

# MBL RUO LETTER



**MBL**

**vol.2**

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- ① **Highlight Products: p62 and phospho-p62 ELISA Kits**
- ② **New & Renewal Website**
  - **Epitope Tag Antibodies**
  - **Autophagy**
  - **Principle and method of the experiment**



Japan is getting cold and my favorite red scarf is keeping me warm☺  
Have you ever visited Japan? If you like winter sports, this is the place you should come!

# ① Highlight Product: p62 and phospho-p62 ELISA Kits

**3 ELISA kits were launched on May, 2017 !**

**CycLex<sup>®</sup> Total p62 ELISA Kit (code# CY-7055)**

<http://ruo.mbl.co.jp/bio/g/dtl/P/?pcd=CY-7055>

**CycLex<sup>®</sup> Phospho-p62 Ser349 ELISA Kit (code# CY-7056)**

<http://ruo.mbl.co.jp/bio/g/dtl/P/?pcd=CY-7056>

**CycLex<sup>®</sup> Phospho-p62 Ser403 ELISA Kit (code# CY-7057)**

<http://ruo.mbl.co.jp/bio/g/dtl/P/?pcd=CY-7057>



## ① Highlight Product:

# p62 and phospho-p62 ELISA Kits



## Features and benefits

- **The market's first ELISA kit for phosphorylated p62!**
- Comes with lysis buffer. Easy to prepare cell lysate!
- Useful for drug screening!
- Human and mouse cell lysate can be used.
- Same sample can be used for 3 kits.

## Who will/might use them?

Researchers who...

- research on neurodegenerative disease and cancer
- do drug discovery related in autophagy in pharmaceutical companies

# ① Highlight Product: p62 and phospho-p62 ELISA Kits

## Special notes

- Announced the new website for Autophagy field!

<http://ruo.mbl.co.jp/bio/g/product/autophagy/index.html>

- Posted an article about p62 written by Dr. Masaaki Komatsu and Dr. Yoshinobu Ichimura who are key opinion leaders on Autophagy in world wide

<http://ruo.mbl.co.jp/bio/g/product/autophagy/special-talk/p62.html>

- These ELISA kits were evaluated by Dr. Masaaki Komatsu and Dr. Yoshinobu Ichimura before these kits were launched.

Visit to our web site!



# ① Highlight Product: p62 and phospho-p62 ELISA Kits

## Products information

### <Kits>

Code No.	Product	Size
<b>NEW!</b> CY-7055	CycLex® Total p62 ELISA Kit	96 Assay
<b>NEW!</b> CY-7056	CycLex® Phospho-p62 Ser349 ELISA Kit	96 Assay
<b>NEW!</b> CY-7057	CycLex® Phospho-p62 Ser403 ELISA Kit	96 Assay

