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POLYCLONAL ANTIBODY

Anti-Atg5 pAb

Code No.	Quantity	Form
PM050	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. 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 rabbit serum using affinity column. The rabbit was immunized with the synthetic peptide corresponding to C-terminus of human Atg5.

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 Atg5-Atg12 complex (55 kDa) on Western blotting.

APPLICATIONS:

Western blotting; 1:500 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**.

SPECIES CROSS REACTIVITY:

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

INTENDED USE:

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REFERENCES:

1) Hanada, T., *et al., J. Biol. Chem.* **282**, 37298-37302 (2007)
2) Pyo, J. O., *et al., J. Biol. Chem.* **280**, 20722-20729 (2005)
3) Mizushima, N., *et al., J. Cell Biol.* **152**, 657-667 (2001)

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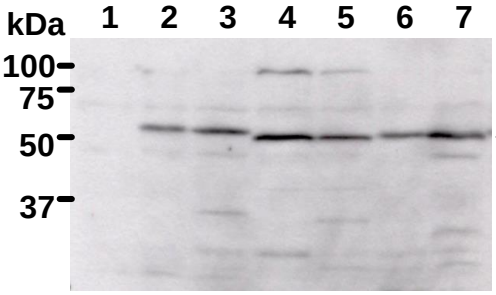

Western blot analysis of Atg5 expression in Atg5$^{-/-}$MEF (1), MEF (2), NIH/3T3 (3), HeLa (4), 293T (5), NRK (6) and PC12 (7) using PM050.

Atg5$^{-/-}$MEF cell 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% Methanol). 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 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
7) Wash the membrane with PBS-T (5 minutes x 3 times).
8) 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.

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 3 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; HeLa, 293T, MEF, NIH/3T3, NRK and PC12)

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