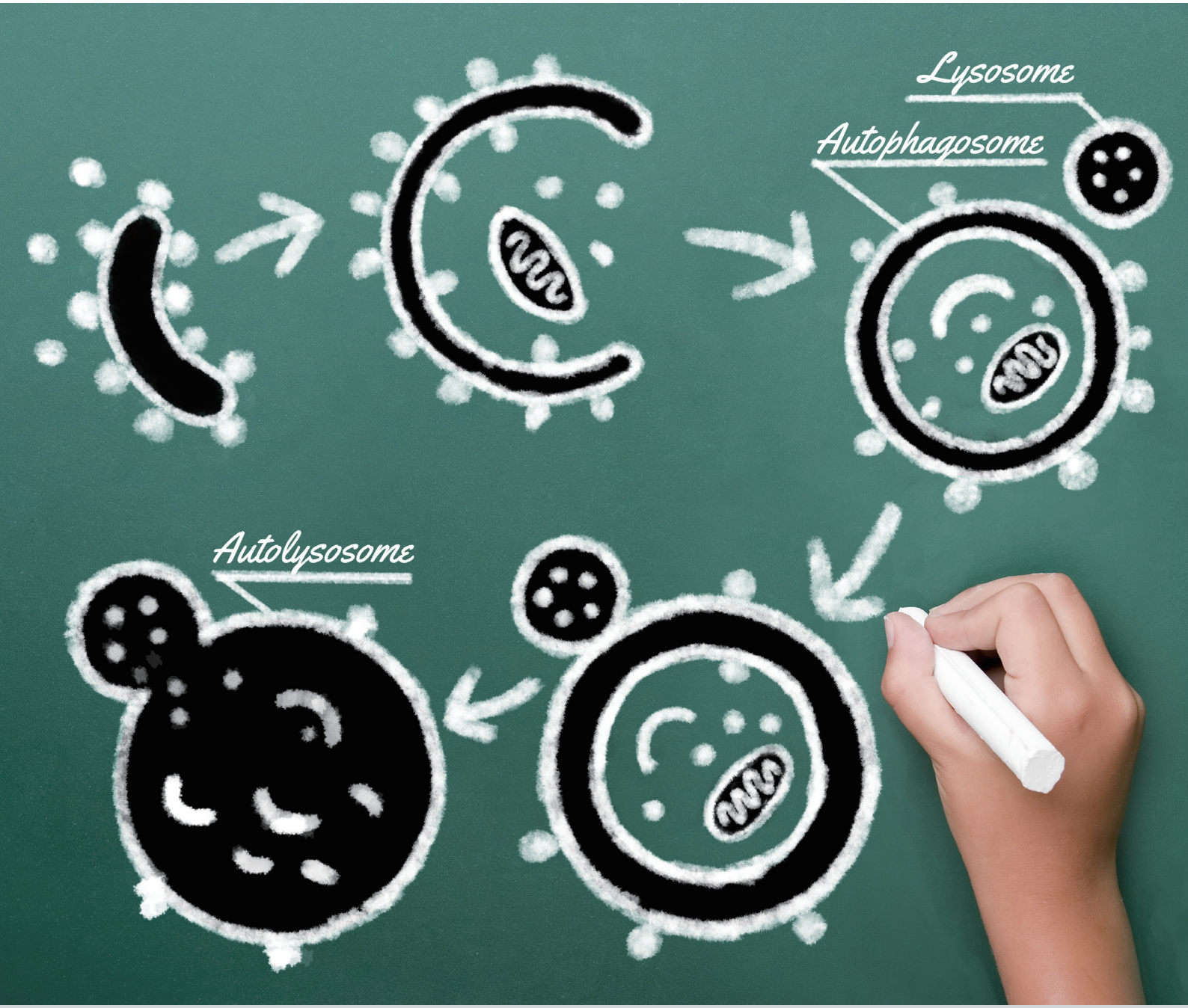
Research Reagents

MBL
A JSR Life Sciences Company

Autophagy-Related Products Catalog



MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.

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, Zeb: Zebrafish
(-): 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.

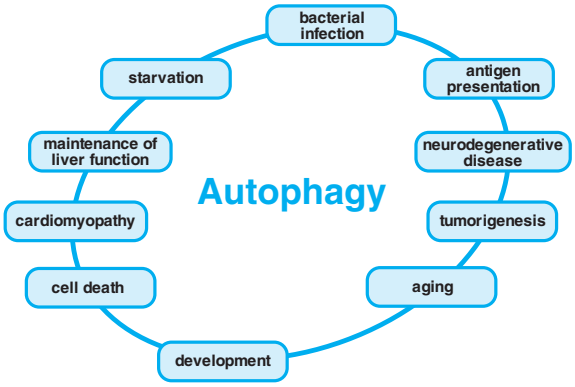
While proteasomes target and selectively degrade ubiquitinated proteins, autophagy degrades all the contents engulfed by autophagosomes, and, therefore, is called “the bulk degradation system.” In addition, selective autophagy pathways target cellular organelles, such as mitochondria and peroxisomes. 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.

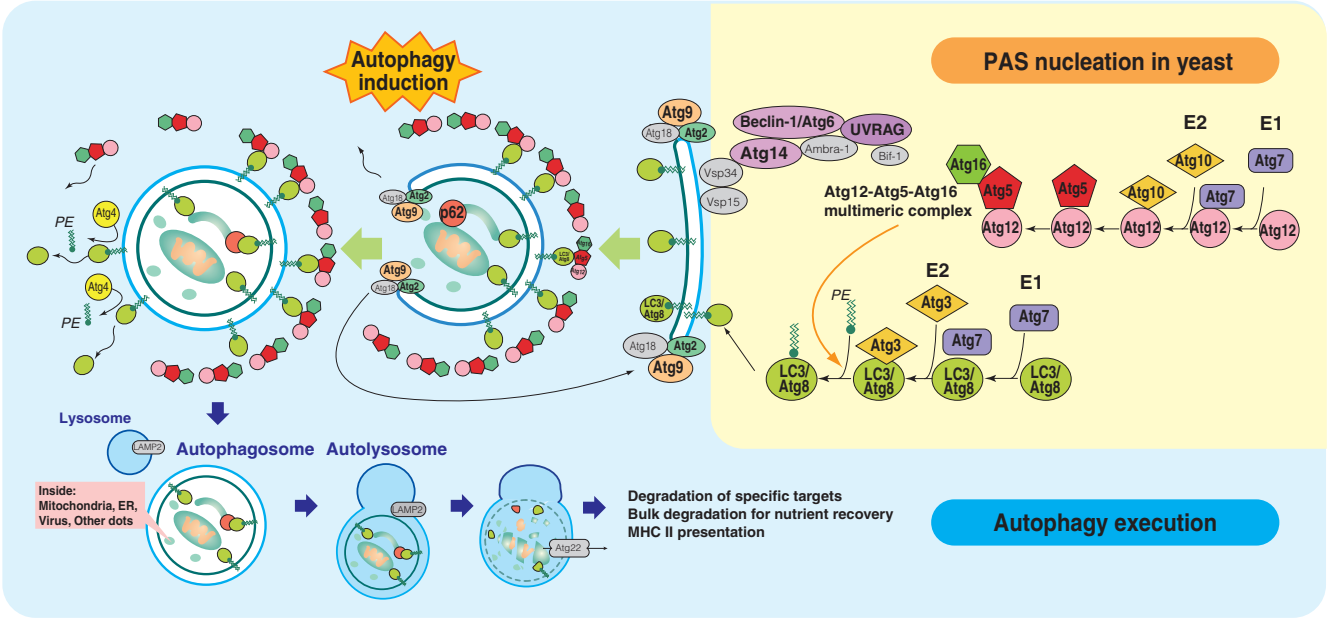


Article written by researcher

“Autophagy research: Current status and future perspectives”
Dr. Noboru Mizushima
https://www.mblbio.com/bio/g/product/autophagy/special-talk.html

“p62/Sqstm1: The molecule that links autophagy to the Keap1-Nrf2 system”
Dr. Masaaki Komatsu Dr. Yoshinobu Ichimura
https://ruo.mbl.co.jp/bio/g/product/autophagy/special-talk/p62.html

“Role of the autophagy regulator Rubicon in the pathogenesis of fatty liver disease”
Dr. Satoshi Tanaka Dr. Tamotsu Yoshimori
https://www.mblbio.com/bio/g/product/autophagy/special-talk/Rubicon.html



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

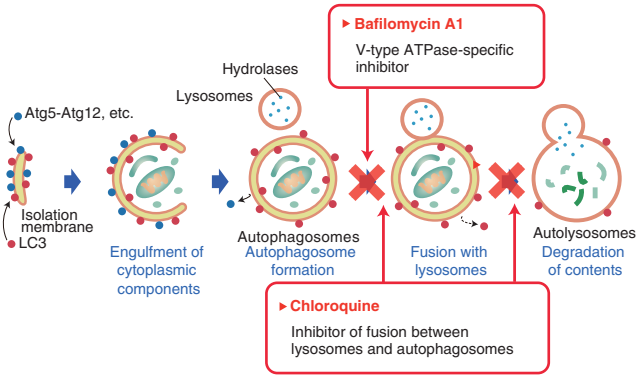
◎ **The antibody for WB is conjugated to HRP, and does not require a secondary antibody. Advantages include a shorter assay time and the absence of nonspecific signal from the secondary antibody.**

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

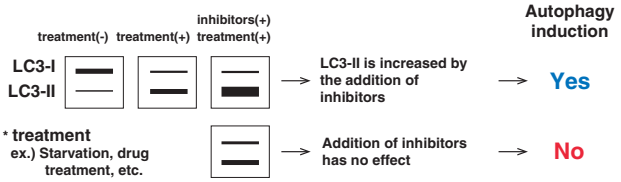
◎ **An antibody for cell staining is also included in this kit. Autophagosomes in the cell can be visualized and monitored by staining with a fluorescence-labeled secondary antibody.**

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.

