For Research Use Only.
Not for use in diagnostic procedures.

MBL
A JSR Life Sciences Company

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

CODE No. D344-3

CLONALITY Monoclonal
CLONE 4C8
ISOTYPE Rat IgG2a κ
QUANTITY 100 µL, 1 mg/mL

SOURCE Purified IgG from hybridoma supernatant
IMMUNOGEN KLH conjugated synthetic peptide, CKKKESLSQMLpSMGFSDEGKKK (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

Western blotting 5 µg/mL for chemiluminescence detection system
Immunohistochemistry 5 µg/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$^{Atg5-/-}$	Not tested	Not tested
Reactivity	+	+		

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

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

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

RELATED PRODUCTS

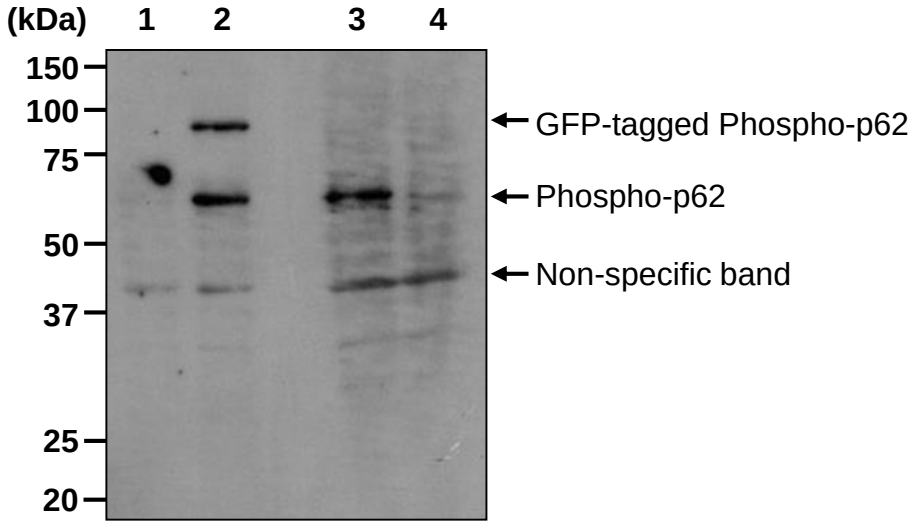
Please visit our web site https://ruo.mbl.co.jp/.

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

SDS-PAGE & Western blotting

1) Wash 1 x 10^7 cells 3 times with PBS and suspends 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 μL 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^2 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$^{Atg5-/-}$)



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 μM, 24 hr.)
Lane 3: MEF$^{Atg5-/-}$
Lane 4: MEF

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

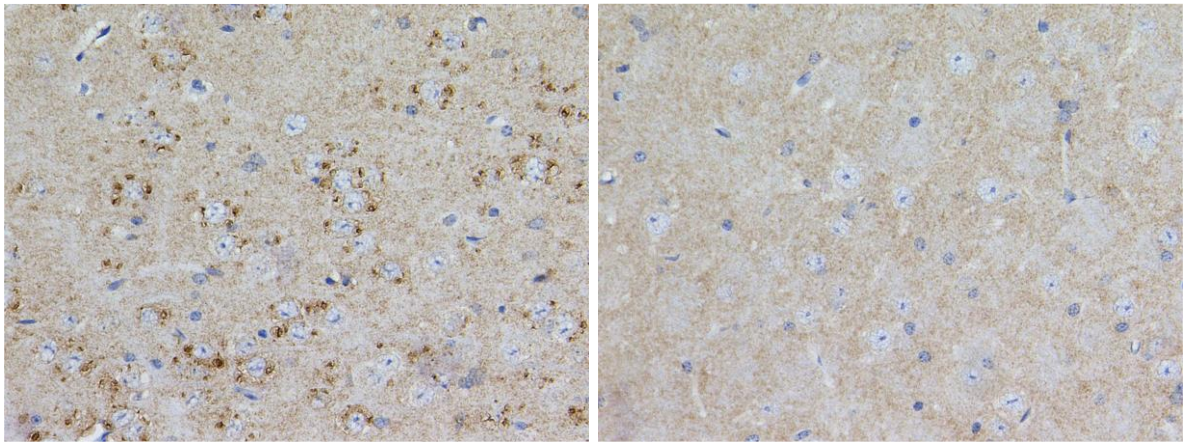
Non-treated and Bafilomycin A1-treated transfectants were provided by Drs. Gen Matsumoto and Nobuyuki Nukina. (Department of Neuroscience for Neurodegenerative Disorders, Juntendo University Graduate School of Medicine)

MEF$^{Atg5-/-}$ 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% H_2O_2 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)