

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

CODE No.	M217-3
CLONALITY	Monoclonal
CLONE	5D5
ISOTYPE	Mouse IgG1 κ
QUANTITY	100 μ L, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	KLH conjugated synthetic peptide, CKEVDP(pS)TGELQSLQ (corresponding to amino acid residues 346-359 of mouse 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-CONFIRMED

<u>Western blotting</u>	0.5 μ g/mL
<u>Immunohistochemistry</u>	1 μ g/mL (paraffin section) Heat treatment for paraffin embedded section: microwave oven, for 20 min. in 10 mM citrate buffer (pH 6.3)
<u>Immunocytochemistry</u>	0.1 μ g/mL

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Cells	huH-1	sodium arsenite-treated MEF, MEF ^{Atg5^{-/-}}	Not tested	Not tested
Reactivity	+	+		

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

- REFERENCES**
- 1) Inoue, H., *et al.*, *Biomed Res.* **38**, 343-350 (2017) [WB]
 - 2) Santarino, I. B., *et al.*, *Sci. Rep.* **7**, 5812 (2017) [WB, IC]
 - 3) Watanabe, Y., *et al.*, *Autophagy* **13**, 133-148 (2017) [IC]
 - 4) Kageyama, S., *et al.*, *J. Biol. Chem.* **289**, 24944-24955 (2014)
 - 5) Ichimura, Y., *et al.*, *Mol. Cell* **51**, 618-631 (2013)

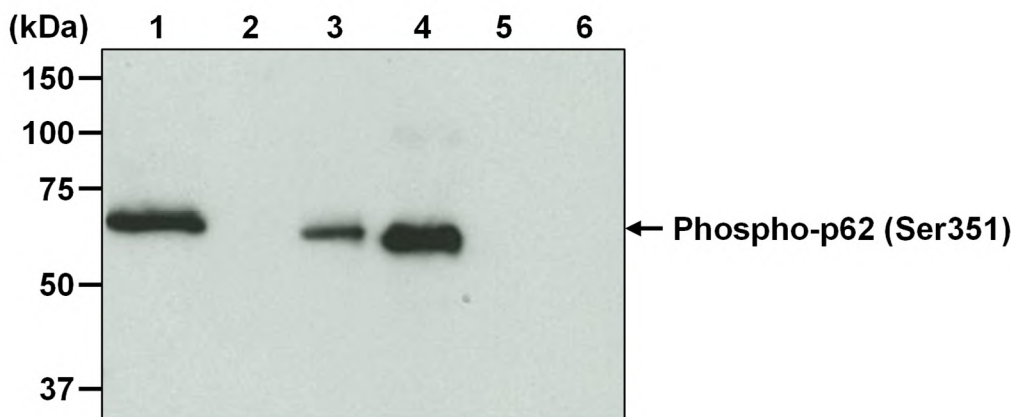
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The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

SDS-PAGE & Western blotting

- 1) Wash 1×10^7 cells 3 times with PBS and suspend with 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 20 sec.).
- 2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
- 3) Boil the samples for 3 min. and centrifuge. Load 10 μ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (10% acrylamide) for electrophoresis.
- 4) 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.
- 5) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
- 6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
- 7) 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.)
- 8) Wash the membrane with PBS-T (5 min. x 3 times).
- 9) Incubate the membrane with 1:10,000 Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 10) Wash the membrane with PBS-T (5 min. x 3 times).
- 11) Wipe excess buffer on the membrane, 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.
- 12) Expose to an X-ray film in a dark room for 3 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; sodium arsenite-treated MEF, MEF^{Atg5^{-/-}} and huH-1)



Western blot analysis of Phospho-p62 (SQSTM1) (Ser351)

- Lane 1: MEF, sodium arsenite-treated (10 μ M, 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

Immunoblotted with Anti-Phospho-p62 (SQSTM1) (Ser351) mAb (M217-3)

