

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



## Anti-LC3 mAb

<b>CODE No.</b>	M186-3
<b>CLONALITY</b>	Monoclonal
<b>CLONE</b>	8E10
<b>ISOTYPE</b>	Mouse IgG2a $\kappa$
<b>QUANTITY</b>	100 $\mu$ L, 1 mg/mL
<b>SOURCE</b>	Purified IgG from hybridoma supernatant
<b>IMMUNOGEN</b>	Human LC3 (MAP1LC3B), 1-120 aa (recombinant)
<b>REACTIVITY</b>	This clone reacts with LC3B and does not cross-react with LC3A, LC3C, GATE-16 and GABARAP
<b>FORMULATION</b>	1 mg/mL in PBS containing 50% Glycerol (pH 7.2). No preservative is contained.
<b>STORAGE</b>	This antibody solution is stable for one year from the date of purchase when stored at -20°C.

### APPLICATIONS-CONFIRMED

<u>Western blotting</u>	1 $\mu$ g/mL
<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 <sup>-/-</sup> MEF Brain	PC12	CHO
Reactivity	+	+	+	+

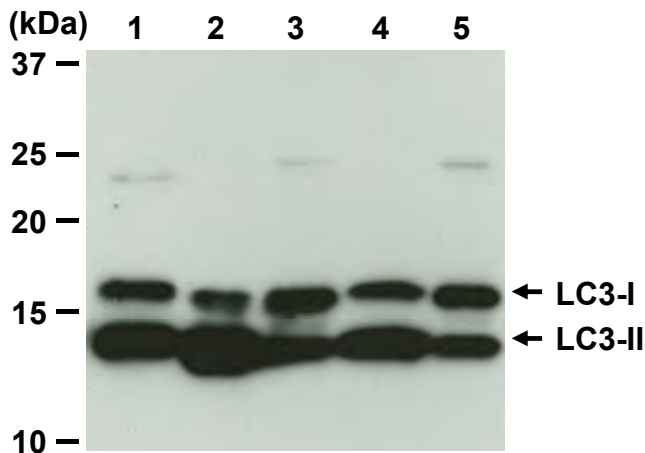
**Entrez Gene ID** 81631 (Human), 67443 (Mouse), 64862 (Rat)

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

### SDS-PAGE & Western blotting

- 1) Wash  $1 \times 10^7$  cells 3 times with PBS and suspend 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  $\mu$ 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/cm<sup>2</sup> 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.

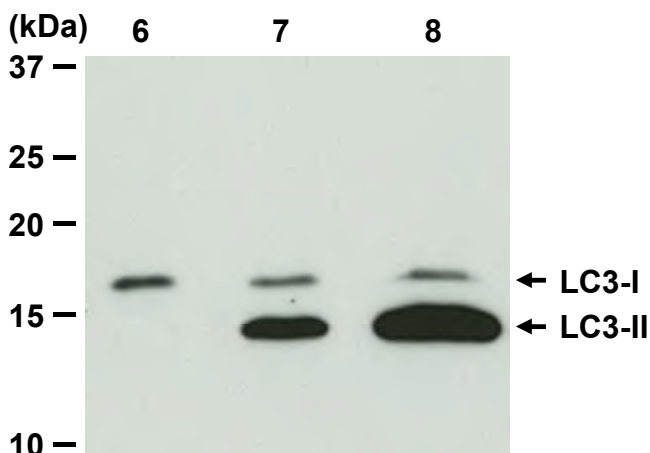
(Positive controls for Western blotting; HeLa, NIH/3T3, PC12, CHO, mouse brain tissue, MEF, Atg5<sup>-/-</sup> MEF)



#### **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<sup>-/-</sup> MEF
- Lane 7: MEF
- Lane 8: MEF (6 hr. treatment with 50  $\mu$ M Chloroquine)

Immunoblotted with Anti-LC3 mAb  
(MBL, code no. M186-3)



Atg5<sup>-/-</sup> 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)