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MBL International Corporation

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Autophagy-Related Products
Catalog

Research Reagents

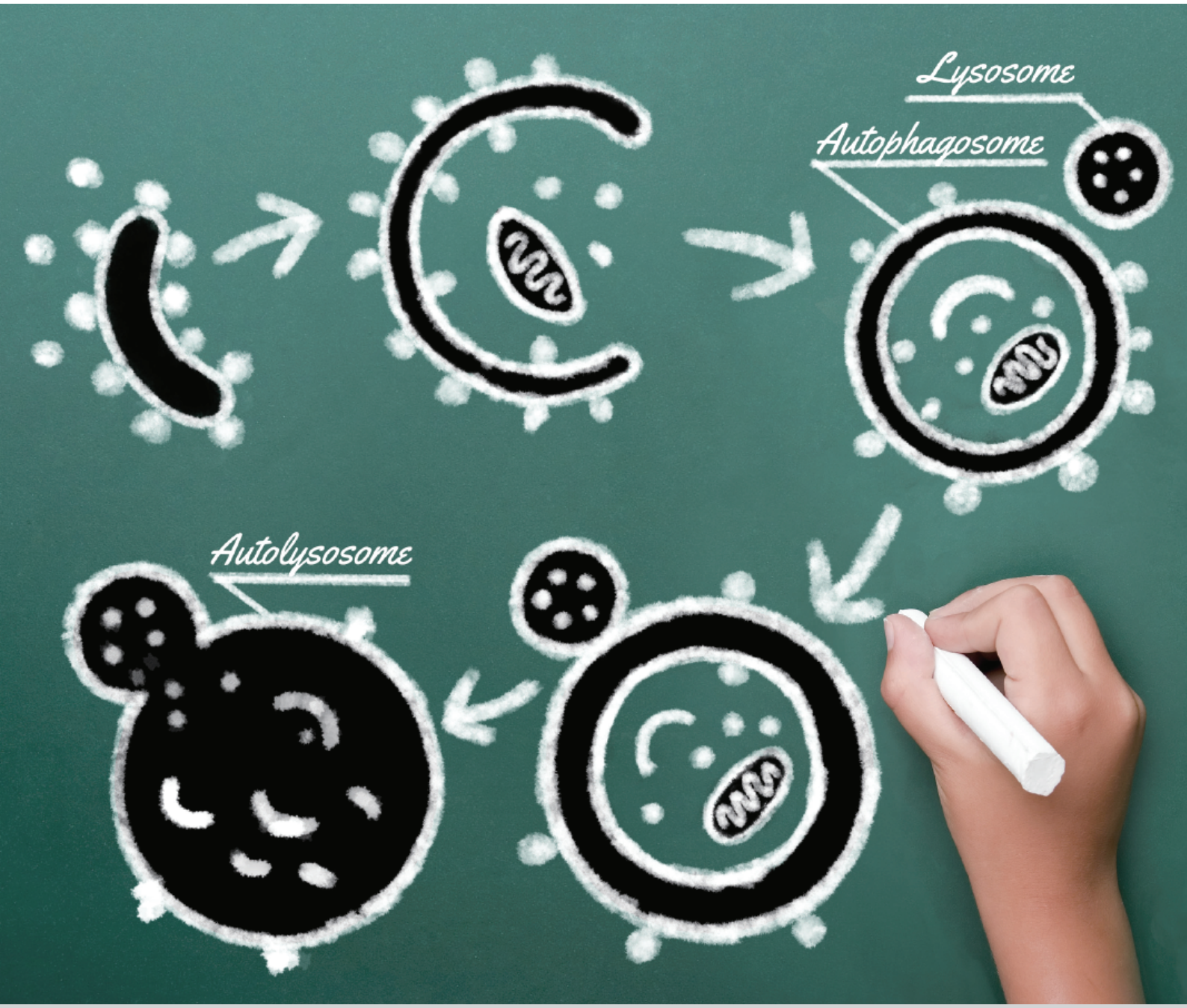


Table of contents

Abbreviations and other product notes

(aff.): affinity purified
Species cross-reactivity: Hu: Human, Mo: Mouse, Rab: Rabbit, Hm: Hamster, Chi: Chicken, Mky: Monkey, Bov: Bovine
(-): No cross-reactivity, (w): weak cross-reactivity
Application: WB: Western Blotting, IP: Immunoprecipitation, FCM: Flow Cytometry, IC: Immunocytochemistry,
IF: Immunofluorescence, IH: Immunohistochemistry, Immuno-EM: Immuno-electron microscopy
*: reported in articles(not confirmed by MBL).

HRP-DirecT series antibodies are directly conjugated to HRP.

What is autophagy?

Autophagy is generally considered as a process to supply nutrients by self-digestion for cells to survive starvation. However, autophagy, along with the proteasome system, is also involved in the turnover of cellular components under normal conditions.

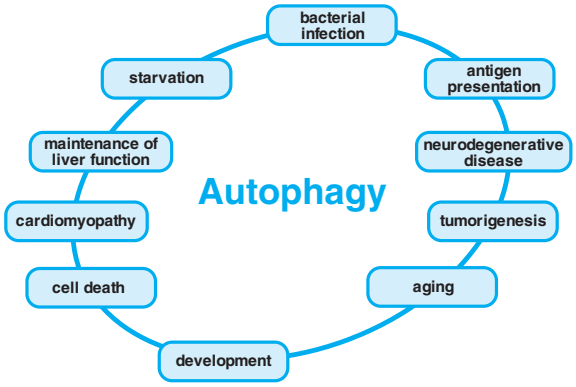
While proteasomes target and selectively degrade ubiquitinated proteins, autophagy degrades all the contents engulfed by autophagosomes, and, therefore, is called “the bulk degradation system.” In addition, selective autophagy pathways target cellular organelles, such as mitochondria and peroxisomes. These degradation mechanisms are respectively known as “mitophagy” and “pexophagy.” Various other autophagic mechanisms are also under investigation.

Although in the limelight in recent years, autophagy was first observed by electron microscopy over 40 years ago. Nevertheless, functional studies of autophagy did not progress rapidly because factors involved in the process remained unknown for a long period of time.

Dr. Yoshinori Ohsumi (currently of the Tokyo Institute of Technology) and his colleagues at the National Institute for Basic Biology isolated yeast strains that were unable to degrades the contents of autophagosomes, and successfully cloned the autophagy-related (APG/ATG) genes (Tsukada and Ohsumi, 1993). As of 2016, the number of ATG genes in budding yeast stands at 41. Many of these genes are conserved in mammals and plants (the amino acid sequence homology among species is limited, but the 3D structures are similar).

With the discovery of APG/ATG genes, functions of the gene products have been extensively studied, and details of the mechanism and physiological role of autophagy are being elucidated one after another.

Atg proteins, discovered in yeast, are conserved in a wide range of organisms, such as the slime molds, nematodes, flies, mammals, and plants. The functions of these proteins, however, have been highly diversified in each species. Further, recent studies have demonstrated that mammalian autophagy is involved not only in the starvation response, but also in antigen presentation, cell death, development, aging, tumorigenesis, and in the defense against bacterial infection. Thus, autophagy research will be increasingly important in understanding these processes in the body.

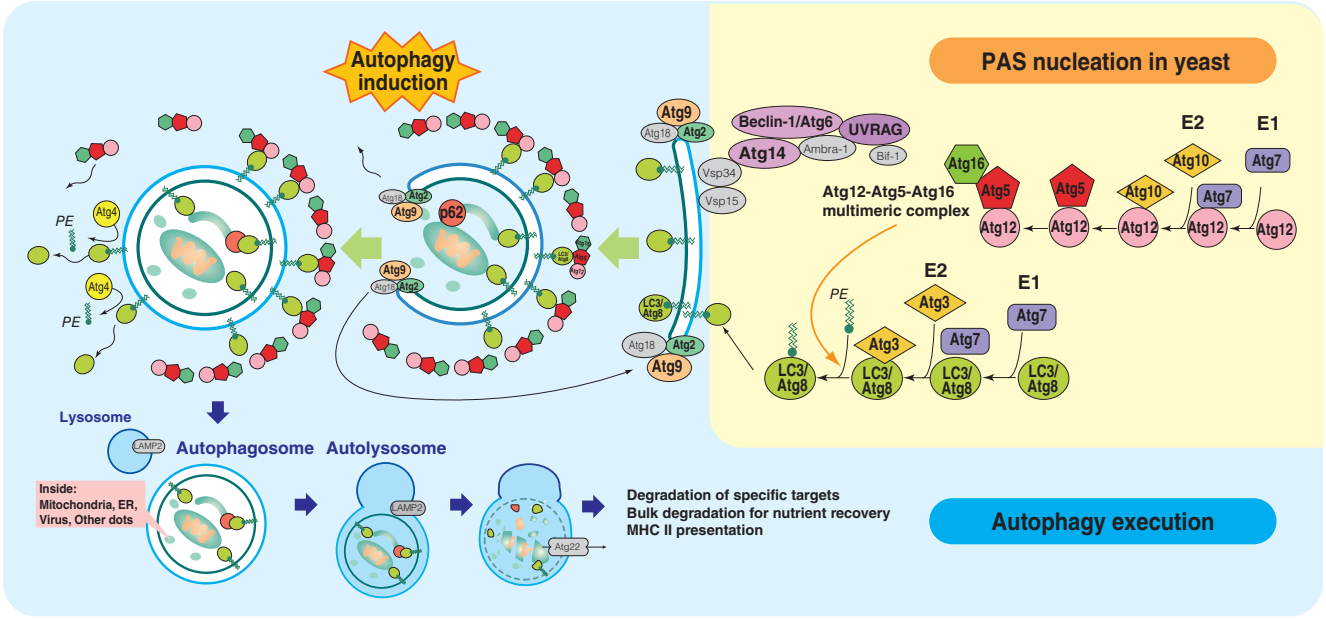


Article written by researcher

“Autophagy research: Current status and future perspectives”

Dr. Noboru Mizushima of the University of Tokyo

Please see page 22 – 25 for the article.



Autophagy Watch for Autophagy Flux Assay and LC3 Immunostaining

The Simple "Autophagy Flux Assay" Kit

Autophagy Watch contains a set of anti-LC3 antibodies and autophagy inhibitors. The Western blotting (WB)-based Autophagy Flux Assay can detect the induction of autophagy.

- Anti-LC3 Antibodies (2)
- Antibody for Loading Control (α-Tubulin)
- Positive Control protein for WB
- Autophagy Inhibitors (2)
- Cell Lysis Buffer

Features

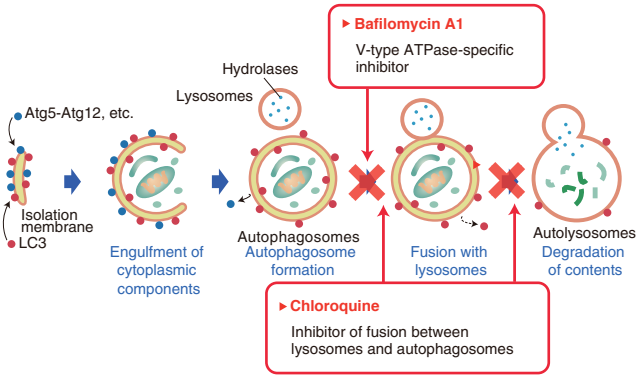
◎ **The antibody for WB is conjugated to HRP, and does not require a secondary antibody. Advantages include a shorter assay time and the absence of nonspecific signal from the secondary antibody.**

◎ **The lysosomal inhibitors chloroquine and bafilomycin A1 are included as autophagy inhibitors. Simply dilute 1,000-fold with culture medium.**

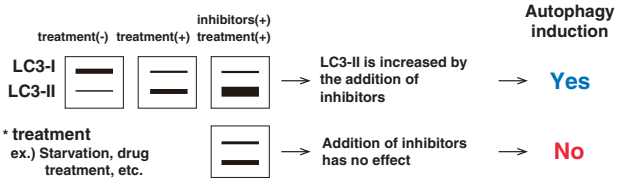
◎ **An antibody for cell staining is also included in this kit. Autophagosomes in the cell can be visualized and monitored by staining with a fluorescence-labeled secondary antibody.**

What Is the Autophagy Flux Assay?

LC3-II is localized to the isolation membrane (phagophore) and the autophagosomal membrane. Induction of autophagy cannot be determined by simply detecting an increase in LC3-II band intensity on Western blotting. The Autophagy Flux Assay compares samples treated with or without lysosomal inhibitors to allow assessment of the induction of autophagy.

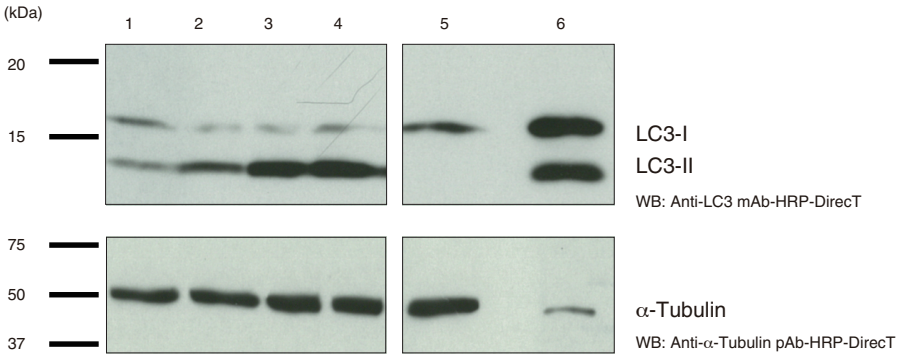


How to interpret the LC3 bands on WB



(Source) Mizushima, N. and Yoshimori, T. How to Interpret LC3 Immunoblotting. Autophagy 3, 542-545 (2007) (PMID:17611390)

Detection of autophagy induction using *Autophagy Watch*: WB



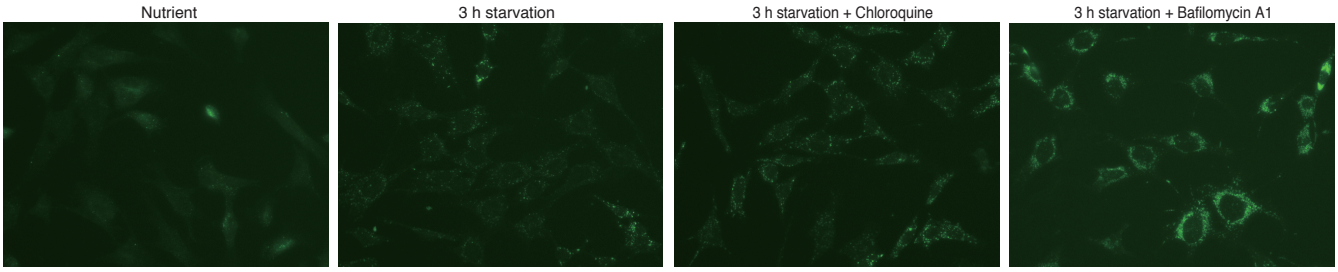
Cell: MEF

Lane 1: Nutrient	Lane 4: 3 h starvation + Bafilomycin A1
Lane 2: 3 h starvation	Lane 5: *Atg5-/-MEF
Lane 3: 3 h starvation + Chloroquine	Lane 6: Positive control

LC3-II is increased in cells under starvation conditions, compared with cells under control (nutrient) conditions (Lanes 1, 2). When starved cells were treated with the lysosomal inhibitor chloroquine or bafilomycin A1, LC3-II band intensity is further increased (Lanes 3, 4). This increase indicates an accumulation of autophagosomes caused by the inhibition of their degradation. Induction of autophagy in starved cells can be confirmed by comparing these results.

*MEF$^{Atg5-/-}$cells were kindly provided by Dr. Noboru Mizushima (The University of Tokyo).

Detection of autophagy induction using *Autophagy Watch*: IC



Microscope: BZ-9000 Generation II (Keyence), Cell : MEF

Autophagosomes can be seen as punctate staining inside the cells starved in HBSS (Hank's Balanced Salt Solution). The addition of the inhibitors increases the number of autophagosomes.

Products

Code No.	Product Name
8486	Autophagy Watch

Kit Components

Product Name	Clone	Isotype	Application	Size	Species Cross-Reactivity
Anti-LC3 mAb-HRP-DirecT	8E10	Mo IgG2aκ	WB	50 µL	Hu, Mo, Rat, Hm
Anti-LC3 mAb	4E12	Mo IgG1κ	WB(weak), IC, IP, FCM, Immuno-EM	50 µL, 2 mg/mL	Hu, Mo, Rat, Hm
Anti-α-Tubulin pAb-HRP-DirecT	Polyclonal	Rab IgG(aff.)	WB Positive Control	50 µL	Hu, Mo, Rat, Hm, Chi
Positive control for anti-LC3 antibody				100 µL (20 tests)	
Chloroquine solution (x1000)				100 µL	
Bafilomycin A1 solution (x1000)				100 µL	
Cell lysis buffer (x5)				1 mL x2	

Autophagy Watch FAQ

Q1. What can I do to induce starvation?

➡ In NRK cells, starvation can be induced by changing the media to Hank's Balanced Salt Solution (serum-free) and incubating for 2 – 4 hours. Serum-free DMEM (Dulbecco's modified Eagle's medium) can be used, but the induction is weaker because DMEM contains amino acids.

