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

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

CODE No. PM074

CLONALITY Polyclonal
ISOTYPE Rabbit Ig, affinity purified
QUANTITY 100 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 for chemiluminescence detection system
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)

REFERENCES

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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RELATED PRODUCTS

PM074	Anti-Phospho-p62 (SQSTM1) (Ser351) pAb
M217-3	Anti-Phospho-p62 (SQSTM1) (Ser351) mAb (5D5)
D343-3	Anti-Phospho-p62 (SQSTM1) (Ser403) mAb (4F6)
D344-3	Anti-Phospho-p62 (SQSTM1) (Ser403) mAb (4C8)
PM045	Anti-p62 (SQSTM1) 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)
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]
M186-7	Anti-LC3 mAb-HRP-DirecT (8E10)
PD014	Anti-LC3 pAb [WB]
PD017	Anti-Beclin 1 pAb
PM037	Anti-GABARAP pAb
M135-3	Anti-GABARAP mAb (1F4)
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)
PM050	Anti-Atg5 pAb
M153-3	Anti-Atg5 mAb (4D3)
PM039	Anti-Atg7 (Human) pAb
PD042	Anti-Atg9A pAb
M151-3	Anti-Atg10 (Human) mAb (5A7)
M154-3	Anti-Atg12 (Human) mAb (6E5)
PD036	Anti-Atg13 (Human) pAb
M183-3	Anti-Atg13 mAb (5G4)
PD026	Anti-Atg14 pAb
M184-3	Anti-Atg14 (Human) mAb (4H8)
PM040	Anti-Atg16L pAb
M150-3	Anti-Atg16L mAb (1F12)
M160-3	Anti-UVRAG mAb (1H4)
PD027	Anti-Rubicon (Human) pAb
M170-3	Anti-Rubicon (Human) mAb (1H6)
PD037	Anti-Tel2 pAb
PM069	Anti-NRF2 pAb
M200-3	Anti-NRF2 mAb (1F2)
PM072	Anti-VMP1 pAb
PM076	Anti-Syntaxin-17 (Human) pAb
M212-3	Anti-Syntaxin-17 (Human) mAb (2F8)
M224-3	Anti-KEAP1 mAb (KP1)
M230-3	Anti-Parkin mAb (Par6)
M175-3	Anti-α-Tubulin mAb (2F9)
M175-A48	Anti-α-Tubulin mAb-Alexa Fluor® 488 (2F9)
M175-A59	Anti-α-Tubulin mAb-Alexa Fluor® 594 (2F9)
M175-A64	Anti-α-Tubulin mAb-Alexa Fluor® 647 (2F9)
PM054	Anti-α-Tubulin pAb
PM054-7	Anti-α-Tubulin pAb-HRP-DirecT
M176-3	Anti-EEA1 mAb (3C10)
M176-A48	Anti-EEA1 mAb-Alexa Fluor® 488 (3C10)
M176-A59	Anti-EEA1 mAb-Alexa Fluor® 594 (3C10)
M176-A64	Anti-EEA1 mAb-Alexa Fluor® 647 (3C10)
PM062	Anti-EEA1 pAb
M178-3	Anti-Calnexin mAb (4F10)
M178-A48	Anti-Calnexin mAb-Alexa Fluor® 488 (4F10)
M178-A59	Anti-Calnexin mAb-Alexa Fluor® 594 (4F10)
M178-A64	Anti-Calnexin mAb-Alexa Fluor® 647 (4F10)
PM060	Anti-Calnexin pAb
M181-3	Anti-KDEL mAb (1D5)
PM059	Anti-KDEL pAb
M179-3	Anti-GM130 mAb (5G8)
M179-A48	Anti-GM130 mAb-Alexa Fluor® 488 (5G8)
M179-A59	Anti-GM130 mAb-Alexa Fluor® 594 (5G8)
M179-A64	Anti-GM130 mAb-Alexa Fluor® 647 (5G8)
PM061	Anti-GM130 pAb
PM063	Anti-COX4 pAb
PM064	Anti-Lamin B1 pAb