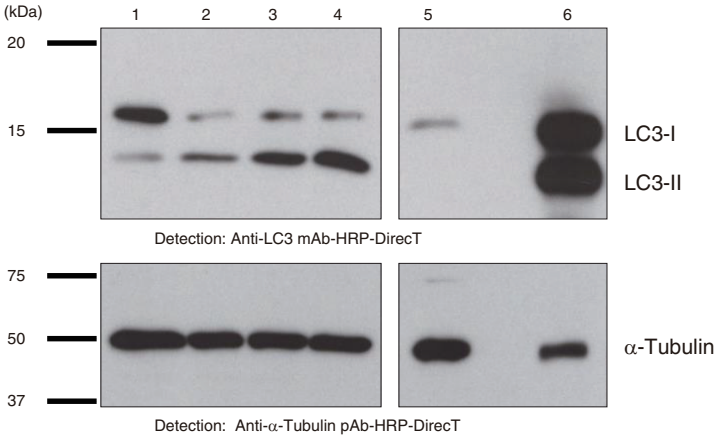


How to interpret the LC3 bands on WB



(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



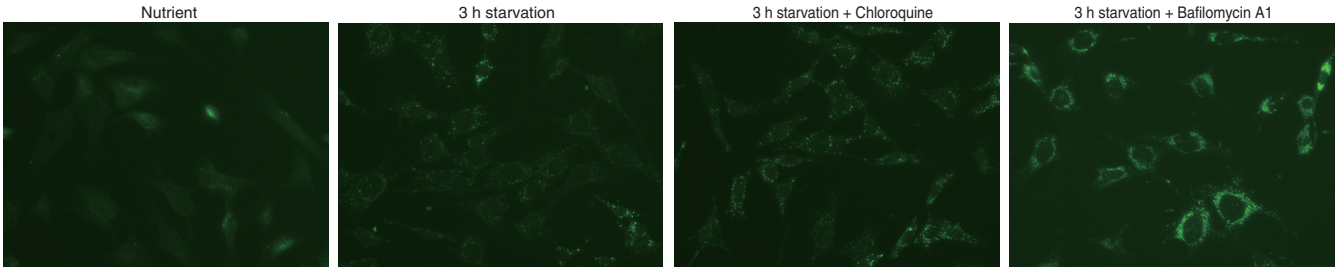
Cell: MEF

Lane 1: 10% FCS/RPMI-1640	Lane 4: HBSS + Bafilomycin A1
Lane 2: HBSS	Lane 5: *MEF Atg5$^{-/-}$
Lane 3: HBSS + Chloroquine	Lane 6: Positive control (PM036-PNY)

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	100 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	100 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?

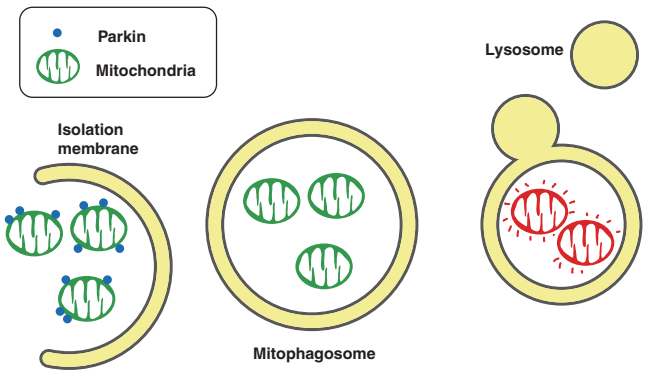
➡ Anti-LC3 antibody, clone 8E10 is conjugated to HRP and suitable for WB. For other applications, such as IC and IP, use clone 4E12.

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

➡ Please refer to the FAQ on page 21 – 22.

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.

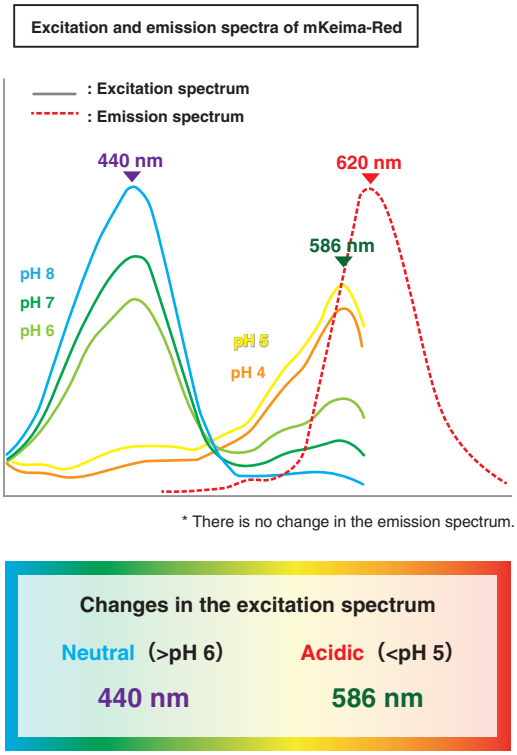
Features

◎ 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

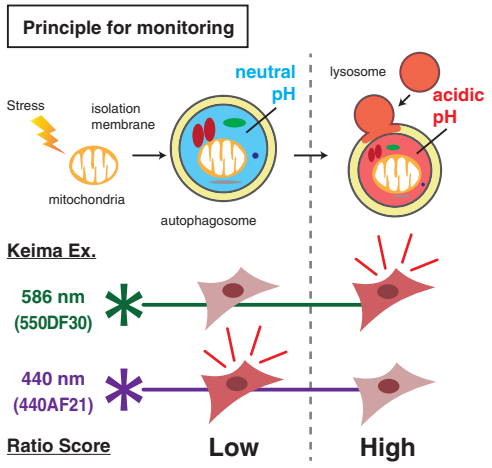
mKeima-Red is a fluorescent protein with an emission maximum at 620 nm. The excitation spectrum changes depending on the pH of the environment.

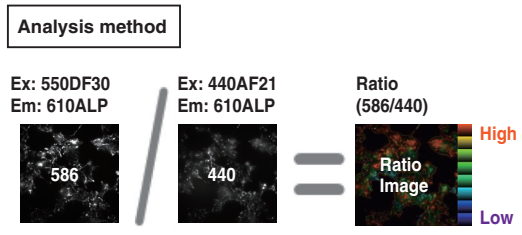


* There is no change in the emission spectrum.

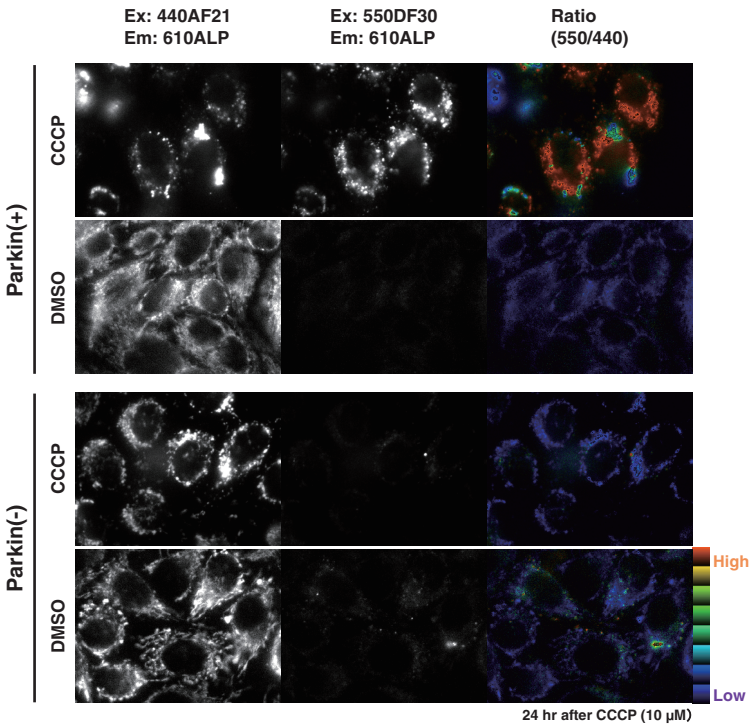
◎ The principle for monitoring and the analysis method

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.