Q2. Tell me more about the inhibitors.

➡ The well-known anti-malarial drug chloroquine has long been used as an inhibitor of lysosomal activity. Today, its efficacy as an anti-cancer drug is being studied. Bafilomycin A1 is a specific autophagy inhibitor used by many autophagy researchers. Another commonly used inhibitor wortmannin (not included in this kit) blocks autophagy at an earlier stage.

Q3. Two anti-LC3 antibodies are included. Are they used for different purposes?

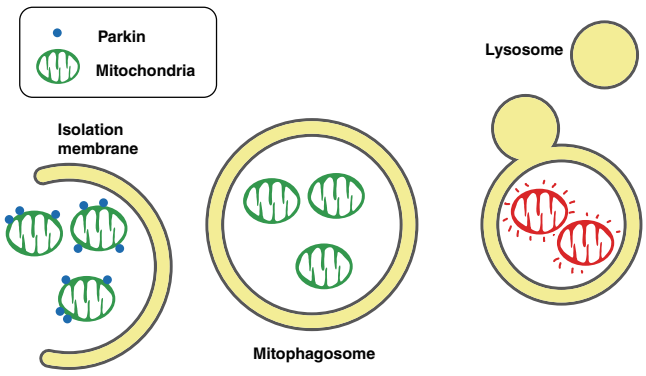
➡ Anti-LC3 antibody, clone 8E10 is conjugated to HRP and suitable for WB. For other applications, such as IC and IP, use clone 4E12.

Q4. Can you tell me the details of the experimental protocol for LC3 detection by Western blotting?

➡ Please refer to the FAQ on page 19 – 20.

What is mitophagy?

Mitophagy is a type of autophagy that selectively degrades mitochondria, and is involved in the turnover of damaged mitochondria. This process is thought to defend the body from diseases resulting from mitochondrial dysfunction. The Parkinson's disease gene product, Parkin (ubiquitin ligase), plays a critical role in the induction of mitophagy. Parkin is recruited to the outer membrane of damaged and depolarized mitochondria. Ubiquitin is subsequently added to the outer membrane of damaged mitochondria by the ubiquitin ligase activity of Parkin. Mitophagy is induced through the recognition of the ubiquitin modification.



Plasmid vector for monitoring mitophagy activity, pMitophagy Keima-Red mPark2

This vector is designed for labeling mitochondria with the fluorescent protein mKeima-red (monomeric with an emission maximum at 620 nm). mKeima-Red is tagged with a mitochondrial localization signal, and is co-expressed in the cells with Parkin (ubiquitin ligase), which plays a critical role in the induction of mitophagy. Mitophagy can be detected and visualized due to the changes in the excitation spectrum of mKeima-Red before and after induction of mitophagy by drug treatment.

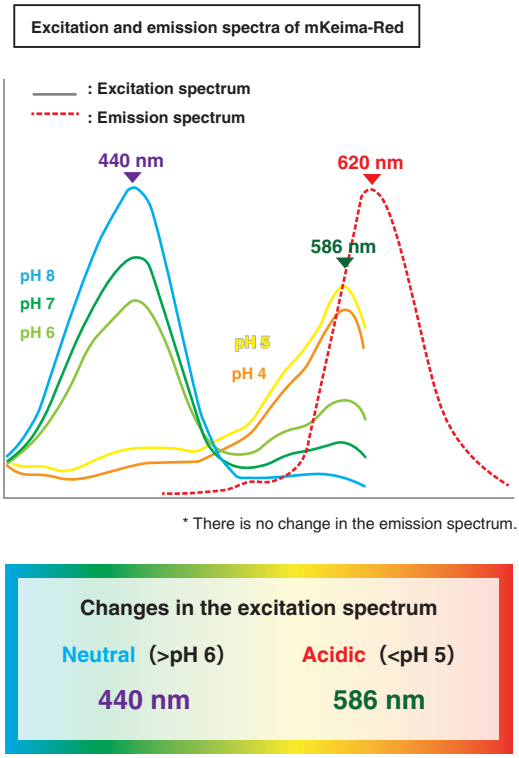
Features

◎ **mKeima-Red (tagged with a mitochondrial localization signal) and Parkin are co-expressed from a single construct.**

* MT-mKeima-Red expression vectors without Parkin are also available.

◎ Features of Keima-Red: pH biosensor

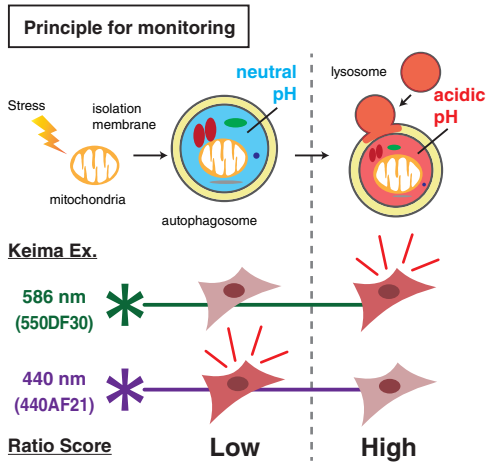
mKeima-Red is a fluorescent protein with an emission maximum at 620 nm. The excitation spectrum changes depending on the pH of the environment.

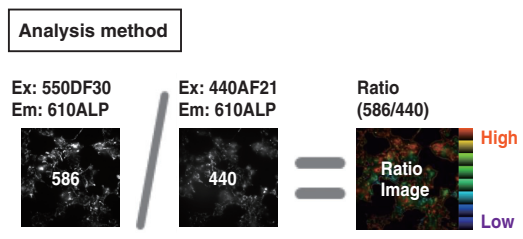


* There is no change in the emission spectrum.

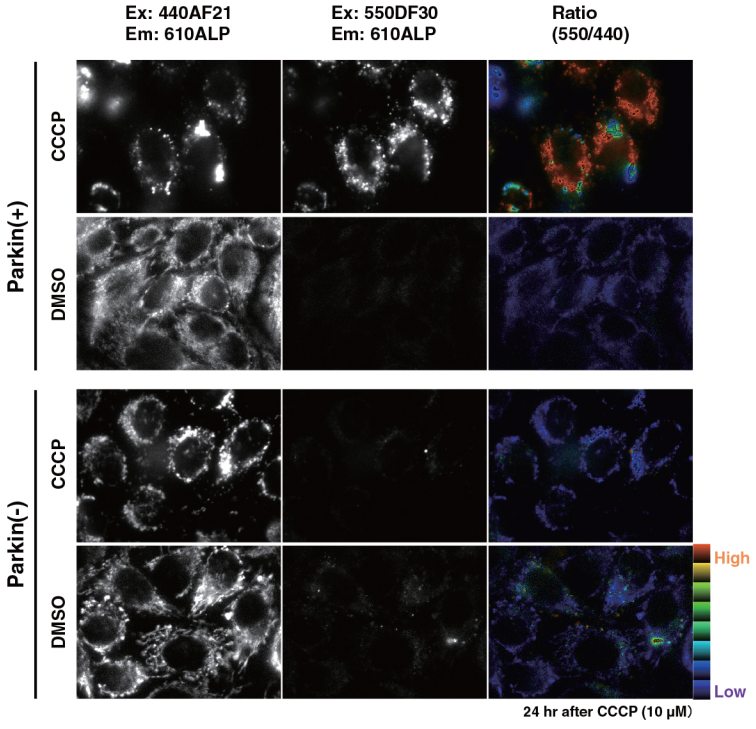
◎ The principle for monitoring and the analysis method

Images are taken with excitation wavelengths of 440 and 586 nm (the excitation maxima in a neutral and acidic environment, respectively) and processed for ratio imaging (586 nm/440 nm). The high ratio is shown in red, and the low ratio is shown in blue. Keima has a low ratio score (colored in blue) in a neutral environment and has a high ratio score (colored in red) in an acidic environment. The change from blue to red indicates the induction of mitophagy.



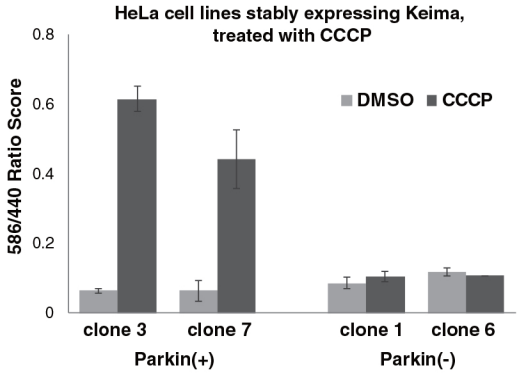


◎ Ratio imaging



The Ratio (586/440) panels show the ratio of fluorescence intensities observed with excitation filters 550DF30 and 440AF21. A higher ratio indicates greater activation of mitophagy.

◎ Quantitative analysis



CCCP: M.P. inducer (membrane depolarizer)
DMSO: Control

■ Stable cell lines (HeLa cells)

Parkin(+): Transfected with MT-mKeima-Red-IRES-Park2
Parkin(-): Transfected with MT-mKeima-Red

■ Assay method

Cells were imaged 24 hours after treatment with CCCP (10 μM) or DMSO.

■ Filter settings

440 nm (Ex: 440AF21, Em: 610ALP, DM: 590DRLP)
586 nm (Ex: 550DF30, Em: 610ALP, DM: 590DRLP)

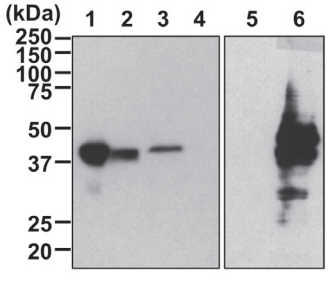
Product list

Code No.	Product name	Size
AM-V0259M	pMitophagy Keima-Red mPark2 (Kan)	20 μg
AM-V0259HM	pMitophagy Keima-Red mPark2 (Hyg)	20 μg
AM-V0251M	*CoralHue*® Mitochondria-targeted mKeima-Red (pMT-mKeima-Red)	20 μg
AM-V0251HM	*CoralHue*® Mitochondria-targeted monomeric Keima-Red (Hyg)	20 μg

Anti-Parkin mAb

Code No.	Product name	Clone	Isotype	Size	Application	Species cross-reactivity
M230-3	Anti-Parkin mAb	Par6	Mouse IgG2aκ	100 μg/100 μL	WB	Hu, Mo, Rat

■Western blotting



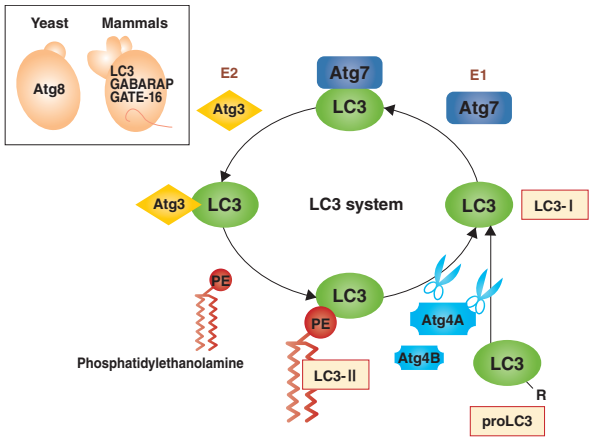
Lane 1: Rat brain lysate, 20 μg
Lane 2: Mouse brain lysate, 20 μg
Lane 3: PC12
Lane 4: HeLa
Lane 5: HEK293T
Lane 6: Human Parkin/ HEK293T

LC3 antibodies

The gold standard for autophagy research

The three proteins, LC3, GABARAP (GABAA receptor-associated protein), and GATE-16 (Golgi-associated ATPase enhancer), are mammalian homologues of yeast Atg8.

Among them, LC3 has been studied most extensively and frequently used as an autophagy marker in mammals. Newly translated LC3 (proLC3) is immediately processed at the C-terminus by Atg4B or Atg4A, forming LC3-I. Upon induction of autophagy, LC3-I is sequentially transferred to E1 and E2, and conjugated to the substrate, PE (phosphatidylethanolamine). The resulting PE-conjugated LC3 is called LC3-II. Although LC3-II has a higher molecular weight than LC3-I, the mobility of LC3-II is greater than LC3-I on SDS-PAGE, due to higher hydrophobicity. GABARAP and GATE-16 are also conjugated to PE in a similar process.



Code No.	Clone	Host species	Application						Conjugation
			WB	IP	IC	IH	FCM	Immuno-EM	
PM036	Polyclonal	Rabbit	★★★	★★★	★★★	★★★	★★★		
M186-3	8E10	Mouse	★★★★						
M186-7	8E10	Mouse	★★★★						HRP
M152-3	4E12	Mouse	★	★★★	★★★★	★*	★★★	★★	
PD014	Polyclonal	Rabbit	★★★						

*: reported in articles

Anti-LC3 pAb

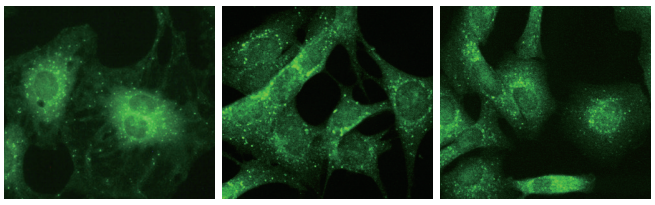
Code No.	Clone	Isotype	Size
PM036	Polyclonal	Rab IgG	100 μL

◎ Suitable for various applications and has been used in a large number of studies!

[Immunogen] Recombinant human LC3 (MAP1LC3B: 1–120 a.a.)
[Species cross-reactivity] Hu, Mo, Rat, Hm
[Form] PBS/50% glycerol, pH 7.2
[Application] WB: 1:1,000
IP: 2 μL/300 μL of cell extract from 1x10^7 cells
IC: 1:500-1:1,000
IH: 1:1,000-1:2,000 (Heat treatment is necessary for paraffin embedded sections.)
FCM: 1:200
[Note] This antibody reacts with LC3 (MAP1LC3A, B, C).
This antibody does not react with GATE-16 or GABARAP.

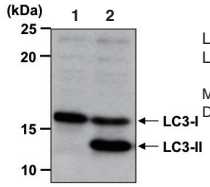
<References>
1) Saitoh, T., *et al.*, Nature 456, 264-268 (2008) [WB]
2) Jing, L., *et al* ., J. Biol. Chem. 291, 13175-13193 (2016) [WB, IC]

■ Immunocytochemistry



NRK (starved condition) MEF (starved condition) A549 (starved condition)

■Western Blotting



Lane 1: MEF$^{Atg5-/-}$
Lane 2: Wild-type MEF

MEF$^{Atg5-/-}$cells were kindly provided by Dr. Noboru Mizushima (The University of Tokyo).

Anti-LC3 mAb

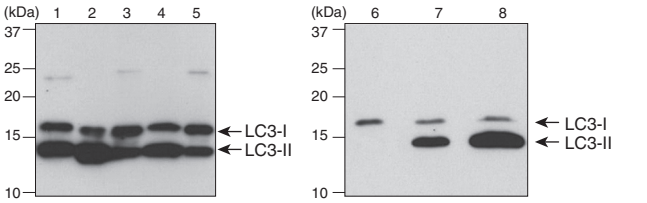
Code No.	Clone	Isotype	Size
M186-3	8E10	Mo IgG2aκ	100 μg/100 μL

◎ The best choice for WB.

[Immunogen] Recombinant human LC3 (MAP1LC3B: 1–120 a.a.)
[Species cross-reactivity] Hu, Mo, Rat, Hm
[Form] 1 mg/mL in PBS/50% glycerol, pH 7.2
[Application] WB: 1 μg/mL
[Note] This antibody reacts with LC3B.
This antibody does not react with LC3A, LC3C, GATE-16, or GABARAP.

<References>
1) Margariti, A., *et al.*, J. Biol. Chem. 288, 859-872 (2013) [WB]
2) Maejima, Y., *et al.*, Nat. Med. 19, 1478-1488 (2013) [WB]

■Western blotting



Lane 1: HeLa
Lane 2: NIH/3T3
Lane 3: PC12
Lane 4: CHO
Lane 5: Mouse brain tissue

Lane 6: MEF$^{Atg5-/-}$
Lane 7: MEF
Lane 8: MEF (6 hr. treatment with 50 μM Chloroquine)

MEF$^{Atg5-/-}$cells were kindly provided by Dr. Noboru Mizushima (The University of Tokyo).

Anti-LC3 mAb-HRP-DirecT

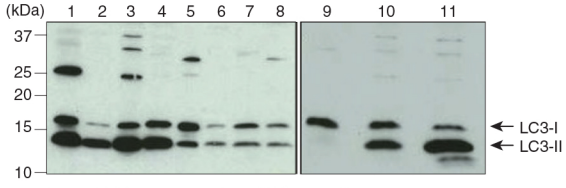
HRP-conjugated

Code No.	Clone	Isotype	Size
M186-7	8E10	Mo IgG2aκ	50 μL

◎ This antibody does not require a secondary antibody.