Kits

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

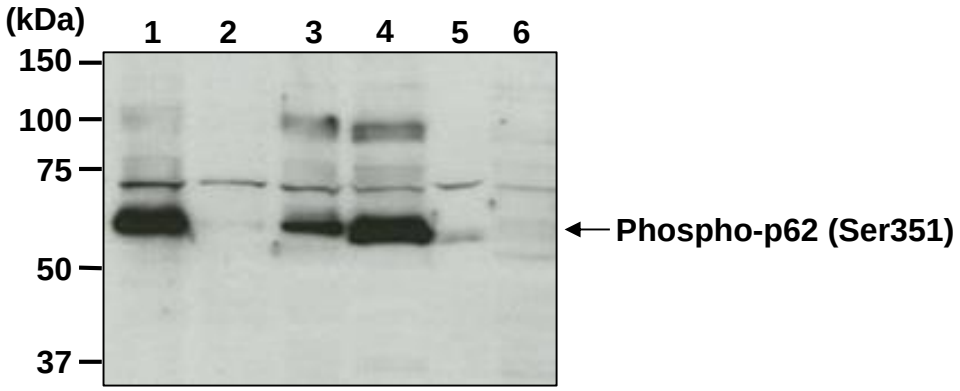
WB: Western blotting
IP: Immunoprecipitation
IC: Immunocytochemistry
IHC: Immunohistochemistry
FCM: Flow cytometry
EM: Immuno-electron microscopy

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

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% 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 (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 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 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 (PM074)

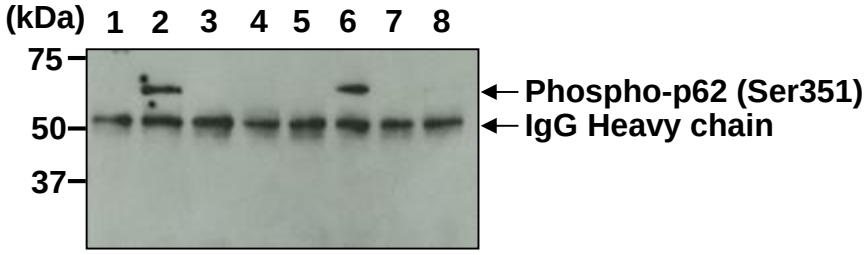
Sodium arsenite-treated MEF and p62-knockout huH-1 were provided by Dr. Yoshinobu Ichimura[1] and Dr. Masaaki Komatsu[2]. ([1]Protein Metabolism Project, Tokyo Metropolitan Institute of Medical Science, [2]Department of Biochemistry, School of Medicine, Niigata University)

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

Immunoprecipitation

1) Resuspend 5 x 10^6 cells with 1 mL of ice-cold Extraction 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 1 time 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) Wash the beads 4 times with 1 mL of IP buffer.
7) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 5 min. and centrifuge.
8) Load 5 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
9) 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.
10) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
11) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
12) 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.)
13) Wash the membrane with PBS-T (5 min. x 3 times).
14) Incubate the membrane with the 1:1,000 True blot®: Anti-Rabbit IgG HRP (Rockland Immunochemicals; code no. 18-8816-31) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
15) Wash the membrane with PBS-T (5 min. x 3 times)
16) 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.
    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 (PM035)
Lane 2, 4, 6, 8: Anti-Phospho-p62 (SQSTM1) (Ser351) pAb (PM074)

Immunoblotted with PM074

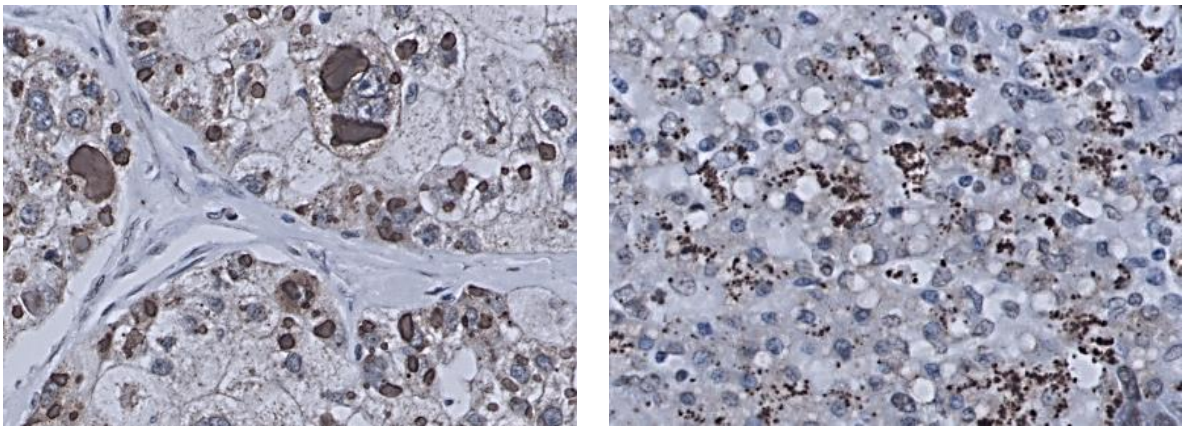
p62-knockout huH-1 was provided by Dr. Yoshinobu Ichimura[1] and Dr. Masaaki Komatsu[2].
([1]Protein Metabolism Project, Tokyo Metropolitan Institute of Medical Science, [2]Department of Biochemistry, School of Medicine, Niigata University)

