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

My select sampler set

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

CODE No. PM074MS

CLONALITY Polyclonal
ISOTYPE Rabbit Ig, affinity purified
QUANTITY 20 µL

SOURCE Purified IgG from rabbit serum
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

Western blotting 1:500
Immunoprecipitation 2 µL/sample
Immunohistochemistry 1:1,000 (paraffin section)
Heat treatment for paraffin embedded section: microwave oven, for 20 min. in 10 mM citrate buffer (pH 6.3)
Immunocytochemistry 1:500

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)

REFERENCE

1) Mizunoe, Y., *et al.*, *Redox Biol.* **15**, 115-124 (2017) [WB]
2) Yanagisawa, H., *et al., Sci. Rep*. **7**, 15994 (2017) [WB]
3) Watanabe, Y., *et al., Autophagy* **13**, 133-148 (2017) [WB, IC, IHC]
4) Yoshii, S. R., *et al., Dev. Cell* **39**, 116-130 (2016) [WB]
5) Johansson, I., *et al. Autophagy* **11**, 1636-1651 (2015) [WB]
6) Kageyama, S., *et al.*, *J. Biol. Chem*. **289**, 24944-24955 (2014)
7) Ichimura, Y., *et al.*, *Mol. Cell* **51**, 618-631 (2013)

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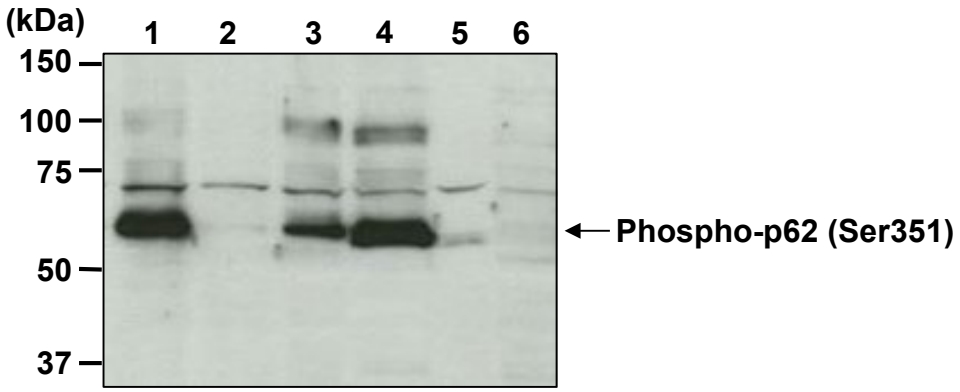
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

SDS-PAGE & Western blotting

1) Wash 1 x 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 μL 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/cm^2 for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). 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).
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).
9) Incubate the membrane with the 1:10,000 anti-IgG (Rabbit) pAb-HRP (MBL, code no. 458) 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)
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 blotting analysis of Phospho-p62 (SQSTM1) (Ser351)

Lane 1: MEF, sodium arsenite-treated (10 μM, 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) pAb (MBL, code no. PM074)

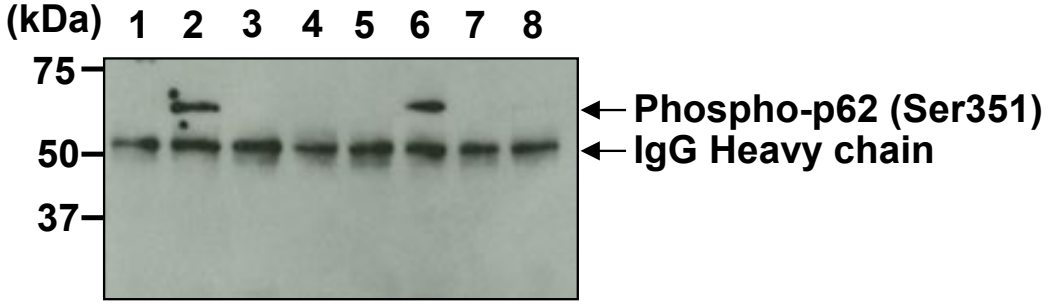
Sodium arsenite-treated MEF and p62-knockout huH-1 were provided by Dr. Yoshinobu Ichimura, Ph.D. and Dr. Masaaki Komatsu, Ph.D. (Protein Metabolism Project, Tokyo Metropolitan Institute of Medical Science)

