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

My select sampler set

Anti-LC3 pAb

Code No.	Quantity	Form
PD014MS	20 µL	Purified IgG

BACKGROUND: Macroautophagy mediates the bulk degradation of cytoplasmic components. These components are delivered to lysosomes via autophagosomes. The rat microtubule-associated protein 1 light chain 3 (LC3), a homologue of yeast Atg8 (Aut7/Apg8), localizes to autophagosomal membranes after post-translational modifications. The C-terminal fragment of LC3 is cleaved immediately following synthesis to yield a cytosolic form called LC3-I (18 kDa). A subpopulation of LC3-I is further converted to an autophagosome-associating form, LC3-II (16 kDa). This antibody can detect both forms of LC3.

SOURCE: This antibody was purified from rabbit serum using protein A agarose. The rabbit was immunized with the recombinant full-length rat LC3.

FORMULATION: 20 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with LC3-I (18 kDa) and LC3-II (16 kDa) on Western blotting.

APPLICATIONS:
Western blotting; 1:1,000
*Blocking; 10% skimmed milk overnight at 4°C
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

REFERENCES:
1) Kabeya, Y., *et al.*, *J. Cell Sci.* **117**, 2805-2812 (2004)
2) Mizushima, N., *et al.*, *Mol. Biol. Sci.* **15**, 1101-1111 (2004)
3) Mizushima, N., *et al.*, *J. Cell Biol.* **152**, 657-667 (2001)
4) Kabeya, Y., *et al.*, *EMBO J.* **19**, 1720-5728 (2000)

This antibody is used in these references.

INTENDED USE:
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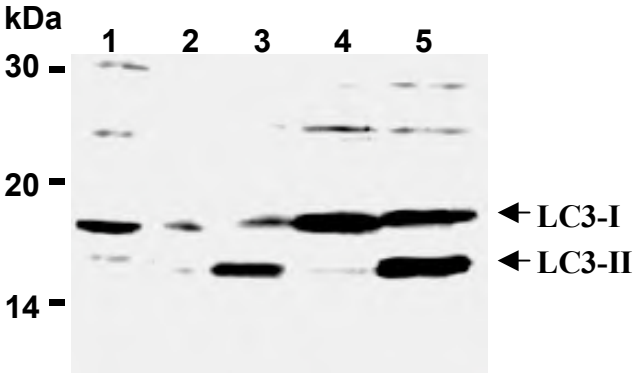
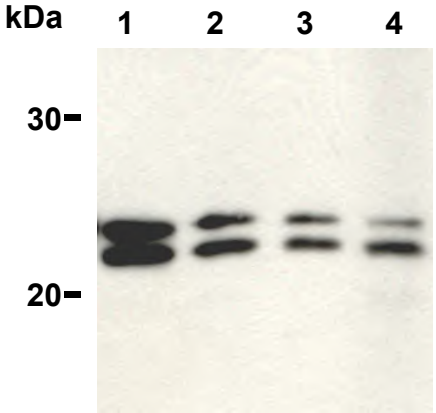
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster
Cells	HeLa, A431	NIH/3T3	PC12	CHO
Reactivity on WB	+	+	+	+



Western blot analysis of LC3-I (18kDa) and LC3-II (16kDa) expression in HeLa (1), A431 (2), NIH/3T3 (3), PC12 (4) and CHO (5) using PD014. LC3-II is modified form a subpopulation of LC3-I.

PROTOCOL:



Western blot analysis of overexpressed HA-tagged LC3 in 293T cells.
Lane 1: Anti-HA Tag (Code no. 561)
Lane 2: Anti-LC3 (polyclonal) (Code no. PM036)
Lane 3: Anti-LC3 (51-11) (Code no. M115-3)
Lane 4: Anti-LC3 (polyclonal) (Code no. PD014)

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SDS-PAGE & Western Blotting

To obtain starved or nutrient condition, cells were incubated with Hank's solution or DMEM respectively for 2 hours at 37°C.

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) 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. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 3 minutes and centrifuge. Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel for electrophoresis.
5) 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% MeOH). See the manufacture's manual for precise transfer procedure.
6) To reduce nonspecific binding, place the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
7) 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.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
9) 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.
10) Wash the membrane with PBS-T (10 minutes x 3 times).
11) 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.
12) 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; HeLa, A431, NIH/3T3, PC12, CHO)

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