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

MBL International Corporation

Autophagy Ab Sampler Set

CODE No. MBL- 8485

DESCRIPTION

Autophagy is a process of intracellular bulk degradation in which cytoplasmic components including organelles are sequestered within double-membrane vesicles. Autophagosomes deliver the contents to the lysosome/vacuole for degradation. This process aids in cell survival during starvation periods by eliminating nutrients being needed for unnecessary cellular processes. Recently, autophagy has been suggested to play an important role in development, differentiation, neurodegeneration, infection and cancer.

MBL provides a useful set of antibodies that can detect endogenous cellular factors for autophagy. The Autophagy Ab Sample Set includes popular autophagy targets for Beclin1, Atg16L, Atg5, p62 and LC3. The MBL LC3 antibodies can clearly detect both LC3-I and LC3-II in western blotting. The MBL Autophagy Antibody sample set is an economical approach to testing autophagy across multiple targets for many different applications.

COMPONENTS

Code No.	Products	Clone	Isotype	Quantity
PM036Y	Anti-LC3 pAb	Polyclonal	Rabbit IgG	25 µL
M186-3Y	Anti-LC3 mAb	8E10	Mouse IgG2a κ	25 µL, 1 mg/mL
M152-3Y	Anti-LC3 mAb	4E12	Mouse IgG1 κ	25 µL, 2 mg/mL
PD017Y	Anti-Beclin 1 pAb	Polyclonal	Rabbit Ig(aff.)	25 µL
PM040Y	Anti-Atg16L pAb	Polyclonal	Rabbit Ig(aff.)	25 µL
PM045Y	Anti-p62 (SQSTM1) pAb	Polyclonal	Rabbit Ig(aff.)	25 µL
PM050Y	Anti-Atg5 pAb	Polyclonal	Rabbit Ig(aff.)	25 µL
PM036-PNY	Positive control for anti-LC3 antibody			100 µL (10 tests)

FORMULATION

Antibodies: PBS containing 50% Glycerol (pH 7.2). No preservative is contained.
PM036-PNY: human LC3B overexpressed 293T
$1x10^5$ cells were suspended with 100 µL of Laemmli's sample buffer.
This product contains 5% 2-mercaptoethanol. Handle with care.

STORAGE

This set is stable for one year from the date of purchase when stored at -20°C.

APPLICATIONS-CONFIRMED

Code No.	WB	IP	IC	IHC	FCM	Immuno-EM
PM036Y	1:1,000	2 μL/sample	1:500-1:1,000	1:1,000-1:2,000	1:200	NT
M186-3Y	1 μg/mL	NT	NR	NR	NT	NT
M152-3Y	5 μg/mL	5 μg/sample	40 μg/mL	NT	40 μg/mL	20 μg/mL
PD017Y	1:1,000	2.5 μL/sample	1:100	NT	NT	NT
PM040Y	1:1,000	2.5 μL/sample	1:200-1:500	NT	NT	NT
PM045Y	1:1,000	2 μL/sample	1:500	1:1,000	NT	NT
PM050Y	1:500	NT	NT	NT	NT	NT
PM036-PNY	10 μL/lane					

NT: Not Tested NR: Not Recommended

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

For detail protocols of each product, please refer to our website.

SPECIES CROSS REACTIVITY on WB

Code No.	Products	Human	Mouse	Rat	Hamster
PM036Y	Anti-LC3 pAb	+	+	+	+
M186-3Y	Anti-LC3 mAb	+	+	+	+
M152-3Y	Anti-LC3 mAb	+	+	+	+
PD017Y	Anti-Beclin 1 pAb	+	+	+	+
PM040Y	Anti-Atg16L pAb	+	+	+	+
PM045Y	Anti-p62 (SQSTM1) pAb	+	+	+	+
PM050Y	Anti-Atg5 pAb	+	+	+	–

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PM036

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PM050	1) Myeku, N., *et al.*, *J. Biol. Chem.* **286**, 22426-22440 (2011)	[WB]

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

RELATED PRODUCTS

PM036	Anti-LC3 (polyclonal)	[WB, IP, IC, IHC, FCM]
M152-3	Anti-LC3 (4E12)	[WB, IP, IC, FCM, EM]
M186-3	Anti-LC3 (8E10)	[WB]
PM045	Anti-p62/SQSTM1 (polyclonal)	
PD017	Anti-Beclin 1 (polyclonal)	
PM050	Anti-Atg5 (polyclonal)	
PM036-PN	Positive control for anti-LC3 antibody	
PD014	Anti-LC3 (polyclonal)	[WB]
PD015	Anti-LC3 (polyclonal)	[IC]
PM046	Anti-LC3 (polyclonal)	[WB, IC]
M115-3	Anti-LC3 (51-11)	[WB]
M162-3	Anti-p62/SQSTM1 (5F2)	
M162-A48	Anti-p62/SQSTM1-Alexa Fluor®488 (5F2)	
M162-A59	Anti-p62/SQSTM1-Alexa Fluor®594 (5F2)	
M162-A64	Anti-p62/SQSTM1-Alexa Fluor®647 (5F2)	
PM066	Anti-p62 C-terminal (polyclonal)	
PM037	Anti-GABARAP (polyclonal)	
M135-3	Anti-GABARAP (1F4)	
PM038	Anti-GATE-16 (polyclonal)	
PM034	Anti-Atg3 (polyclonal)	
M133-3	Anti-Atg3 (3E8)	
M134-3	Anti-Atg4B (9H5)	
M153-3	Anti-Atg5 (4D3)	
PM039	Anti-Atg7 (polyclonal)	
M151-3	Anti-Atg10 (5A7)	
M154-3	Anti-Atg12 (6E5)	
PD036	Anti-Atg13 (polyclonal)	
M183-3	Anti-Atg13 (5G4)	
PD026	Anti-Atg14 (polyclonal)	

