

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

Positive control for anti-LC3 antibody

CODE No.	PM036-PN
QUANTITY	100 μ L (10 tests)
FORMULATION	1x10 ⁵ cells of human LC3B/293T were suspended with 100 μ L of Laemmli's sample buffer. This product contains 5% 2-mercaptoethanol. Handle with care.
STORAGE	This product is stable for one year from the date of purchase when stored at -20°C. Avoid repeated freeze/thaw cycles.
APPLICATION-CONFIRMED	
<u>Western blotting</u>	10 μ L/lane (ready-to-use)

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

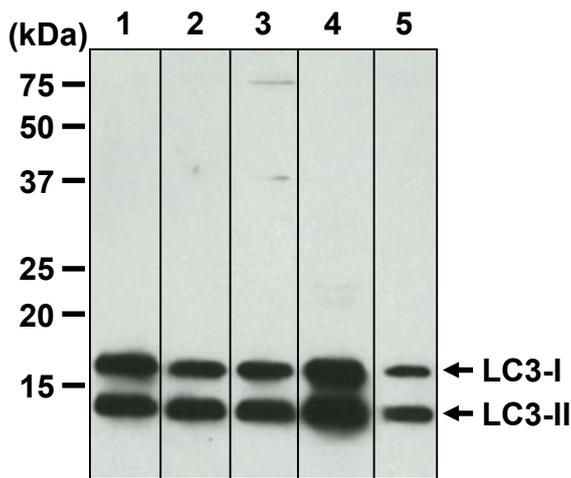
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The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

SDS-PAGE & Western blotting

- 1) Load 10 μ L of PM036-PN per lane in a 1-mm-thick SDS-polyacrylamide gel (15% acrylamide) for electrophoresis.
- 2) 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.
- 3) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
- 4) Wash the membrane with PBS-T (0.05% Tween-20 in PBS) (5 min. x 3 times).
- 5) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 6) Wash the membrane with PBS-T (5 min. x 3 times).
- 7) Incubate the membrane with 1:10,000 Anti-IgG (Rabbit) pAb-HRP (MBL; code no. 458) or 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.
- 8) Wash the membrane with PBS-T (5 min. x 3 times).
- 9) 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.
- 10) 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.



Western blot analysis of overexpressed human LC3B in 293T

- Lane 1: IB with anti-LC3 pAb (code no. PM036), 1:1,000
- Lane 2: IB with anti-LC3 pAb (code no. PD014), 1:1,000
- Lane 3: IB with anti-LC3 pAb (code no. PM046), 1:1,000
- Lane 4: IB with anti-LC3 mAb (clone: 8E10) (code no. M186-3), 1 μ g/mL
- Lane 5: IB with anti-LC3 mAb (clone: 4E12) (code no. M152-3), 10 μ g/mL