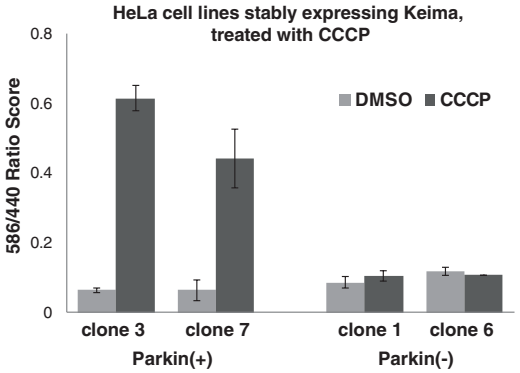


◎ Ratio imaging



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)

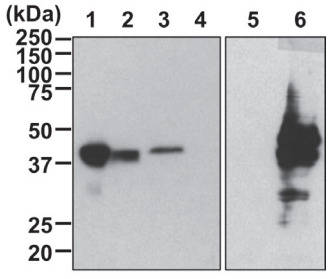
Product list

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



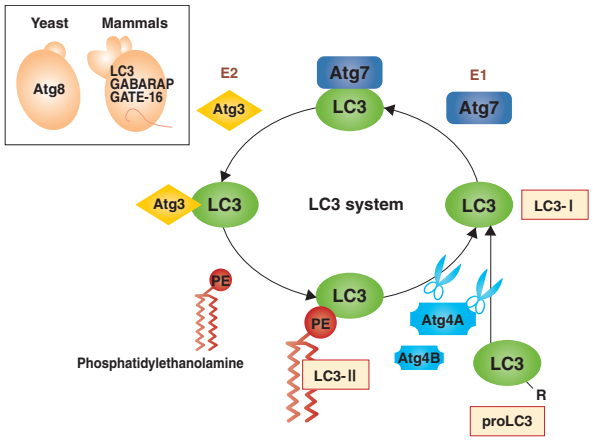
Lane 1: Rat brain lysate, 20 μg
Lane 2: Mouse brain lysate, 20 μg
Lane 3: PC12
Lane 4: HeLa
Lane 5: HEK293T
Lane 6: Human Parkin/ HEK293T

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

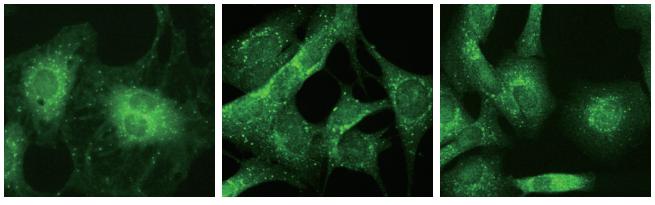
Code No.	Clone	Isotype	Size
PM036MS	Polyclonal	Rab IgG	20 μL
PM036	Polyclonal	Rab IgG	100 μL

◎ 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, Zeb*
[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.

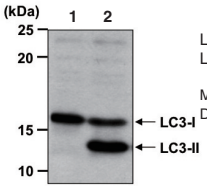
<References>
1) Saitoh, T., *et al*., Nature 456, 264-268 (2008) [WB]
2) Jing, L., *et al*., J. Biol. Chem. 291, 13175-13193 (2016) [WB, IC]

■ **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

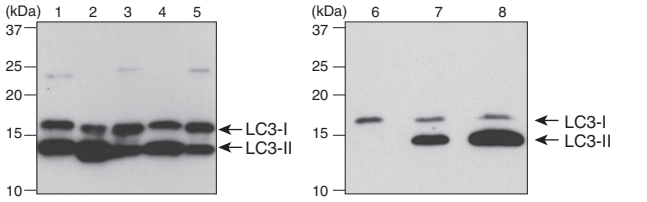
Code No.	Clone	Isotype	Size
M186-3MS	8E10	Mo IgG2aκ	20 μg/20 μL
M186-3	8E10	Mo IgG2aκ	100 μg/100 μL

◎ 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
IP*: reported in articles
[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]
3) Meng, XH., *et al*., Int J Biol Sci. 13(8), 985-995 (2017) [IP]

■ **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

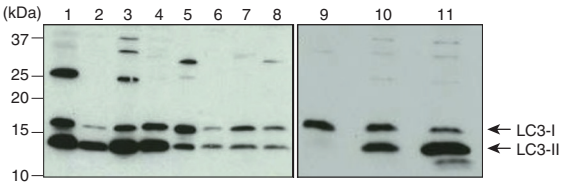
HRP-conjugated

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**



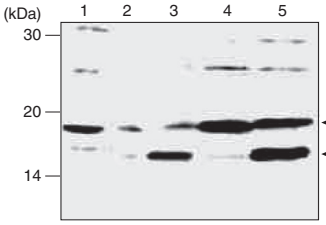
Lane 1: HeLa
Lane 2: NIH/3T3
Lane 3: PC12
Lane 4: CHO
Lane 5: Mouse brain
Lane 6: Mouse liver
Lane 7: Mouse spleen
Lane 8: Mouse kidney
Lane 9: MEF$^{Atg5-/-}$
Lane 10: MEF
Lane 11: MEF (6 hr. treatment with 50 μM Chloroquine)
MEF$^{Atg5-/-}$cells were kindly provided by Dr. Noboru Mizushima (The University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

Anti-LC3 pAb

Code No.	Clone	Isotype	Size
PD014MS	Polyclonal	Rab IgG	20 μL
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

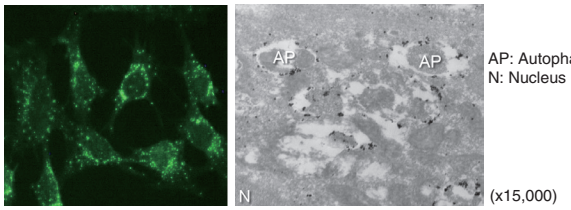
Anti-LC3 mAb

Code No.	Clone	Isotype	Size
M152-3MS	4E12	Mo IgG1κ	40 μg/20 μL
M152-3	4E12	Mo IgG1κ	200 μg/100 μL

◎ The best choice for cell staining.

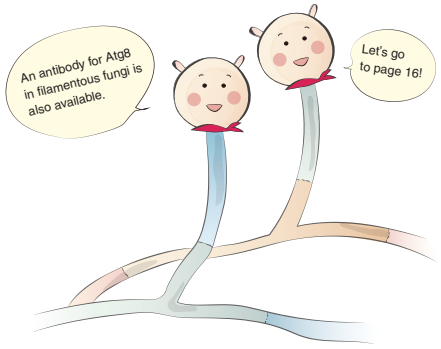
[Immunogen] Recombinant human LC3 (MAP1LC3B: 1-120 a.a.)
[Species cross-reactivity] Hu, Mo, Rat, Hm
[Form] 2 mg/mL in PBS/50% glycerol, pH 7.2
[Application] IP: 5 μg/300 μL of cell extract from 1x10^7 cells
IC: 40 μg/mL
IH*: reported in articles
FCM: 40 μg/mL
Immuno-EM: 20 μg/mL
Image-based FCM*: reported 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**



AP: Autophagosome
N: Nucleus
(x15,000)

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



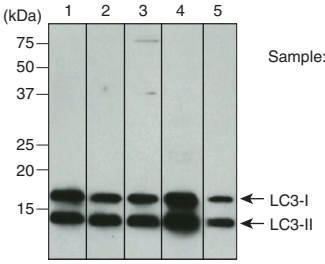
Positive control for anti-LC3 antibody

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

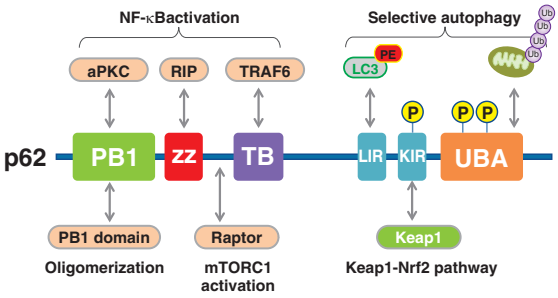
See page 21 - 22 for FAQ about anti-LC3 antibodies.

