

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

CODE No.	D343-3
CLONALITY	Monoclonal
CLONE	4F6
ISOTYPE	Rat IgG2a κ
QUANTITY	100 μ L, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	KLH conjugated synthetic peptide, CKKESLSQMLpSMGFSDEGKKK (corresponding to amino acid residues 396-410 of human p62 (SQSTM1))
FORMULATION	PBS containing 50% Glycerol (pH 7.2). No preservative is contained.
STORAGE	This antibody solution is stable for one year from the date of purchase when stored at -20°C .

APPLICATIONS

<u>Western blotting</u>	5 $\mu\text{g}/\text{mL}$ for chemiluminescence detection system
<u>Immunohistochemistry</u>	10 $\mu\text{g}/\text{mL}$ (paraffin section)
Heat treatment for paraffin embedded section: microwave oven, for 20 min. in 10 mM citrate buffer (pH 6.3)	

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Cell	Transfectant	MEF ^{Atg5^{-/-}}	Not tested	Not tested
Reactivity	+	+		

Entrez Gene ID 8878 (Human), 18412 (Mouse)

REFERENCE 1) Matsumoto, G., *et al.*, *Mol. Cell* **44**, 279-289 (2011) [WB, IHC]

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

RELATED PRODUCTS

Antibodies

D343-3	Anti-Phospho-p62 (SQSTM1) (Ser403) mAb (4F6)
D344-3	Anti-Phospho-p62 (SQSTM1) (Ser403) mAb (4C8)
M217-3	Anti-Phospho-p62 (SQSTM1) (Ser351) mAb (5D5)
PM074	Anti-Phospho-p62 (SQSTM1) (Ser351) pAb
M162-3	Anti-p62 (SQSTM1) (Human) mAb (5F2)
M162-A48	Anti-p62 (SQSTM1) (Human) mAb -Alexa Fluor®488 (5F2)
M162-A59	Anti-p62 (SQSTM1) (Human) mAb -Alexa Fluor®594 (5F2)
M162-A64	Anti-p62 (SQSTM1) (Human) mAb -Alexa Fluor®647 (5F2)
PM045	Anti-p62 (SQSTM1) pAb
PM066	Anti-p62 C-terminal pAb
PM066-7	Anti-p62 C-terminal pAb-HRP-Direct
PM036	Anti-LC3 pAb [WB, IP, IC, IHC, FCM]
M152-3	Anti-LC3 mAb (4E12) [WB, IP, IC, FCM, EM]
M186-3	Anti-LC3 mAb (8E10) [WB]
PD014	Anti-LC3 pAb [WB]
PD017	Anti-Becn1 pAb
M135-3	Anti-GABARAP mAb (1F4)
PM037	Anti-GABARAP pAb
PM038	Anti-GATE-16 pAb
PD041	Anti-Atg2A pAb
PM034	Anti-Atg3 pAb
M133-3	Anti-Atg3 mAb (3E8)
M134-3	Anti-Atg4B mAb (9H5)
M153-3	Anti-Atg5 mAb (4D3)
PM050	Anti-Atg5 pAb
PM039	Anti-Atg7 (Human) pAb
PD042	Anti-Atg9A pAb
M151-3	Anti-Atg10 (Human) mAb (5A7)
M154-3	Anti-Atg12 (Human) mAb (6E5)
M183-3	Anti-Atg13 mAb (5G4)
PD036	Anti-Atg13 (Human) pAb
M184-3	Anti-Atg14 (Human) mAb (4H8)
PD026	Anti-Atg14 pAb
M150-3	Anti-Atg16L mAb (1F12)
PM040	Anti-Atg16L pAb
M160-3	Anti-UVRAG mAb (1H4)
M170-3	Anti-Rubicon (Human) mAb (1H6)
PD027	Anti-Rubicon (Human) pAb
PD037	Anti-Tel2 pAb
M200-3	Anti-NRF2 mAb (1F2)
PM069	Anti-NRF2 pAb
PM072	Anti-VMP1 pAb
M212-3	Anti-Syntaxin-17 (Human) mAb (2F8)
PM074	Anti-Syntaxin-17 pAb
M224-3	Anti-KEAP1 mAb (KP1)
M230-3	Anti-Parkin mAb (Par6)

