10 functions as E2-like enzyme, plays a role to form conjugates of Atg12 and Atg5. Atg12-Atg5 complex interacts with Atg16L and forms 800 kDa complex that elongate autophagic isolation membrane.

**SOURCE:** This antibody was purified from hybridoma (clone 5A7) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with C3H mouse lymphocyte immunized with the full-length human Atg10 (1-220 aa).

**FORMULATION:** 20  $\mu$ g IgG in 20  $\mu$ 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  $-20^{\circ}\text{C}$ .

**REACTIVITY:** This antibody reacts with human Atg10 (26 kDa) on Western blotting.

### APPLICATIONS:

Western blotting: 2  $\mu$ g/mL for chemiluminescence detection system

Immunoprecipitation: Not recommended

Immunohistochemistry: Not tested

Immunocytochemistry: Not tested

Flow cytometry: Not tested

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

### SPECIES CROSS REACTIVITY:

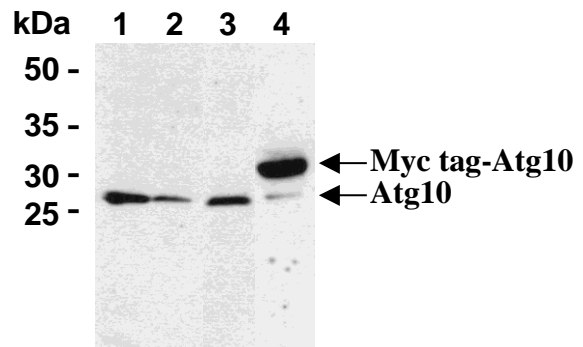
Species	Human	Mouse	Rat
Cells	HeLa, 293T, A431	Not Tested	Not Tested
Reactivity on WB	+		

### INTENDED USE:

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

### REFERENCES:

- 1) Nemoto, T., *et al.*, *J. Biol. Chem.* **278**, 39517-39526 (2003)
- 2) Kabeya, Y., *et al.*, *EMBO J.* **19**, 5720-5728 (2000)
- 3) Mizushima, N., *et al.*, *J. Biol. Chem.* **273**, 33889-33892 (1998)



**Western blot analysis of Atg10 expression in HeLa (1), 293T (2), A431 (3) and Myc tagged Atg10 (4) using M151-3.**

### PROTOCOL:

#### SDS-PAGE & Western Blotting

- 1) Wash the  $1 \times 10^7$  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 20  $\mu$ L 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  $1 \text{ mA/cm}^2$  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  $4^{\circ}\text{C}$ .
- 5) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
- 6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).

- 7) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG (MBL; code no. 330) 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) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
- 10) 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 10 minutes.
- 12) Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; HeLa, 293T, A431, transfectant)

## RELATED PRODUCTS

### Antibodies

PM036	Anti-LC3 pAb	[WB, IP, IC, IHC, FCM]
M152-3	Anti-LC3 mAb (4E12)	[WB, IP, IC, FCM, EM]
M186-3	Anti-LC3 mAb (8E10)	[WB]
PD014	Anti-LC3 pAb	[WB]
PD015	Anti-LC3 pAb	[IC]
PM046	Anti-LC3 pAb	[WB, IC]
M115-3	Anti-LC3 mAb (51-11)	[WB]
PM045	Anti-p62 (SQSTM1) pAb	
M162-3	Anti-p62 (SQSTM1) (Human) mAb (5F2)	
M162-A48	Anti-p62 (SQSTM1) (Human) mAb -Alexa Fluor <sup>®</sup> 488 (5F2)	
M162-A59	Anti-p62 (SQSTM1) (Human) mAb -Alexa Fluor <sup>®</sup> 594 (5F2)	
M162-A64	Anti-p62 (SQSTM1) (Human) mAb -Alexa Fluor <sup>®</sup> 647 (5F2)	
PM066	Anti-p62 C-terminal 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	
M200-3	Anti-NRF2 mAb (1F2)	
PM069	Anti-NRF2 pAb	
PM072	Anti-VMP1 pAb	

### Kits

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

WB: Western blotting  
IP: Immunoprecipitation  
IC: Immunocytochemistry  
IHC: Immunohistochemistry  
FCM: Flow cytometry  
EM: Immuno-electron microscopy

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