

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



Anti-LC3 mAb

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| CODE No. | M186-3 |
| CLONALITY | Monoclonal |
| CLONE | 8E10 |
| ISOTYPE | Mouse IgG2a κ |
| QUANTITY | 100 μ L, 1 mg/mL |
| SOURCE | Purified IgG from hybridoma supernatant |
| IMMUNOGEN | Human LC3 (MAP1LC3B), 1-120 aa (recombinant) |
| REACTIVITY | This clone reacts with LC3B and does not cross-react with LC3A, LC3C, GATE-16 and GABARAP |
| FORMURATION | 1 mg/mL in 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 . |

APPLICATIONS-CONFIRMED

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| <u>Western blotting</u> | 1 μ g/mL for chemiluminescence detection system |
| <u>Immunohistochemistry</u> | Not recommended |
| <u>Immunocytochemistry</u> | Not recommended |

SPECIES CROSS REACTIVITY on WB

| Species | Human | Mouse | Rat | Hamster |
|------------|-------|--|------|---------|
| Cells | HeLa | NIH/3T3, MEF, Atg5 ^{-/-} MEF Brain | PC12 | CHO |
| Reactivity | + | + | + | + |

Entrez Gene ID 81631 (Human), 67443 (Mouse), 64862 (Rat)

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- 5) Tabata, K., *et al.*, *Mol. Biol. Cell* **21**, 4162-4172 (2010)
- 6) Mookerjee, S., *et al.*, *J. Neurosci.* **29**, 15134-15144 (2009)
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- 11) Schmitz, J., *et al.*, *Immunity* **23**, 479-490 (2005)

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RELATED PRODUCTS

Antibodies

| | | |
|----------|--|------------------------|
| PM036 | Anti-LC3 pAb | [WB, IP, IC, IHC, FCM] |
| M152-3 | Anti-LC3 mAb (4E12) | [WB, IP, IC, FCM, EM] |
| M186-3 | Anti-LC3 mAb (8E10) | [WB] |
| M186-7 | Anti-LC3 mAb-HRP-Direct (8E10) | |
| PD014 | Anti-LC3 pAb | [WB] |
| PD015 | Anti-LC3 pAb | [IC] |
| PM046 | Anti-LC3 pAb | [WB, IC] |
| M115-3 | Anti-LC3 mAb (51-11) | [WB] |
| PM045 | Anti-p62 (SQSTM1) pAb | |
| M162-3 | Anti-p62 (SQSTM1) (Human) mAb (5F2) | |
| M162-A48 | Anti-p62 (SQSTM1) (Human) mAb -Alexa Fluor [®] 488 (5F2) | |
| M162-A59 | Anti-p62 (SQSTM1) (Human) mAb -Alexa Fluor [®] 594 (5F2) | |
| M162-A64 | Anti-p62 (SQSTM1) (Human) mAb -Alexa Fluor [®] 647 (5F2) | |
| PM066 | Anti-p62 C-terminal pAb | |
| PM066-7 | Anti-p62 C-terminal pAb-HRP-Direct | |
| D343-3 | Anti-Phospho-p62 (SQSTM1) (Ser403) mAb (4F6) | |
| D344-3 | Anti-Phospho-p62 (SQSTM1) (Ser403) mAb (4C8) | |
| PM074 | Anti-Phospho-p62 (SQSTM1) (Ser351) pAb | |
| PD017 | Anti-Beclin 1 pAb | |
| PM037 | Anti-GABARAP pAb | |
| M135-3 | Anti-GABARAP mAb (1F4) | |
| PM038 | Anti-GATE-16 pAb | |
| PD041 | Anti-Atg2A pAb | |
| PM034 | Anti-Atg3 pAb | |
| M133-3 | Anti-Atg3 mAb (3E8) | |
| M134-3 | Anti-Atg4B mAb (9H5) | |
| PM050 | Anti-Atg5 pAb | |
| M153-3 | Anti-Atg5 mAb (4D3) | |
| PM039 | Anti-Atg7 (Human) pAb | |
| PD042 | Anti-Atg9A pAb | |
| M151-3 | Anti-Atg10 (Human) mAb (5A7) | |
| M154-3 | Anti-Atg12 (Human) mAb (6E5) | |
| PD036 | Anti-Atg13 (Human) pAb | |
| M183-3 | Anti-Atg13 mAb (5G4) | |
| PD026 | Anti-Atg14 pAb | |
| M184-3 | Anti-Atg14 (Human) mAb (4H8) | |
| PM040 | Anti-Atg16L pAb | |
| M150-3 | Anti-Atg16L mAb (1F12) | |
| M160-3 | Anti-UVRAG mAb (1H4) | |
| PD027 | Anti-Rubicon (Human) pAb | |
| M170-3 | Anti-Rubicon (Human) mAb (1H6) | |
| PM069 | Anti-NRF2 pAb | |
| M200-3 | Anti-NRF2 mAb (1F2) | |
| PD037 | Anti-Tel2 pAb | |
| PM072 | Anti-VMP1 pAb | |
| M175-3 | Anti- α -Tubulin mAb (2F9) | |
| M175-A48 | Anti- α -Tubulin mAb-Alexa Fluor [®] 488 (2F9) | |
| M175-A59 | Anti- α -Tubulin mAb-Alexa Fluor [®] 594 (2F9) | |
| M175-A64 | Anti- α -Tubulin mAb-Alexa Fluor [®] 647 (2F9) | |
| PM054 | Anti- α -Tubulin pAb | |
| PM054-7 | Anti- α -Tubulin pAb-HRP-Direct | |

