

POLYCLONAL ANTIBODY

# Anti-Atg16L pAb

Code No.	Quantity	Form
PM040	100 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 Atg16L-Atg12-Atg5 forms 800 kDa complex that elongate 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 rabbit serum using affinity column. The rabbit was immunized with the recombinant human ATG16L1 TV2 (85-588 aa).

**FORMULATION:** 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, Immunoprecipitation and Immunocytochemistry.

**APPLICATIONS:**

Western blotting: 1:1,000

Immunoprecipitation: 2.5 µL/300 µL of cell extract from 3 x 10<sup>6</sup> cells

Immunohistochemistry: Not tested

Immunocytochemistry: 1:200-1:500

Flow cytometry: Not tested

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

**SPECIES CROSS REACTIVITY:**

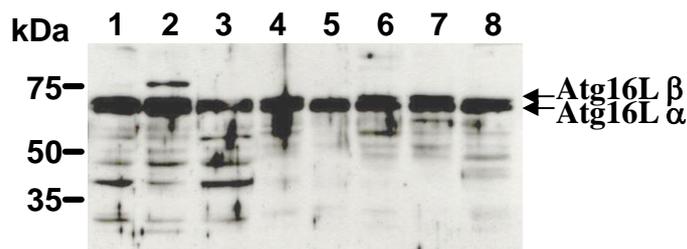
Species	Human	Mouse	Rat	Hamster
Cells	293T, HeLa, Raji	NIH/3T3, WR19L	Rat-1, PC12	CHO
Reactivity on WB	+	+	+	+

**INTENDED USE:**

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

**REFERENCES:**

- 1) Sakamoto, S., *et al.*, *Sci. Rep.* **7**, 46668 (2017) [IC]
- 2) Erbil, S., *et al.*, *J. Biol. Chem.* **291**, 16753-16765 (2016) [WB]
- 3) Ohshima, J., *et al.*, *J. Immunol.* **192**, 3328-3335 (2014) [WB]
- 4) Murthy, A., *et al.*, *Nature* **506**, 456-462 (2014) [IP]
- 5) Seto, S., *et al.*, *PLoS One* **8**, e86017 (2013) [WB]
- 6) Young, M. M., *et al.*, *J. Biol. Chem.* **287**, 12455-12468 (2012) [IC]
- 7) Moreau, K., *et al.*, *J. Cell Biol.* **196**, 483-496 (2012) [IC]
- 8) Takahashi, Y., *et al.*, *Autophagy* **7**, 61-73 (2011) [IC]
- 9) Matsunaga, K., *et al.*, *J. Cell Biol.* **190**, 511-521 (2010) [IC]
- 10) Matsushita, M., *et al.*, *J. Biol. Chem.* **282**, 6763-6772 (2007)



**Western blotting analysis of Atg16L expression in 293T (1), HeLa (2), Raji (3), NIH/3T3 (4), WR19L (5), Rat-1 (6), PC12 (7) and CHO (8) using PM040.**

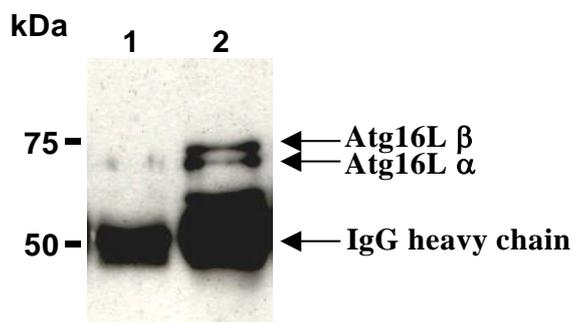
**PROTOCOLS:**

**SDS-PAGE & Western blotting**

- 1) Wash the 1x10<sup>7</sup> 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<sup>2</sup> 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 Anti-IgG (Rabbit) pAb-HRP (MBL; code no. 458) 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, Raji, NIH/3T3, WR19L, Rat-1, PC12 and CHO)



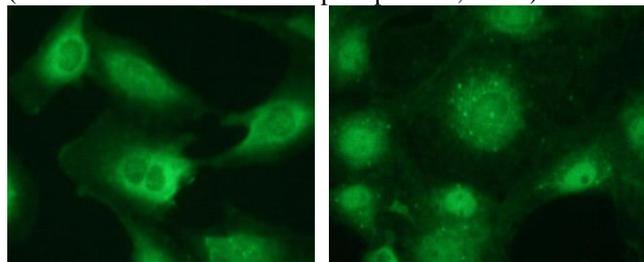
**Immunoprecipitation of HeLa with normal rabbit IgG (1) or PM040 (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with PM040.**

#### 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 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 control for Immunoprecipitation; HeLa)



**Immunocytochemical detection of Atg16L on 4% PFA fixed nutrient normal rat kidney cell line (NRK, left) and starved NRK (right) with PM040.**

#### Immunocytochemistry

- 1) Spread the cells in the nutrient condition on a glass slide, then incubate in a CO<sub>2</sub> incubator overnight.
- 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) Incubate the slide with 0.1% Gelatin in PBS to block the nonspecific staining for 30 minutes at room temperature.
- 7) Immerse the slide in 100 µg/mL of Digitonin for 15 minutes at room temperature.
- 8) Wash the slide in a plenty of PBS as in the step 5).
- 9) Add the primary antibody diluted with 0.1% Gelatin in 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.)
- 10) Wash the slide in a plenty of PBS as in the step 5).
- 11) Add FITC-conjugated anti-rabbit IgG antibody diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
- 12) Wash the slide in a plenty of PBS as in the step 5).
- 13) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
- 14) 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)

#### Related Products:

Please visit our website at <https://ruo.mbl.co.jp/>.