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

Other related antibodies and kits are also available.
Please visit our website at <http://ruo.mbl.co.jp/>

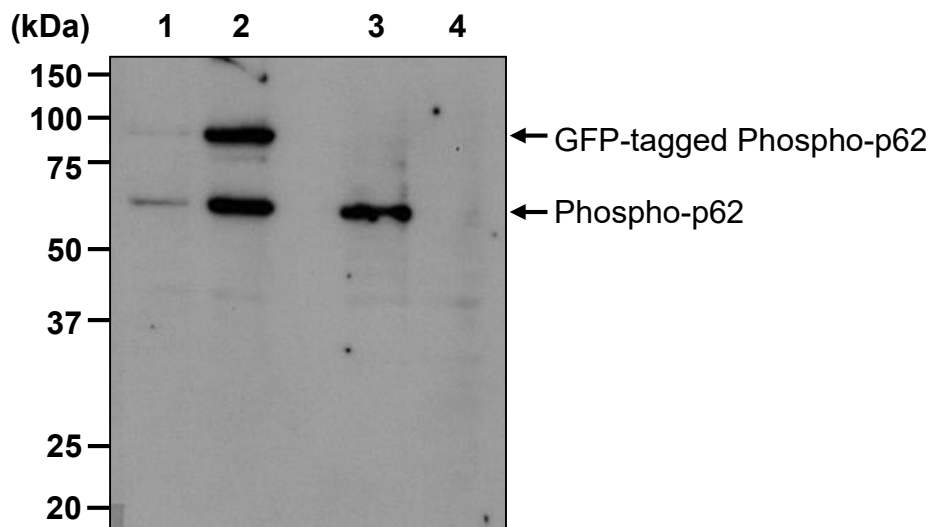
Kits

CY-7055	CycLex® Total p62 ELISA Kit
CY-7056	CycLex® Phospho-p62 Ser349 ELISA Kit
CY-7057	CycLex® Phospho-p62 Ser403 ELISA Kit
8485	Autophagy Ab Sampler Set
8486	Autophagy Watch
PM036-PN	Positive control for anti-LC3 antibody

SDS-PAGE & Western blotting

- 1) Wash 1×10^7 cells 3 times with PBS and suspend them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 10 sec.).
- 2) Boil the samples for 2 min. and centrifuge. Load 10 μ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
- 3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer's manual for precise transfer procedure.
- 4) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature. *2% of goat serum, horse serum or BSA (in PBS, pH 7.2) may also be used for blocking.
- 5) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.) *1% BSA (in PBS, pH 7.2) may also be used as antibody diluent.
- 6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
- 7) Incubate the membrane with HRP-conjugated anti-rat IgG antibody diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature. *1% BSA (in PBS, pH 7.2) may also be used as antibody diluent.
- 8) Wash the membrane with PBS-T (5 min. x 3 times).
- 9) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 10) Expose to an X-ray film in a dark room for 10 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Neuro2a transfectant and MEF^{Atg5^{-/-}})



Western blot analysis of Phospho-p62 (SQSTM1) (Ser403)

Lane 1: GFP-tagged human p62/Neuro2a

Lane 2: GFP-tagged human p62/Neuro2a, Bafilomycin A1-treated (1 μ M, 24 hr.)

Lane 3: MEF^{Atg5^{-/-}}

Lane 4: MEF

Immunoblotted with Anti-Phospho-p62 (SQSTM1) (Ser403) mAb (D343-3)