### <Antibodies>

Code No.	Product	Clone	Isotype	Size	Application	Reactivity
PM045	Anti-p62 (SQSTM1) pAb	Polyclonal	Rabbit Ig (aff.)	100 µL	WB / IP / IC / IH	Hu / Mo / Rat / Ham
PM066	Anti-p62 C-terminal pAb	Polyclonal	Guinea pig Ig (aff.)	100 µL	WB / IP / IC / IH	Hu / Mo / Rat / Ham
PM066-7	Anti-p62 C-terminal pAb-HRP-Direct	Polyclonal	Guinea pig Ig (aff.)	50 µL	WB	Hu / Mo / Rat / Ham
M162-3	Anti-p62 (SQSTM1) (Human) mAb	5F2	Mouse IgG1 κ	100 µg/100 µL	WB / IP / FCM / IC / IH	Hu
M162-A48	Anti-p62 (SQSTM1) (Human) mAb-Alexa Fluor®488	5F2	Mouse IgG1 κ	100 µg/100 µL	FCM / IC	Hu
M162-A59	Anti-p62 (SQSTM1) (Human) mAb-Alexa Fluor®594	5F2	Mouse IgG1 κ	100 µg/100 µL	FCM / IC	Hu
M162-A64	Anti-p62 (SQSTM1) (Human) mAb-Alexa Fluor®647	5F2	Mouse IgG1 κ	100 µg/100 µL	FCM / IC	Hu
PM074	Anti-Phospho-p62 (SQSTM1) (Ser351) pAb	Polyclonal	Rabbit Ig (aff.)	100 µL	WB / IP / IC / IH	Hu / Mo
M217-3	Anti-Phospho-p62 (SQSTM1) (Ser351) mAb	5D5	Mouse IgG1 κ	100 µg/100 µL	WB / IC / IH	Hu / Mo
D343-3	Anti-Phospho-p62 (SQSTM1) (Ser403) mAb	4F6	Rat IgG2a κ	100 µg/100 µL	WB / IH	Hu / Mo
D344-3	Anti-Phospho-p62 (SQSTM1) (Ser403) mAb	4C8	Rat IgG2a κ	100 µg/100 µL	WB / IH	Hu / Mo
PM069	Anti-NRF2 pAb	Polyclonal	Rabbit Ig (aff.)	100 µL	WB / IP / IC / IH	Hu / Mo(w) / Rat(w) / Ham(w)
M200-3	Anti-NRF2 mAb	1F2	Mouse IgG1 κ	100 µg/100 µL	WB / IP / IC / IH	Hu / Mo / Rat / Ham
M224-3	Anti-KEAP1 mAb	KP1	Mouse IgG2a κ	100 µg/100 µL	WB	Hu / Mo / Rat / Ham
MK-11-3	Anti-Ubiquitin mAb	1B3	Mouse IgG1	100 µg/100 µL	WB / IC* / IH* / Other*	Hu / Mo* / Bov*
MK-12-3	Anti-Ubiquitin mAb	2C5	Mouse IgG1	100 µg/100 µL	WB / IP* / IC*	Hu / Mo / Rat / Bov
D058-3	Anti-Multi Ubiquitin mAb	FK2	Mouse IgG1 κ	100 µg/100 µL	WB / IC* / ELISA*	Hu / Mo* / Mky* / Yeast*

\*Application : WB : Western Blotting, IP : Immunoprecipitation, IH : Immunohistochemistry, IC : Immunocytochemistry, FCM : Flow Cytometry

Reactivity : Hu : Human, Mo : Mouse, Ham : Hamster, Bov : Bovine, Mky : Monkey, (w) : (weak)

(aff.) : affinity purified \* : The use is reported in a research article (Not tested by MBL). Please check the data sheet for detailed information.

# ① Highlight Product: p62 and phospho-p62 ELISA Kits

## Sales promotion materials

For Research Use Only

**MBL**

### p62 and phospho-p62 ELISA Kit

**CyclLex® Total p62 ELISA Kit**  
**CyclLex® Phospho-p62 Ser349 ELISA Kit**  
**CyclLex® Phospho-p62 Ser403 ELISA Kit**

The market's first ELISA kit for phosphorylated p62!

**Features**

- Comes with lysis buffer. Easy to prepare cell lysate!
- Useful for drug screening!
- Human and mouse cell lysate can be used.

**What is Selective autophagy?**

Autophagy was initially thought to be a non-selective degradation mechanism, because the entire vesicle contents were digested. However, recent findings have revealed the selective degradation of mitochondria and other specific organelles, bacteria, and aggregates of proteins with attached ubiquitin chains (polyubiquitinated proteins). This mechanism is called "selective autophagy".

**p62/SQSTM1**

\*Adaptor proteins\* are necessary to link autophagosomes to proteins destined for selective degradation. One of these adaptor proteins is p62/SQSTM1. p62 is a scaffolding protein that interacts with various signaling molecules. 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 polyubiquitinated protein aggregates and depolarized mitochondria to the autophagic machinery.

There is increasing interest about impairment of autophagic degradation in neurodegenerative diseases (such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis), alcoholic hepatitis, hepatic steatosis, and liver cancer.

**Domain structure of p62/SQSTM1**

**p62-Keap1-Nrf2 pathway**

This illustration was made under the supervision of Dr. Masashi Komatsu and Dr. Yoshitaka Saitoh (Nagoya University).

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### Phospho-p62

p62 has several phosphorylation sites. Two biophytaxis systems described below are effectively activated by the continuous phosphorylation on these sites.

