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Not for use in diagnostic procedures.

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Anti-Atg4B mAb

Code No.	Clone	Subclass	Quantity	Concentration
M134-3MS	9H5	Mouse IgG1	20 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. 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: 20 µg IgG in 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 Atg4B on Western blotting.

APPLICATIONS:

Western blotting; 1 µg/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:

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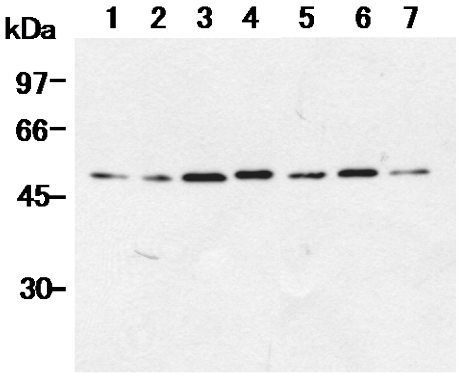
SPECIES CROSS REACTIVITY:

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

REFERENCES:

1) Yoshimura, K., *et al., Autophagy* **2**, 200-208 (2006)
2) Kumanomidou, T., *et al*., *J. Mol. Biol.* **355**, 612-618 (2006)
3) 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.

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

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.
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 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. 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, CHO)

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