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| M176-3 | Anti-EEA1 mAb (3C10) |
| M176-A48 | Anti-EEA1 mAb-Alexa Fluor [®] 488 (3C10) |
| M176-A59 | Anti-EEA1 mAb-Alexa Fluor [®] 594 (3C10) |
| M176-A64 | Anti-EEA1 mAb-Alexa Fluor [®] 647 (3C10) |
| PM062 | Anti-EEA1 pAb |
| M178-3 | Anti-Calnexin mAb (4F10) |
| M178-A48 | Anti-Calnexin mAb-Alexa Fluor [®] 488 (4F10) |
| M178-A59 | Anti-Calnexin mAb-Alexa Fluor [®] 594 (4F10) |
| M178-A64 | Anti-Calnexin mAb-Alexa Fluor [®] 647 (4F10) |
| PM060 | Anti-Calnexin pAb |
| M181-3 | Anti-KDEL mAb (1D5) |
| PM059 | Anti-KDEL pAb |
| M179-3 | Anti-GM130 mAb (5G8) |
| M179-A48 | Anti-GM130 mAb-Alexa Fluor [®] 488 (5G8) |
| M179-A59 | Anti-GM130 mAb-Alexa Fluor [®] 594 (5G8) |
| M179-A64 | Anti-GM130 mAb-Alexa Fluor [®] 647 (5G8) |
| PM061 | Anti-GM130 pAb |
| PM063 | Anti-COX4 pAb |
| PM064 | Anti-Lamin B1 pAb |

Kits

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| 8485 | Autophagy Ab Sampler Set |
| PM036-PN | Positive control for anti-LC3 antibody |

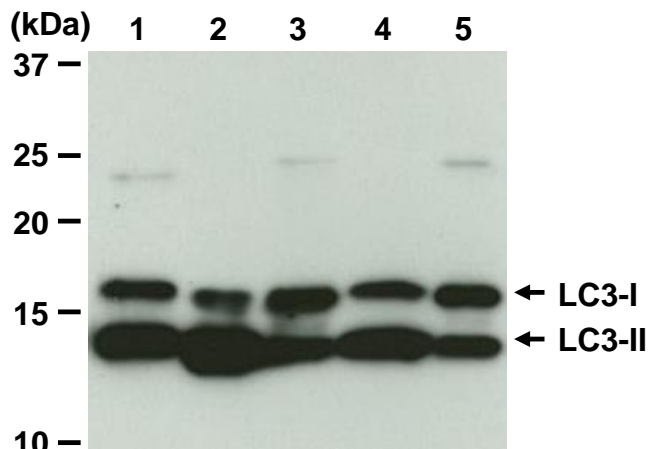
WB: Western blotting
IP: Immunoprecipitation
IC: Immunocytochemistry
IHC: Immunohistochemistry
FCM: Flow cytometry
EM: Immuno-electron microscopy

Other related antibodies and kits are also available.
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SDS-PAGE & Western blotting

- 1) Wash 1×10^7 cells 3 times with PBS and suspend them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 10 sec.).
- 2) Boil the samples for 3 min. and centrifuge. Load 10 μ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (15% acrylamide) for electrophoresis.
- 3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer'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) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
- 6) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
- 7) Wash the membrane with PBS-T (5 min. x 3 times).
- 8) Incubate the membrane with the 1:10,000 anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 9) Wash the membrane with PBS-T (5 min. x 3 times).
- 10) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 11) Expose to an X-ray film in a dark room for 1 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; HeLa, NIH/3T3, PC12, CHO, mouse brain tissue, MEF, Atg5^{-/-} MEF)



Western blot analysis of LC3

Lane 1: HeLa
Lane 2: NIH/3T3
Lane 3: PC12
Lane 4: CHO
Lane 5: mouse brain tissue

Lane 6: Atg5^{-/-} MEF
Lane 7: MEF
Lane 8: MEF (6 hr. treatment with 50 μ M Chloroquine)
Immunoblotted with Anti-LC3 mAb (M186-3)

Atg5^{-/-} MEF was kindly provided by Dr. Noboru Mizushima, *M.D., Ph.D.* (Department of Biochemistry and Molecular Biology, Graduate School and Faculty of Medicine, The University of Tokyo)

