

 **My select** sampler set

Anti-Atg3 mAb

Code No.	Clone	Subclass	Quantity	Concentration
M133-3MS	3E8	Mouse IgG2b κ	20 μ L	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: 20 μ g IgG in 20 μ L 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, Immunoprecipitation and Immunocytochemistry.

APPLICATIONS:

Western blotting; 1 μ g/mL for chemiluminescence detection system

Immunoprecipitation; 2.5 μ g/300 μ L of cell extract from 3×10^6 cells

Immunohistochemistry; Not tested

Immunocytochemistry; 0.5 μ g/mL

Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

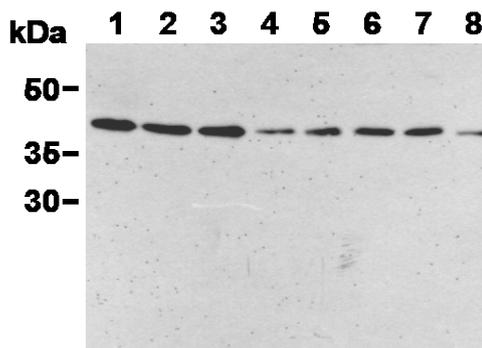
Species	Human	Mouse	Rat	Hamster
Cells	293T, HeLa, Jurkat	NIH/3T3, WR19L	Rat-1, PC12	CHO
Reactivity on WB	+	+	+	+

INTENDED USE:

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

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)



Western blot 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.

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

PROTOCOLS:

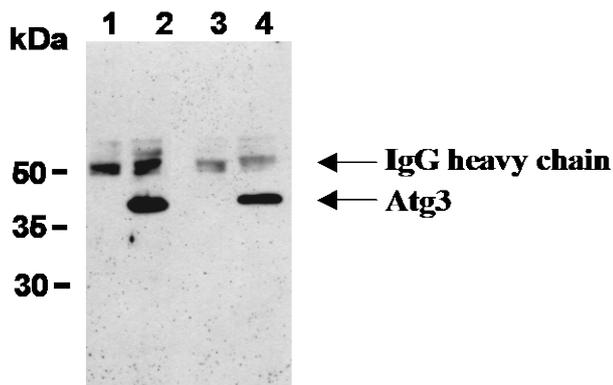
SDS-PAGE & Western Blotting

- 1) Wash the 1×10^7 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 10 μ L 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/cm² 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) 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 [0.05% Tween-20 in PBS] (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; 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.

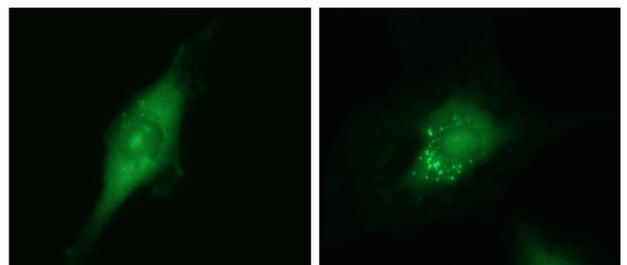
Immunoprecipitation

- 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 suggest 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) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
- 5) 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 CO₂ 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 PBS containing 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 another 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 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; NRK)

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