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

MBL
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MONOCLONAL ANTIBODY

Anti-Atg16L mAb

Code No.	Clone	Subclass	Quantity	Concentration
M150-3	1F12	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 Atg16L-Atg12-Atg5 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. In recent study, nonsynonymous SNP analysis has indicated that ATG16L1 is a Crohn's disease susceptibility gene.

SOURCE: This antibody was purified from hybridoma (clone 1F12) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with C3H mouse lymphocyte immunized with the recombinant human ATG16L1 TV2 (85-588 a.a.).

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 Atg16L on Western blotting.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not recommended
Immunohistochemistry; Not tested*
*It is reported that this antibody can be used in the reference number 4).
Immunocytochemistry; Not recommended
Flow cytometry; Not tested*
*It is reported that this antibody can be used in the reference number 2).

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

INTENDED USE:
For Research Use Only. Not for use in diagnostic procedures.

REFERENCES:

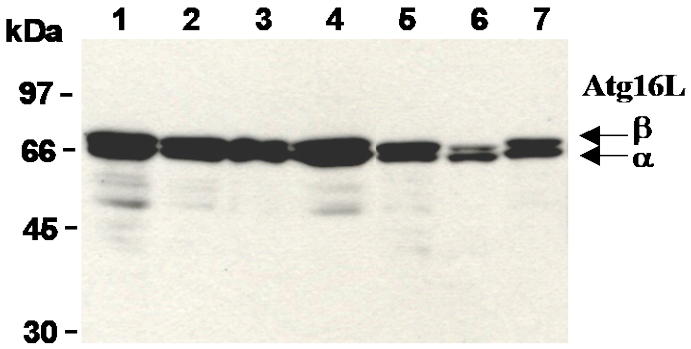
1) Boada-Romero, E., *et al., Nat. Commun.* **7**, 11821 (2016) [WB]
2) Morozova, *et al., Nat. Commun.* **6**, 5856 (2015) [IF, FCM]
3) Murthy, A., *et al., Nature* **506**, 456-462 (2014) [WB]
4) Adolph, T. E., *et al*., *Nature* **503**, 272-276 (2013) [WB, IHC]
5) Myeku, N. and Figueiredo-Pereira, M. E., *J. Biol. Chem.* **286**, 22426-22440 (2011) [WB]
6) Matsushita, M., *et al., J. Biol. Chem.* **282**, 6763-6772 (2007)

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	HeLa, 293T, Raji, Jurkat	NIH/3T3, WR19L	Rat1
Reactivity on WB	+	+	+

The descriptions of the following protocols are examples.

Each user should determine the appropriate condition.



Western blot analysis of Atg16L expression in HeLa (1), 293T (2), Jurkat (3), Raji (4), NIH/3T3 (5), WR19L (6) and Rat1 (7) using M150-3.

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 1:10,000 of Anti-IgG (Mouse) pAb-HRP (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; HeLa, 293T, Jurkat, Raji, NIH/3T3, WR19L and Rat1)

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