[Immunogen] Recombinant human LC3 (MAP1LC3B: 1-120 a.a.)
[Species cross-reactivity] Hu, Mo, Rat, Hm
[Form] PBS/Preservative/Stabilizer
[Application] WB: 1:1,000
[Note] This antibody reacts with LC3B.
This antibody does not cross-react with LC3A, LC3C, GATE-16, and GABARAP.
<References>
1) Jia, W., and He, Y. W., J. Immunol. 186, 5313-5322 (2011)
2) Tabata, K., *et al.*, Mol. Biol. Cell 21, 4162-4172 (2010)

■ **Western blotting**



Lane 1: HeLa
Lane 2: NIH/3T3
Lane 3: PC12
Lane 4: CHO
Lane 5: Mouse brain
Lane 6: Mouse liver
Lane 7: Mouse spleen
Lane 8: Mouse kidney
Lane 9: MEF$^{Atg5-/-}$
Lane 10: MEF
Lane 11: MEF (6 hr. treatment with 50 μM Chloroquine)
MEF$^{Atg5-/-}$cells were kindly provided by Dr. Noboru Mizushima (The University of Tokyo).

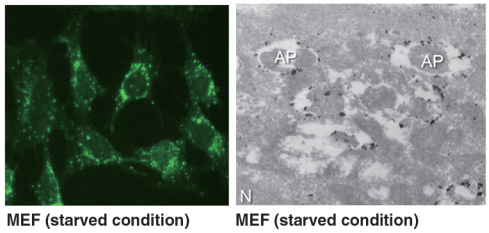
Anti-LC3 mAb

Code No.	Clone	Isotype	Size
M152-3	4E12	Mo IgG1κ	200 μg/100 μL

◎ The best choice for cell staining.

[Immunogen] Recombinant human LC3 (MAP1LC3B: 1-120 a.a.)
[Species cross-reactivity] Hu, Mo, Rat, Hm
[Form] 2 mg/mL in PBS/50% glycerol, pH 7.2
[Application] WB: 5 μg/mL
IP: 5 μg/300 μL of cell extract from 1x10^7 cells
IC: 40 μg/mL
IH*: reported in articles
FCM: 40 μg/mL
Immuno-EM: 20 μg/mL
Image-based FCM*: reported in articles
[Note] This antibody reacts with LC3 (MAP1LC3A, B).
<References>
1) Moreau, K., *et al.*, Cell 146, 303-317 (2011) [IC]
2) McKnight, N.C., *et al.*, EMBO J. 31, 1931-1946 (2012) [IC]

■ **Immunocytochemistry** ■ **Immuno-EM**



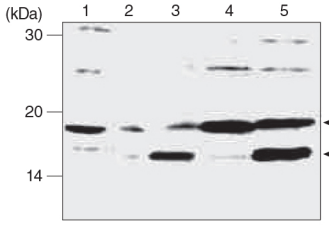
MEF (starved condition) **MEF (starved condition)**
The immuno-EM data was kindly provided by Dr. Noboru Mizushima (The University of Tokyo).

Anti-LC3 pAb

Code No.	Clone	Isotype	Size
PD014	Polyclonal	Rab IgG	100 μL

[Immunogen] Recombinant rat LC3 (1-142 a.a.)
[Species cross-reactivity] Hu, Mo, Rat, Hm
[Form] PBS/50% glycerol, pH 7.2
[Application] WB: 1:1,000
IC*: reported in articles
IH*: reported in articles
<References>
1) Tsuchiya, Y., *et al.*, Mol. Cell. Biol. 33, 3461-3472 (2013) [WB]
2) Kobayashi, S., *et al.*, PNAS 112, 7027-32 (2015) [IC]

■ **Western blotting**



Lane 1: HeLa
Lane 2: A431
Lane 3: NIH/3T3
Lane 4: PC12
Lane 5: CHO

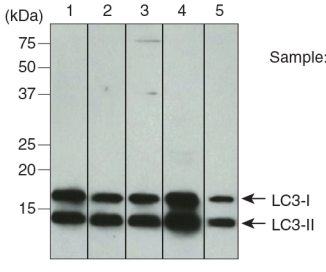
Positive control for anti-LC3 antibody

Code No.	Size
PM036-PN	100 μL (10 tests)

◎ Migrates at the same level as the endogenous human LC3 in WB.

[Application] Positive control in WB with anti-LC3 antibody
[Note] Since this product is using human LC3 without any tag, its molecular weight is the same as the endogenous LC3.
<References>
1) Zadra, G., *et al.*, EMBO Mol. Med. 6, 519-538 (2014) [WB]

■ **Western blotting**



Sample: PM036-PN (10 μL/lane)

Lane 1: Anti-LC3 pAb (MBL; code no. PM036), 1:1,000
Lane 2: Anti-LC3 pAb (MBL; code no. PD014), 1:1,000
Lane 3: Anti-LC3 pAb (MBL; code no. PM046), 1:1,000
Lane 4: Anti-LC3 mAb (clone: 8E10) (MBL; code no. M186-3), 1 μg/mL
Lane 5: Anti-LC3 mAb (clone: 4E12) (MBL; code no. M152-3), 10 μg/mL

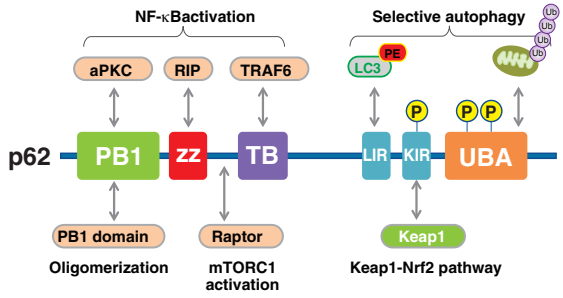
See page 19 - 20 for FAQ about anti-LC3 antibodies.

p62 Antibodies

A link between the ubiquitin-proteasome system and autophagy

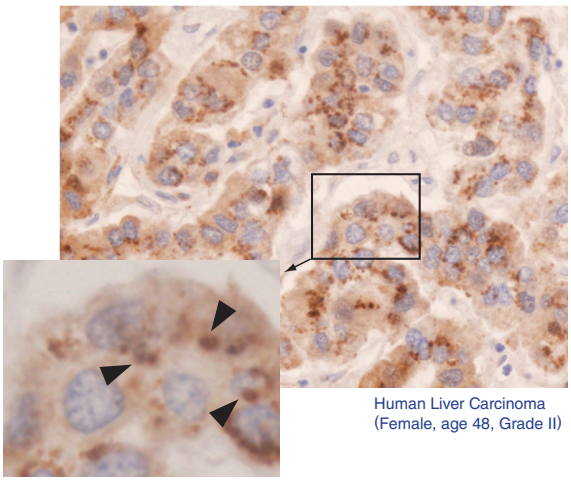
p62/SQSTM1 is a scaffolding protein that interacts with various signaling molecules such as TRAF6, RIP, and aPKC (figure below, left). p62 contains an LC3-interacting region and is believed to be a substrate for selective autophagy. In addition, p62 contains a domain that binds ubiquitin chains, and mediates the recruitment of poly ubiquitinated protein aggregates and depolarized mitochondria to the autophagic machinery (see page 11 for the details of selective autophagy). In fact, in liver- and brain-specific autophagy-deficient mice, overaccumulation of p62 occurs, and ubiquitin- and p62-positive inclusion bodies are observed (figure below, right). Importantly, ubiquitin- and p62-positive inclusion bodies are also observed in tissues of patients with neurodegenerative diseases (such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis), alcoholic hepatitis, hepatic steatosis, and liver cancer. There is increasing interest in the involvement of impaired autophagic degradation of p62 in these diseases.

■ Domain structure of p62/SQSTM1



This illustration was made with the supervision of
Dr. Masaaki Komatsu and Dr. Yoshinobu Ichimura (Niigata University).

■ Immunohistochemistry



Human Liver Carcinoma
(Female, age 48, Grade II)

Antibody: Anti-p62 pAb (Code No. PM045)

p62-positive inclusion bodies are observed in human liver cancer tissue.

Code No.	Clone	Immunized host	Immunogen	Species cross-reactivity	Application					Conjugation
					WB	IP	IC	IH	FCM	
M162-3	5F2	Mouse	Human p62 (120-440 a.a.)	Hu	★★★	★★★	★★★	★★★	★★	
M162-A48	5F2	Mouse	Human p62 (120-440 a.a.)	Hu			★★★★		★★★	Alexa 488
M162-A59	5F2	Mouse	Human p62 (120-440 a.a.)	Hu			★★★			Alexa 594
M162-A64	5F2	Mouse	Human p62 (120-440 a.a.)	Hu			★★★		★★★	Alexa 647
PM045	Polyclonal	Rabbit	Human p62 (120-440 a.a.)	Hu, Mo, Rat, Hm	★★★	★★★	★★★	★★★		
PM066 C-terminal	Polyclonal	Guinea Pig	Human p62, C-terminal region	Hu, Mo, Rat, Hm	★★★★	★★★	★★★	★★★★		
PM066-7 C-terminal	Polyclonal	Guinea Pig	Human p62, C-terminal region	Hu, Mo, Rat, Hm	★★★★					HRP

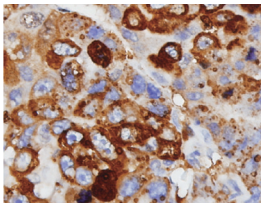
Anti-p62 (SQSTM1) (Human) mAb

Code No.	Clone	Isotype	Size
M162-3	5F2	Mo IgG1κ	100 μg/100 μL

[Immunogen] Recombinant human p62 (120–440 a.a.)
[Species cross-reactivity] Hu, Mo(-), Rat(-), Hm(-)
[Form] 1 mg/mL in PBS/50% glycerol, pH 7.2
[Application] WB: 1 μg/mL
IP: 2 μg/250 μL of cell extract from 2.5×10^6 cells
IC: 5 μg/mL
IH: 2 – 10 μg/mL (Heat treatment is necessary for paraffin embedded sections.)
FCM: 2 μg/mL

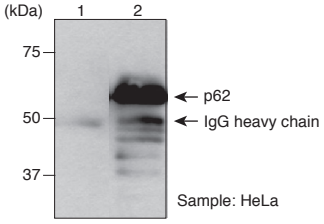
<References>
1) Janda, E., *et al.*, Autophagy 11, 1063-80 (2015) [IC]
2) Matsumoto, G., *et al.*, Mol. Cell. 44, 279-89 (2011) [WB]

■ Immunohistochemistry



Human liver carcinoma

■ Immunoprecipitation



Lane 1: Isotype control (M075-3)
Lane 2: Anti-p62 mAb (M162-3)
Immunoblotted with Anti-p62 pAb (PM045)

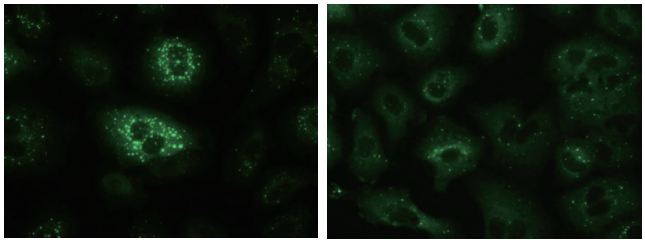
Anti-p62 (SQSTM1) (Human) mAb-Alexa Fluor® 488

Alexa Fluor® 488

Code No.	Clone	Isotype	Size
M162-A48	5F2	Mo IgG1κ	100 μg/100 μL

[Immunogen] Recombinant human p62 (120–440 a.a.)
[Species cross-reactivity] Hu
[Form] 1 mg/mL in PBS/1% BSA/0.09% NaN_3
[Application] IC: 2 μg/mL
FCM: 1 μg/mL
<References>
1) Ichimura, Y., *et al.*, J. Biol. Chem. 283, 22847-22857 (2008)
2) Komatsu, M., *et al.*, Cell 131, 1149-1163 (2007)

■Immunocytochemistry



A549 (starved condition) A549 (nutrient-rich condition)

Anti-p62 (SQSTM1) (Human) mAb-Alexa Fluor® 594

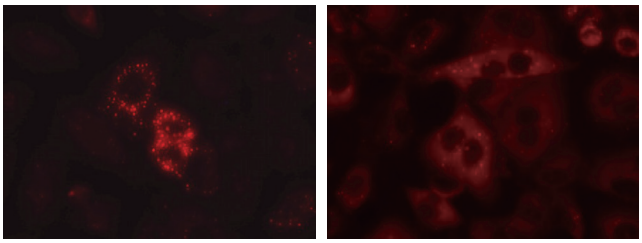
Alexa Fluor® 594

Code No.	Clone	Isotype	Size
M162-A59	5F2	Mo IgG1κ	100 μg/100 μL

[Immunogen] Recombinant human p62 (120–440 a.a.)
[Species cross-reactivity] Hu
[Form] 1 mg/mL in PBS/1% BSA/0.09% NaN_3
[Application] IC: 5 μg/mL

<References>
1) Ichimura, Y., *et al.*, J. Biol. Chem. 283, 22847-22857 (2008)
2) Komatsu, M., *et al.*, Cell 131, 1149-1163 (2007)

■Immunocytochemistry



A549 (starved condition) A549 (nutrient-rich condition)

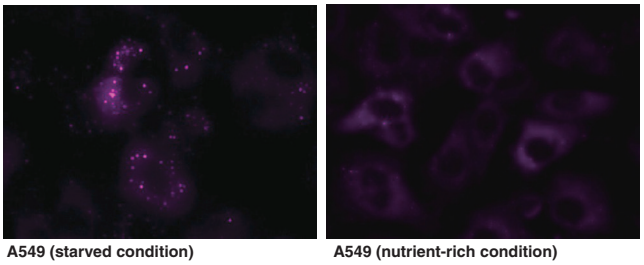
Anti-p62 (SQSTM1) (Human) mAb-Alexa Fluor® 647

Alexa Fluor® 647

Code No.	Clone	Isotype	Size
M162-A64	5F2	Mo IgG1κ	100 μg/100 μL

[Immunogen] Recombinant human p62 (120–440 a.a.)
[Species cross-reactivity] Hu
[Form] 1 mg/mL in PBS/1% BSA/0.09% NaN_3
[Application] IC: 5 μg/mL
FCM: 1 μg/mL
<References>
1) Ichimura, Y., *et al.*, J. Biol. Chem. 283, 22847-22857 (2008)
2) Komatsu, M., *et al.*, Cell 131, 1149-1163 (2007)

■Immunocytochemistry



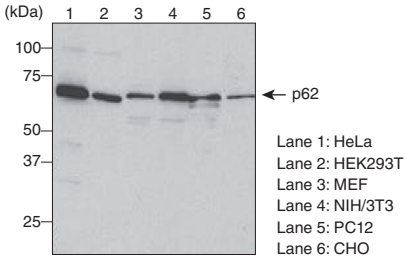
A549 (starved condition) A549 (nutrient-rich condition)

Anti-p62 (SQSTM1) pAb

Code No.	Clone	Isotype	Size
PM045	Polyclonal	Rab Ig (aff.)	100 μL

[Immunogen] Recombinant human p62 (120–440 a.a.)
[Species cross-reactivity] Hu, Mo, Rat, Hm
[Form] PBS/50% glycerol, pH 7.2
[Application] WB: 1:1,000
IP: 2 μL/300 μL of cell extract from $1x10^7$ cells
IC: 1:500
IH: 1:1,000 (Heat treatment is necessary for paraffin embedded sections.)
<References>
1) Hasegawa J., *et al.*, EMBO J. 35, 1853-1867 (2016) [WB]
2) Chen, H., *et al.*, J. Cell Biol. 211, 795-805 (2015) [IH]
3) Takasaka, N., *et al.*, J. Immunol. 192, 958-968 (2014) [WB]

■ Western blotting

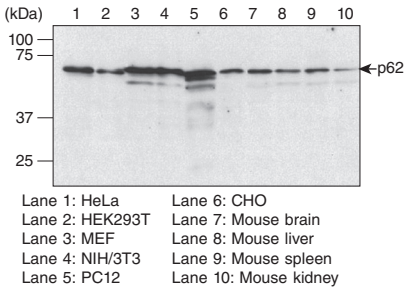


Anti-p62 C-terminal pAb

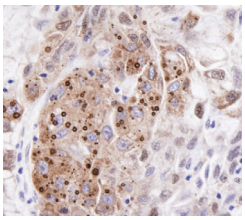
Code No.	Clone	Isotype	Size
PM066	Polyclonal	Guinea Pig Ig (aff.)	100 μL

[Immunogen] Human p62 C-terminal region (synthetic peptide)
[Species cross-reactivity] Hu, Mo, Rat, Hm
[Form] PBS/50% glycerol, pH 7.2
[Application] WB: 1:1,000
IP: 5 μL/300 μL of cell extract from $3x10^6$ cells
IC: 1:500
IH: 1:100
<References>
1) Komatsu, M., *et al.*, Cell 131, 1149-1163 (2007)
2) Moscat, J., *et al.*, Mol. Cell 23, 631-640 (2006)

■ Western blotting



■ Immunohistochemistry



Human liver carcinoma

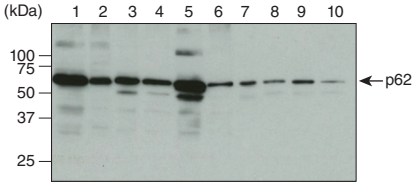
Anti-p62 C-terminal pAb-HRP-DirecT

HRP-conjugated

Code No.	Clone	Isotype	Size
PM066-7	Polyclonal	Guinea Pig Ig (aff.)	50 μL

[Immunogen] Human p62 C-terminal region (synthetic peptide)
[Species cross-reactivity] Hu, Mo, Rat, Hm
[Form] PBS/Preservative/Stabilizer
[Application] WB: 1:1,000
<References>
1) Komatsu, M., *et al.*, Cell 131, 1149-1163 (2007)
2) Moscat, J., *et al.*, Mol. Cell 23, 631-640 (2006)

■ Western blotting

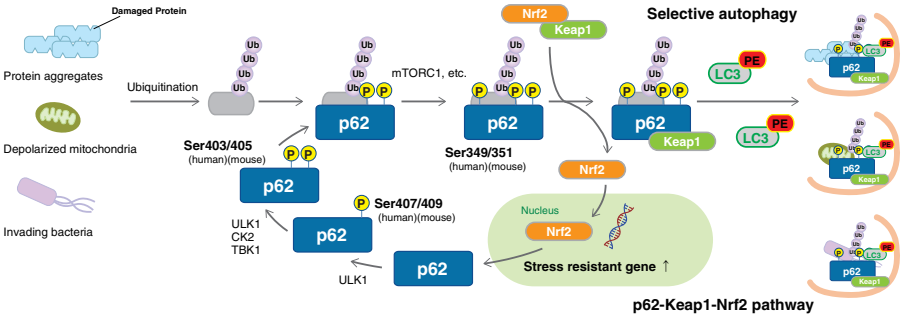


Phospho-p62 antibody

Hot topic in research on neurodegenerative disease and cancer

p62 contains multiple phosphorylation sites. Sequential phosphorylation of these sites regulates biological defense mechanisms such as selective autophagy.

