

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

<b>CODE No.</b>	D344-3
<b>CLONALITY</b>	Monoclonal
<b>CLONE</b>	4C8
<b>ISOTYPE</b>	Rat IgG2a $\kappa$
<b>QUANTITY</b>	100 $\mu$ L, 1 mg/mL
<b>SOURCE</b>	Purified IgG from hybridoma supernatant
<b>IMMUNOGEN</b>	KLH conjugated synthetic peptide, CKKESLSQMLpSMGFSDEGKKK (corresponding to amino acid residues 396-410 of human p62 (SQSTM1))
<b>FORMULATION</b>	PBS containing 50% Glycerol (pH 7.2). No preservative is contained.
<b>STORAGE</b>	This antibody solution is stable for one year from the date of purchase when stored at -20°C.

## APPLICATIONS

Western blotting 5  $\mu$ g/mL for chemiluminescence detection system

Immunohistochemistry 5  $\mu$ g/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
Cells	Bafilomycin A1-treated transfectant	MEF <sup>Atg5<sup>-/-</sup></sup>	Not tested	Not tested
Reactivity	+	+		

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

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

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

## RELATED PRODUCTS

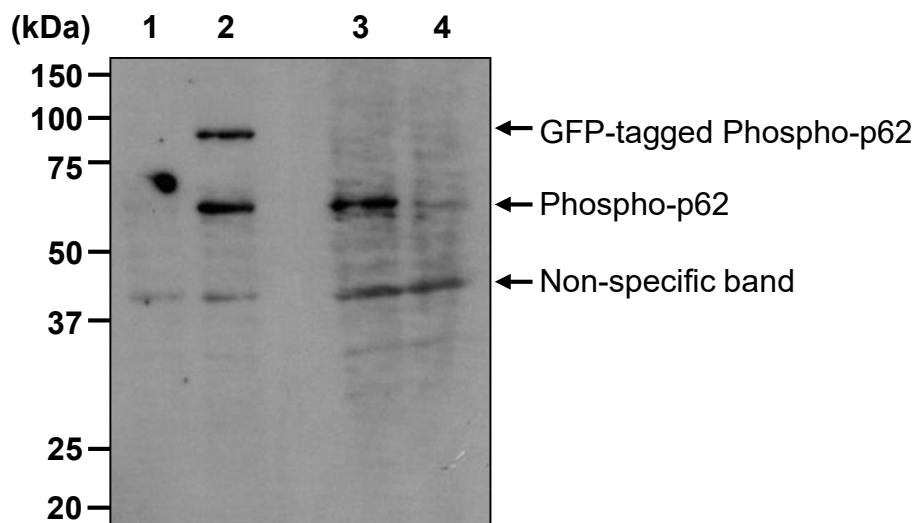
Please visit our web site <https://ruo.mbl.co.jp/>

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

### **SDS-PAGE & Western blotting**

- 1) Wash  $1 \times 10^7$  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  $\mu$ 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/cm<sup>2</sup> 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: Bafilomycin A1-treated Neuro2a transfectant and MEF<sup>Atg5<sup>-/-</sup></sup>)



### **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  $\mu$ M, 24 hr.)

Lane 3: MEF<sup>Atg5<sup>-/-</sup></sup>

Lane 4: MEF

Immunoblotted with Anti-Phospho-p62 (SQSTM1) (Ser403) mAb (D344-3)

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<sup>Atg5<sup>-/-</sup></sup> 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% H<sub>2</sub>O<sub>2</sub> 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 (D344-3)

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)