

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



Anti-LC3 mAb-HRP-Direct

CODE No. M186-7

CLONALITY Monoclonal
CLONE 8E10
ISOTYPE Mouse IgG2a κ
QUANTITY 50 μ L

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 PBS/Preservative/Stabilizer
STORAGE This antibody solution is stable for one year from the date of purchase when stored at 4°C.

APPLICATION-CONFIRMED

Western blotting 1:1,000 for chemiluminescence detection system

SPECIES CROSS REACTIVITY on WB

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

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

REFERENCES

- 1) Jia, W., *et al.*, *J. Immunol.* **186**, 5313-5322 (2011)
- 2) Tabata, K., *et al.*, *Mol. Biol. Cell* **21**, 4162-4172 (2010)
- 3) Mookerjee, S., *et al.*, *J. Neurosci.* **29**, 15134-15144 (2009)
- 4) Saitoh, T., *et al.*, *Nature* **456**, 264-268 (2008)
- 5) Kabeya, Y., *et al.*, *J. Cell Sci.* **117**, 2805-2812 (2004)
- 6) Mizushima, N., *et al.*, *Mol. Biol. Cell* **15**, 1101-1111 (2004)
- 7) Mizushima, N., *et al.*, *J. Cell Biol.* **152**, 657-668 (2001)

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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]
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	
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	

M186-7	Anti-LC3 mAb-HRP-Direct (8E10)
PM066-7	Anti-p62 C-terminal pAb-HRP-Direct
PM054-7	Anti- α -Tubulin pAb-HRP-Direct
PM053-7	Anti- β -Actin pAb-HRP-Direct
M185-7	Anti-DDDDK-tag mAb-HRP-Direct (FLA-1)
PM020-7	Anti-DDDDK-tag pAb-HRP-Direct
M171-7	Anti-GAPDH mAb-HRP-Direct (3H12)
598-7	Anti-GFP pAb-HRP-Direct
PM013-7	Anti-GST-tag pAb-HRP-Direct
M180-7	Anti-HA-tag mAb-HRP-Direct (TANA2)
561-7	Anti-HA-tag pAb-HRP-Direct
D291-7	Anti-His-tag mAb-HRP-Direct (OGHis)
M047-7	Anti-Myc-tag mAb-HRP-Direct (PL14)
M192-7	Anti-Myc-tag mAb-HRP-Direct (My3)

PM005-7	Anti-RFP pAb-HRP-Direct
PM003-7	Anti-V5-tag pAb-HRP-Direct

Kits

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

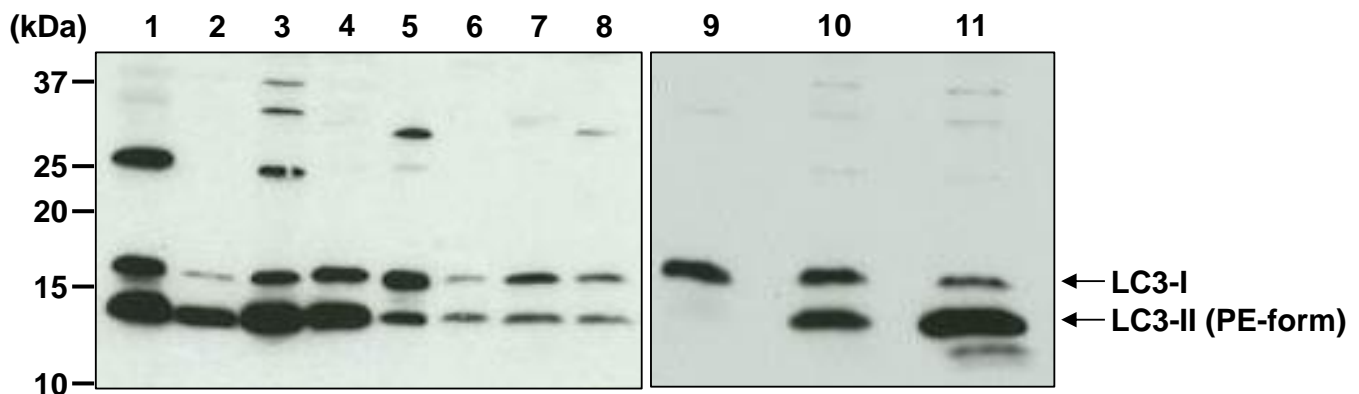
WB: Western blotting
IP: Immunoprecipitation
IC: Immunocytochemistry
IHC: Immunohistochemistry
FCM: Flow cytometry
EM: Immuno-electron microscopy

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) and carry out 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) for 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 PBS, pH 7.2 containing 1% skimmed milk as suggested in the **APPLICATION** 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) 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.
- 9) Expose to an X-ray film in a dark room for 3 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, liver, spleen, kidney, MEF, MEF^{Atg5^{-/-}} and chloroquine-treated MEF)



Western blot analysis of LC3

- Lane 1: HeLa
- Lane 2: NIH/3T3
- Lane 3: PC12
- Lane 4: CHO
- Lane 5: Mouse brain (20 μ g of tissue lysate)
- Lane 6: Mouse liver (20 μ g of tissue lysate)
- Lane 7: Mouse spleen (20 μ g of tissue lysate)
- Lane 8: Mouse kidney (20 μ g of tissue lysate)
- Lane 9: MEF^{Atg5^{-/-}}
- Lane 10: MEF
- Lane 11: MEF (6 hr. treatment with 50 μ M Chloroquine)

Immunoblotted with anti-LC3 mAb-HRP-Direct (M186-7)

MEF^{Atg5^{-/-}} 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)