The phosphorylation of Ser407 (human)/Ser409 (mouse) precedes the phosphorylation of Ser403 (human)/Ser405 (mouse) in p62, which increases its affinity for poly ubiquitin chains. Consequently, ubiquitinated abnormal protein aggregates, depolarized mitochondria, and invading intracellular bacteria are sequestered by phospho-p62. Further phosphorylation of Ser349 (human)/Ser351 (mouse) by mTORC1 increases the affinity of p62 for Keap1, inducing dissociation of Nrf2 from Keap1 and nuclear translocation of Nrf2 (the p62-Keap1-Nrf2 pathway). Nrf2 is a stress-response transcription factor and activates the transcription of various stress resistance genes. Nrf2 also induces p62 gene expression, forming a positive feedback loop. Phospho-p62 with bound Keap1 interacts with LC3 through the LIR (LC3-interacting region) and is degraded by the autophagy pathway. Thus, the cells under stress conditions effectively overcome their negative environment by activating two biological defense mechanisms through the phosphorylation of p62.



This illustration was made under the supervision of Dr. Masaaki Komatsu and Dr. Yoshinobu Ichimura (Niigata University).

Impaired selective autophagy is implicated in various diseases. For example, neurons in familial parkinsonism fail to clear protein aggregates and depolarized mitochondria, resulting in neuronal damage and compromised brain function. In hepatocarcinoma cells, p62 is constitutively phosphorylated at Ser349, causing continuous activation of Nrf2. Hence, inhibitors of p62 phosphorylation and inhibitors of the interaction between phospho-p62 and Keap1 have the potential to be novel cancer therapeutics.
(Reference: Saito, T., *et al*., Nat. Commun. 7, 12030 (2016) PMID: 27345495).

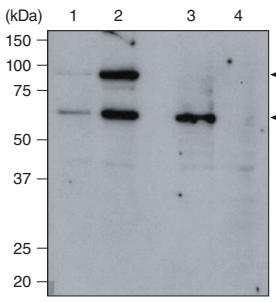
Anti-Phospho-p62 (SQSTM1) (Ser403) mAb

Code No.	Clone	Isotype	Size
D343-3	4F6	Rat IgG2aκ	100 μg/100 μL

[Immunogen] Human p62 (396–410 a.a.) (synthetic peptide)
[Species cross-reactivity] Hu, Mo
[Form] 1 mg/mL in PBS/50% glycerol, pH 7.2
[Application] WB: 5 μg/mL
IH: 10 μg/mL

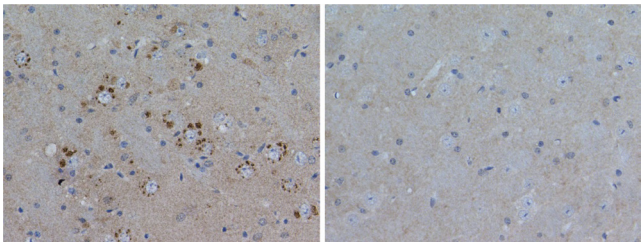
<References>
1) Kurosawa, M., *et al*., Hum. Mol. Genet., 24, 1092-1105 (2015) [IH]
2) Matsumoto, G., *et al*., Mol. Cell 44, 279-289 (2011) [WB, IH]

■**Western blotting**



←GFP-tagged Phospho-p62
←Phospho-p62
Lane 1: GFP-tagged human p62/Neuro2a
Lane 2: GFP-tagged human p62/Neuro2a, Bafilomycin A1-treated (1 μM, 24 hr.)
Lane 3: MEF$^{Atg5-/-}$
Lane 4: MEF
The Neuro2a cells expressing GFP-tagged human p62 were kindly provided by Dr. Nobuyuki Nukina and Dr. Gen Matsumoto (Juntendo University).
MEF$^{Atg5-/-}$cells were kindly provided by Dr. Noboru Mizushima (The University of Tokyo).

■**Immunohistochemistry**



Atg5 conditional knockout mouse brain　Wild type mouse brain

Brown: Anti-Phospho-p62 (SQSTM1) (Ser403) mAb (D343-3)
Blue: Hematoxylin

The tissue samples were kindly provided by Dr. Nobuyuki Nukina and Dr. Gen Matsumoto (Juntendo University).

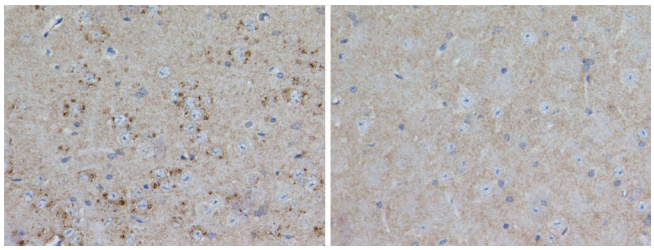
Anti-Phospho-p62 (SQSTM1) (Ser403) mAb

Code No.	Clone	Isotype	Size
D344-3	4C8	Rat IgG2aκ	100 μg/100 μL

[Immunogen] Human p62 (396–410 a.a.) (synthetic peptide)
[Species cross-reactivity] Hu, Mo
[Form] 1 mg/mL in PBS/50% glycerol, pH 7.2
[Application] WB: 5 μg/mL
IH: 5 μg/mL

<References>
1) Matsumoto, G., *et al*., Mol. Cell 44, 279-289 (2011)

■**Immunohistochemistry**



Atg5 conditional knockout mouse brain　Wild type mouse brain

Brown: Anti-Phospho-p62 (SQSTM1) (Ser403) mAb (D344-3)
Blue: Hematoxylin

The tissue samples were kindly provided by Dr. Nobuyuki Nukina and Dr. Gen Matsumoto (Juntendo University).

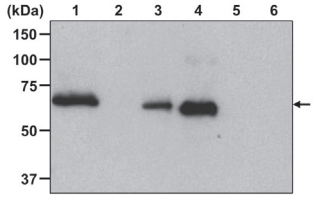
Anti-Phospho-p62 (SQSTM1) (Ser351) mAb

Code No.	Clone	Isotype	Size
M217-3	5D5	Mo IgG1κ	100 μg/100 μL

[Immunogen] Mouse p62 (346–359 a.a.) (synthetic peptide)
[Species cross-reactivity] Hu, Mo
[Form] 1 mg/mL in PBS/50% glycerol, pH7.2
[Application] WB: 0.5 μg/mL
IC: 0.1 μg/mL
IH: 1 μg/mL

< References>
1) Ichimura, Y., *et al*., Mol. Cell 51, 618-31 (2013)
2) Kageyama, S., *et al*., J. Biol. Chem. 289, 24944-55 (2014)

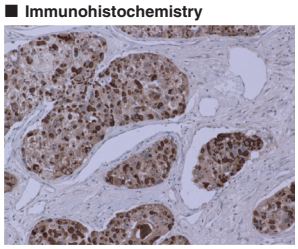
■ **Western blotting**



Lane 1: MEF, sodium arsenite-treated (10 μM, 12 hr.)
Lane 2: MEF
Lane 3: MEF$^{Atg5-/-}$
Lane 4: huH-1
Lane 5: huH-1, λ-phosphatase-treated
Lane 6: p62-knockout huH-1

Sodium arsenite-treated MEF cells and p62-knockout huH-1 cells were kindly provided by Dr. Masaaki Komatsu and Dr. Yoshinobu Ichimura (Niigata University).
MEF$^{Atg5-/-}$cells were kindly provided by Dr. Noboru Mizushima (The University of Tokyo).

■ **Immunohistochemistry**



Human liver carcinoma

Brown: Anti-Phospho-p62 (SQSTM1) (Ser351) mAb (M217-3)
Blue: Hematoxylin

Anti-Phospho-p62 (SQSTM1) (Ser351) pAb

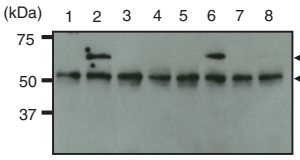
Code No.	Clone	Isotype	Size
PM074	Polyclonal	Rab Ig (aff.)	100 μL

[Immunogen] Mouse p62 (346–359 a.a.) (synthetic peptide)
[Species cross-reactivity] Hu, Mo
[Form] PBS/50% glycerol, pH7.2
[Application] WB: 1:500
IP: 2 μL/sample
IC: 1:500
IH: 1:1,000

<References>

1) Kageyama, S., *et al*., J. Biol. Chem. 289, 24944-55 (2014)
2) Ichimura, Y., *et al*., Mol. Cell 51, 618-31 (2013)

■ **Immunoprecipitation**

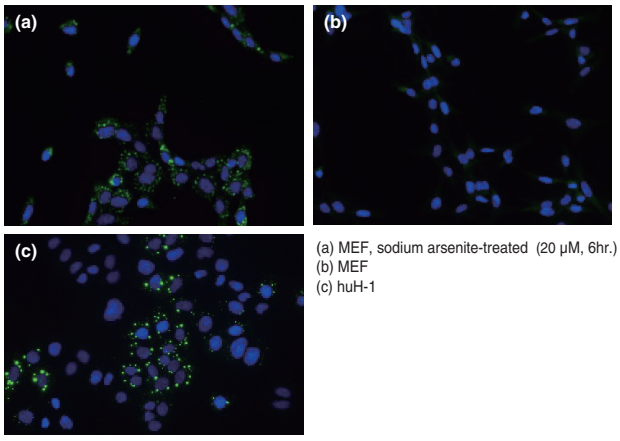


Immunoblotted with Anti-Phospho-p62 (Ser351) pAb (PM074)

Sample:
Lane 1, 2: huH-1
Lane 3, 4: p62-knockout huH-1
Lane 5, 6: MEF$^{Atg5-/-}$
Lane 7, 8: MEF

IP:
Lane 1, 3, 5, 7: Normal Rabbit IgG (PM035)
Lane 2, 4, 6, 8: Anti-Phospho-p62 (SQSTM1) (Ser351) pAb (PM074)

■ **Immunocytochemistry**



(a) MEF, sodium arsenite-treated (20 μM, 6hr.)
(b) MEF
(c) huH-1

Sodium arsenite-treated MEF cells and p62-knockout huH-1 cells were kindly provided by Dr. Masaaki Komatsu and Dr. Yoshinobu Ichimura (Niigata University).
MEF$^{Atg5-/-}$cells were kindly provided by Dr. Noboru Mizushima (The University of Tokyo).

Antibodies for phospho-p62-related proteins

Anti-NRF2 mAb

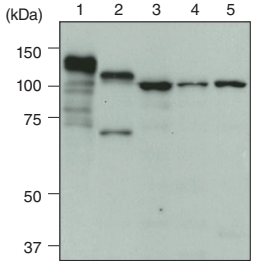
Code No.	Clone	Isotype	Size
M200-3	1F2	Mo IgG1κ	100 μg/100 μL

[Immunogen] Recombinant human NRF2 (1–605 a.a.)
[Species cross-reactivity] Hu, Mo, Rat, Hm
[Form] 1 mg/mL in PBS/50% glycerol, pH 7.2
[Application] WB: 1 μg/mL
IP: 5 μg/300 μL of cell extract from 3x10^6 cells
IC: 0.5 μg/mL
IH: 1 μg/mL (for paraffin embedded sections)

<References>

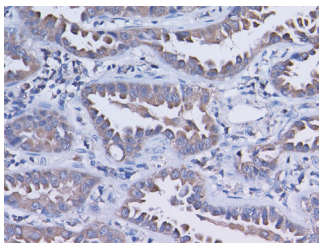
1) Nguyen, T., *et al*., J. Biol. Chem. 284, 13291-13295 (2009)

■ **Western blotting**



Lane 1: NRF2 transfectant (HEK293T)
Lane 2: HeLa
Lane 3: PC12
Lane 4: CHO
Lane 5: NIH/3T3

■ **Immunohistochemistry**



Human lung carcinoma

Brown: Anti-NRF2 mAb (M200-3)
Blue: Hematoxylin

Anti-NRF2 pAb

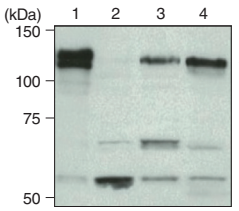
Code No.	Clone	Isotype	Size
PM069	Polyclonal	Rab Ig (aff.)	100 μL

[Immunogen] Recombinant human NRF2 (1–605 a.a.)
[Species cross-reactivity] Hu, Mo(w), Rat(w), Hm(w)
[Form] PBS/50% glycerol, pH 7.2
[Application] WB: 1:1,000
IP: 5 μL/300 μL of cell extract from 3x10^6 cells
IC: 1:1,000
IH: 1:1,000

<References>

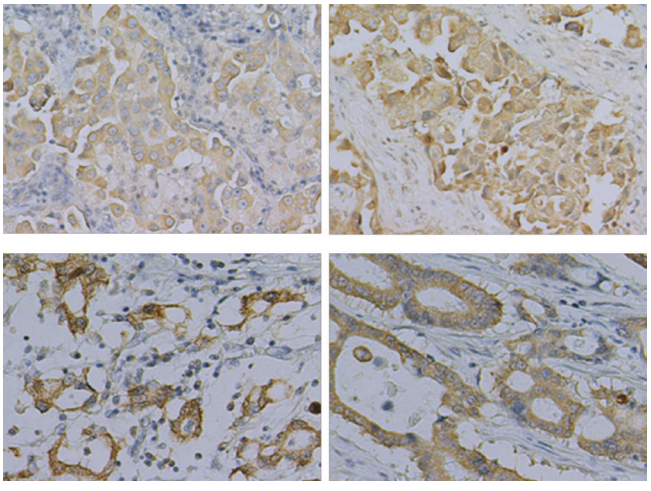
1) Taguchi, K., *et al*., Genes Cell 16, 123-140 (2011)
2) Komatsu, M., *et al*., Nat. Cell Biol. 12, 213-223 (2010)
3) Nguyen, T., *et al*., J. Biol. Chem. 284, 13291-13295 (2009)

■ **Western blotting**



Lane 1: NRF2 transfectant (HEK293T)
Lane 2: HEK293T
Lane 3: HeLa
Lane 4: A549

■ **Immunohistochemistry**



Human cancer tissue

Upper: Lung carcinoma (different fields)
Lower: Colon carcinoma (different fields)

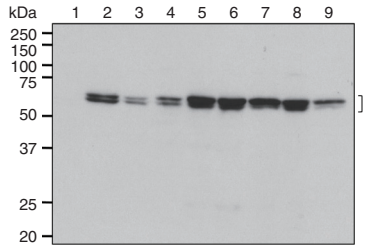
Anti-KEAP1 mAb

Code No.	Clone	Isotype	Size
M224-3	KP1	Mo IgG2aκ	100 μg/100 μL

◎ High affinity for KEAP1 and does not cross-react with other proteins in WB.

[Immunogen] Recombinant human KEAP1
[Species cross-reactivity] Hu, Mo, Rat, Hm
[Form] 1 mg/mL in PBS/50% glycerol, pH7.2
[Application] WB: 1 μg/mL

■ **Western blotting**



Lane 1: HeLa
Lane 2: HEK293T
Lane 3: A549
Lane 4: HepG2
Lane 5: MEF
Lane 6: NIH/3T3
Lane 7: Rat1
Lane 8: NRK
Lane 9: CHO

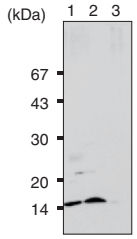
Anti-Ubiquitin mAb

Code No.	Clone	Isotype	Size
MK-11-3	1B3	Mo IgG1	100 μg/100 μL

◎ Specific for mono ubiquitin.