p62 antibodies

A link between the ubiquitin-proteasome system and autophagy

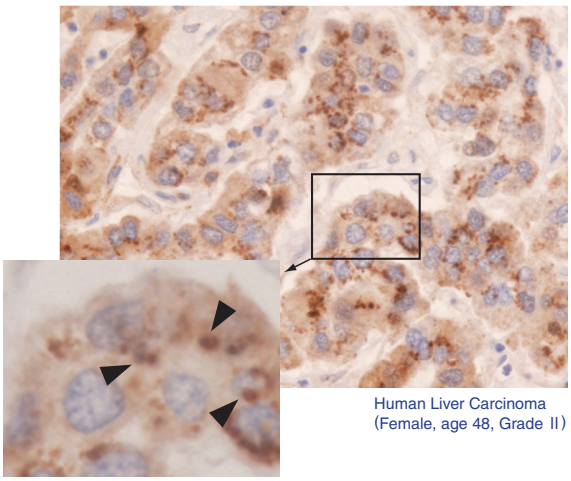
p62/SQSTM1 is a scaffolding protein that interacts with various signaling molecules such as TRAF6, RIP, and aPKC (figure below, left). p62 contains an LC3-interacting region and is believed to be a substrate for selective autophagy. In addition, p62 contains a domain that binds ubiquitin chains, and 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	Immuno-EM	
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	★★★★	★★★	★★★	★★★★			

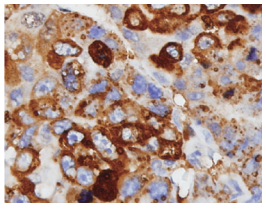
Anti-p62 (SQSTM1) (Human) mAb

Code No.	Clone	Isotype	Size
M162-3MS	5F2	Mo IgG1κ	20 μg/20 μL
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

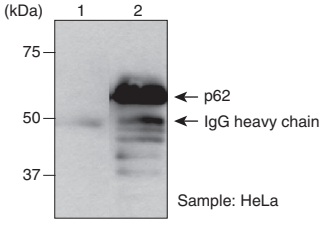
<References>
1) Janda, E., *et al.*, Autophagy 11, 1063-80 (2015) [IC]
2) Matsumoto, G., *et al.*, Mol. Cell. 44, 279-89 (2011) [WB]

■ **Immunohistochemistry**



Human liver carcinoma

■ **Immunoprecipitation**



Lane 1: Isotype control (Code No. M075-3)
Lane 2: Anti-p62 mAb (Code No. M162-3)
Immunoblotted with Anti-p62 pAb (Code No. PM045)

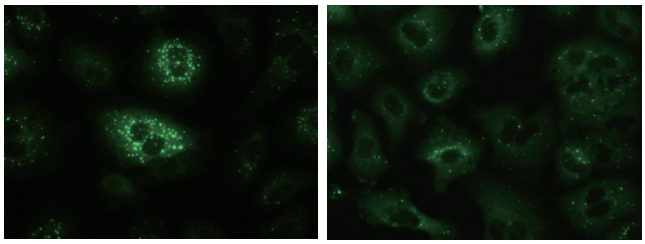
Anti-p62 (SQSTM1) (Human) mAb-Alexa Fluor™ 488

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)

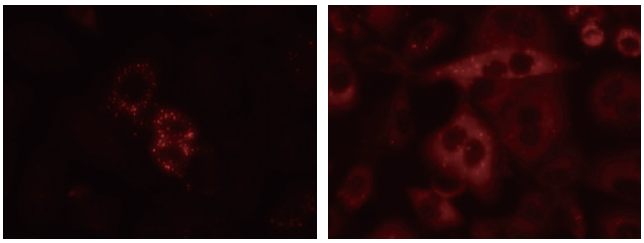
Anti-p62 (SQSTM1) (Human) mAb-Alexa Fluor™ 594

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)

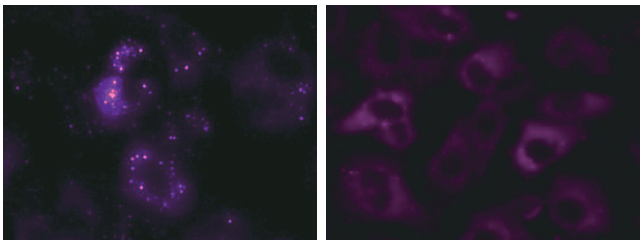
Anti-p62 (SQSTM1) (Human) mAb-Alexa Fluor™ 647

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/0.1% ProClin 150
[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)

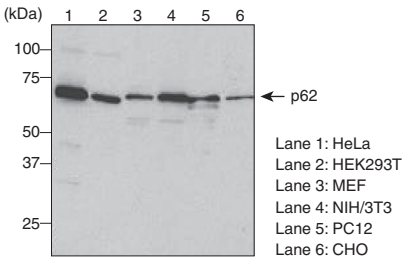
Anti-p62 (SQSTM1) pAb

Code No.	Clone	Isotype	Size
PM045MS	Polyclonal	Rab Ig (aff.)	20 μL
PM045	Polyclonal	Rab Ig (aff.)	100 μL

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

[Immunogen] Recombinant human p62 (120–440 a.a.)
[Species cross-reactivity] Hu, Mo, Rat, Hm, Zeb*
[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**

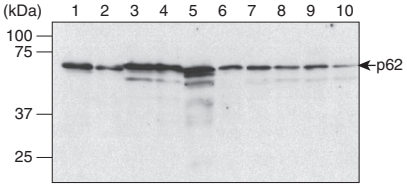


Anti-p62 C-terminal pAb

Code No.	Clone	Isotype	Size
PM066MS	Polyclonal	Guinea Pig Ig (aff.)	20 μL
PM066	Polyclonal	Guinea Pig Ig (aff.)	100 μL

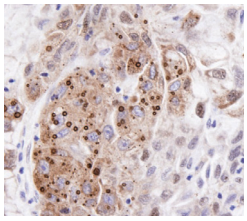
[Immunogen] Human p62 C-terminal region (synthetic peptide)
[Species cross-reactivity] Hu, Mo, Rat, Hm
[Form] PBS/50% glycerol, pH 7.2
[Application] WB: 1:1,000
IP: 5 μL/300 μL of cell extract from $3x10^6$ cells
IC: 1:500
IH: 1:100
<References>
1) Komatsu, M., *et al.*, Cell 131, 1149-1163 (2007)
2)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

■ **Western blotting**



Lane 1: HeLa
Lane 2: HEK293T
Lane 3: MEF
Lane 4: NIH/3T3
Lane 5: PC12
Lane 6: CHO
Lane 7: Mouse brain
Lane 8: Mouse liver
Lane 9: Mouse spleen
Lane 10: Mouse kidney

■ **Immunohistochemistry**



Human liver carcinoma

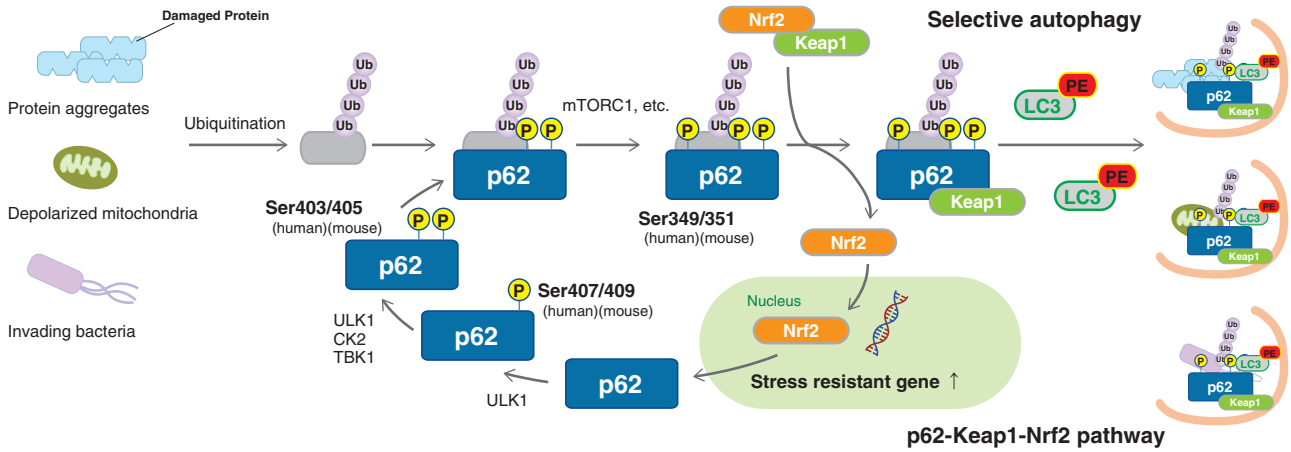
Phospho-p62 antibodies

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.

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).