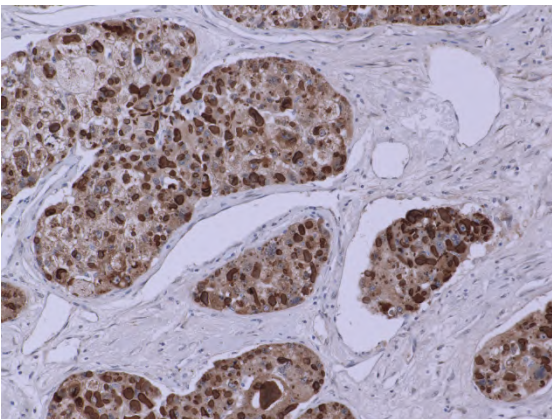
Sodium arsenite-treated MEF and p62-knockout huH-1 were provided by Dr. Yoshinobu Ichimura¹ and Dr. Masaaki Komatsu². (¹Protein Metabolism Project, Tokyo Metropolitan Institute of Medical Science, ²Department of Biochemistry, School of Medicine, Niigata University)

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 5 min. each.
- 2) Wash the slides with Ethanol 3 times for 5 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 (pH 6.3) 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 2 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 5 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 the blocking buffer 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 2 times in PBS for 5 min. each.
- 12) Wipe gently around each section and cover tissues with Histostar™ (Ms + Rb) (MBL; code no. 8460). Incubate for 30 min. at room temperature.
- 13) Wash the slides 2 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; Human liver carcinoma)



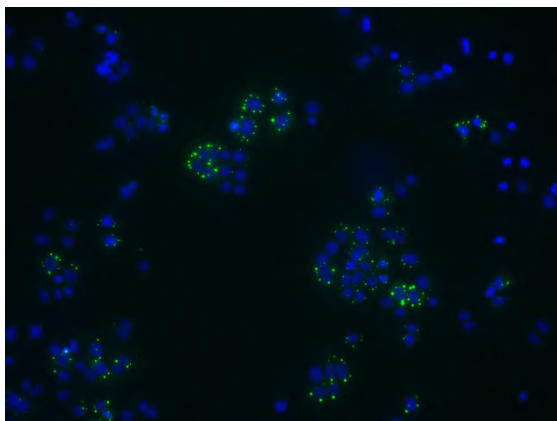
Immunohistochemical detection of Phospho-p62 (SQSTM1) (Ser351) in human liver carcinoma

Brown: Anti-Phospho-p62 (SQSTM1) (Ser351) mAb (M217-3)
Blue: Hematoxylin

Immunocytochemistry

- 1) Spread the cells on a glass slide, then incubate in a CO₂ incubator for one night.
- 2) Remove the culture supernatant by careful aspiration.
- 3) Wash the slide 2 times with PBS.
- 4) Fix the cells with 4% paraformaldehyde (PFA)/PBS for 10 min. at room temperature (20~25°C).
- 5) Wash the slide 2 times with PBS.
- 6) Permeabilize the cells with 100 µg/mL of Digitonin/PBS for 10 min. at room temperature.
- 7) Wash the slide 2 times with PBS.
- 8) Add 200 µL of the primary antibody diluted with PBS as suggested in the **APPLICATIONS** onto the cells. Incubate for 1 hr. at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
- 9) Wash the slide 2 times with PBS.
- 10) Add 200 µL of 1:500 Alexa Fluor®488 anti-mouse IgG (Invitrogen; code no. A11001) diluted with PBS onto the cells. Incubate for 30 min. at room temperature. Keep out light by aluminum foil.
- 11) Wash the slide 2 times with PBS.
- 12) Counter stain with DAPI for 5 minutes at room temperature.
- 13) Wash the glass slide 2 times with PBS.
- 14) Wipe excess liquid off the slide but take care not to touch the cells. Never leave the cells to dry.
- 15) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; huH-1)



Immunocytochemical detection of Phospho-p62 (SQSTM1) (Ser351)

Green: Anti-Phospho-p62 (SQSTM1) (Ser351) mAb (M217-3)

Blue: DAPI