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

MBL
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POLYCLONAL ANTIBODY

Anti-GABARAP pAb

Code No.	Quantity	Form
PM037	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. 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 rabbit serum using affinity column. The rabbit was immunized with carrier protein conjugated synthetic peptide at the N-terminus region of GABARAP.

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 GABARAP on Western blotting and Immunocytochemistry. It does not react with LC3 and GATE16.

APPLICATIONS:
Western blotting; 1:1,000
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; 1:100
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, WR19L	Rat1, PC12	CHO
Reactivity on WB	+	+	+	+

REFERENCES:
1) Polletta, L., *et al., Autophagy* **11**, 253-270 (2015) [WB]
2) Tanida, I., *et al., Curr. Protoc. Cell Biol.* **646**, 11.20.1-13 (2014)
3) Maruyama, Y., *et al., Biochem. Biophys. Res. Commun.* **446**, 309-315 (2014)
4) Mariño, G., *et al., J. Clin. Invest.* **120**, 2331-2344 (2010) [WB]
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)

This antibody has been used in the reference number 1)-4).

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

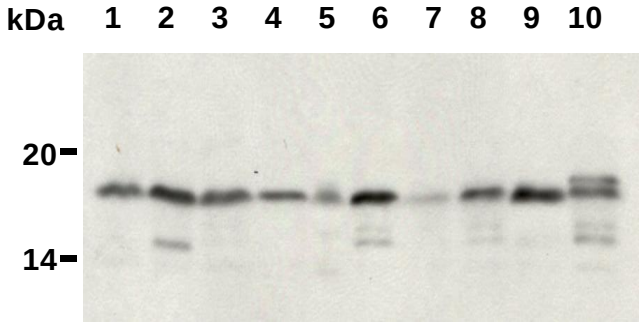
PROTOCOLS:

SDS-PAGE & Western Blotting

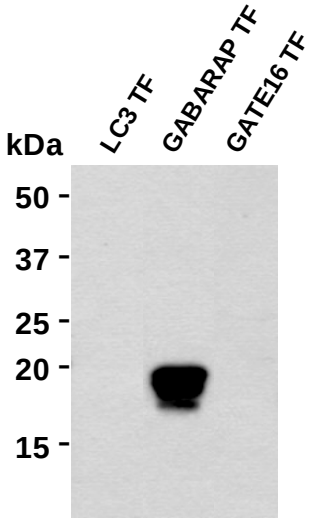
1) Wash the 1 x 10^7 cells 3 times with PBS and suspend with 1 mL of Laemmli's sample buffer.
2) Boil the samples for 2 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane 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) 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 (Rabbit) pAb-HRP (MBL; code no. 458) 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 3 minutes.
12) Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; 293T, HeLa, Raji, HL60, Jurkat, NIH/3T3, WR19L, Rat1, PC12 and CHO)



Western blot analysis of GABARAP expression in 293T (1), HeLa (2), Raji (3), HL-60 (4), Jurkat (5), NIH/3T3 (6), WR19L (7), Rat1 (8), PC12 (9) and CHO (10) using PM037.

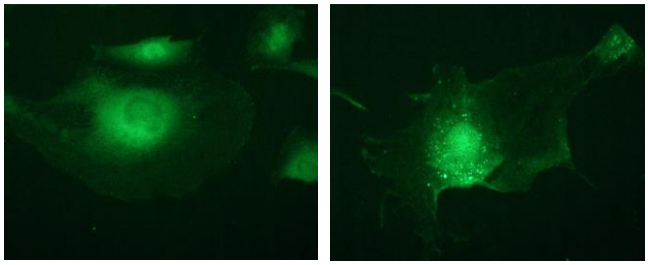


Western blot analysis of GABARAP expression on transfectant (TF) of human Atg8 homologs using PM037.

Immunocytochemistry

1) Spread the cells in the nutrient condition on a glass slide, then incubate in a CO_2 incubator for one night.
2) Remove the culture supernatant by careful aspiration.
3) To obtain serum-starved conditions, culture the cells with Hank's solution or DMEM for 2-4 hours at 37°C.
4) Fix the cells by immersing the slide in 4% paraformaldehyde (PFA)/PBS for 10 minutes at room temperature (20~25°C).
5) Prepare a wash container such as a 500 mL beaker with a magnetic stirrer. Then wash the fixed cells on the glass slide by soaking the slide with a plenty of PBS in the wash container for 5 minutes. Take care not to touch the cells. Repeat another wash once more.
6) Immerse the slide in 100 μg/mL of Digitonin for 15 minutes at room temperature.
7) Wash the slide in a plenty of PBS as in the step 5).
8) Add the primary antibody diluted with PBS as suggest in the **APPLICATIONS** onto the cells and incubate for 1 hour at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
9) Wash the slide in a plenty of PBS as in the step 5).
10) Add 200 μL of FITC conjugated anti-rabbit IgG antibody diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
11) Wash the slide in a plenty of PBS as in the step 5).
12) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
13) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; NRK)



Immunocytochemical detection of GABARAP on 4% PFA fixed nutrient normal rat kidney cell line (NRK, left) and starved NRK (right) with PM037.

RELATED PRODUCTS:

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