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

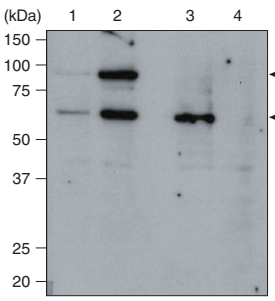
Anti-Phospho-p62 (SQSTM1) (Ser403) mAb

Code No.	Clone	Isotype	Size
D343-3MS	4F6	Rat IgG2aκ	20 μg/20 μL
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

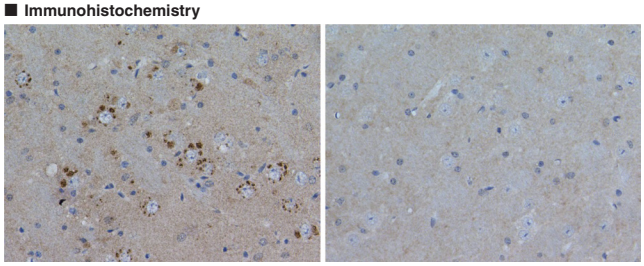
<References>
1) Kurosawa, M., *et al.*, Hum. Mol. Genet., 24, 1092-1105 (2015) [IH]
2) Matsumoto, G., *et al.*, Mol. Cell 44, 279-289 (2011) [WB, IH]

■ **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 (Code No. D343-3)
Blue: Hematoxylin

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

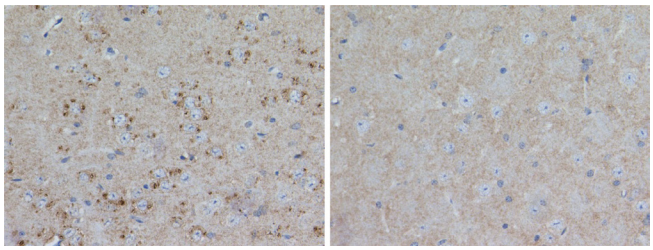
Anti-Phospho-p62 (SQSTM1) (Ser403) mAb

Code No.	Clone	Isotype	Size
D344-3MS	4C8	Rat IgG2aκ	20 μg/20 μL
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 (Code No. D344-3)
Blue: Hematoxylin

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

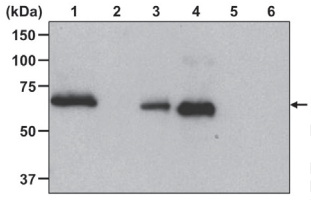
Anti-Phospho-p62 (SQSTM1) (Ser351) mAb

Code No.	Clone	Isotype	Size
M217-3MS	5D5	Mo IgG1κ	20 μg/20 μL
M217-3	5D5	Mo IgG1κ	100 μg/100 μL

[Immunogen] Mouse p62 (346–359 a.a.) (synthetic peptide)
[Species cross-reactivity] Hu, Mo, Rat*
[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)

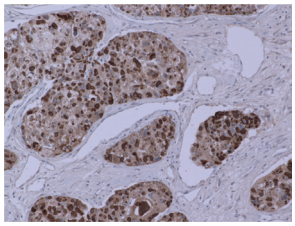
■ **Western blotting**



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 (Code No. M217-3)
Blue: Hematoxylin

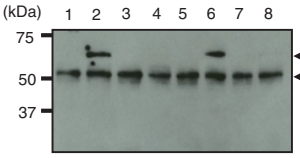
Anti-Phospho-p62 (SQSTM1) (Ser351) pAb

Code No.	Clone	Isotype	Size
PM074MS	Polyclonal	Rab Ig (aff.)	20 μL
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**

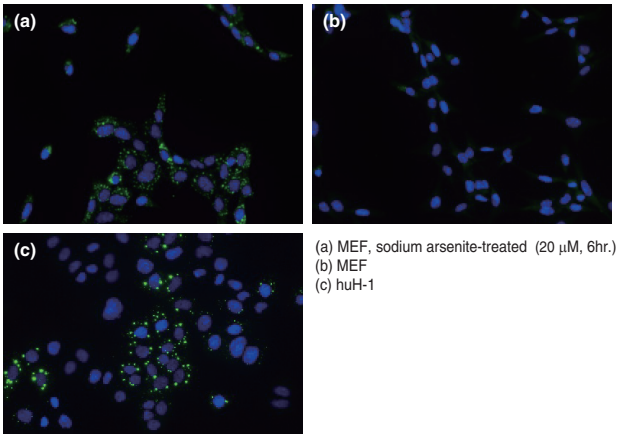


Immunoblotted with Anti-Phospho-p62 (Ser351) pAb (Code No. PM074)

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 (Code No. PM035)
Lane 2, 4, 6, 8: Anti-Phospho-p62 (SQSTM1) (Ser351) pAb (Code No. 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).

p62 ELISA Kit

Features

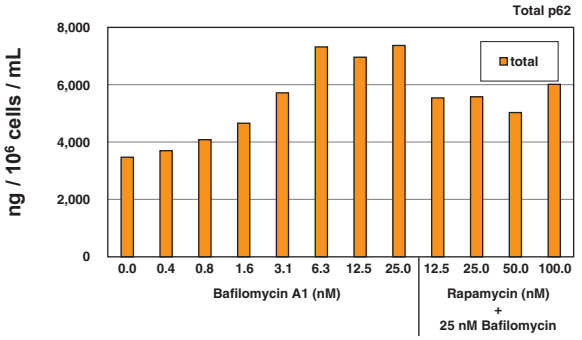
◎ **Comes with lysis buffer. Easy to prepare cell lysate!**

◎ **Useful for drug screening!**

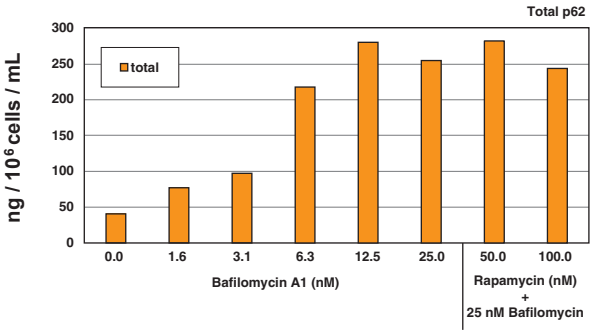
◎ **Human and mouse cell lysate can be used.**

■ **Example data of HeLa and MEF cells.**

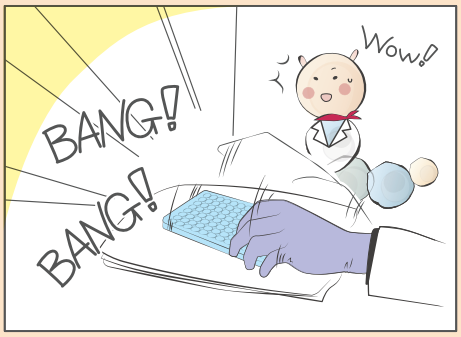
HeLa cells



MEF cells



Code No.	Product name	Size
CY-7055	CycLex® Total p62 ELISA Kit	96 Assay



The principle and method of ELISA

https://ruo.mbl.co.jp/bio/e/support/method/elisa.html

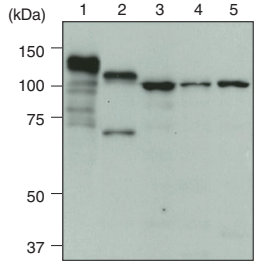
Antibodies for phospho-p62-related proteins

Anti-NRF2 mAb

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

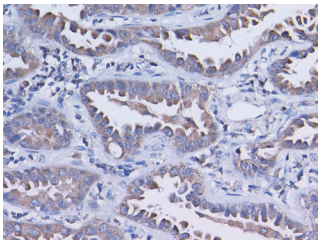
[Immunogen] Recombinant human NRF2 (1–605 a.a.)
[Species cross-reactivity] Hu, Mo, Rat, Hm
[Form] 1 mg/mL in PBS/50% glycerol, pH 7.2
[Application] WB: 1 μg/mL
IP: 5 μg/300 μL of cell extract from 3x10^6 cells
IC: 0.5 μg/mL
IH: 1 μg/mL (for paraffin embedded sections)
<References>
1) Nguyen, T., *et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

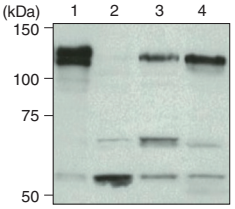
Brown: Anti-NRF2 mAb (Code No. M200-3)
Blue: Hematoxylin

Anti-NRF2 pAb

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

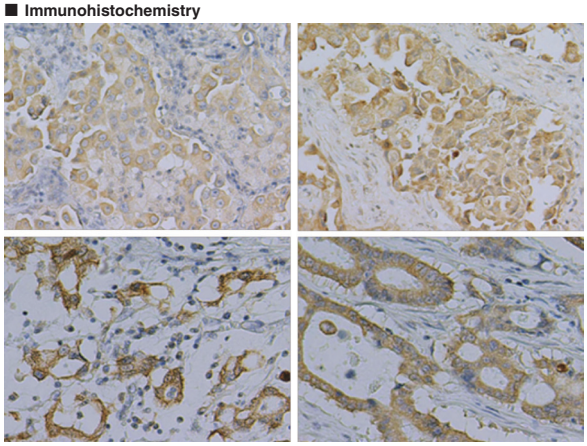
[Immunogen] Recombinant human NRF2 (1–605 a.a.)
[Species cross-reactivity] Hu, Mo(w), Rat(w), Hm(w)
[Form] PBS/50% glycerol, pH 7.2
[Application] WB: 1:1,000
IP: 5 μL/300 μL of cell extract from 3x10^6 cells
IC: 1:1,000
IH: 1:1,000
<References>
1) Taguchi, K., *et al.*, Genes Cell 16, 123-140 (2011)
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)

