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## Anti-Beclin 1 pAb

<b>Code No.</b>	<b>Quantity</b>	<b>Form</b>
PD017MS	20 $\mu$ L	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. Beclin 1, the mammalian homologue of yeast Atg6, was first identified Bcl-2-interacting protein. Beclin 1 localizes to the trans-Golgi network, and forms a complex with phosphatidylinositol 3-kinase. Beclin 1 is essential for early autophagosome formation.

**SOURCE:** This antibody was purified from rabbit serum using affinity column. The rabbit was immunized with the recombinant full-length human Beclin 1.

**FORMULATION:** 20  $\mu$ 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  $-20^{\circ}\text{C}$ .

**REACTIVITY:** This antibody reacts with Beclin 1 on Western blotting, Immunoprecipitation and Immunocytochemistry.

### APPLICATIONS:

Western blotting: 1:1,000 for chemiluminescence detection system

Immunoprecipitation: 2.5  $\mu$ L/200  $\mu$ L of cell extract from  $5 \times 10^6$  cells

Immunohistochemistry: Not tested

Immunocytochemistry: 1:100

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	PC12	CHO
Reactivity on WB	+	+	+	+

### INTENDED USE:

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

### REFERENCES:

- 1) Yu, L., *et al.*, *Science* **304**, 1500-1502 (2004)
- 2) Kihara, A., *et al.*, *EMBO Rep*, **2**, 330-335 (2001)
- 3) Liang, X. H., *et al.*, *Nature* **402**, 672-676 (1999)
- 4) Liang, X. H., *et al.*, *J. Virol.* **72**, 8586-8596 (1998)

**kDa**    1    2    3    4    5    6    7



**Western blot analysis of Beclin 1 expression in 293T (1), HeLa (2), Raji (3), NIH/3T3 (4), WR19L (5), PC12 (6) and CHO (7) using PD017.**

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

### PROTOCOLS:

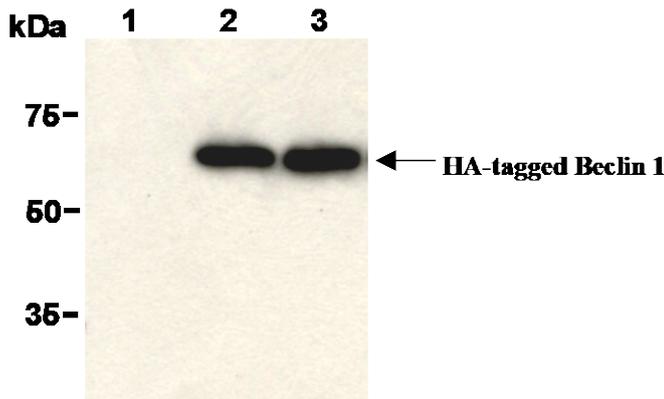
#### SDS-PAGE & Western Blotting

- 1) Wash the  $1 \times 10^7$  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  $\mu$ 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/cm<sup>2</sup> 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 the 1:10,000 HRP-conjugated anti-rabbit IgG (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 2 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, PC12, CHO)

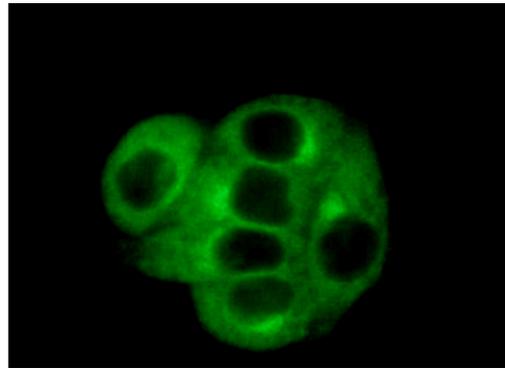


**Immunoprecipitation of HA tagged Beclin 1 with normal rabbit IgG (1), anti-HA-tag (2) and PD017 (3). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with anti-HA-tag monoclonal antibody (MBL; code no. M132-3).**

#### **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 suggest in the APPLICATIONS into 200 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20 µL of resuspended 50% protein A agarose beads 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 control for Immunoprecipitation; transfectant)



**Immunocytochemical detection of Beclin 1 on 4% PFA fixed HeLa cells with PD017.**

#### **Immunocytochemistry**

- 1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 1x10<sup>4</sup> cells for one slide, then incubate in a CO<sub>2</sub> incubator for one night.)
- 2) Wash the cells 3 times with PBS.
- 3) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde (PFA) for 10 minutes at room temperature.
- 4) The glass slide was washed with PBS 3 times.
- 5) 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 are recommended if necessary).
- 6) The glass slide was washed 2 times with PBS.
- 7) Add 100 µL of 1:100 FITC conjugated anti-rabbit IgG (MBL; code no. 234) diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
- 8) The glass slide was washed 3 times with PBS.
- 9) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
- 10) Promptly add Permafluor™ aqueous mounting medium (MBL; code no. IM-0752) onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; HeLa)

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