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POLYCLONAL ANTIBODY

Anti-Beclin 1 pAb

Code No.	Quantity	Form
PD017	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. 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: 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 Beclin 1 on Western blotting, Immunoprecipitation and Immunocytochemistry.

APPLICATIONS:

Western blotting; 1:1,000
Immunoprecipitation; 2.5 µL/200 µL of cell extract from 5 x 10^6 cells
Immunohistochemistry; Not tested*
*It is reported that this antibody can be used in Immunohistochemistry in the reference number 3).
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:
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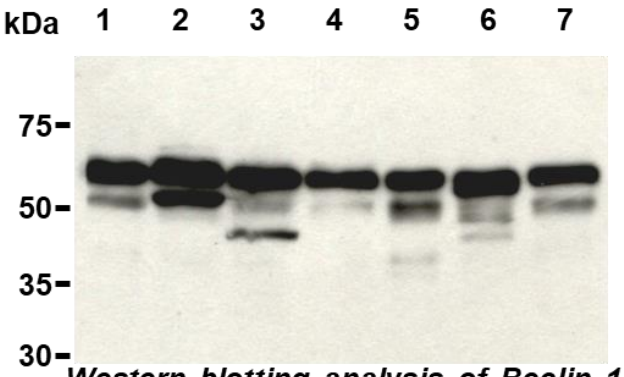
REFERENCES:

1) Hamasaki, M., *et al.*, *Nature* **495**, 389-393 (2013) [WB]
2) Berliocchi, L., *et al.*, *Mol. Pain* **7**, 83 (2011) [WB]
3) Russo, R., *et al.*, *Cell Death Dis.* **2**, e144 (2011) [WB, IHC]
4) Matsunaga, K., *et al.*, *J. Cell Biol.* **190**, 511-521 (2010) [WB]
5) Yu, L., *et al.*, *Science* **304**, 1500-1502 (2004)
6) Kihara, A., *et al.*, *EMBO Rep.* **2**, 330-335 (2001)
7) Liang, X. H., *et al.*, *Nature* **402**, 672-676 (1999)
8) Liang, X. H., *et al.*, *J. Virol.* **72**, 8586-8596 (1998)



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

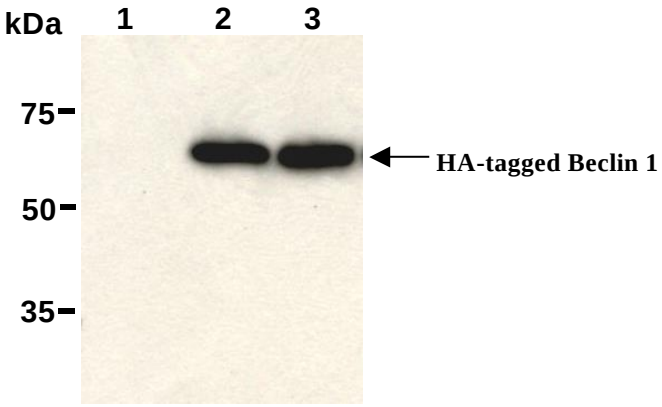
PROTOCOLS:

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, 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 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 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)

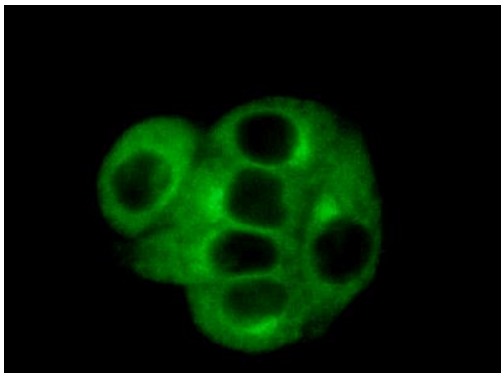


Immunoprecipitation of HA tagged Beclin 1 with normal rabbit IgG (1), anti-HA-tag (2) and PD017 (3). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with anti-HA-tag monoclonal antibody (MBL; code no. M132-3).*

Immunoprecipitation

1) Wash the cells 3 times with PBS and suspend with 10 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 200 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20 µL of resuspend 50% protein A agarose beads 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; 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 $1x10^4$ cells for one slide, then incubate in a CO_2 incubator overnight.)
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 suggested 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 twice with PBS.
7) Add 100 µL of 1:100 Anti-IgG (Rabbit) pAb-FITC (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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