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

MBL A JSR Life Sciences Company

My select sampler set

Anti-Atg5 pAb

Code No.	Quantity	Form
PM050MS	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. 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: 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 -20ºC.

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:

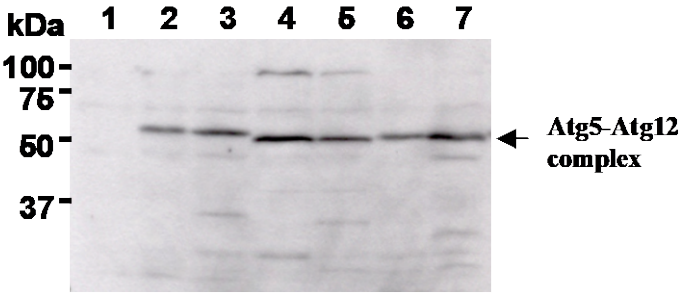
Species	Human	Mouse	Rat	Hamster
Cells	HeLa, 293T	MEF, NIH/3T3	NRK, PC12	CHO
Reactivity on WB	+	+	+	-

INTENDED USE:

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

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)



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)

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 4ºC.
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 suggested 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-rabbit IgG (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, PC12)

RELATED PRODUCTS

Please visit our web site https://ruo.mbl.co.jp/