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POLYCLONAL ANTIBODY

Anti-LC3 pAb

Code No.	Quantity	Form
PD014	100 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. A subpopulation of LC3-I is further converted to an autophagosome-associating form, LC3-II. 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: 100 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 and LC3-II 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
*It is reported that this antibody can be used in immunohistochemistry and immunocytochemistry in the reference number 4)-5) and 1), 3).

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

SPECIES CROSS REACTIVITY:

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

INTENDED USE:
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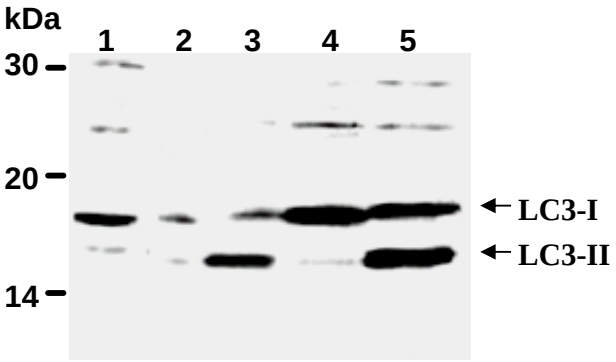
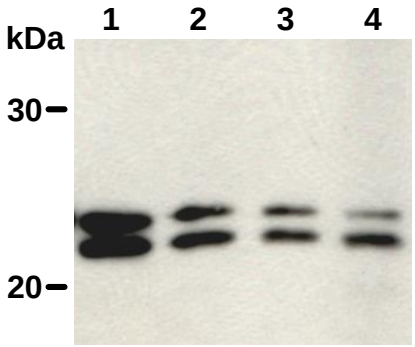
REFERENCES:
1) Kobayashi, S., *et al.*, *PNAS* **112**, 7027-7032 (2015) [IC]
2) Delpeut, S., *et al.*, *J. Virol.* **86**, 8527-8535 (2012) [WB]
3) Brown, J. A., *et al.*, *J. Neurosci.* **30**, 5242-5252 (2010) [IC]
4) Wang, J., *et al.*, *J. Clin. Exp. Hematop.* **49**, 97-108 (2009) [IHC]
5) Takamura, A., *et al.*, *Biochem. Biophys. Res. Commun.* **367**, 616-622 (2008) [WB, IHC]



Western blotting analysis of LC3-I and LC3-II 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.



Western blotting analysis of overexpressed HA-tagged LC3 in 293T cells
Lane 1: Anti-HA Tag (Code: 561)
Lane 2: Anti-LC3 (polyclonal) (Code: PM036)
Lane 3: Anti-LC3 (51-11) (Code: M115-3)
Lane 4: Anti-LC3 (polyclonal) (Code: PD014)

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

PROTOCOL:

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 volumes 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% methanol). 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 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 the conditions.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
9) 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.
10) Wash the membrane with PBS-T (10 minutes x 3).
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, PC1 and CHO)

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