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

MONOCLONAL ANTIBODY

Anti-GABARAP mAb

Code No.	Clone	Subclass	Quantity	Concentration
M135-3	1F4	Mouse IgG1	100 µL	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. LC3, GABARAP (γ-aminobutyric-acid-type-A-receptor-associated protein), and GATE-16 (Golgi-associated ATPase enhancer of 16 kDa) have been identified as a homologue of yeast Atg8. These homologues have been characterized as modifiers in reactions mediated by hAtg7 (an E1-like enzyme) and hAtg3 (an E2-like enzyme) as in yeast Atg8 lipidation. These homologues also generate form II, which are recovered in membrane fractions. Generation of the form II correlates with autophagosome association. These results suggest that all mammalian Atg8 homologues receive a common modification to associate with autophagosomal membrane as the form II.

SOURCE: This antibody was purified from hybridoma (clone 1F4) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse lymphocyte immunized with the N-terminus of human GABARAP.

FORMULATION: 100 µg IgG in 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 GABARAP on Western blotting.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested*
*It is reported that clone 1F4 can be used in Immunohistochemistry in the reference number 3).
Immunocytochemistry; Not tested*
*It is reported that clone 1F4 can be used in Immunocytochemistry in the reference number 2).
Flow cytometry; Not tested

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

INTENDED USE:
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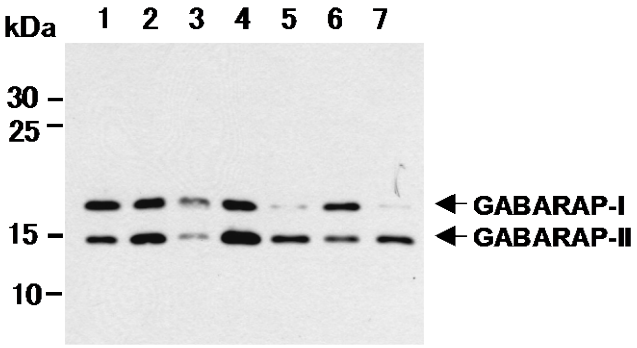
SPECIES CROSS REACTIVITY:

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

*The expression of GABARAP on chicken embryo fibroblasts has been reported in the reference number 1). Not tested by MBL.

REFERENCES:

1) Maynard, S., *et al., J. Cell Physiol.* **230**, 1475-1488 (2015) [WB]
2) Zhang, Z., *et al., J. Immunol.* **190**, 3517-3524 (2013) [WB]
3) Colecchia, D., *et al., Autophagy* **8**, 1724-1740 (2012) [IC]
4) Tanji, K., *et al., Neurobiol. Dis.* **43**, 690-697 (2011) [WB, IH]
5) Klionsky, D. J., *et al., J. Cell Sci.* **118**, 7-18 (2005)
6) Tanida, I., *et al., J. Biol. Chem.* **277**, 13739-13744 (2002)



Western blot analysis of GABARAP expression in 293T (1), HeLa (2), Raji (3), NIH/3T3 (4), Rat1 (5), PC12 (6) and CHO (7) using M135-3.

PROTOCOL:

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.

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e-mail support@mbl.co.jp, TEL 052-238-1904

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, 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 the 1:10,000 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)

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]
M186-7	Anti-LC3 mAb-HRP-DirecT (8E10)	
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 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	
M217-3	Anti-Phospho-p62 (SQSTM1) (Ser351) mAb (5D5)	
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)	
PM069	Anti-NRF2 pAb	
M200-3	Anti-NRF2 mAb (1F2)	
PD037	Anti-Tel2 pAb	
PM072	Anti-VMP1 pAb	
PM076	Anti-Syntaxin-17 (Human) pAb	
M212-3	Anti-Syntaxin-17 (Human) mAb (2F8)	
M224-3	Anti-KEAP1 mAb	
M230-3	Anti-Parkin mAb	

Kits

8485	Autophagy Ab Sampler Set
8486	Autophagy Watch
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 web site at http://ruo.mbl.co.jp