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

Immunoprecipitation

1) Resuspend 5 x 10^6 cells with 1 mL of ice-cold Lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% NP-40, 1:1,000 of Phosphatase Inhibitor Cocktail 2 (Sigma-Aldrich, code no. P5726)] containing appropriate protease inhibitors.
2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
3) Mix 20 µL of 50% protein A agarose beads slurry resuspended in 400 µL of IP buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40] with primary antibody as suggested in the **APPLICATIONS**. Incubate with gentle agitation for 30 min. at room temperature.
4) Wash the beads once with 1 mL of IP buffer.
5) Add 500 µL of cell lysate (prepared sample from step 2), then incubate with gentle agitation for 1 hr. at 4°C.
6) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
7) Resuspend the agarose with 1ml IP Buffer.
8) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
9) Repeat steps 7)-8) 4 times.
10) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 5 min. and centrifuge.
11) Load 5 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
12) 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% methanol). See the manufacturer's manual for precise transfer procedure.
13) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
14) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3).
15) 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.)
16) Wash the membrane with PBS-T (5 min. x 3).
17) Incubate the membrane with the 1:1,000 TrueBlot® Anti-Rabbit IgG HRP (Rockland, code no. 18-8816-31) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
18) Wash the membrane with PBS-T (5 min. x 3)
19) 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.
20) 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 Immunoprecipitation; huH-1 and $MEF^{Atg5-/-}$)



Immunoprecipitation of Phospho-p62 (SQSTM1) (Ser351)

Lane 1, 2: huH-1
Lane 3, 4: p62-knockout huH-1
Lane 5, 6: MEF$^{Atg5-/-}$
Lane 7, 8: MEF

Lane 1, 3, 5, 7: Normal Rabbit IgG (MBL, code no. PM035)
Lane 2, 4, 6, 8: Anti-Phospho-p62 (SQSTM1) (Ser351) pAb (MBL, code no. PM074)

Immunoblotted with PM074

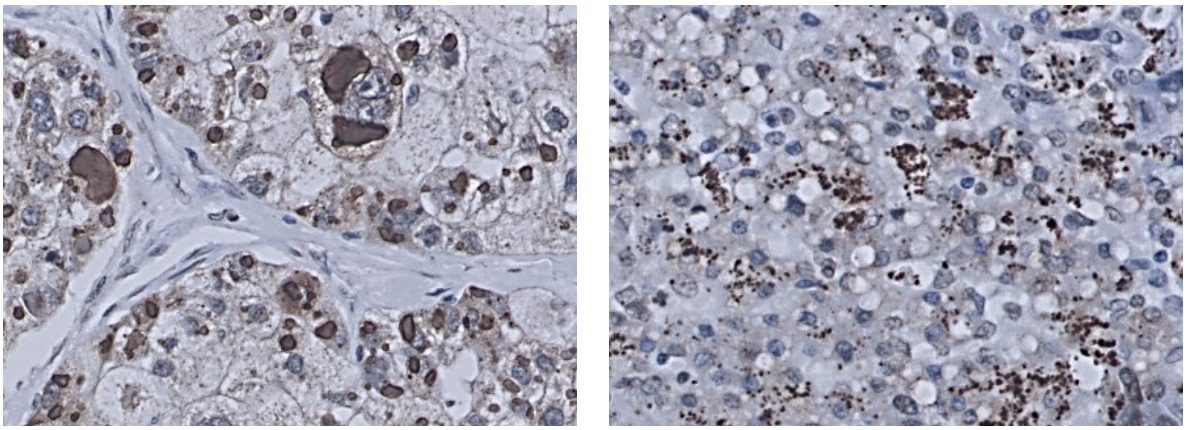
p62-knockout huH-1 was provided by Dr. Yoshinobu Ichimura[1] and Dr. Masaaki Komatsu[2]. ([1]Protein Metabolism Project, Tokyo Metropolitan Institute of Medical Science, [2]Department of Biochemistry, School of Medicine, Niigata University)

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 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-treat with 10 mM citrate buffer (pH 6.3) for 20 min. using microwave oven.
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 twice 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 twice 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 1 hr. at room temperature.
13) Wash the slides twice 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)



