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Anti-GATE-16 pAb

Code No.	Quantity	Form
PM038MS	20 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 GATE-16.

FORMULATION: 20 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 GATE-16 on Western blotting. It does not react with LC3 and GABARAP.

APPLICATIONS:

Western blotting; 1:1,000 for chemiluminescence detection system
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; Not recommended
Flow cytometry; Not tested

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

INTENDED USE:

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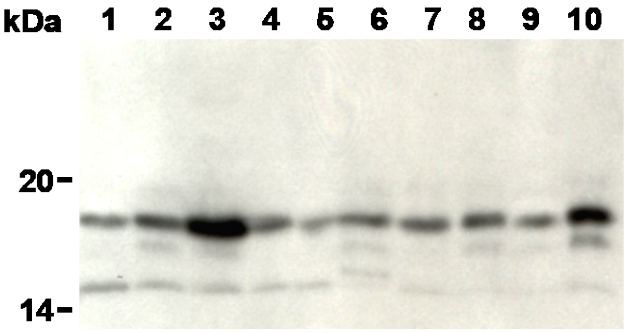
SPECIES CROSS REACTIVITY:

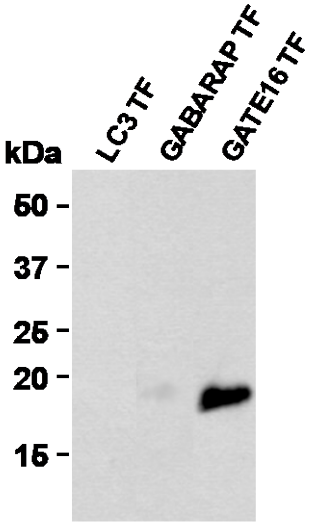
Species	Human	Mouse	Rat	Hamster
Cells	293T, HeLa, Raji, HL-60, Jurkat	NIH/3T3, WR19L	Rat1, PC12	CHO
Reactivity on WB	+	+	+	+

REFERENCES:

1) Klionsky, D. J., *et al*., *J. Cell Sci*. **118**, 7-18 (2005)
2) Tanida, I., *et al., J. Biol. Chem*. **277**, 13739-13744 (2002)



Western blot analysis of GATE-16 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 PM038.



Western blot analysis of GATE16 expression on transfectant (TF) of human Atg8 homologs using PM038.

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

PROTOCOL:

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) for 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 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.
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, HL-60, Jurkat, NIH/3T3, WR19L, Rat1, PC12, CHO)

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