

MONOCLONAL ANTIBODY

Anti-Atg4B mAb

Code No.	Clone	Subclass	Quantity	Concentration
M134-3	9H5	Mouse IgG1	100 μ 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. Atg4B is a cysteine protease required for autophagy process, which cleaves the carboxyl-terminus of LC3, to produce LC3-I. 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 hybridoma (clone 9H5) 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 of human ATG4B (1-393 aa).

FORMULATION: 100 μ g IgG in 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 Atg4B on Western blotting.

APPLICATIONS:

- Western blotting; 1 μ g/mL for chemiluminescence detection system
- Immunoprecipitation; Not tested
- Immunohistochemistry; Not tested
- Immunocytochemistry; Not tested
- Flow cytometry; Not tested

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

INTENDED USE:

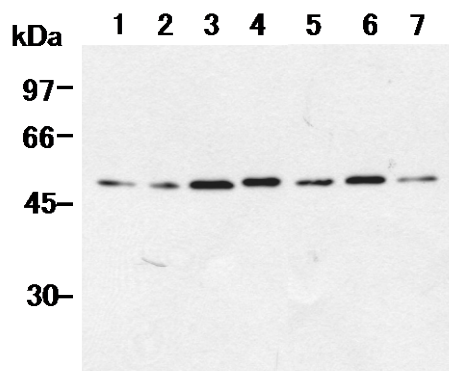
For Research Use Only. Not for use in diagnostic procedures.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster
Cells	293T, HeLa, Raji	NIH/3T3	Rat1, PC12	CHO
Reactivity on WB	+	+	+	+

REFERENCES:

- 1) Maejima, Y., *et al.*, *Nat. Med.* **19**, 1478-1488 (2013) [WB]
- 2) Kang, Y. A., *et al.*, *Mol. Cell Biol.* **32**, 226-239 (2012) [WB]
- 3) Yoshimura, K., *et al.*, *Autophagy* **2**, 200-208 (2006)
- 4) Kumanomidou, T., *et al.*, *J. Mol. Biol.* **355**, 612-618 (2006)
- 5) Sugawara, K., *et al.*, *J. Biol. Chem.* **280**, 40058-40065 (2005)



Western blot analysis of Atg4B expression in 293T (1), HeLa (2), Raji (3), NIH/3T3 (4), Rat1 (5), PC12 (6) and CHO (7) using M134-3.

PROTOCOL:

SDS-PAGE & Western Blotting

- 1) Wash the 1×10^7 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 10 μ 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 mA/cm² 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, place the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°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 1:10,000 of Anti-IgG (Mouse) pAb-HRP (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. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
 - 10) Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; 293T, HeLa, Raji, NIH/3T3, Rat1, PC12 and CHO)

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
PM076	Anti-Syntaxin-17 (Human) pAb
M224-3	Anti-KEAP1 mAb (KP1)
M230-3	Anti-Parkin mAb (Par6)

Kits

8485	Autophagy Ab Sampler Set
8486	Autophagy Watch
CY-7057	CycLex [®] Phospho-p62 Ser403 ELISA Kit
CY-7056	CycLex [®] Phospho-p62 Ser349 ELISA Kit
CY-7055	CycLex [®] Total p62 ELISA Kit
PM036-PN	Positive control for anti-LC3 antibody

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]
PM045	Anti-p62 (SQSTM1) pAb	
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)	
PM066	Anti-p62 C-terminal pAb	
PM066-7	Anti-p62 C-terminal (Human) pAb-HRP-Direct	
D343-3	Anti-Phospho-p62 (SQSTM1) (Ser403) mAb (4F6)	
D344-3	Anti-Phospho-p62 (SQSTM1) (Ser403) mAb (4C8)	
PM074	Anti-Phospho-p62 (SQSTM1) (Ser351) 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	

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 web site at <http://ruo.mbl.co.jp>