$MEF^{Atg5-/-}$ 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-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 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 (Rb) (MBL; code no. 8466). Incubate for 1 hr. 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)



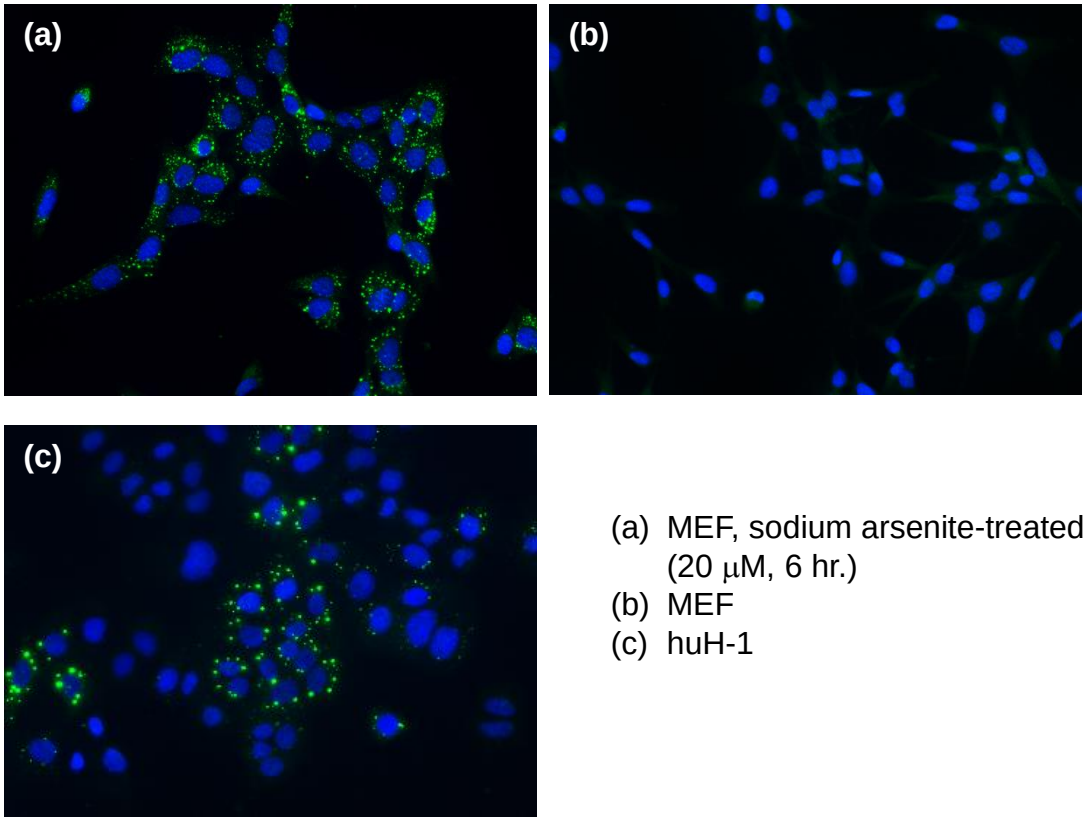
Immunohistochemical detection of Phospho-p62 (SQSTM1) (Ser351) in human liver carcinoma

Brown: Anti-Phospho-p62 (SQSTM1) (Ser351) pAb (PM074)
Blue: Hematoxylin

Immunocytochemistry

1) Spread the cells on a glass slide, then incubate in a CO_2 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 Gigitonin/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-rabbit IgG (Invitrogen; code no. A110374) diluted with PBS onto the cells. Incubate for 30 min. at room temperature. Keep out light by aluminum foil.
11) Wash the slide 2 time 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 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 (PM074)
Blue: DAPI

Sodium arsenite-treated MEF 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)