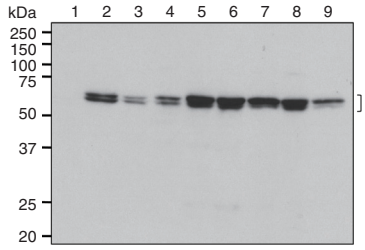
Anti-KEAP1 mAb

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**



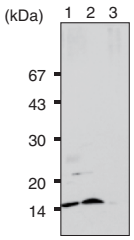
Lane 1: HeLa
Lane 2: HEK293T
Lane 3: A549
Lane 4: HepG2
Lane 5: MEF
Lane 6: NIH/3T3
Lane 7: Rat1
Lane 8: NRK
Lane 9: CHO

Anti-Ubiquitin mAb

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

[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] Clone IB3 and 2C5(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*

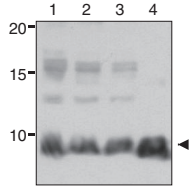
*PPUb4: partially purified multi ubiquitin chains in ubiquitin-protein conjugates

Anti-Ubiquitin mAb

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

[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] Clone 2C5 and 1B3(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

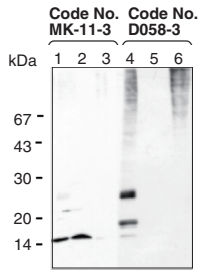
Anti-Multi Ubiquitin mAb

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*, Yeast*, Fruit fly*
[Form] 1 mg/mL in PBS/50% glycerol, pH7.2
[Application] WB: 1-5 μg/mL
IC*: reported in articles
IH*: 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
D058-11	Magnetic Beads	IP	20 tests (Slurry: 1 mL)

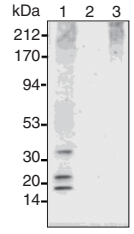
Anti-Multi Ubiquitin mAb

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

◎ This antibody is specific for multi ubiquitin.

[Immunogen] Partially purified poly-ubiquitin-lysozyme
[Species cross-reactivity] Hu, Mo*
[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

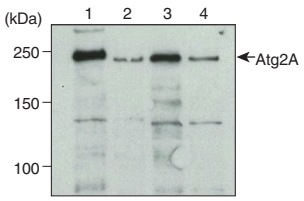
Atg antibody series

Anti-Atg2A pAb

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

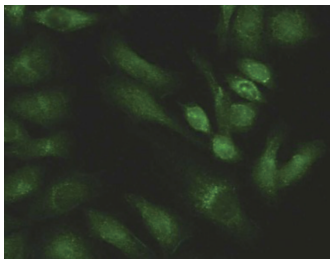
[Immunogen] Recombinant human Atg2A (700–1,400 a.a.)
[Species cross-reactivity] Hu, Mo, Rat, Hm
[Form] PBS/50% glycerol, pH 7.2
[Application] WB: 1:1,000
IP: 5 μL/300 μL of cell extract from $3x10^6$ cells
IC: 1:400
<References>
1) Velikkakath, A. K., *et al.*, Mol. Biol. Cell 23, 896-909 (2012)

■ **Western blotting**



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

■ **Immunocytochemistry**



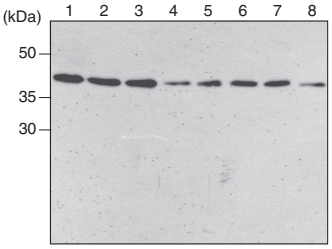
HeLa (starved condition)

Anti-Atg3 mAb

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

[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**



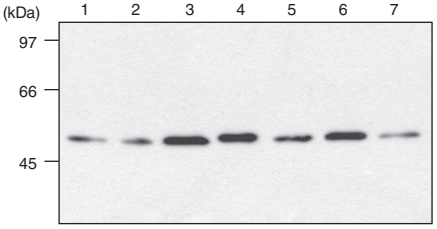
Lane 1: HEK293T
Lane 2: HeLa
Lane 3: Jurkat
Lane 4: NIH/3T3
Lane 5: WR19L
Lane 6: Rat1
Lane 7: PC12
Lane 8: CHO

Anti-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**



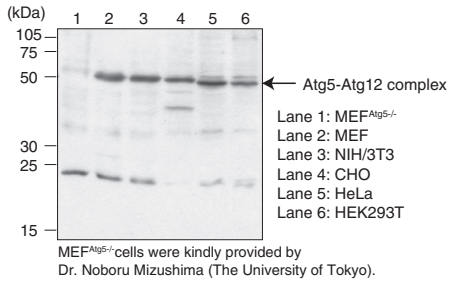
Lane 1: HEK293T
Lane 2: HeLa
Lane 3: Raji
Lane 4: NIH/3T3
Lane 5: Rat1
Lane 6: PC12
Lane 7: CHO

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**



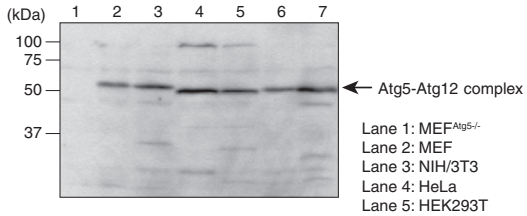
MEF$^{Atg5-/-}$cells were kindly provided by
Dr. Noboru Mizushima (The University of Tokyo).

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**



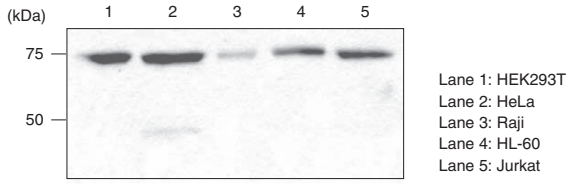
MEF$^{Atg5-/-}$cells were kindly provided by
Dr. Noboru Mizushima (The University of Tokyo).

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**

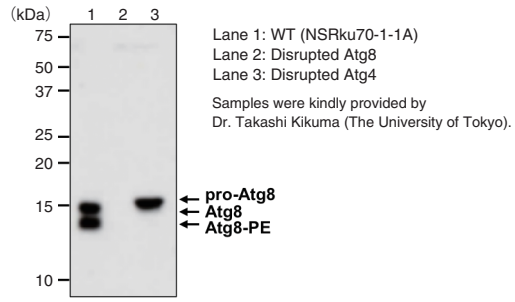


Anti-Atg8 (Filamentous fungi) pAb

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] Filamentous fungi
[Form] PBS/50% glycerol, pH7.2
[Application] WB: 1:1,000

■ **Western blotting**

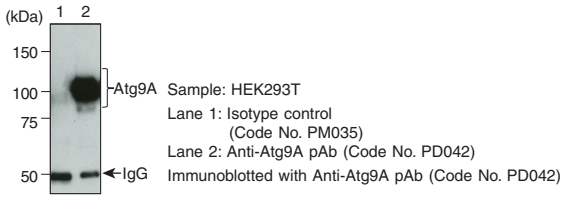


Anti-Atg9A pAb

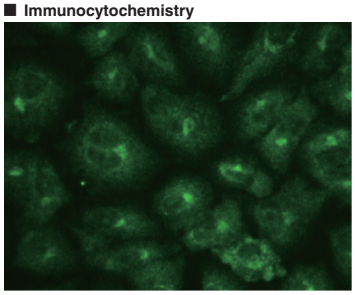
Code No.	Clone	Isotype	Size
PD042	Polyclonal	Rab Ig (aff.)	100 μL

[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
IH*: reported in articles
<References>
1) Itakura, E., *et al.*, J. Cell Sci. 125, 1488-1499 (2012)

■ **Immunoprecipitation**



■ **Immunocytochemistry**



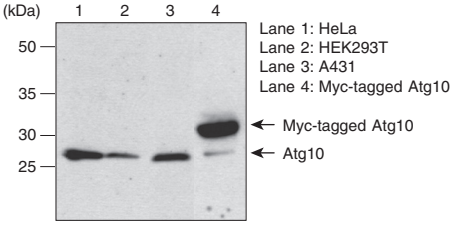
A549
Green: Anti-Atg9A pAb
(Code No. PD042)

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**

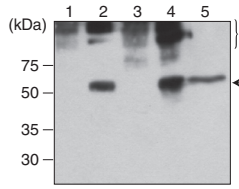


Anti-Atg12 (Human) mAb

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**



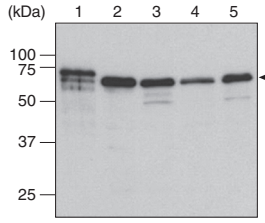
*2-Mercaptoethanol was not included in the sample buffer.
Lane 1: HeLa + mouse IgG1
Lane 2: HeLa + Anti-Atg12 (Code No. M154-3)
Lane 3: HEK293T + mouse IgG1
Lane 4: HEK293T + Anti-Atg12 (Code No. M154-3)
Lane 5: HeLa (WB positive control)
Immunoblotted with Anti-Atg12 mAb (Code No. M154-3)

