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

MBL
A JSR Life Sciences Company

Anti-LC3 mAb

CODE No.	M186-3
CLONALITY	Monoclonal
CLONE	8E10
ISOTYPE	Mouse IgG2a κ
QUANTITY	100 µL, 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
FORMULATION	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

Western blotting	1 µg/mL
Immunohistochemistry	Not recommended
Immunocytochemistry	Not recommended

SPECIES CROSS REACTIVITY on WB

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

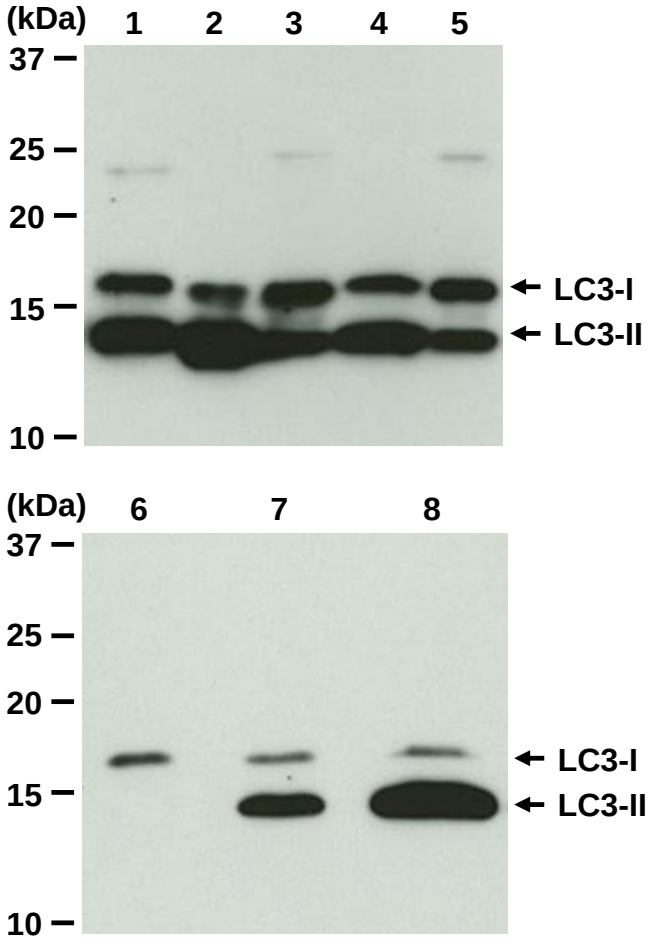
Entrez Gene ID 81631 (Human), 67443 (Mouse), 64862 (Rat)

For more information, please visit our web site **https://ruo.mbl.co.jp/.**

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 10sec.).
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) for 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) 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 **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).
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).
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 blotting 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 μM Chloroquine)

Immunoblotted with Anti-LC3 mAb
(MBL, code no. 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)