M184-3	Anti-Atg14 (4H8)
PM040	Anti-Atg16L (polyclonal)
M150-3	Anti-Atg16L (1F12)
M160-3	Anti-UVRAG (1H4)
PD027	Anti-Rubicon (polyclonal)
M170-3	Anti-Rubicon (1H6)
PM069	Anti-NRF2 (polyclonal)
PD037	Anti-Tel2 (polyclonal)

WB: Western blotting

IP: Immunoprecipitation

IC: Immunocytochemistry

IHC: Immunohistochemistry

FCM: Flow cytometry

EM: Immuno-electron microscopy

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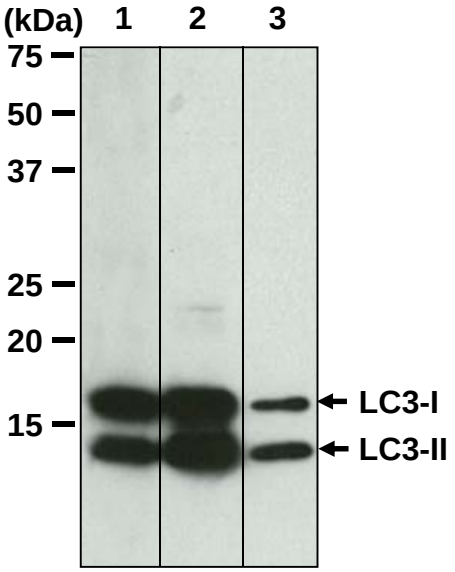
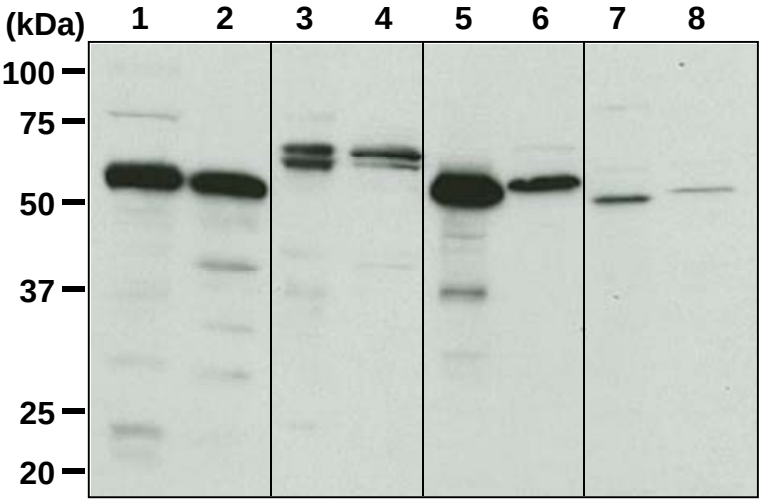
SDS-PAGE & Western blotting

1) Wash 1 x 10^6 cells 3 times with PBS and suspends them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 15 seconds).
2) Boil the samples for 2 min. and centrifuge. Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis. In the case of LC3 detection, load 10 µL of the PM036-PNY 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% 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 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 times).
8) Incubate the membrane with the 1:10,000 anti-IgG (Mouse)-HRP (MBL; code no. 330) or anti-IgG (Rabbit)-HRP (MBL; code no. 458) 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 times).
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 3 min. Develop the film as usual. The condition for exposure and development may vary.





Western blot analysis

Left figure
Sample; human LC3B overexpressed in 293T (code no. PM036-PNY)
Antibody;
Lane 1: Anti-LC3 pAb (code no. PM036Y)
Lane 2: Anti-LC3 mAb (code no. M186-3Y)
Lane 3: Anti-LC3 mAb (code no. M152-3Y)

Right figure
Sample;
Lane 1, 3, 5, 7: HeLa
Lane 2, 4, 6, 8: NIH/3T3
Antibody;
Lane 1, 2: Anti-Beclin 1 pAb (code no. PD017Y)
Lane 3, 4: Anti-Atg16L pAb (code no. PM040Y)
Lane 5, 6: Anti-p62 (SQSTM1) pAb (code no. PM045Y)
Lane 7, 8: Anti-Atg5 pAb (code no. PM050Y)

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