[Immunogen] Bovine erythrocyte ubiquitin
[Species cross-reactivity] Hu, Mo*, Bov*
[Form] 1 mg/mL in PBS/50% glycerol, pH7.2
[Application] WB: 5 μg/mL
IC*: reported in articles
IH*: reported in articles
Immuno-EM*: reported in articles
[Note] This antibody does not react with multi ubiquitin. Clone IB3 and 2C5 (MBL; Code No. MK-12-3) recognize different epitope sites each other.
<References>
1) Hara, T., *et al*., Nature 441, 885-889 (2006) [IH]
2) Yamanaka, A., *et al*., Mol. Biol. Cell 11, 2821-2831 (2000) [WB]

■ **Western blotting**



Lane 1: Raji cell
Lane 2: free ubiquitin
Lane 3: PPUb4*

*PPUb4: partially purified multi ubiquitin chains in ubiquitin-protein conjugates

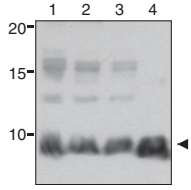
Anti-Ubiquitin mAb

Code No.	Clone	Isotype	Size
MK-12-3	2C5	Mo IgG1	100 μg/100 μL

◎ Specific for mono ubiquitin.

[Immunogen] Bovine erythrocyte ubiquitin
[Species cross-reactivity] Hu, Mo, Rat, Bov
[Form] 1 mg/mL in PBS/50% glycerol, pH7.2
[Application] WB: 5 μg/mL
IP*: reported in articles
IC*: reported in articles
[Note] This antibody does not react with multi ubiquitin. Clone 2C5 and 1B3 (MBL; Code No. MK-11-3) recognize different epitope sites each other.
<References>
1) Sutovsky, P., *et al*., Biol. Reprod. 63, 582-90 (2000) [WB, IC]
2) Hiyama, H., *et al*., J. Biol. Chem. 274, 28019-25 (1999) [IP]

■ **Western blotting**



Lane 1: Raji cell
Lane 2: HeLa cell
Lane 3: HL-60
Lane 4: ubiquitin purified protein

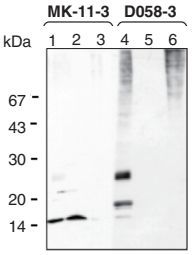
Anti-Multi Ubiquitin mAb

Code No.	Clone	Isotype	Size
D058-3	FK2	Mo IgG1κ	100 μg/100 μL

◎ This antibody recognizes both multi ubiquitin and mono ubiquitin.

[Immunogen] Partially purified poly-ubiquitin-lysozyme
[Species cross-reactivity] Hu, Mo*, Mky*
[Form] 1 mg/mL in PBS/50% glycerol, pH7.2
[Application] WB: 1-5 μg/mL
IC*: reported in articles
ELISA*: reported in articles
[Note] This antibody recognizes K29-, K48-, and K63-linked poly ubiquitinated and mono ubiquitinated proteins but not free ubiquitin.
<References>
1) Sin, Y., *et al*., J. Biol. Chem. 291, 1387-1397 (2016) [WB]
2) Choi, U.Y., *et al*., Exp. Mol. Med. 47, e159 (2015) [IC]

■ **Western blotting**



Lane 1: Raji cell
Lane 2: free ubiquitin
Lane 3: PPUb4*

*PPUb4: partially purified multi ubiquitin chains in ubiquitin protein conjugates

◎ Anti-Multi Ubiquitin mAb (clone FK2)-conjugated agarose and magnetic beads. Recommended for IP.

Code No.	Conjugate	Application	Size
D058-8	Agarose	IP	Gel: 200 μL
D058-9	Magnetic Beads	IP	20 tests (Slurry: 1 mL)

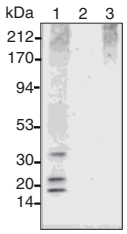
Anti-Multi Ubiquitin mAb

Code No.	Clone	Isotype	Size
D071-3	FK1	Mo IgM	100 μg

◎ This antibody is specific for multi ubiquitin.

[Immunogen] Partially purified poly-ubiquitin-lysozyme
[Species cross-reactivity] Hu
[Form] 1 mg/mL in PBS/50% glycerol, pH7.2
[Application] WB: 1-5 μg/mL
[Note] This antibody recognizes K29-, K48-, and K63-linked poly ubiquitinated proteins but not mono ubiquitinated proteins or free ubiquitin.
<References>
1) Zhou, L., and Yang, H., PLoS One 6, e23936 (2011) [WB]
2) Ledda, F., *et al*., J. Neurosci. 28, 39-49 (2008) [WB]

■ **Western blotting**



Lane 1: Raji cell
Lane 2: free ubiquitin
Lane 3: PPUb4*

*PPUb4: partially purified multi ubiquitin chains in ubiquitin protein conjugates

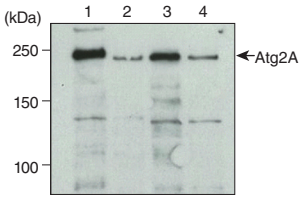
Atg antibody series

Anti-Atg2A pAb

Code No.	Clone	Isotype	Size
PD041	Polyclonal	Rab Ig (aff.)	100 μL

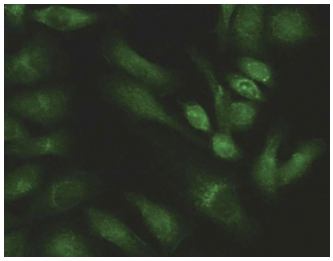
[Immunogen] Recombinant human Atg2A (700–1,400 a.a.)
[Species cross-reactivity] Hu, Mo, Rat, Hm
[Form] PBS/50% glycerol, pH 7.2
[Application] WB: 1:1,000
IP: 5 μL/300 μL of cell extract from 3x10^6 cells
IC: 1:400
<References>
1) Velikkakath, A. K., *et al.*, Mol. Biol. Cell 23, 896-909 (2012)

■ **Western blotting**



Lane 1: HEK293T
Lane 2: MEF
Lane 3: PC12
Lane 4: CHO

■ **Immunocytochemistry**



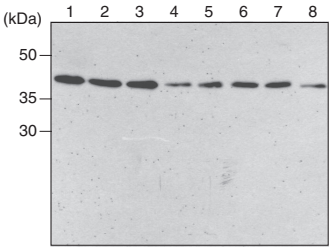
HeLa (starved condition)

Anti-Atg3 mAb

Code No.	Clone	Isotype	Size
M133-3	3E8	Mo IgG2bκ	100 μg

[Immunogen] Recombinant human Atg3
[Species cross-reactivity] Hu, Mo, Rat, Hm
[Form] 1 mg/mL in PBS/50% glycerol, pH 7.2
[Application] WB: 1 μg/mL
IP: 2.5 μg/300 μL of cell extract from 3x10^6 cells
IC: 0.5 μg/mL
<References>
1) Metlagel, Z., *et al.*, PNAS 110, 18844-18849 (2013) [WB]

■ **Western blotting**



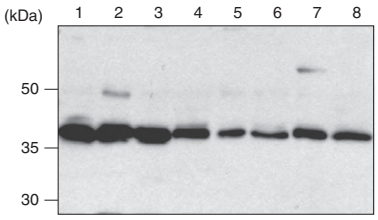
Lane 1: HEK293T
Lane 2: HeLa
Lane 3: Jurkat
Lane 4: NIH/3T3
Lane 5: WR19L
Lane 6: Rat1
Lane 7: PC12
Lane 8: CHO

Anti-Atg3 pAb

Code No.	Clone	Isotype	Size
PM034	Polyclonal	Rab Ig (aff.)	100 μL

Immunogen] Recombinant human Atg3
[Species cross-reactivity] Hu, Mo, Rat, Hm
[Form] PBS/50% glycerol, pH 7.2
[Application] WB: 1:1,000
IC: 1:50
<References>
1) Klionsky, D. J. *et al.*, J. Cell Sci. 118, 7-18 (2005)
2) Tanida, I., *et al.*, J. Biol. Chem. 277, 13739-13744 (2002)

■ **Western blotting**



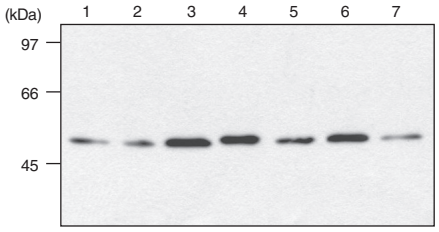
Lane 1: HEK293T
Lane 2: HeLa
Lane 3: Raji
Lane 4: NIH/3T3
Lane 5: WR19L
Lane 6: Rat1
Lane 7: PC12
Lane 8: CHO

Anti-Atg4B mAb

Code No.	Clone	Isotype	Size
M134-3	9H5	Mo IgG1	100 μg/100 μL

[Immunogen] Recombinant human Atg4B (1–393 a.a.)
[Species cross-reactivity] Hu, Mo, Rat, Hm
[Form] 1 mg/mL in PBS/50% glycerol, pH 7.2
[Application] WB: 1 μg/mL
<References>
1) Maejima, Y., *et al.*, Nat. Med. 19, 1478-88 (2013) [WB]
2) Kang, Y.A., *et al.*, Mol. Cell. Biol. 32, 226-239 (2012) [WB]

■ **Western blotting**



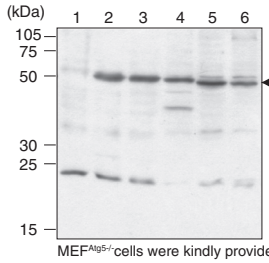
Lane 1: HEK293T
Lane 2: HeLa
Lane 3: Raji
Lane 4: NIH/3T3
Lane 5: Rat1
Lane 6: PC12
Lane 7: CHO

Anti-Atg5 mAb

Code No.	Clone	Isotype	Size
M153-3	4D3	Mo IgG1κ	100 μg/100 μL

[Immunogen] Recombinant human Atg5 (1–275 a.a.)
[Species cross-reactivity] Hu, Mo, Rat(-), Hm
[Form] 1 mg/mL in PBS/50% glycerol, pH 7.2
[Application] WB: 2-5 μg/mL
[Note] This antibody reacts with Atg5-Atg12 complex (55 kDa).
<References>
1) Liu, Y., *et al.*, Sci. Rep. 6, 20453 (2016) [WB]
2) Katagiri, N., *et al.*, Sci. Rep. 5, 8903 (2015) [WB]

■ **Western blotting**



Lane 1: MEF$^{Atg5-/-}$
Lane 2: MEF
Lane 3: NIH/3T3
Lane 4: CHO
Lane 5: HeLa
Lane 6: HEK293T

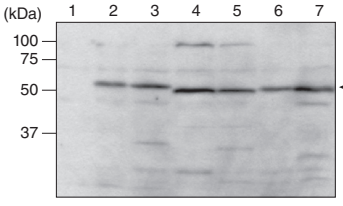
MEF$^{Atg5-/-}$cells were kindly provided by
Dr. Noboru Mizushima (The University of Tokyo).

Anti-Atg5 pAb

Code No.	Clone	Isotype	Size
PM050	Polyclonal	Rab Ig (aff.)	100 μL

[Immunogen] C-terminal region of human Atg5 (synthetic peptide)
[Species cross-reactivity] Hu, Mo, Rat, Hm(-)
[Form] PBS/50% glycerol, pH 7.2
[Application] WB: 1:500
[Note] This antibody recognizes the Atg5-Atg12 complex (55 kDa).
<References>
1) Maejima, Y., *et al.*, Nat. Med. 19, 1478-88 (2013) [WB]
2) Myeku, N., and Figueiredo-Pereira, M.E., J. Biol. Chem. 286, 22426-40 (2011) [WB]

■ **Western blotting**



Lane 1: MEF$^{Atg5-/-}$
Lane 2: MEF
Lane 3: NIH/3T3
Lane 4: HeLa
Lane 5: HEK293T
Lane 6: NRK
Lane 7: PC12

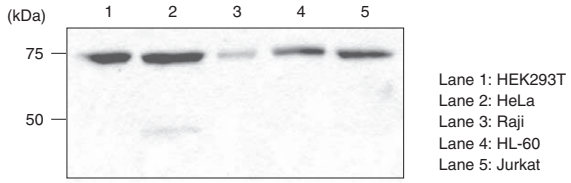
MEF$^{Atg5-/-}$cells were kindly provided by
Dr. Noboru Mizushima (The University of Tokyo).

Anti-Atg7 (Human) pAb

Code No.	Clone	Isotype	Size
PM039	Polyclonal	Rab Ig (aff.)	100 μL

[Immunogen] C-terminal region of human Atg7 (synthetic peptide)
[Species cross-reactivity] Hu, Mo(-), Rat(-), Hm(-)
[Form] PBS/50% glycerol, pH 7.2
[Application] WB: 1:1,000-1:2,000
IP: 5 μL/300 μL of cell extract from 3 x10^6 cells
<References>
1) Maejima, Y., *et al*., Nat. Med. 19, 1478-88 (2013) [WB]
2) Fujita, K., *et al*., PNAS 108, 1427-1432 (2011) [WB]

■ **Western blotting**

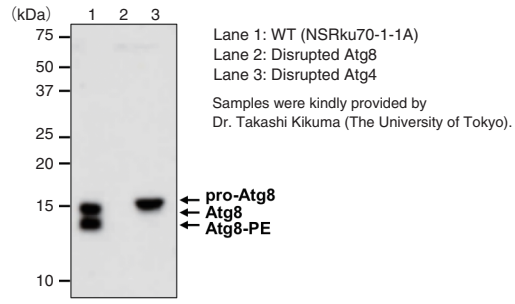


Anti-Atg8 (Filamentous fungi) pAb

Code No.	Clone	Isotype	Size
PM090	Polyclonal	Rab Ig (aff.)	100 μL

[Immunogen] Recombinant rice blast fungus MGG_01062 (Atg8) (1-116 a.a)
[Species cross-reactivity] Other
[Form] PBS/50% glycerol, pH7.2
[Application] WB: 1:1,000

■ **Western blotting**

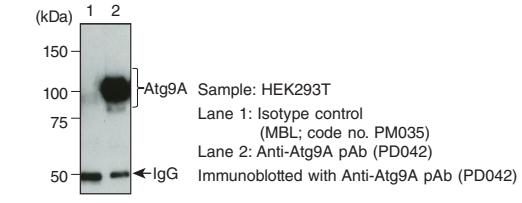


Anti-Atg9A pAb

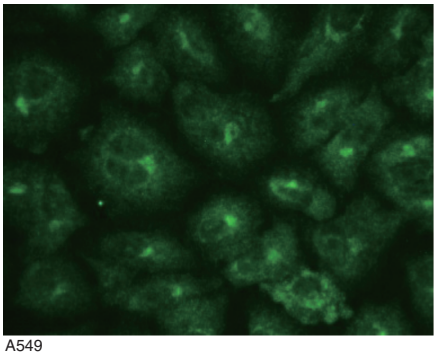
Code No.	Clone	Isotype	Size
PD042	Polyclonal	Rab Ig (aff.)	100 μL

[Immunogen] Recombinant mouse Atg9A (506 – 839 a.a.)
[Species cross-reactivity] Hu, Mo, Rat, Hm
[Form] PBS/50% glycerol, pH 7.2
[Application] WB: 1:500
IP: 2.5 μL/300 μL of cell extract from 3x10^6 cells
IC: 1:400
<References>
1) Itakura, E., *et al*., J. Cell Sci. 125, 1488-1499 (2012)

■ **Immunoprecipitation**



■ **Immunocytochemistry**



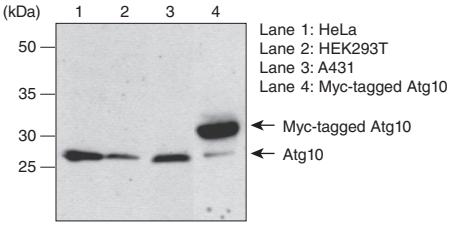
A549
Green: Anti-Atg9A pAb (PD042)

Anti-Atg10 (Human) mAb

Code No.	Clone	Isotype	Size
M151-3	5A7	Mo IgG1κ	100 μg/100 μL

[Immunogen] Recombinant human Atg10 (1–220 a.a.)
[Species cross-reactivity] Hu
[Form] 1 mg/mL in PBS/50% glycerol, pH 7.2
[Application] WB: 2 μg/mL
IH*: reported in articles
<References>
1) Jo, Y.K., *et al*., PLoS One 7, e52705 (2012) [IH]
2) Jiang, H., *et al*., J. Virol. 85, 4720-9 (2011) [WB]

■ **Western blotting**

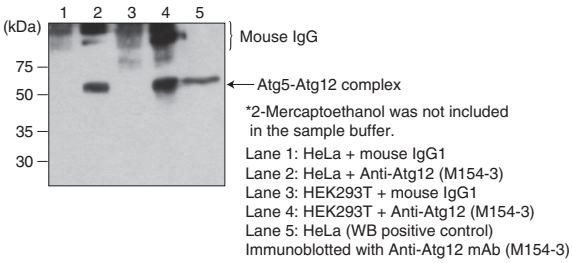


Anti-Atg12 (Human) mAb

Code No.	Clone	Isotype	Size
M154-3	6E5	Mo IgG1κ	100 μg/100 μL

[Immunogen] Internal region of human Atg12 (synthetic peptide)
[Species cross-reactivity] Hu, Mo(-), Rat(-), Hm(-)
[Form] 1 mg/mL in PBS/50% glycerol, pH 7.2
[Application] WB: 1 μg/mL
IP: 5 μg/250 μL of cell extract from 1x10^7 cells
IC: 10 μg/mL
[Note] This antibody reacts with human Atg5-Atg12 complex (55 kDa).
Because almost all Atg12 exist in the form of Atg5-Atg12 complex,
it is difficult to detect the monomeric Atg12.
<References>
1) Mizushima, N., *et al*., J. Cell Sci. 116, 1679-1688 (2003)
2) Mizushima, N., *et al*., FEBS Lett. 532, 450-454 (2002)