- **Selective autophagy**  
 Removing misfolded or aggregated proteins and eliminating intracellular pathogens.  
 Phosphorylation of p62 at Ser407 (Human) / Ser409 (Mouse)  
 Phosphorylation of p62 at Ser403 (Human) / Ser405 (Mouse)  
 → Increases the affinity of p62 for polyubiquitin chains.
- **Nuclear translocation of Nrf2**  
 Exacerbate the expression of stress tolerance gene.  
 Phosphorylation of p62 at Ser349 (Human) / Ser351 (Mouse)  
 → Increases the affinity of p62 for Keap1.

**Example data of HeLa and MEF cells**

**Related product list**

**<Kits>**

Code No.	Product	Size
CV-7055	CyclLex® Total p62 ELISA kit	96 Assay
CV-7056	CyclLex® Phospho-p62 Ser349 ELISA kit	96 Assay
CV-7057	CyclLex® Phospho-p62 Ser403 ELISA kit	96 Assay

**<Antibodies>**

Code No.	Product	Clone	Isotype	Size	Application	Reactivity
PA066	Anti-p62 (SQSTM1) pAb	Polyclonal	Rabbit Ig (pAb)	100 µL	WB / IP / IC / IIF	Hu / Mo / Rat / Ham
PA068	Anti-p62 C-terminal pAb-HRP-Direct	Polyclonal	Goat anti-pig Ig (pAb)	100 µL	WB / IP / IC / IIF	Hu / Mo / Rat / Ham
PA068-7	Anti-p62 C-terminal pAb-HRP-Direct	Polyclonal	Goat anti-pig Ig (pAb)	80 µL	WB / IP / IC / IIF	Hu / Mo / Rat / Ham
MH02-2	Anti-p62 (SQSTM1) (Human) mAb	SP2	Mouse IgG1 κ	100 µg/100 µL	WB / IP / IC / IIF / FM	Hu
MH02-A48	Anti-p62 (SQSTM1) (Human) mAb-Alexa Fluor®488	SP2	Mouse IgG1 κ	100 µg/100 µL	WB / IP / IC / IIF	Hu
MH02-A09	Anti-p62 (SQSTM1) (Human) mAb-Alexa Fluor®488	SP2	Mouse IgG1 κ	100 µg/100 µL	WB / IC / IIF	Hu
MH02-A04	Anti-p62 (SQSTM1) (Human) mAb-Alexa Fluor®488	SP2	Mouse IgG1 κ	100 µg/100 µL	WB / IC / IIF	Hu
PA074	Anti-Phospho-p62 (SQSTM1) (Ser351) pAb	Polyclonal	Rabbit Ig (pAb)	100 µL	WB / IP / IC / IIF	Hu / Mo
MD17-3	Anti-Phospho-p62 (SQSTM1) (Ser351) mAb	5D6	Mouse IgG1 κ	100 µg/100 µL	WB / IC / IIF	Hu / Mo
DS02-2	Anti-Phospho-p62 (SQSTM1) (Ser403) mAb	4F6	Rat IgG2b κ	100 µg/100 µL	WB / IIF	Hu / Mo
DS04-0	Anti-Phospho-p62 (SQSTM1) (Ser403) mAb	4C8	Rat IgG2b κ	100 µg/100 µL	WB / IIF	Hu / Mo
PA069	Anti-Nrf2 pAb	Polyclonal	Rabbit Ig (pAb)	100 µL	WB / IP / IC / IIF	Hu / Mo / Rat / Ham
MD00-3	Anti-Nrf2 mAb	1F7	Mouse IgG1 κ	100 µg/100 µL	WB / IP / IC / IIF	Hu / Mo / Rat / Ham
MD04-3	Anti-Keap1 mAb	NP1	Mouse IgG2a κ	100 µg/100 µL	WB / Mo / Rat / Ham	
MK-11-3	Anti-Ubiquitin mAb	1B3	Mouse IgG1 κ	100 µg/100 µL	WB / IC / IP / IIF / Other	Hu / Mo / Rat / Ham
MK-12-3	Anti-Ubiquitin mAb	2C5	Mouse IgG1 κ	100 µg/100 µL	WB / IP / IC	Hu / Mo / Rat / Bow
DS05-0	Anti-Multi Ubiquitin mAb	FN2	Mouse IgG1 κ	100 µg/100 µL	WB / IC / ELISA*	Hu / Mo / Rat / Ham / Bow

\* Application: WB, Western Blotting; IP, Immunoprecipitation; IIF, Immunofluorescence; IC, Flow Cytometry; ICM, Immunocytochemistry; FM, Fluorescence Microscopy; Hu, Human; Mo, Mouse; Rat, Rat; Bow, Bow; Ham, Ham; MAb, Monoclonal Antibody; pAb, Polyclonal Antibody.