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**



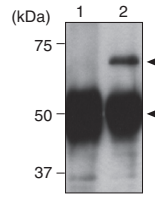
Lane 1: Atg14 transfectant (HEK293T)
Lane 2: HeLa
Lane 3: HEK293T
Lane 4: A549
Lane 5: Jurkat

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**



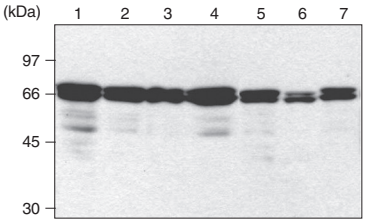
Sample: HeLa
Lane 1: Isotype control (Code No. PM035)
Lane 2: Anti-Atg14 pAb (Code No. PD026)
Immunoblotted with Anti-Atg14 pAb (Code No. PD026)

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
<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**



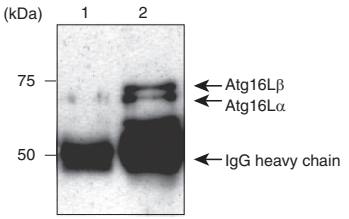
Lane 1: HeLa
Lane 2: HEK293T
Lane 3: Jurkat
Lane 4: Raji
Lane 5: NIH/3T3
Lane 6: WR19L
Lane 7: Rat1

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**



Sample: HeLa
Lane 1: Isotype control
Lane 2: Anti-Atg16L pAb (Code No. PM040)
Immunoblotted with Anti-Atg16L pAb (Code No. PM040)

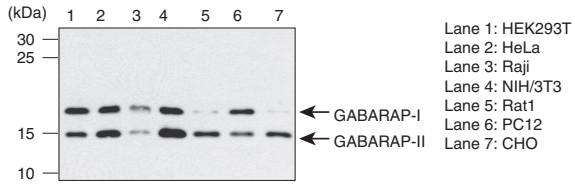
Antibodies for autophagy-related proteins

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, Chi*
[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**

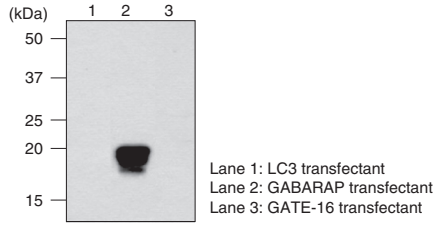


Anti-GABARAP pAb

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**

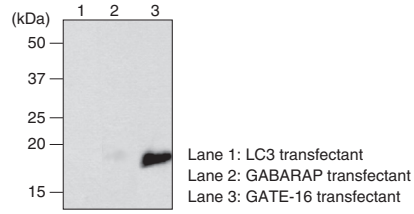


Anti-GATE-16 pAb

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**

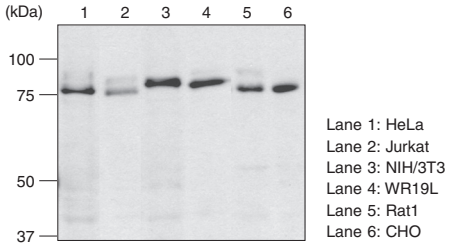


Anti-UVRAG mAb

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**

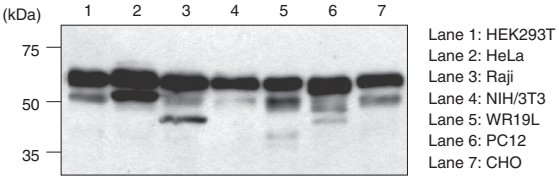


Anti-Beclin 1 pAb

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**

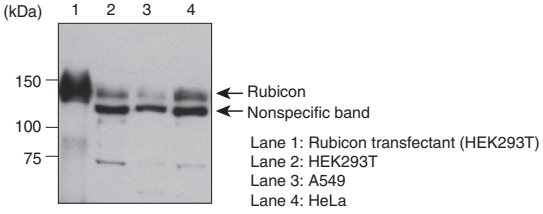


Anti-Rubicon (Human) pAb

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**

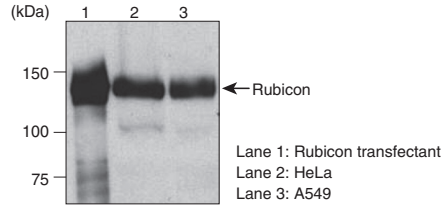


Anti-Rubicon (Human) mAb

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**



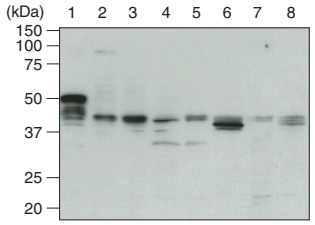
Lane 1: Rubicon transfectant
Lane 2: HeLa
Lane 3: A549

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**



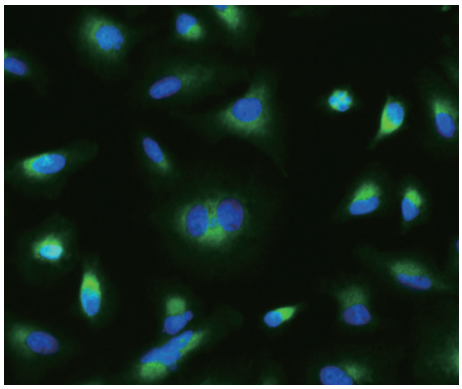
Lane 1: VMP1 transfectant (HEK293T)
Lane 2: HEK293T
Lane 3: A431
Lane 4: NIH/3T3
Lane 5: MEF
Lane 6: NRK
Lane 7: PC12
Lane 8: CHO

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 μL/sample
IC: 1:2,000
<References>
1) Itakura, E., *et al*., Cell 151, 1256–1269 (2012)

■ **Immunocytochemistry**



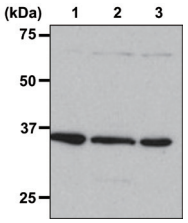
A549
Green: Anti-Syntaxin-17 (Human) pAb (Code No. PM076)
Blue: DAPI

Anti-Syntaxin-17 (Human) mAb

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

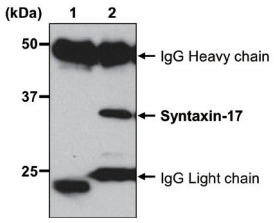
[Immunogen] Recombinant human Syntaxin-17 (1-302 a.a.)
[Species cross-reactivity] Hu, Mo(-), Rat(-), Hm(-)
[Form] PBS/50% glycerol, pH7.2
[Application] WB: 1 μg/mL
IP: 2 μg/sample
IC: reported in articles
<References>
1) Hamasaki, M., *et al*., Nature 495, 389-93 (2013)
2) Itakura, E., *et al*., Cell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

■ **Western blotting**



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

■ **Immunoprecipitation**



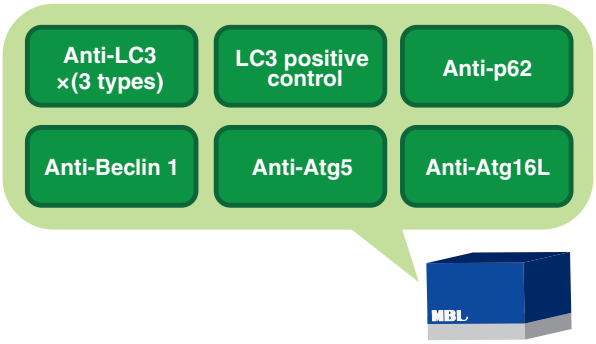
Sample : HeLa
Lane 1: Mouse IgG2a (Code No. M076-3)
Lane 2: Anti-Syntaxin-17 (Human) mAb (Code No. M212-3)
Immunoblotted with Anti-Syntaxin-17 mAb (Code No. M212-3)

Antibody sampler set

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	

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 (WB) ?

➡ 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 (IH)?

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

Q8 Can I stain frozen sections?

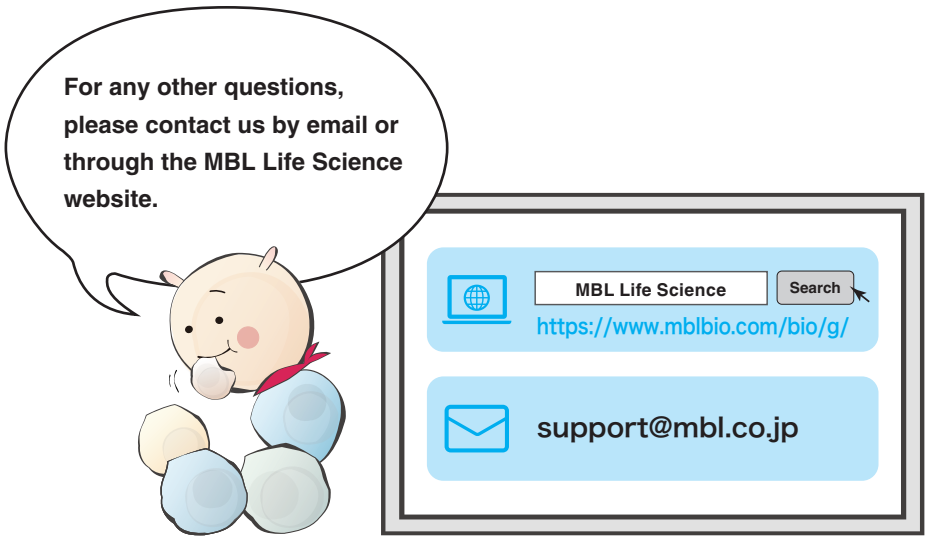
➡ Use of cryosections has not been evaluated by MBL.

