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MONOCLONAL ANTIBODY

Anti-Atg3 mAb

Code No.	Clone	Subclass	Quantity	Concentration
M133-3	3E8	Mouse IgG2b	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. Microtubule-associated protein light chain 3 (LC3) is a homologue of yeast Atg8, an essential component of autophagy. Following synthesis, the C-terminus of LC3 is cleaved by a cysteine protease-Atg4, to produce LC3-I, which is located in cytosolic fraction. LC3-I is activated by the E1-like enzyme Atg7 and forms a Atg7-LC3-I thioester. Atg7-LC3-I is transferred to Atg3 to form Atg3-LC3-I thioester. Atg3 is an E2-like enzyme that catalyzes the conjugation of LC3-I and phosphatidylethanolamine (PE) to form LC3-II. The LC3-II-PE conjugate is essential for binding tightly to autophagosomal membrane.

SOURCE: This antibody was purified from hybridoma (clone 3E8) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse lymphocyte immunized with the recombinant human Atg3.

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 Atg3 on Western blotting.

APPLICATIONS:

Western blotting; 1 µg/mL
Immunoprecipitation; 2.5 µg/300 µL of cell extract from 3 x 10^6 cells
Immunohistochemistry; Not tested
Immunocytochemistry; 0.5 µg/mL
Flow cytometry; Not tested

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

INTENDED USE:

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SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster
Cells	293T, HeLa, Jurkat	NIH/3T3, WR19L	Rat-1, PC12	CHO
Reactivity on WB	+	+	+	+

REFERENCES:

1) Klionsky, D. J., *et al.*, *J. Cell Sci.* **118**, 7-18 (2005)
2) Tanida, I., *et al.*, *J. Biol. Chem.* **277**, 13739-13744 (2002)

RELATED PRODUCTS:

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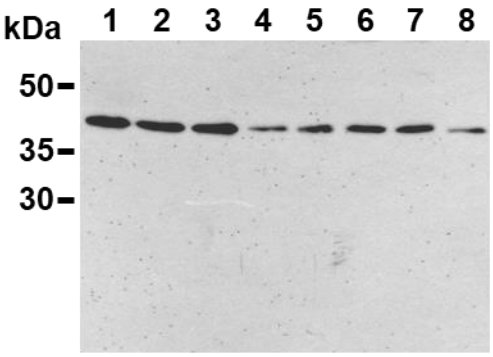


Western blotting analysis of Atg3 expression in 293T (1), HeLa (2), Jurkat (3), NIH/3T3 (4), WR19L (5), Rat-1 (6), PC12 (7) and CHO (8) using M133-3.

PROTOCOLS:

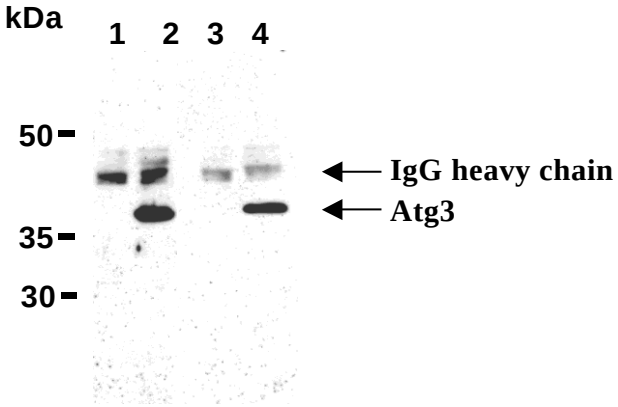
SDS-PAGE & Western blotting

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% 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) 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 condition.)

6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
7) 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.
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; 293T, HeLa, Jurkat, NIH/3T3, WR19L, Rat-1, PC12, CHO)

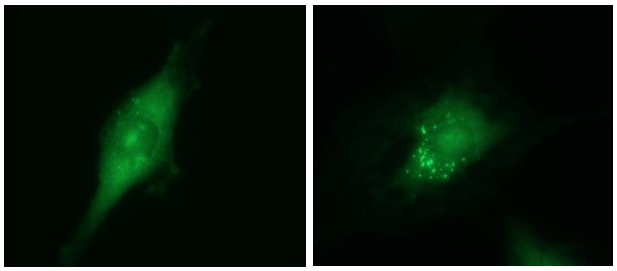


Immunoprecipitation of HeLa (1, 2) and NIH/3T3 (3, 4) with mouse IgG2b (1, 3) or M133-3 (2, 4). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with PM034.*

Immunoprecipitation

1) Wash the cells 3 times with PBS and suspend with 10 volume 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 4°C 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 suggested in the **APPLICATIONS** into 300 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. 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.
4) Centrifuge the tube at 2,500 x g for 10 seconds, and carefully discard the supernatant using a pipettor without disturbing the beads.
5) Resuspend the beads with cold Lysis buffer.
6) Centrifuge the tube at 2,500 x g for 10 seconds, and carefully discard the supernatant.
7) Repeat steps 5)-6) 3-5 times
8) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 µL/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting**.)

(Positive controls for Immunoprecipitation; HeLa, NIH/3T3)



Immunocytochemical detection of Atg3 on 4% PFA fixed nutrient normal rat kidney cell line (NRK, left) and starved NRK (right) with M133-3.

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) 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 wash once more.
6) Immerse the slide in 100 µg/mL of Digitonin for 15 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 suggested 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 (Beckman Coulter; code no. IM0819) 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 Permafluor™ aqueous mounting medium (Beckman Coulter; code no. IM0752) onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; NRK)