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 URL: http://www.mbl.co.jp

We prepared leaflet in October. Ask us for the editable PDF file!



[http://ruo.mbl.co.jp/bio/e/literature/pdf/349203\\_p62ELISA.pdf](http://ruo.mbl.co.jp/bio/e/literature/pdf/349203_p62ELISA.pdf)

## What is p62 ?

p62/SQSTM1 is a scaffolding protein that interacts with various signaling molecules.

p62 contains an LC3-interacting region and is believed to be a substrate for selective autophagy.

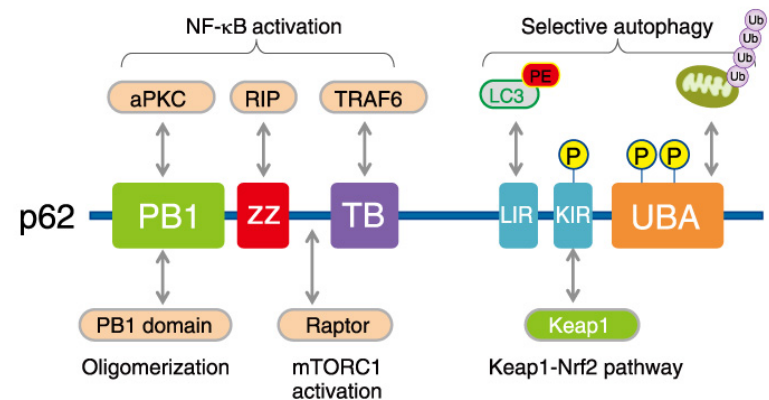
In addition, p62 contains a domain that binds ubiquitin chains, and mediates the recruitment of poly ubiquitinated protein aggregates and depolarized mitochondria to the autophagic machinery.

There is increasing interest about impairment of autophagic degradation in neurodegenerative diseases (such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis), alcoholic hepatitis, hepatic steatosis, and liver cancer.

Access the web site for the detail of p62.

<http://ruo.mbl.co.jp/bio/g/product/autophagy/pickup/p62.html>

■ Domain structure of p62/SQSTM1



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

# Review of p62 and phospho-p62

## What is phospho-p62 ?

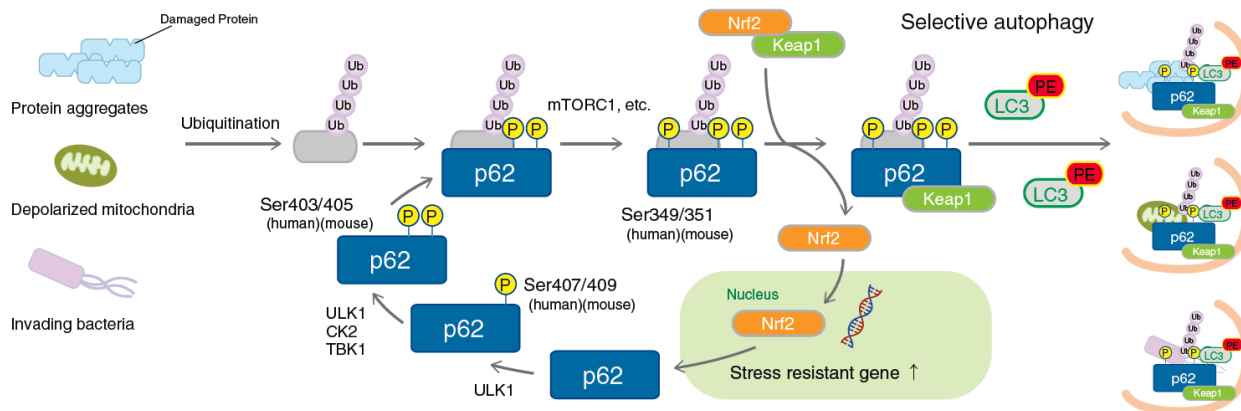
p62 has several phosphorylation sites. Two biophylaxis systems described below are effectively activated by the continuous phosphorylation on these sites.

Impaired selective autophagy is implicated in various diseases.

