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 µL
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.
FORMULATION	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

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), 100769810 (Hamster)

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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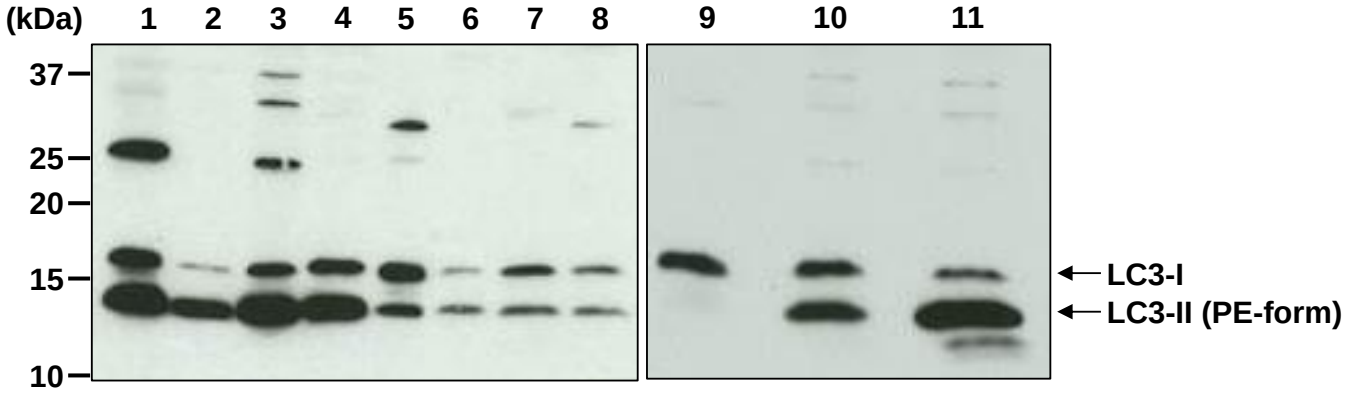
SDS-PAGE & Western blotting

1) Wash 1 x 10^7 cells 3 times with PBS and suspends 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 μL 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/cm^2 for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). 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).
6) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) 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).
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 μg of tissue lysate)
Lane 6: Mouse liver (20 μg of tissue lysate)
Lane 7: Mouse spleen (20 μg of tissue lysate)
Lane 8: Mouse kidney (20 μg of tissue lysate)
Lane 9: $MEF^{Atg5-/-}$
Lane 10: MEF
Lane 11: MEF (6 hr. treatment with 50 μM Chloroquine)

Immunoblotted with anti-LC3 mAb-HRP-DirecT (MBL, code no. 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)