■ **Immunoprecipitation**

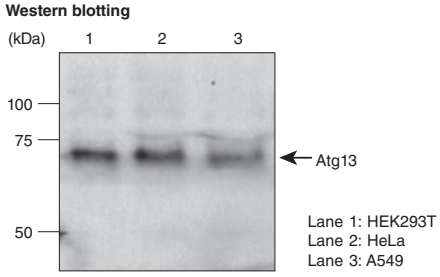


Anti-Atg13 (Human) pAb

Code No.	Clone	Isotype	Size
PD036	Polyclonal	Rab Ig (aff.)	100 μL

[Immunogen] Recombinant human Atg13
[Species cross-reactivity] Hu, Mo(-)
[Form] PBS/50% glycerol, pH 7.2
[Application] WB: 1:500
IP: 4 μL/300 μL of cell extract from 3x10^6 cells
<References>
1) Hosokawa, N., *et al*., Mol. Biol. Cell 20, 1981-91 (2009) [WB, IP]

■ **Western blotting**

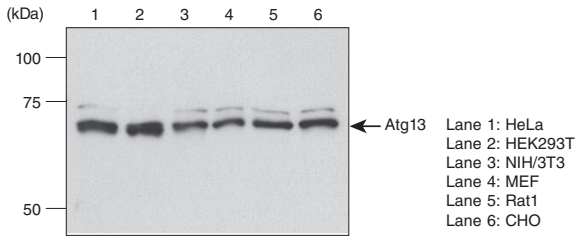


Anti-Atg13 mAb

Code No.	Clone	Isotype	Size
M183-3	5G4	Mo IgG2aκ	100 μg/100 μL

[Immunogen] Recombinant human Atg13
[Species cross-reactivity] Hu, Mo, Rat, Hm
[Form] 1 mg/mL in PBS/50% glycerol, pH 7.2
[Application] WB: 1 μg/mL
IP: 2 μg/300 μL cell extract from 3x10^6 cells
<References>
1) Ganley, I. G., *et al*., J. Biol. Chem. 284, 12297-12305 (2009)
2) Hosokawa, N., *et al*., Mol. Biol. Cell 20, 1981-1991 (2009)

■ **Western blotting**

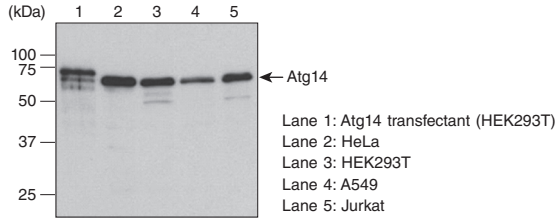


Anti-Atg14 (Human) mAb

Code No.	Clone	Isotype	Size
M184-3	4H8	Mo IgG2aκ	100 μg/100 μL

[Immunogen] Recombinant human Atg14 (167–404 a.a.)
[Species cross-reactivity] Hu, Mo(-), Rat(-)
[Form] 1 mg/mL in PBS/50% glycerol, pH 7.2
[Application] WB: 1 μg/mL
IP: 2 μg/300 μL cell extract from 3x10^6 cells
<References>
1) Zhong, Y., *et al*., Nat. Cell Biol. 11, 468-476 (2009)
2) Matsunaga, K., *et al*., Nat. Cell Biol. 11, 385-396 (2009)

■ **Western blotting**

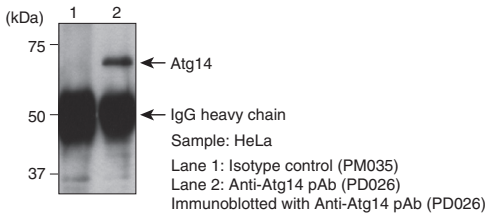


Anti-Atg14 pAb

Code No.	Clone	Isotype	Size
PD026	Polyclonal	Rab Ig (aff.)	100 μL

[Immunogen] Recombinant human Atg14 (167–404 a.a.)
[Species cross-reactivity] Hu, Mo, Rat, Hm(-)
[Form] PBS/50% glycerol, pH 7.2
[Application] WB: 1:500
IP: 5 μL/300 μL of cell extract from 3 x10^6 cells
IC*: reported in articles
<References>
1) Nemazanyy, I., *et al*., Nat. Commun. 6, 8283 (2015) [IP]
2) Bejarano, E., *et al*., Nat. Cell Biol. 16, 401-14 (2014) [WB, IC]

■ **Immunoprecipitation**

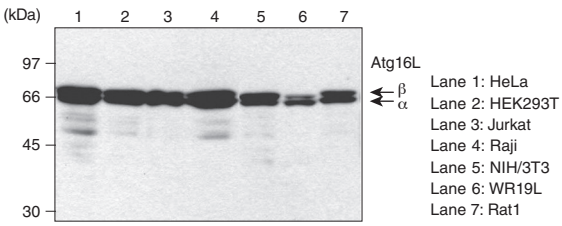


Anti-Atg16L mAb

Code No.	Clone	Isotype	Size
M150-3	1F12	Mo IgG1κ	100 μg/100 μL

[Immunogen] Recombinant human Atg16L1 TV2 (85–588 a.a.)
[Species cross-reactivity] Hu, Mo, Rat
[Form] 1 mg/mL in PBS/50% glycerol, pH 7.2
[Application] WB: 1 μg/mL
IH*: reported in articles
FCM*: reported in articles
IF*: reported in articles
<References>
1) Boada-Romero, E., *et al*., Nat. Commun. 7, 11821 (2016) [WB]
2) Morozova, K., *et al*., Nat. Commun. 6, 5856 (2015) [FCM, IF]
3) Adolph, T.E., *et al*., Nature 503, 272-6 (2013) [IH]

■ **Western blotting**

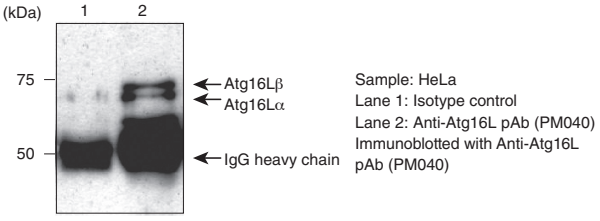


Anti-Atg16L pAb

Code No.	Clone	Isotype	Size
PM040	Polyclonal	Rab Ig (aff.)	100 μL

[Immunogen] Recombinant human Atg16L1 TV2 (85–588 a.a.)
[Species cross-reactivity] Hu, Mo, Rat, Hm
[Form] PBS/50% glycerol, pH 7.2
[Application] WB: 1:1,000
IP: 2.5 μL/300 μL of cell extract from 3x10^6 cells
IC: 1:200-1:500
Image-based FCM*: reported in articles
<References>
1) Erbil, S., *et al*., J. Biol. Chem. 291, 16753-16765 (2016) [WB]
2) Murthy, A., *et al*., Nature 506, 456-62 (2014) [IP, Image-based FCM]

■ **Immunoprecipitation**



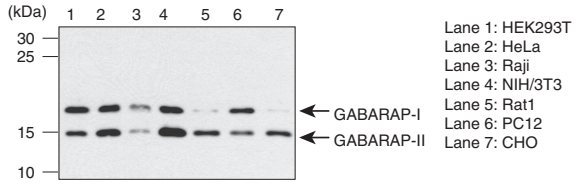
Antibodies for autophagy-related proteins

Anti-GABARAP mAb

Code No.	Clone	Isotype	Size
M135-3	1F4	Mo IgG1	100 μg/100 μL

[Immunogen] N-terminal region of human GABARAP (synthetic peptide)
[Species cross-reactivity] Hu, Mo, Rat, Hm
[Form] 1 mg/mL in PBS/50% glycerol, pH 7.2
[Application] WB: 1 μg/mL
IC*: reported in articles
IH*: reported in articles
<References>
1) Zhang, Z., *et al*., J. Immunol. 190, 3517-24 (2013) [WB]
2) Colecchia, D., *et al*., Autophagy 8, 1724-40 (2012) [IC]

■ **Western blotting**

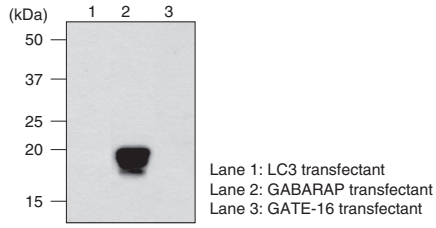


Anti-GABARAP pAb

Code No.	Clone	Isotype	Size
PM037	Polyclonal	Rab Ig (aff.)	100 μL

[Immunogen] N-terminal region of GABARAP (synthetic peptide)
[Species cross-reactivity] Hu, Mo, Rat, Hm
[Form] PBS/50% glycerol, pH 7.2
[Application] WB: 1:1,000
IC: 1:100
[Note] This antibody does not react with GATE-16 and LC3.
<References>
1) Polletta, L., *et al*., Autophagy 11, 253-70 (2015) [WB]
2) Mariño, G., *et al*., J. Clin. Invest. 120, 2331-44 (2010) [WB]

■ **Western blotting**

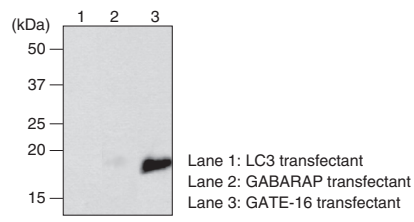


Anti-GATE-16 pAb

Code No.	Clone	Isotype	Size
PM038	Polyclonal	Rab Ig (aff.)	100 μL

[Immunogen] N-terminal region of GATE-16 (synthetic peptide)
[Species cross-reactivity] Hu, Mo, Rat, Hm
[Form] PBS/50% glycerol, pH 7.2
[Application] WB: 1:1,000
IH*: reported in articles
[Note] This antibody does not react with LC3 and GABARAP.
<References>
1) Niso-Santano, M., *et al*., EMBO J. 34, 1025-1041 (2015) [WB]
2) Tanji, K., *et al*., Neurobiol. Dis. 43, 690-7 (2011) [WB, IH]

■ **Western blotting**

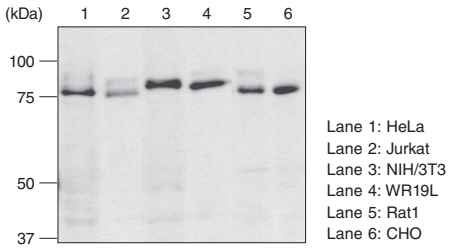


Anti-UVRAG mAb

Code No.	Clone	Isotype	Size
M160-3	1H4	Mo IgG1κ	100 μg/100 μL

[Immunogen] Recombinant human UVRAG (389–699 a.a.)
[Species cross-reactivity] Hu, Mo, Rat, Hm
[Form] 1 mg/mL in PBS/50% glycerol, pH 7.2
[Application] WB: 1 μg/mL
IP*: reported in articles
IC*: reported in articles
<References>
1) Nemazanyy, I., *et al*., Nat. Commun. 6, 8283 (2015) [IP]
2) Niso-Santano, M., *et al*., EMBO J. 34, 1025-1041 (2015) [WB]

■ **Western blotting**

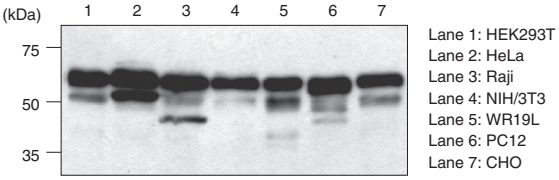


Anti-Beclin 1 pAb

Code No.	Clone	Isotype	Size
PD017	Polyclonal	Rab Ig (aff.)	100 μL

[Immunogen] Recombinant human Beclin 1 (1–450 a.a.)
[Species cross-reactivity] Hu, Mo, Rat, Hm
[Form] PBS/50% glycerol, pH 7.2
[Application] WB: 1:1,000
IP: 2.5 μL/200 μL of cell extract from $5x10^6$ cells
IC: 1:100
IH*: reported in articles
<References>
1) Munson, M.J., *et al*., EMBO J. 34, 2272-2290 (2015) [WB]
2) Hamasaki, M., *et al*., Nature 495, 389-93 (2013) [WB]

■ **Western blotting**

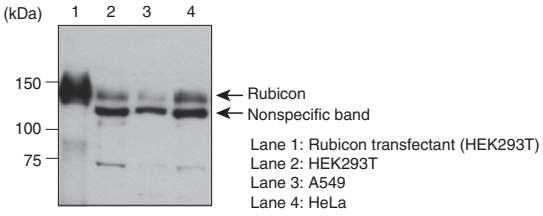


Anti-Rubicon (Human) pAb

Code No.	Clone	Isotype	Size
PD027	Polyclonal	Rab Ig (aff.)	100 μL

[Immunogen] Recombinant human Rubicon (722–972 a.a.)
[Species cross-reactivity] Hu, Mo(-)
[Form] PBS/50% glycerol, pH 7.2
[Application] WB: 1:1,000
IP: 5 μL/300 μL of cell extract from $3x10^6$ cells
<References>
1) Bejarano, E., *et al*., Nat. Cell Biol. 16, 401-14 (2014) [WB]
2) Maejima, Y., *et al*., Nat. Med. 19, 1478-88 (2013) [WB]

■ **Western blotting**



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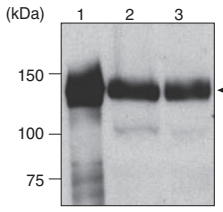
Anti-Rubicon (Human) mAb

Code No.	Clone	Isotype	Size
M170-3	1H6	Mo IgG2aκ	100 μg/100 μL

[Immunogen] Recombinant human Rubicon (722–972 a.a.)
[Species cross-reactivity] Hu, Mo(-)
[Form] 1 mg/mL in PBS/50% glycerol, pH 7.2
[Application] WB: 1 μg/mL
<References>
1) Matsunaga, K., *et al.*, Nat. Cell Biol. 11, 385-396 (2009)
2) Zhong, Y., *et al.*, Nat. Cell Biol. 11, 468-476 (2009)

■ **Western blotting**



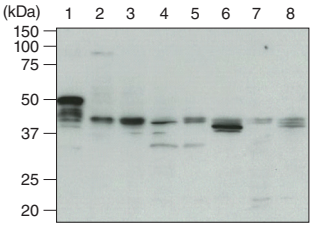
Lane 1: Rubicon transfectant
Lane 2: HeLa
Lane 3: A549

Anti-VMP1 pAb

Code No.	Clone	Isotype	Size
PM072	Polyclonal	Rab Ig (aff.)	100 μL

[Immunogen] Recombinant human VMP1 (131–217 a.a.)
[Species cross-reactivity] Hu, Mo, Rat, Hm
[Form] PBS/50% glycerol, pH 7.2
[Application] WB: 1:500
IP: 5 μL/2x10^6 cells/sample
<References>
1) Itakura, E., *et al.*, Autophagy. 6, 764-76 (2010)
2) Itakura, E., *et al.*, J. Cell Biol. 192, 17-27 (2011)

■ **Western blotting**



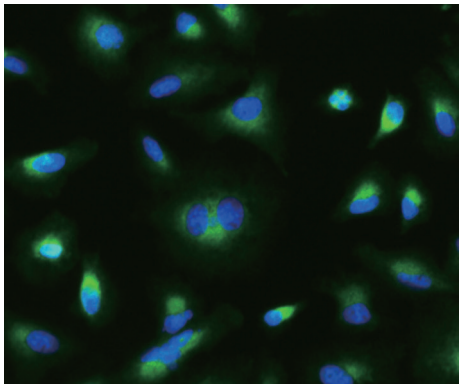
Lane 1: VMP1 transfectant (HEK293T)
Lane 2: HEK293T
Lane 3: A431
Lane 4: NIH/3T3
Lane 5: MEF
Lane 6: NRK
Lane 7: PC12
Lane 8: CHO

Anti-Syntaxin-17 (Human) pAb

Code No.	Clone	Isotype	Size
PM076	Polyclonal	Rab Ig (aff.)	100 μL

[Immunogen] Recombinant human Syntaxin-17 (1–302 a.a.)
[Species cross-reactivity] Hu, Mo(-), Rat(-)
[Form] PBS/50% glycerol, pH 7.2
[Application] WB: 1:1,000
IP: 2.5 μg/sample
IC: 1:2,000
<References>
1) Itakura, E., *et al.*, Cell 151, 1256–1269 (2012)

■ **Immunocytochemistry**



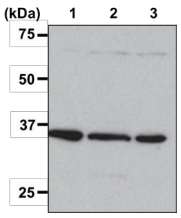
A549
Green: Anti-Syntaxin-17 (Human) pAb (PM076)
Blue: DAPI

Anti-Syntaxin-17 (Human) mAb

Code No.	Clone	Isotype	Size
M212-3	2F8	Mo IgG2aκ	100 μg/100 μL

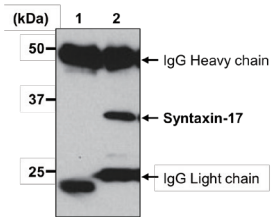
[Immunogen] Recombinant human Syntaxin-17 (1-302 a.a.)
[Species cross-reactivity] Hu, Mo(-), Rat(-), Hm(-)
[Form] PBS/50% glycerol, pH7.2
[Application] WB: 1 μg/mL
IP: 2 μg/sample
<References>
1) Hamasaki, M., *et al.*, Nature 495, 389-93 (2013)
2) Itakura, E., *et al.*, Cell 151, 1256-69 (2012)

■ **Western blotting**



Lane 1: Jurkat
Lane 2: A549
Lane 3: HeLa

■ **Immunoprecipitation**



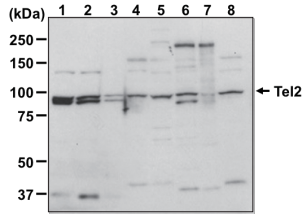
Sample : HeLa
Lane 1: Mouse IgG2a (M076-3)
Lane 2: Anti-Syntaxin-17 (Human) mAb (M212-3)
Immunoblotted with Anti-Syntaxin-17 mAb (M212-3)

Anti-Tel2 pAb

Code No.	Clone	Isotype	Size
PD037	Polyclonal	Rab Ig (aff.)	100 μL

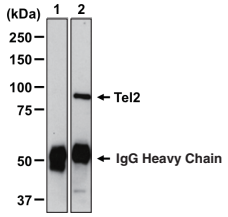
[Immunogen] Recombinant human Tel2 (618–838 a.a.)
[Species cross-reactivity] Hu, Mo, Rat, Hm
[Form] PBS/50% glycerol, pH7.2
[Application] WB: 1:2,000
IP: 1 μL/250 μL of cell extract from 2.5x10^6 cells
<References>
1) Kaizuka, T., *et al.*, J. Biol. Chem. 285, 20109-16 (2010) [WB]

■ **Western blotting**



Lane 1: HeLa
Lane 2: HEK293T
Lane 3: A549
Lane 4: NIH/3T3
Lane 5: MEF
Lane 6: Rat1
Lane 7: NRK
Lane 8: CHO

■ **Immunoprecipitation**



Sample : HeLa
Lane 1: Normal rabbit IgG (PM035)
Lane 2: Anti-Tel2 pAb (PD037)
Immunoblotted with Anti-Tel2 pAb (PD037)

FAQs (LC3 antibody)

Q1 What can I do to induce starvation?