<http://ruo.mbl.co.jp/bio/g/product/autophagy/pickup/phospho-p62.html>



■ p62-Keap1-Nrf2 pathway



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



② New & Renewal Website  
Now Available!

**MBL**

**Want to Use Epitope Tag Antibodies?**

**Interested in Autophagy?**

**Need a Guide for Your Experiment?**

**Want to Know the Basic Principle and Method of the Experiment?**

**CHECK OUR RE-NEWED WEBSITE!!**

# ② New & Renewal Website Epitope Tag Antibodies

**“Examples of use” and “FAQ” are totally NEW!**

Click the contents to find out more information!

Our renewal website is much more easier to find what you want!

<http://ruo.mbl.co.jp/bio/g/product/tag/index.html>

## Epitope tags

MBL offers a large selection of products, from unlabeled and labeled antibodies to purification kits. In addition to supplying antibodies to common epitope tags (DDDDK, HA, Myc, His, V5, etc.), we have introduced antibodies to fusion proteins (GFP, RFP, etc.) and special purpose tags (mini-AID-tag and others). We invite you to try out our products.



- 1 I cannot purify epitope-tagged target proteins from my samples.
- 2 My epitope-tagged target protein does not bind to the tag beads.
- 3 My epitope-tagged target protein binds to tag beads, but I cannot elute the protein with peptide.
- 4 Which lysis buffer should I use?
- 5 Does the epitope tag location affect purification efficiency?
- 6 Can I elute the epitope-tagged target protein at 4°C ?
- 7 Can I purify the epitope-tagged target protein from inclusion bodies of *E. coli* ?
- 8 Can I elute the epitope-tagged target protein using buffers other than Elution Peptide Solution?

### Contents

What are epitope tags?	Featured products	Examples of use	Citations
Products list	Brochures & leaflets	FAQ	

# ② New & Renewal Website Autophagy

**Not just the information about Autophagy, but you can also find the white papers written by the famous researchers of Autophagy!**

Whoever wants to try Autophagy, check our Website and learn more about Autophagy!

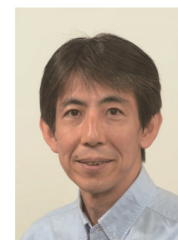
<http://ruo.mbl.co.jp/bio/g/product/autophagy/index.html>

## Autophagy

The role of autophagy is generally understood to be securing nutrient sources through autodigestion in order to survive starvation conditions. However, in recent years, it has been found to work along with the proteasome system in the metabolism of cell components even in normal environments. Compared to proteasomes, which selectively degrade ubiquitinated proteins as their targets, in autophagy the space within the cell is entirely digested; therefore it is called a bulk degradation system. Recent researches and advances have shown an association of mammalian autophagy with diseases such as neurodegenerative disease, infection disease, cardiac disease as well as cancers.



### Autophagy research: Current status and future perspectives

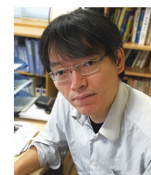


**Dr. Noboru Mizushima**  
of the University of Tokyo

Autophagy research:  
Current status and future perspectives  
by Dr. Noboru Mizushima of the University of Tokyo

"p62/Sqstm1 : The molecule that links autophagy to the Keap1-Nrf2 system"  
by Drs. Komatsu and Ichimura of Niigata University

### "p62/Sqstm1 : The molecule that links autophagy to the Keap1-Nrf2 system"



**Dr. Masaaki Komatsu**  
Department of Biochemistry,  
Niigata University Graduate School  
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**Dr. Yoshinobu Ichimura**  
Department of Biochemistry,  
Niigata University Graduate School  
of Medical and Dental Sciences

## Contents

<b>What is Autophagy?</b> 	<b>Featured products</b> 	<b>Citations</b> 	<b>Products list</b> 
<b>Brochures &amp; leaflets</b> 	<b>FAQ</b> 		

**Help your experiments keep going!**  
**This can be a guide for people who are going to do an experiment for the first time as well!!**

There are **4 categories** you can check!

- ✓ Antibody basics
- ✓ Antibodies as a research tool
- ✓ Qualitative and quantitative measurements of proteins using antibodies
- ✓ Fractionation and purification of proteins

<http://ruo.mbl.co.jp/bio/g/support/method/index.html>

