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

MBL
A JSR Life Sciences Company

MONOCLONAL ANTIBODY

Anti-Atg12 (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
M154-3	6E5	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 6E5) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with C3H mouse lymphocyte immunized with the KLH conjugated synthetic peptide corresponding to internal region of human Atg12.

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 Atg5-Atg12 complex (55 kDa) on Western blotting. Because almost all Atg12 exist in the form of Atg5-Atg12 complex, it is difficult to detect the monomeric Atg12.

APPLICATIONS:

Western blotting; 1 µg/mL
Immunoprecipitation; 5 µg/250 µL of cell extract from 1 x 10^7 cells
Immunohistochemistry; Not tested
Immunocytochemistry; 10 µg/mL
Flow cytometry; Not tested

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

INTENDED USE:

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

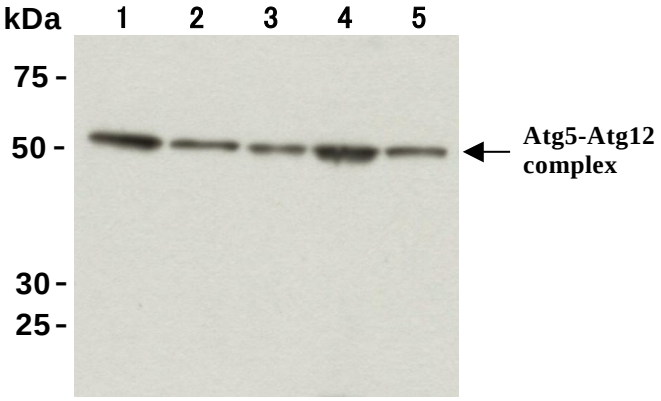
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster
Cells	HeLa, 293T, Jurkat, Raji, T24	NIH/3T3, MEF	PC12	CHO
Reactivity on WB	+	-	-	-

REFERENCES:

1) Mizushima, N., *et al., J. Cell Sci*. **116**, 1679-1688 (2003)
2) Mizushima, N., *et al., FEBS Lett*. **532**, 450-454 (2002)
3) Tanida, I., *et al., J. Biol. Chem*. **276**, 1701-1706 (2001)

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



Western blot analysis of Atg12 expression in HeLa (1), 293T (2), Jurkat (3), Raji (4) and T24 (5) using M154-3.

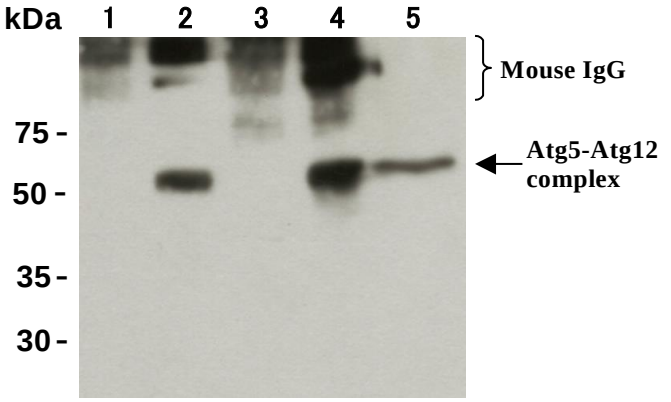
PROTOCOLS:

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

(Positive controls for Western blotting; HeLa, 293T, Jurkat, Raji, T24)

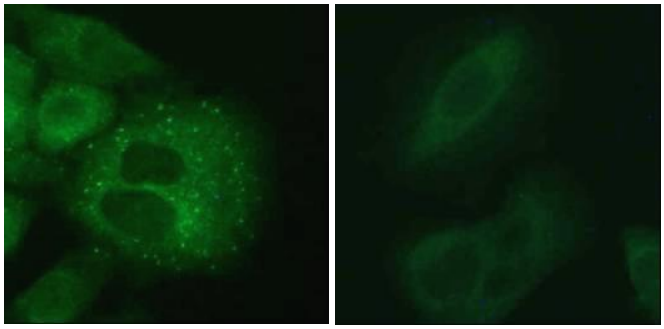


Immunoprecipitation of Atg12 from HeLa (1, 2) and 293T (3, 4) with Mouse IgG1 (1, 3) or M154-3 (2, 4). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with M154-3. Lane 5 is positive control for Western blotting.*

Immunoprecipitation

1) Wash the cells 3 times with PBS and suspend with 10 volumes of cold Lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.05% NP-40) containing appropriate protease inhibitors. Incubate it at 4ºC with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
3) Add primary antibody as suggest in the **APPLICATIONS** into 250 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C.
4) Add 20 µL of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
5) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
6) Resuspend the beads in 20 µL of Laemmli's sample buffer (2-ME free), boil for 3-5 minutes, and centrifuge for 5 minutes. Use 20 µL/lane for the SDS-PAGE analysis. (See **SDS-PAGE & Western blotting**.)

(Positive controls for Immunoprecipitation; HeLa, 293T)



Immunocytochemical detection of Atg12 on 4% PFA fixed starved A549 (left) and nutrient A549 (right) with M154-3.

Immunocytochemistry

1) Spread the cells in the nutrient condition on a glass slide, then incubate in a CO_2 incubator for one night.
2) Remove the culture supernatant by careful aspiration.
3) To obtain serum-starved conditions, culture the cells with Hank's solution or DMEM for 2-4 hours at 37°C.
4) Fix the cells by immersing the slide in 4% paraformaldehyde (PFA)/PBS for 10 minutes at room temperature (20~25°C).
5) Prepare a wash container such as a 500 mL beaker with a magnetic stirrer. Then wash the fixed cells on the glass slide by soaking the slide with a plenty of PBS in the wash container for 5 minutes. Take care not to touch the cells. Repeat another wash once more.
6) Immerse the slide in 100 µg/mL of Digitonin for 10 minutes at room temperature.
7) Wash the slide in a plenty of PBS as in the step 5).
8) Add the primary antibody diluted with PBS as suggest in the **APPLICATIONS** onto the cells and incubate for 1 hour at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
9) Wash the slide in a plenty of PBS as in the step 5).
10) Add 200 µL of 1:100 FITC conjugated anti-mouse IgG (MBL; code no. IM-0819) diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
11) Wash the slide in a plenty of PBS as in the step 5).
12) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
13) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; A549)

RELATED PRODUCTS:

Other related antibodies and kits are also available.
Please visit our website at **https://ruo.mbl.co.jp/**.