➡ In NRK cells, starvation can be induced by changing the media to Hank's Balanced Salt Solution (serum-free) and incubating for 2 – 4 hours. Serum-free DMEM (Dulbecco's modified Eagle's medium) can be used, but the induction is weaker because DMEM contains amino acids. Since optimal conditions depend on the cell type, experimental conditions should be determined for your cells of interest by thorough evaluation.

Q2 What percentage of gel should I use to detect LC3 by Western blotting?

➡ We recommend 15%. The LC3-I and LC3-II bands overlap on a 10% gel, which makes them difficult to distinguish from each other.

Q3 LC3 bands are not detectable in Western blotting.

➡ Please refer to the datasheet and check for the following issues:

- Use a buffer containing SDS for sample preparation.
  We recommend the SDS-PAGE sample buffer (Laemmli's sample buffer).
- The washing step after blocking is essential when using a monoclonal antibody for detection.
  LC3-II bands become more intense if 0.05% Tween-20/PBS is used for the washing (three times for 5 minutes each).
- A positive control for WB (cell lysates expressing human LC3B) is available (Code No. PM036-PN).

Q4 Do you have any information about interpretation of LC3-I and LC3-II bands detected by WB?

➡ Please refer to the following publications for a detailed explanation of WB data for LC3.

Mizushima, N. and Yoshimori, T., How to interpret LC3 immunoblotting. Autophagy 3 (6), 542-545 (2007) PMID:17611390

Klionsky, DJ., *et al*., Guidelines for the use and interpretation of assays for monitoring autophagy in higher eukaryotes, Autophagy 4(2), 151-175 (2008) PMID: 18188003

Klionsky, DJ., *et al*., Guidelines for the use and interpretation of assays for monitoring autophagy, Autophagy 8(4), 445-544 (2012) PMID: 22966490

Q5 Are there any issues I should be aware of when performing immunocytochemistry?

➡ We use Digitonin (Sigma, D141) to permeabilize the membranes. The solvent is PBS (freshly prepared at a final concentration of 100 µg/mL). We do not recommend using Triton X-100 for membrane permeabilization.

Q6 What fixatives should I use for immunocytochemistry (IC)?

➡ We use 4% PFA/PBS. Fixation with methanol or acetone is not recommended.

Q7 What fixatives should I use for immunohistochemistry (IHC)?

➡ We recommend 10% formalin solution (3.7% formaldehyde) or 4% PFA/PBS.

Q8 Can I stain frozen sections?

➡ Use of cryosections has not been evaluated by MBLI.

Q9 Which antibody is most recommended?

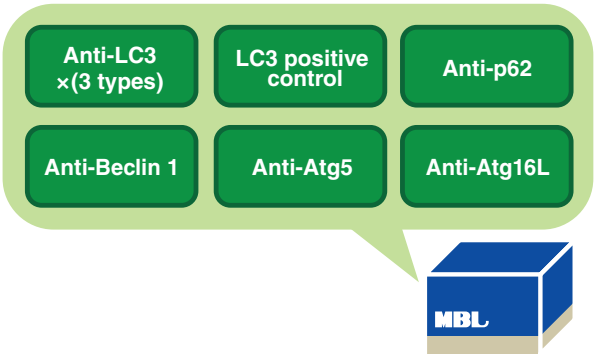
➡ We recommend different antibodies depending on the application.

Below is a guideline:

WB: Code No. M186-3, PM036
IP: Code No. M152-3, PM036
IC: Code No. M152-3, PM036
FCM: Code No. M152-3, PM036
IHC: Code No. PM036

Autophagy Ab Sampler Set

Popular MBL antibodies for autophagy-related proteins are available in a set.



- ◎ For customers planning to start autophagy research.
- ◎ For customers interested in trying MBL autophagy antibodies.
- ◎ For customers interested in purchasing a small amount of several antibodies.

Code No.	Product name	Size
8485	Autophagy Ab Sampler Set	Antibodies: 25 μL each, Positive control: 10 tests

Components

Code No.	Product name	Clone	Isotype	Application	Size	Species cross-reactivity
PM036Y	Anti-LC3 pAb	Polyclonal	Rabbit IgG	WB, IP, FCM, IC, IH	25 μL	Hu, Mo, Rat, Hm
M186-3Y	Anti-LC3 mAb	8E10	Mouse IgG2aκ	WB	25 μL	Hu, Mo, Rat, Hm
M152-3Y	Anti-LC3 mAb	4E12	Mouse IgG1κ	WB, IP, FCM, IC, IH*, Immuno-EM, Immug-based-FCM*	25 μL	Hu, Mo, Rat, Hm
PD017Y	Anti-Beclin 1 pAb	Polyclonal	Rabbit Ig (aff.)	WB, IP, IC	25 μL	Hu, Mo, Rat, Hm
PM040Y	Anti-Atg16L pAb	Polyclonal	Rabbit Ig (aff.)	WB, IP, IC	25 μL	Hu, Mo, Rat, Hm
PM045Y	Anti-p62 (SQSTM1) pAb	Polyclonal	Rabbit Ig (aff.)	WB, IP, IC, IH	25 μL	Hu, Mo, Rat, Hm
PM050Y	Anti-Atg5 pAb	Polyclonal	Rabbit Ig (aff.)	WB	25 μL	Hu, Mo, Rat
PM036-PNY	Positive control for anti-LC3 antibody			WB	100 μL	

Autophagy research: Current status and future perspectives

Dr. Noboru Mizushima
of the University of Tokyo

1. What is autophagy?

The lysosome is a cellular organelle whose main function is degradation. It is primarily known as the place where extracellular materials and plasma membrane proteins internalized by endocytosis are degraded. But, it can certainly degrade intracellular components as well (Figure 1). Autophagy is a "cellular function in which the cell degrades its own components in the lysosome." Although often mistaken for a type of cell death, autophagy is a degradative process that mostly protects the cell from cell death.

Autophagy is broadly classified into "macroautophagy," "microautophagy," and "chaperone-mediated autophagy" (Figure 1). Among them, macroautophagy has the largest degradative capacity and has been extensively studied in many species from yeast to animals and plants. In contrast, microautophagy and chaperone-mediated autophagy have been studied primarily in yeast and mammals, respectively, and their occurrence and molecular mechanisms are not fully understood (microautophagy might be the same process as the formation of multivesicular bodies in mammals). For this reason, macroautophagy is commonly referred to by the term autophagy, which will be used hereafter in this article.

Many of the molecules involved in autophagy were identified in genetic studies of the budding yeast in early 1990s by Dr. Yoshinori Ohsumi (currently of the Tokyo

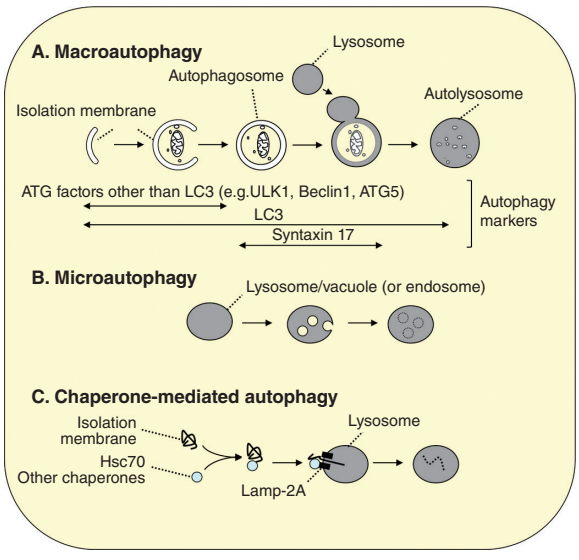


Figure 1. Three types of autophagy

A. Macroautophagy. As autophagosomes (approximately 1 μm in diameter) are formed, a portion of the cytoplasm is enclosed. Subsequently, the autophagosomes fuse with the lysosome, resulting in degradation of the inner autophagosomal membrane along with its contents. Because LC3 family proteins are localized to most membranes formed in this process, LC3 is used as a general marker for autophagy-associated membranes (in autolysosomes, LC3 on the inner membrane is degraded, and LC3 on the outer membrane is gradually detached from the membrane). In contrast, most ATG factors other than LC3 are localized only to the isolation membrane prior to closure, and syntaxin 17, which is necessary for fusion with the lysosome, is localized to autophagosomes after closure. Thus, these molecules serve as specific markers for these stages.

B. Microautophagy. Lysosomal or vacuolar membranes invaginate and detach, thereby engulfing the cytoplasmic components. In yeast, peroxisomes and a portion of the nucleus are degraded in this process (in microautophagy of peroxisomes, vacuolar membrane is not sufficient for the engulfment, and *de novo* membrane synthesis is required). In mammals, late endosomal membranes undergo similar membrane dynamics known as "endosomal microautophagy" to degrade cytoplasmic components.

C. Chaperone-mediated autophagy. Cytoplasmic proteins with the pentapeptide "KFERQ motif" are recognized by Hsc70 and other co-chaperones, and directly transported into the lumen of the lysosome via binding to the LAMP2A receptor on the lysosome.

Institute of Technology) and his colleagues [1]. The *ATG1* through *ATG41* genes have been known as of August 2016. Most of these genes are required for selective autophagy, which targets specific substrates for degradation. For example, ATG30 is required only for autophagy of peroxisomes (pexophagy), and ATG32 is required only for autophagy of mitochondria (mitophagy). In contrast, the 15 genes known as the "core *ATG* genes" (ATG1 – 10, 12 – 14, 16, 18) are required for all types of autophagy, including the non-selective "ordinary autophagy" that is induced during starvation. These genes are highly conserved in other organisms including mammals. The detailed functions of these genes have been reviewed elsewhere [2, 3].

2. Current status of autophagy research

The discovery of yeast *ATG* genes opened an entirely new chapter of autophagy research, in which three main features have to be considered together.

First, functional analysis of the yeast Atg molecules was conducted using powerful yeast genetics as the main tool. Studies using genetic, biochemical, morphological, and structural biology approaches broadly advanced our understanding of various aspects of autophagy, such as the function of individual Atg proteins and complexes, their genetic hierarchy, the mechanism of regulation of their activities, and the mechanism of substrate recognition. In yeast, the autophagosome is formed from the "pre-autophagosomal structure (PAS)," which typically exists as a single entity near the vacuole. Through details of autophagosome formation have been actively studied, many questions remain unanswered, including the origin of the autophagosomal membrane, composition of proteins and lipids in the membrane, mechanism of autophagosome membrane closure, and mechanism of fusion with the vacuole.

Second, ATG homologues have been studied in species other than yeast. Even before genome sequences were completed, it was clear that many species had ATG homologues, including mammals and plants. Although red algae and some protozoan species lack some or all ATG homologues [4], *ATG* genes are conserved in nearly all eukaryotes. In addition, autophagy factors that are absent in yeast, such as VMP1, EPD5, and Ei24, were discovered in higher animals. These discoveries complement those made by yeast studies on the molecular mechanism of autophagy, and at the same time made important contributions to the development of markers essential for autophagy research. A good example is the autophagosome marker LC3B, which was discovered by Dr. Tamotsu Yoshimori (currently of Osaka University), and is most widely used today (Figure 1) [5, 6]. In many organisms other than the budding yeast, autophagosomes are simultaneously formed at multiple locations in the cell. These locations are closely associated at least with the endoplasmic reticulum.

Third, phenotypes of *atg* null mutants have been investigated in various species using the reverse genetics approach. Numerous tissue-specific *ATG* knockout mice have been generated in addition to simple knockout mice. For example, the *ATG5flox* mice, generated in the author's laboratory, alone have been shared with more than 400 laboratories. Presumably, this strain has been crossed with all publicly available Cre-expressing mice. As a result, it has become clear that autophagic degradation essentially plays two main roles [7]. One is to supply degradation products, which is important in maintaining the intracellular amino acid pool during starvation and early embryogenesis, and for self-antigen presentation. The first evidence of this role was the isolation of yeast autophagy mutants that had lost resistance to starvation [1]. This role of autophagy is believed to be common to all organisms. The second role is intracellular quality control via substrate-selective as well as non-selective bulk degradation. Substrates of selective autophagy include LC3-binding proteins such as p62/SQSTM1, damaged organelles such as depolarized mitochondria, and intracellular pathogens (in some cases, damaged endosomes surrounding bacteria). As demonstrated by Dr. Masaaki Komatsu (currently of Niigata University), it is especially important to maintain p62/SQSTM1 at a low level in order to prevent accumulation of protein aggregates and uncontrolled activation of the oxidative stress-responsive Nrf2 pathway. Insufficient quality control is generally associated with cellular degeneration in the nervous system and the liver, acceleration of age-related changes, tumor formation, and exacerbation of infection in mice. These quality control functions have a greater impact on long-lived cells and little impact on cells (such as budding yeast) whose doubling time is much shorter than the average half-life of proteins. Similar physiological studies in many non-mouse models have been reported, but are not included in this article due to limited space.

3. Autophagy and human disease

Basic research on autophagy has dramatically expanded in the past 10 years. In contrast, our understanding of the relevance of autophagy in medicine has lagged far behind. Only recently, association of autophagy with human disease has begun to emerge through studies in human genetics.

In 2007, *ATG16L1* was identified as a risk allele associated with the inflammatory bowel disease, Crohn's disease [8, 9]. This allele does not encode a null mutation, but has a threonine-to-alanine substitution at position 300 (T300A), which is located close to the center of ATG16L1. Healthy individuals often carry this allele, and the odds ratio for Crohn's disease is about 2-fold in patients with the homozygous mutation. The C-terminal domain that includes the mutated region is not present in yeast Atg16, and the function of this domain remains largely unknown in mammals. There are conflicting reports about the T300A mutation; some claimed that the mutation affected the activity of autophagy, and others reported the opposite. Regardless, given that the high-frequency mutation was identified through statistical analyses of multiple populations, it would be very challenging to define the significance of the mutation in tissue culture cells or experimental animals.

Dr. Richard Youle and his colleagues in the United States reported in 2008 that a causative gene for familial Parkinson's disease, *Parkin*/*PARK2*, was involved in autophagic degradation of depolarized mitochondria (also called mitophagy) [10]. Parkin is a ubiquitin ligase localized to depolarized/damaged mitochondria. The localization process requires another factor associated with familial Parkinson's disease, PINK1/PARK6, and phosphorylated ubiquitin [11]. Based on these findings, Parkin-associated Parkinson's disease and PINK1-associated Parkinson's disease are thought to be caused by impaired mitophagy, resulting in accumulation of damaged mitochondria that should have been degraded. However, Parkin has other functions unrelated to mitophagy, such as induction of outer membrane protein degradation while maintaining intact mitochondria [12]. Additional *in vivo* experiments are necessary to demonstrate that impaired mitophagy itself is the cause of Parkinson's disease.