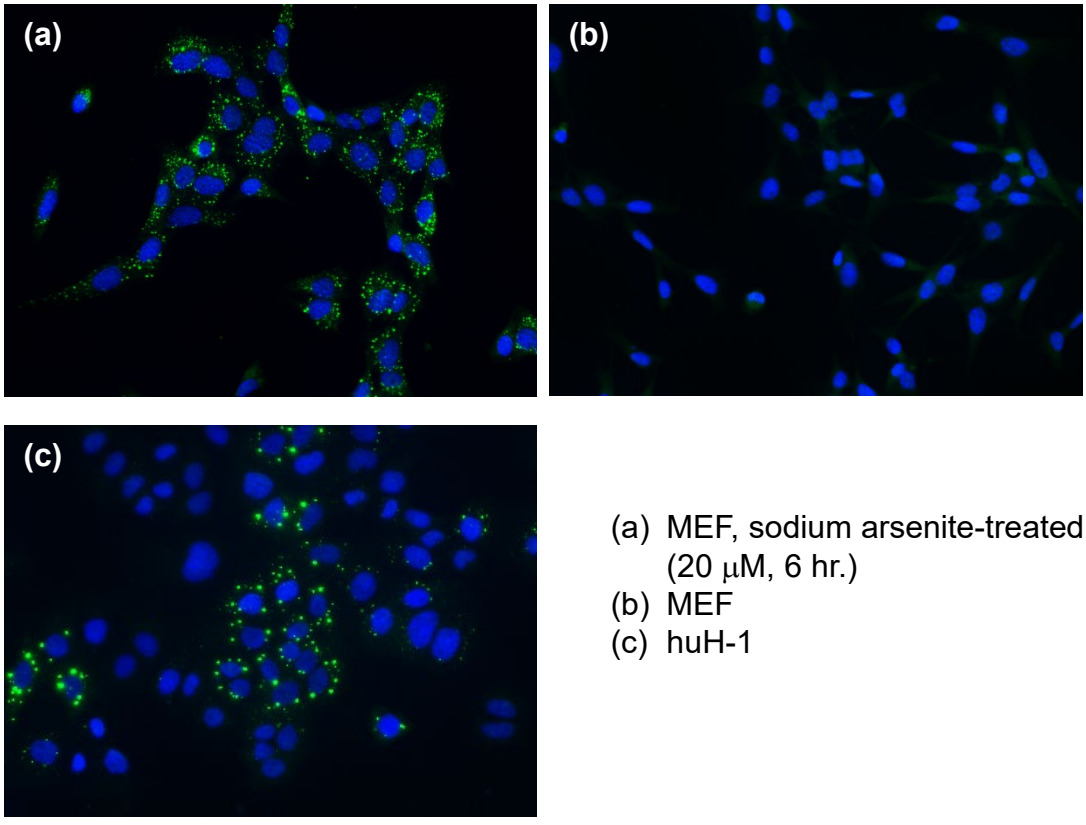
Immunohistochemical detection of Phospho-p62 (SQSTM1) (Ser351) in human liver carcinoma

Brown: Anti-Phospho-p62 (SQSTM1) (Ser351) pAb (MBL, code no. PM074)
Blue: Hematoxylin

Immunocytochemistry

1) Spread the cells on a glass slide, then incubate in a CO_2 incubator overnight.
2) Remove the culture supernatant by careful aspiration.
3) Wash the slide twice with PBS.
4) Fix the cells with 4% paraformaldehyde /PBS for 10 min. at room temperature (20~25°C).
5) Wash the slide twice with PBS.
6) Permeabilize the cells with 100 μg/mL of Gigitonin/PBS for 10 min. at room temperature.
7) Wash the slide twice 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 twice with PBS.
10) Add 200 μL of 1:500 Alexa Fluor™ 488 conjugated anti-rabbit IgG (Thermo Fisher Scientific, code no. A-11008) diluted with PBS onto the cells. Incubate for 30 min. at room temperature. Keep out light by aluminum foil.
11) Wash the slide twice with PBS.
12) Counter stain with DAPI for 5 min. at room temperature.
13) Wash the glass slide twice 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 controls for Immunocytochemistry; huH-1 and arsenite-treated MEF)



(a) MEF, sodium arsenite-treated (20 μM, 6 hr.)
(b) MEF
(c) huH-1

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

Green: Anti-Phospho-p62 (SQSTM1) (Ser351) pAb (MBL, code no. PM074)
Blue: DAPI

Sodium arsenite-treated MEF was provided by Dr. Yoshinobu Ichimura, Ph.D. and Dr. Masaaki Komatsu, Ph.D. (Protein Metabolism Project, Tokyo Metropolitan Institute of Medical Science)