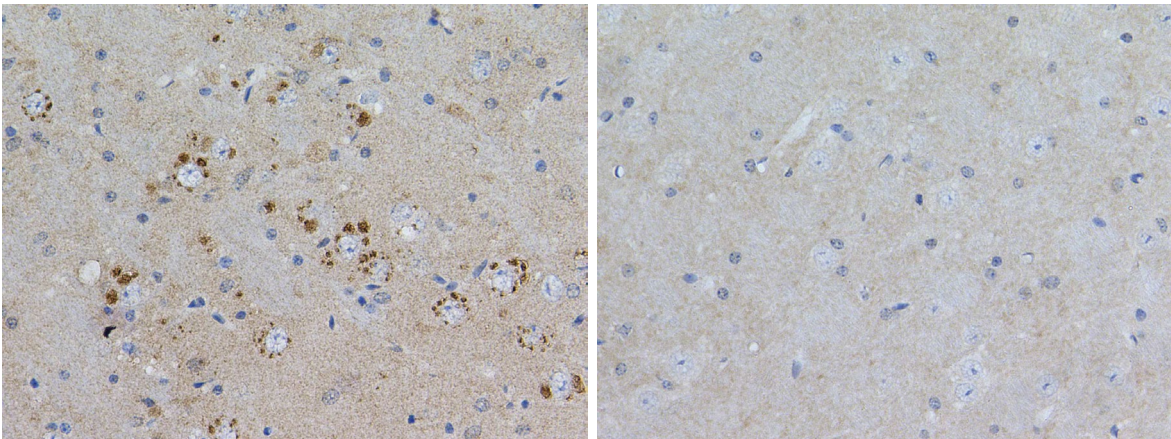
Non-treated and Bafilomycin A1-treated transfectants were provided by Drs, Gen Matsumoto and Nobuyuki Nukina. (Department of Neuroscience for Neurodegenerative Disorders, Juntendo University Graduate School of Medicine)

MEF^{Atg5^{-/-}} was provided by Dr. Noboru Mizushima. (Department of Biochemistry and Molecular Biology, Graduate School and Faculty of Medicine, The University of Tokyo)

Immunohistochemistry

- 1) Deparaffinize the sections with Xylene 3 times for 3 min. each.
- 2) Wash the slides with Ethanol 3 times for 3 min. each.
- 3) Wash the slides with PBS 3 times for 5 min. each.
- 4) Remove the slides from PBS and heat-treated with 10 mM Citrate buffer (pH6.3) for 20 min. using microwave.
- 5) Let the slides cool down at room temperature in the Citrate buffer.
- 6) Wash the slides with running water for 5 min., then wash with PBS for 5 min.
- 7) Remove the slides from PBS and inactivate endogenous peroxidase with 3% H₂O₂ in PBS for 10 min.
- 8) Wash the slides 3 times in PBS for 5 min. each.
- 9) Remove the slides from PBS, wipe gently around each section and cover tissues with blocking buffer [1% BSA/20 mM HEPES/135 mM NaCl (pH 7.4)] for 10 min. at room temperature to block non-specific staining. Do not wash.
- 10) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with 1% BSA/PBS as suggested in the **APPLICATION**. (The concentration of antibody will depend on the conditions.) Incubate the sections for 1 hr. at room temperature.
- 11) Wash the slides 3 times in PBS for 5 min. each.
- 12) Wipe gently around each section and cover tissues with Histostar (Rat) (MBL; code no. 8463). Incubate for 30 min. at room temperature.
- 13) Wash the slides 3 times in PBS for 5 min. each.
- 14) Visualize by reacting for 5 min. with Histostar DAB Substrate Solution (MBL; code no. 8469). *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
- 15) Wash the slides in water for 5 min.
- 16) Counterstain in hematoxylin for 1 min., wash the slides 3 times in water for 5 min. each, and then immerse the slides in PBS for 5 min.
- 17) Dehydrate by immersing in Ethanol 3 times for 3 min. each, followed by immersing in Xylene 3 times for 3 min. each. Now ready for mounting.

(Positive control for Immunohistochemistry; Brain from *Atg5* conditional knockout mouse)



Immunohistochemical detection of Phospho-p62 (SQSTM1) (Ser403) in mouse brain

Left: *Atg5* conditional knockout
Right: Wild type

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

The samples were provided by Drs, Gen Matsumoto and Nobuyuki Nukina. (Department of Neuroscience for Neurodegenerative Disorders, Juntendo University Graduate School of Medicine)