Since 2012, mutations in autophagy-related genes have been identified one after another through exome analyses of patients' families. Dr. Hayflick's group in the United States and Drs. Naomichi Matsumoto and Hirotomo Saitsu (currently of Yokohama City University) independently discovered mutations in *WDR45*/*WIPI4* gene as a cause for SENDA (static encephalopathy of childhood with neurodegeneration in adulthood) [13, 14]. WIPI4 is the human homologue of yeast ATG18 or ATG21 (and there are four WIPI proteins 1 – 4). Moreover, this is the first report of human disease associated with mutations in core *ATG* genes. *WDR45* gene is located on the X chromosome, and most cases occur in women with mosaicism. SENDA (also known as BPAN) is a neurodegenerative disease characterized by iron accumulation in the basal ganglia in the brain. Patients present with non-progressive intellectual and motor deficits in childhood, and their Parkinson-like symptoms rapidly progress after 20 – 30 years of age. We demonstrated that patients' lymphoblasts had reduced autophagic activity [14]. However, the exact function of WIPI4 remains unclear. Among the WIPI family proteins, WIPI2 has the most important role in typical culture cells such as HeLa cells. In contrast, p62, which is a substrate of selective autophagy, is found to accumulate in the nerves of the recently generated WIPI4 knockout mice, suggesting the possibility that WIPI4 plays an important role in the nervous system [15]. As for mutations in other core *ATG* genes, an *ATG5* mutation has been reported in patients with congenital ataxia with mental retardation [16]. This mutation results in partially reduced activity of autophagy due to impaired ATG5-ATG12 conjugation. Mutations in other genes have also been identified through exome analyses. For example, mutations in a gene involved in autolysosome degradation, *EPG5*, were identified in patients with Vici syndrome (characterized by agenesis of the corpus callosum, cataracts, cardiomyopathy, immunodeficiency, and hypopigmentation) [17]; a mutation in *TECPR2* gene, encoding an LC3-binding protein, was identified in patients with hereditary spastic paraparesis [18]; and mutations in lysosomal PI(3,5,)P_2-binding protein SNX14 were identified in patients with cerebellar atrophy [19]. These mutations cause reduced activity of autophagy as well. However, it is likely that these effects are primarily caused by lysosomal abnormalities rather than the autophagy pathway *per se*. See another review for details [20].

4. Therapeutic approaches targeting autophagy

To date, autophagy is known as the direct cause of human disease in only a limited number of cases. Nevertheless, efforts to target autophagy as a therapeutic strategy have already begun. In fact, a drug chloroquine (or hydroxychloroquine) is being tested in clinical trials for malignant tumors [20]. Chloroquine inhibits lysosomal function. This drug is not necessarily specific for autophagy. A multicenter clinical trial is currently underway with the University of Pennsylvania in the United States as the coordinating center. As of August 2016, 27 studies with hydroxychloroquine and 8 studies with chloroquine have been registered on the NIH's website ClinicalTrials.gov (https://clinicaltrials.gov/ct2/home) as Phase 1 or 2 trials. Most of the studies are combination trials. Detailed outcomes of some of the trials have been reported in 6 articles published in the August 2014 issue of Autophagy. According to the reports, treatments were effective in some patients. Several theories have been proposed to explain why inhibition of autophagy can be effective for cancer treatment. The therapeutic effects could be attributed to inhibition of a broad range of autophagic functions, such as cellular remodeling and a quality control mechanism, in addition to inhibition of amino acid production [22, 23]. If a subset of cancers could be identified as highly dependent on autophagy, treatment could be more effective. On the other hand, the anticancer effects of chloroquine may be independent of inhibition of autophagy [24]. Thus, effects of autophagy inhibitors with higher specificity should be tested in the future.

Although neurodegenerative disease could possibly be effectively treated by targeting autophagy, large-scale clinical trials are yet to be conducted. Many neurodegenerative diseases are caused, in part, by accumulation of abnormal proteins in the cell. Therefore, efforts are being made to remove abnormal or denatured proteins that are harmful to the cell, by enhancing the intracellular cleansing effect of autophagy. In experiments using neurodegenerative disease models (such as polyglutamine disease in mouse and *Drosophila*), the mTORC1 inhibitor rapamycin and its derivatives were effective in reducing the symptoms. However, mTORC1 inhibitors are not suitable for human use because of strong side effects. Hence, mTORC1-independent activators of autophagy have been sought. One such drug is the antiepileptic medicine carbamazepine. A report showed that carbamazepine was effective in an animal model of alpha1-antitrypsin deficiency, in which liver damage was caused by accumulation of the mutant protein in the endoplasmic reticulum of hepatocytes [25]. Although it is unclear whether the treatment effects were truly mediated by

activation of autophagy, future developments are expected in this area.

5. Future tasks and perspectives

Much has been discovered about the mechanism and physiological significance of autophagy. Although many important issues remain to be addressed, continued progress toward elucidation is expected in the future. While at the same time, autophagy-associated factors have been found to be also involved in physiological pathways other than autophagy. The findings in this area include the "LC3-associated phagocytosis (LAP)" pathway in which autophagy factors (excluding the ULK complex) facilitate the maturation of phagosomes, and a different pathway in which autophagosomes or related structures are used in non-classical secretion [26, 27]. The physiological significance of these processes is currently under investigation, and is thought to be closely associated with immune processes, such as phagocytosis of dead cells, autoimmune disease, and secretion of cytokines.

There is much room for improvement in methods for monitoring autophagy *in vivo*. Even in basic research using tissue culture cells, current methods of autophagy measurement are still complicated and not fully satisfactory. For instance, an increase in the number of autophagosomes and the LC3-II form (the membrane-bound form of LC3) does not unconditionally indicate activation of autophagy. Instead, it could indicate a blockage at a later step of autophagy, such as inhibition of lysosomes [6]. In fact, in previously conducted screening of compounds, increased number of LC3 puncta or LC3-II levels had mainly resulted from blockage at a later stage of the process and not activation of autophagy. Like chloroquine, many weak base compounds have such characteristics. This issue has become well recognized recently, and a more appropriate "flux assay" has been commonly used in parallel. However, it is difficult or impossible to perform a flux assay using fixed samples, and this difficulty is a major impediment in histopathological analyses. Further, because these assays can be done only with dissected tissue samples, it is virtually impossible to measure the activity of autophagy in living human beings at this time. An indirect method to assess the activity of autophagy needs to be developed, even if it might be imperfect. With such a method, it may become possible to identify human disease with partially impaired autophagy. Currently, there are very few ways to detect the involvement of autophagy, other than identifying mutations in autophagy-associated genes.

Lastly, regarding autophagy as the target for drug discovery, there will be plenty of possibilities in this area. A frequently expressed concern is that activating autophagy may have side effects. But, we view this issue optimistically. Notably, knocking out the relatively low steady-state level of autophagy can cause marked accumulation of abnormal proteins. This means that the low steady-state level of autophagy activity has a sufficient intracellular cleansing effect. Therefore, a slight increase in activation can be expected to have significant effects. Also, since there must be a sophisticated feedback mechanism between intracellular degradation and synthesis, activation of degradation is likely to be offset by activation of synthesis. If turnover (and not degradation alone) is increased, toxicity may not be very high. Hence, it would be more important to develop drugs that exclusively activate the autophagy pathway.

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Product list

Kit

Page	Code No.	Product name	Size
P.3	8486	Autophagy Watch	1 kit
P.21	8485	Autophagy Ab Sampler Set	Antibodies: 25 μL each, Positive control: 10 tests

Antibody

Page	Code No.	Product name	Clone	Isotype	Application	Size	Species cross-reactivity
P.14	PD041	Anti-Atg2A pAb	Polyclonal	Rabbit Ig (aff.)	WB, IP, IC	100 μL	Hu, Mo, Rat, Hm
P.14	M133-3	Anti-Atg3 mAb	3E8	Mouse IgG2bκ	WB, IP, IC	100 μg	Hu, Mo, Rat, Hm
P.14	PM034	Anti-Atg3 pAb	Polyclonal	Rabbit Ig (aff.)	WB, IC	100 μL	Hu, Mo, Rat, Hm
P.14	M134-3	Anti-Atg4B mAb	9H5	Mouse IgG1	WB	100 μg/100 μL	Hu, Mo, Rat, Hm
P.14	M153-3	Anti-Atg5 mAb	4D3	Mouse IgG1κ	WB	100 μg/100 μL	Hu, Mo, Hm
P.14	PM050	Anti-Atg5 pAb	Polyclonal	Rabbit Ig (aff.)	WB	100 μL	Hu, Mo, Rat
P.15	PM039	Anti-Atg7 (Human) pAb	Polyclonal	Rabbit Ig (aff.)	WB, IP	100 μL	Hu
P.15	PM090	Anti-Atg8 (Filamentous fungi) pAb	Polyclonal	Rabbit Ig (aff.)	WB	100 μL	Other
P.15	PD042	Anti-Atg9A pAb	Polyclonal	Rabbit Ig (aff.)	WB, IP, IC	100 μL	Hu, Mo, Rat, Hm
P.15	M151-3	Anti-Atg10 (Human) mAb	5A7	Mouse IgG1κ	WB, IH*	100 μg/100 μL	Hu
P.15	M154-3	Anti-Atg12 (Human) mAb	6E5	Mouse IgG1κ	WB, IP, IC	100 μg/100 μL	Hu
P.15	PD036	Anti-Atg13 (Human) pAb	Polyclonal	Rabbit Ig (aff.)	WB, IP	100 μL	Hu
P.16	M183-3	Anti-Atg13 mAb	5G4	Mouse IgG2aκ	WB, IP	100 μg/100 μL	Hu, Mo, Rat, Hm
P.16	M184-3	Anti-Atg14 (Human) mAb	4H8	Mouse IgG2aκ	WB, IP	100 μg/100 μL	Hu
P.16	PD026	Anti-Atg14 pAb	Polyclonal	Rabbit Ig (aff.)	WB, IP	100 μL	Hu, Mo, Rat
P.16	M150-3	Anti-Atg16L mAb	1F12	Mouse IgG1κ	WB, IH*, FCM*, IF*	100 μg/100 μL	Hu, Mo, Rat
P.16	PM040	Anti-Atg16L pAb	Polyclonal	Rabbit Ig (aff.)	WB, IP, IC, Image-based FCM*	100 μL	Hu, Mo, Rat, Hm
P.17	PD017	Anti-Beclin 1 pAb	Polyclonal	Rabbit Ig (aff.)	WB, IP, IC, IH*	100 μL	Hu, Mo, Rat, Hm
P.17	M135-3	Anti-GABARAP mAb	1F4	Mouse IgG1	WB, IC*, IH*	100 μg/100 μL	Hu, Mo, Rat, Hm
P.17	PM037	Anti-GABARAP pAb	Polyclonal	Rabbit Ig (aff.)	WB, IC	100 μL	Hu, Mo, Rat, Hm
P.17	PM038	Anti-GATE-16 pAb	Polyclonal	Rabbit Ig (aff.)	WB, IH*	100 μL	Hu, Mo, Rat, Hm
P.13	M224-3	Anti-KEAP1 mAb	KP1	Mouse IgG2aκ	WB	100 μg/100 μL	Hu, Mo, Rat, Hm
P.8	M152-3	Anti-LC3 mAb	4E12	Mouse IgG1κ	WB, IP, FCM, IC, IH*, Immuno-EM*, Image-based FCM*	200 μg/100 μL	Hu, Mo, Rat, Hm
P.7	M186-3	Anti-LC3 mAb	8E10	Mouse IgG2aκ	WB	100 μg/100 μL	Hu, Mo, Rat, Hm
P.8	M186-7	Anti-LC3 mAb-HRP-DirecT	8E10	Mouse IgG2aκ	WB	50 μL	Hu, Mo, Rat, Hm
P.7	PM036	Anti-LC3 pAb	Polyclonal	Rabbit IgG	WB, IP, FCM, IC, IH	100 μL	Hu, Mo, Rat, Hm
P.8	PD014	Anti-LC3 pAb	Polyclonal	Rabbit IgG	WB, IC*, IH*	100 μL	Hu, Mo, Rat, Hm
P.13	D058-3	Anti-Multi Ubiquitin mAb	FK2	Mouse IgG1κ	WB, IC*, ELISA*	100 μg/100μL	Hu, Mo*, Mky*
P.13	D071-3	Anti-Multi Ubiquitin mAb	FK1	Mouse IgM	WB	100 μg	Hu
P.12	M200-3	Anti-NRF2 mAb	1F2	Mouse IgG1κ	WB, IP, IC, IH	100 μg/100 μL	Hu, Mo, Rat, Hm
P.12	PM069	Anti-NRF2 pAb	Polyclonal	Rabbit Ig (aff.)	WB, IP, IC, IH	100 μL	Hu, Mo(w), Rat(w), Hm(w)
P.9	M162-3	Anti-p62 (SQSTM1) (Human) mAb	5F2	Mouse IgG1κ	WB, IP, FCM, IC, IH	100 μg/100 μL	Hu
P.10	M162-A48	Anti-p62 (SQSTM1) (Human) mAb-Alexa Fluor® 488	5F2	Mouse IgG1κ	FCM, IC	100 μg/100 μL	Hu
P.10	M162-A59	Anti-p62 (SQSTM1) (Human) mAb-Alexa Fluor® 594	5F2	Mouse IgG1κ	IC	100 μg/100 μL	Hu
P.10	M162-A64	Anti-p62 (SQSTM1) (Human) mAb-Alexa Fluor® 647	5F2	Mouse IgG1κ	FCM, IC	100 μg/100 μL	Hu
P.10	PM045	Anti-p62 (SQSTM1) pAb	Polyclonal	Rabbit Ig (aff.)	WB, IP, IC, IH	100 μL	Hu, Mo, Rat, Hm
P.10	PM066	Anti-p62 C-terminal pAb	Polyclonal	Guinea pig Ig (aff.)	WB, IP, IC, IH	100 μL	Hu, Mo, Rat, Hm
P.10	PM066-7	Anti-p62 C-terminal pAb-HRP-DirecT	Polyclonal	Guinea pig Ig (aff.)	WB	50 μL	Hu, Mo, Rat, Hm
P.6	M230-3	Anti-Parkin mAb	Par6	Mouse IgG2aκ	WB	100 μg/100 μL	Hu, Mo, Rat
P.11	M217-3	Anti-Phospho-p62 (SQSTM1) (Ser351) mAb	5D5	Mouse IgG1κ	WB, IC, IH	100 μg/100 μL	Hu, Mo
P.12	PM074	Anti-Phospho-p62 (SQSTM1) (Ser351) pAb	Polyclonal	Rabbit Ig (aff.)	WB, IP, IC, IH	100 μL	Hu, Mo
P.11	D343-3	Anti-Phospho-p62 (SQSTM1) (Ser403) mAb	4F6	Rat IgG2aκ	WB, IH	100 μg/100 μL	Hu, Mo
P.11	D344-3	Anti-Phospho-p62 (SQSTM1) (Ser403) mAb	4C8	Rat IgG2aκ	WB, IH	100 μg/100 μL	Hu, Mo
P.18	M170-3	Anti-Rubicon (Human) mAb	1H6	Mouse IgG2aκ	WB	100 μg/100 μL	Hu
P.17	PD027	Anti-Rubicon (Human) pAb	Polyclonal	Rabbit Ig (aff.)	WB, IP	100 μL	Hu
P.18	M212-3	Anti-Syntaxin-17 (Human) mAb	2F8	Mouse IgG2aκ	WB, IP	100 μg/100 μL	Hu
P.18	PM076	Anti-Syntaxin-17 (Human) pAb	Polyclonal	Rabbit Ig (aff.)	WB, IP, IC	100 μL	Hu
P.18	PD037	Anti-Tel2 pAb	Polyclonal	Rabbit Ig (aff.)	WB, IP	100 μL	Hu, Mo, Rat, Hm
P.13	MK-11-3	Anti-Ubiquitin mAb	1B3	Mouse IgG1	WB, IC*, IH*, Immuno-EM*	100 μg/100 μL	Hu, Mo*, Bov*
P.13	MK-12-3	Anti-Ubiquitin mAb	2C5	Mouse IgG1	WB, IP*, IC*	100 μg/100 μL	Hu, Mo, Rat, Bov
P.17	M160-3	Anti-UVRAG mAb	1H4	Mouse IgG1κ	WB, IP*, IC*	100 μg/100 μL	Hu, Mo, Rat, Hm
P.18	PM072	Anti-VMP1 pAb	Polyclonal	Rabbit Ig (aff.)	WB, IP	100 μL	Hu, Mo, Rat, Hm
P.8	PM036-PN	Positive control for anti-LC3 antibody			WB	100 μL (10 tests)	

Vector

Page	Code No.	Product name	Size
P.5	AM-V0259M	pMitophagy Keima-Red mPark2 (Kan)	20 μg
P.5	AM-V0259HM	pMitophagy Keima-Red mPark2 (Hyg)	20 μg
P.5	AM-V0251M	*CoralHue*® Mitochondria-targeted mKeima-Red (pMT-mKeima-Red)	20 μg
P.5	AM-V0251HM	*CoralHue*® Mitochondria-targeted monomeric Keima-Red (Hyg)	20 μg

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