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
IH: Code No. PM036



Product list

Kit

Page	Code No.	Product name	Size
P.4	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.15	PD041	Anti-Atg2A pAb	Polyclonal	Rabbit Ig (aff.)	WB, IP, IC	100 µL	Hu, Mo, Rat, Hm
P.15	M133-3	Anti-Atg3 mAb	3E8	Mouse IgG2bκ	WB, IP, IC	100 µg/100 µL	Hu, Mo, Rat, Hm
P.15	M134-3	Anti-Atg4B mAb	9H5	Mouse IgG1	WB	100 µg/100 µL	Hu, Mo, Rat, Hm
P.16	M153-3	Anti-Atg5 mAb	4D3	Mouse IgG1κ	WB	100 µg/100 µL	Hu, Mo, Rat, Hm
P.16	PM050	Anti-Atg5 pAb	Polyclonal	Rabbit Ig (aff.)	WB	100 µL	Hu, Mo, Rat, Hm
P.16	PM039	Anti-Atg7 (Human) pAb	Polyclonal	Rabbit Ig (aff.)	WB, IP	100 µL	Hu, Mo, Rat, Hm
P.16	PM090	Anti-Atg8 (Filamentous fungi) pAb	Polyclonal	Rabbit Ig (aff.)	WB	100 µL	Filamentous fungi
P.16	PD042	Anti-Atg9A pAb	Polyclonal	Rabbit Ig (aff.)	WB, IP, IC, IH*	100 µL	Hu, Mo, Rat, Hm
P.16	M151-3	Anti-Atg10 (Human) mAb	5A7	Mouse IgG1κ	WB, IH*	100 µg/100 µL	Hu
P.17	M154-3	Anti-Atg12 (Human) mAb	6E5	Mouse IgG1κ	WB, IP, IC	100 µg/100 µL	Hu, Mo, Rat, Hm
P.17	M184-3	Anti-Atg14 (Human) mAb	4H8	Mouse IgG2aκ	WB, IP	100 µg/100 µL	Hu, Mo, Rat
P.17	PD026	Anti-Atg14 pAb	Polyclonal	Rabbit Ig (aff.)	WB, IP, IC*	100 µL	Hu, Mo, Rat, Hm
P.17	M150-3	Anti-Atg16L mAb	1F12	Mouse IgG1κ	WB, FCM*, IH*	100 µg/100 µL	Hu, Mo, Rat
P.17	PM040	Anti-Atg16L pAb	Polyclonal	Rabbit Ig (aff.)	WB, IP, IC, Other*	100 µL	Hu, Mo, Rat, Hm
P.18	PD017	Anti-Beclin 1 pAb	Polyclonal	Rabbit Ig (aff.)	WB, IP, IC, IH*	100 µL	Hu, Mo, Rat, Hm
P.18	M135-3	Anti-GABARAP mAb	1F4	Mouse IgG1	WB, IC*, IH*	100 µg/100 µL	Hu, Mo, Rat, Hm, Chi*
P.18	PM037	Anti-GABARAP pAb	Polyclonal	Rabbit Ig (aff.)	WB, IC	100 µL	Hu, Mo, Rat, Hm
P.18	PM038	Anti-GATE-16 pAb	Polyclonal	Rabbit Ig (aff.)	WB, IH*	100 µL	Hu, Mo, Rat, Hm
P.14	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*, Other*, Other*	200 µg/100 µL	Hu, Mo, Rat, Hm
P.7	M186-3	Anti-LC3 mAb	8E10	Mouse IgG2aκ	WB, IP*	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.8	PM036	Anti-LC3 pAb	Polyclonal	Rabbit IgG	WB, IP, FCM, IC, IH, Other*	100 µL	Hu, Mo, Rat, Hm, Zeb*
P.8	PD014	Anti-LC3 pAb	Polyclonal	Rabbit IgG	WB, IC*, IH*	100 µL	Hu, Mo, Rat, Hm
P.15	D071-3	Anti-Multi Ubiquitin mAb	FK1	Mouse IgM	WB	100 µg/100µL	Hu, Mo*
P.15	D058-3	Anti-Multi Ubiquitin mAb	FK2	Mouse IgG1κ	WB, IC*, ELISA*, IH*	100 µg/100µL	Hu, Mo*, Mky*, Yeast*, Fruit fly*
P.15	D058-8	Anti-Multi Ubiquitin mAb-Agarose	FK2	Mouse IgG1κ	IP	Gel: 200 µL	Hu
P.15	D058-11	Anti-Multi Ubiquitin mAb-Magnetic Beads	FK2	Mouse IgG1κ	IP	20 tests (Slurry: 1 mL)	Hu
P.14	M200-3	Anti-NRF2 mAb	1F2	Mouse IgG1κ	WB, IP, IC, IH	100 µg/100 µL	Hu, Mo, Rat, Hm
P.14	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, Mo, Rat, Hm
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, Other*	100 µL	Hu, Mo, Rat, Hm, Zeb*
P.10	PM066	Anti-p62 C-terminal pAb	Polyclonal	Guinea pig Ig (aff.)	WB, IP, IC, IH	100 µL	Hu, Mo, Rat, Hm
P.6	M230-3	Anti-Parkin mAb	Par6	Mouse IgG2aκ	WB	100 µg/100 µL	Hu, Mo, Rat
P.12	M217-3	Anti-Phospho-p62 (SQSTM1) (Ser351) mAb	5D5	Mouse IgG1κ	WB, IC, IH	100 µg/100 µL	Hu, Mo, Rat*
P.12	PM074	Anti-Phospho-p62 (SQSTM1) (Ser351) pAb	Polyclonal	Rabbit Ig (aff.)	WB, IP, IC, IH	100 µL	Hu, Mo
P.12	D344-3	Anti-Phospho-p62 (SQSTM1) (Ser403) mAb	4C8	Rat IgG2aκ	WB, IH	100 µg/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.19	M170-3	Anti-Rubicon (Human) mAb	1H6	Mouse IgG2aκ	WB, IH	100 µg/100 µL	Hu, Mo
P.18	PD027	Anti-Rubicon (Human) pAb	Polyclonal	Rabbit Ig (aff.)	WB, IP, IH	100 µL	Hu, Mo
P.19	M212-3	Anti-Syntaxin-17 (Human) mAb	2F8	Mouse IgG2aκ	WB, IP, IC*	100 µg/100 µL	Hu, Mo, Rat, Hm
P.19	PM076	Anti-Syntaxin-17 (Human) pAb	Polyclonal	Rabbit Ig (aff.)	WB, IP, IC	100 µL	Hu, Mo, Rat
P.14	MK-12-3	Anti-Ubiquitin mAb	2C5	Mouse IgG1	WB, IP*, IC*	100 µg/100 µL	Hu, Mo, Rat, Bov
P.14	MK-11-3	Anti-Ubiquitin mAb	1B3	Mouse IgG1	WB, IC*, IH*, Other*	100 µg/100 µL	Hu, Mo*, Bov*
P.18	M160-3	Anti-UVRAG mAb	1H4	Mouse IgG1κ	WB, IC*	100 µg/100 µL	Hu, Mo, Rat, Hm
P.19	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)	

ELISA Kit

Page	Code No.	Product name	Size
P.13	CY-7055	CycLex® Total p62 ELISA Kit	96 Assay

Vector

Page	Code No.	Product name	Size
P.6	AM-V0259M	pMitophagy Keima-Red mPark2 (Kan)	20 µg
P.6	AM-V0259HM	pMitophagy Keima-Red mPark2 (Hyg)	20 µg
P.6	AM-V0251M	*CoralHue*™ Mitochondria-targeted mKeima-Red (pMT-mKeima-Red)	20 µg
P.6	AM-V0251HM	*CoralHue*™ Mitochondria-targeted monomeric Keima-Red (Hyg)	20 µg

For research use only. Not for use in diagnostic or therapeutic procedures.
The information is as of April 2024. Please contact us for the latest information. Please read the data sheets carefully before use.

2024.04 156002-24041000

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SUMITOMO FUDOSAN SHIBADAIMON NICHOME BLDG.
2-11-8 Shibadaimon, Minato-ku, Tokyo 105-0012 Japan
E-mail: support@mbl.co.jp
URL: https://www.mblbio.com/bio/g/