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MONOCLONAL ANTIBODY

Anti-Atg5 mAb

Code No.	Clone	Subclass	Quantity	Concentration
M153-3	4D3	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, the Atg12-Atg5-Atg16L forms 800 kDa complex that elongates autophagic isolation membrane. After completion of the formation of the autophagosome, the Atg12-Atg5-Atg16L complex dissociates from the membrane.

SOURCE: This antibody was purified from hybridoma (clone 4D3) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with C3H mouse lymphocyte immunized with recombinant full-length human Atg5 (275 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 Atg5-Atg12 complex (55 kDa) on Western blotting.

APPLICATIONS:

Western blotting; 2-5 µg/mL
Immunoprecipitation; Not recommended
Immunohistochemistry; Not tested
Immunocytochemistry; Not recommended
Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

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

INTENDED USE:

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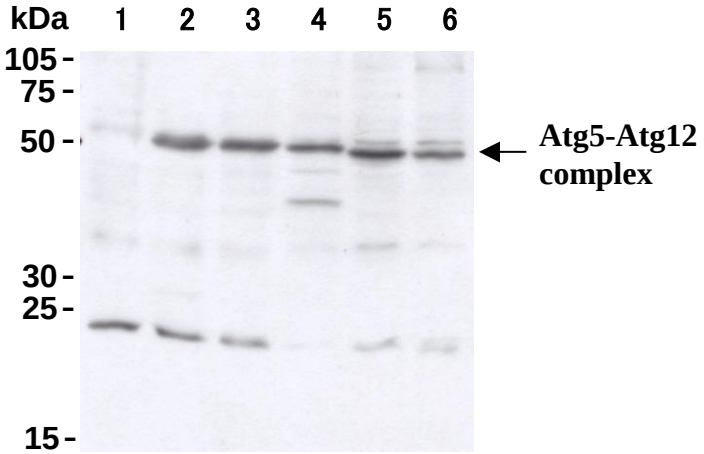
REFERENCES:

1) Young, M., *et al., J. Biol. Chem*. **287**, 12455-68 (2012) [WB]
2) Takaesu, G., *et al., J. Biochem*. **151**, 157-166 (2012)
3) Takahashi, Y., *et al., Autophagy* **7**, 61-73 (2011) [WB]
4) Hanada, T., *et al., J. Biol. Chem*. **282**, 37298-37302 (2007)
5) Pyo, J. O., *et al., J. Biol. Chem*. **280**, 20722-20729 (2005)
6) Mizushima, N., *et al., J. Cell Biol*. **152**, 657-667 (2001)

This antibody is used in the reference 1)-3).

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



Western blot analysis of Atg5 expression in Atg5$^{-/-}$ MEF (1), MEF (2), NIH/3T3 (3), CHO (4), HeLa (5) and 293T (6) using M153-3.

The bands near 25 kDa are nonspecific because they are detected in Atg5$^{-/-}$ MEF (1).

Atg5$^{-/-}$ MEF was kindly provided by Dr. Mizushima M.D. Ph.D. (Department of Physiology and Cell Biology, Tokyo Medical and Dental University, Tokyo)

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) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
6) 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.)
7) Wash the membrane with PBS-T (5 minutes x 3 times).
8) 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.
9) Wash the membrane with PBS-T (5 minutes x 3 times).
10) 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.
11) Expose to an X-ray film in a dark room for 2 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; MEF, NIH/3T3, CHO, HeLa, 293T)

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