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

MONOCLONAL ANTIBODY

Anti-Atg10 (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
M151-3	5A7	Mouse IgG1 κ	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. Autophagy has two ubiquitin-like conjugation systems, the Atg12 and LC3-II systems. In the Atg12 conjugation system, Atg10 functions as E2-like enzyme, plays a role to form conjugates of Atg12 and Atg5. Atg12-Atg5 complex interacts with Atg16L and forms 800 kDa complex that elongate autophagic isolation membrane.

SOURCE: This antibody was purified from hybridoma (clone 5A7) 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 human Atg10 (1-220 aa).

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 human Atg10 (26 kDa) on Western blotting.

APPLICATIONS:
Western blotting; 2 µg/mL
Immunoprecipitation; Not recommended
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	HeLa, 293T, A431	Not Tested	Not Tested
Reactivity on WB	+		

INTENDED USE:
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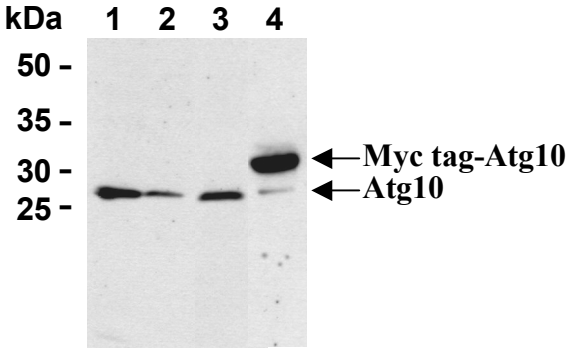
REFERENCES:
1) Nemoto, T., *et al., J. Biol. Chem.* **278**, 39517-39526 (2003)
2) Kabeya, Y., *et al., EMBO J.* **19**, 5720-5728 (2000)
3) Mizushima, N., *et al., J. Biol. Chem.* **273**, 33889-33892 (1998)



Western blot analysis of Atg10 expression in HeLa (1), 293T (2), A431 (3) and Myc tagged Atg10 (4) using M151-3.

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 20 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 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.
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 10 minutes.
12) Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; HeLa, 293T, A431, transfectant)

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