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MONOCLONAL ANTIBODY

Anti-p62 (SQSTM1) (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
M162-3	5F2	Mouse IgG1 κ	100 µL	1 mg/mL

BACKGROUND: p62/SQSTM1 interacts with various molecular groups such as RIP, TRAF6, ERK, aPKCs, and poly-ubiquitin through PB1 domain, Zn finger domain, and UBA domain. This protein directly interacts with LC3, which is localized on autophagosome membrane, and is degradated by autophagic-lysosome pathway. p62 regulates ubiquitin-positive protein aggregates caused by autophagy deficiency.

SOURCE: This antibody was purified from hybridoma (clone 5F2) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with C3H mouse lymphocyte immunized with the recombinant human p62 protein corresponding to amino acids 120-440.

FORMULATION: 100 µg IgG in 100 µL volume of 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.

REACTIVITY: This antibody reacts with human p62 on Western blotting, Immunoprecipitation, Immunohistochemistry and Immunocytochemistry.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; 2 µg/250 µL of cell extract from 2.5 x 10^6 cells
Flow cytometry; 2 µg/mL (final concentration)
Immunocytochemistry; 5 µg/mL
Immunohistochemistry; 2-10 µg/mL
Heat treatment is necessary for paraffin embedded sections.
Microwave oven; 2 times for 10 minutes each in 10 mM citrate buffer (pH 6.0)

Detailed procedure is provided in the following **PROTOCOLS**.

INTENDED USE:
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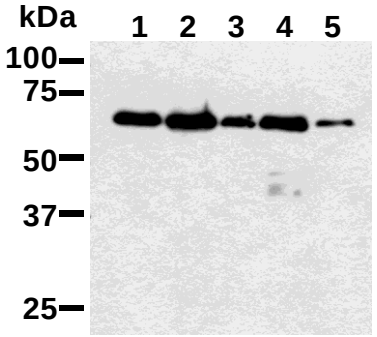
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster
Cells	HeLa, Raji, HepG2, A549, 293T	NIH/3T3, MEF	NRK	CHO
Reactivity on WB	+	-	-	-

REFERENCES:

1) Janda, E., *et al*., *Autophagy* **11**, 1063-1080 (2015) [WB, IC]
2) Zhu, X., *et al*., *J. Clin. Invest*. **125**, 1098-1110 (2015) [WB]
3) Akaishi, R., *et al*., *Placenta* **35**, 974-980 (2014) [IHC, IC]
4) Onodera, Y., *et al*., *Endocr. Relat. Cancer* **21**, 241-252 (2014) [IHC]
5) Guo, X., *et al*., *Cell Death Dis.* **4**, e822 (2013) [WB]
6) Schmeisser, H., *et al*., *Autophagy* **9**, 683-696 (2013) [WB]
7) Park, J. M., *et al*., *Cancer Biol. Ther.* **14**, 100-107 (2013) [IHC]
8) Wang, B. S., *et al*., *Clin. Sci. (Lond).* **124**, 203-214 (2013) [WB, IP, IHC]
9) Rovetta, F., *et al*., *Exp. Cell Res*. **318**, 238-250 (2012) [WB]
10) Matsumoto, G., *et al*., *Mol. Cell* **44**, 279-289 (2011) [WB]
11) Fan, W., *et al*., *Autophagy* **6**, 614-621 (2010) [WB]
12) Soll, C., *et al*., *Hepatology* **51**, 1244-1254 (2010) [WB]
13) Ichimura, Y., *et al*., *J. Biol. Chem.* **283**, 22847-22857 (2008)
14) Komatsu, M., *et al*., *Cell* **131**, 1149-1163 (2007)



Western blot analysis of p62 expression in HeLa (1), Raji (2), HepG2 (3), A549 (4) and 293T (5) using M162-3.

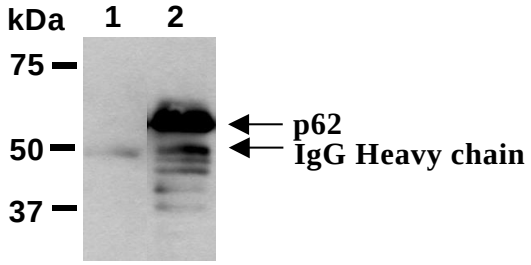
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOLS:

SDS-PAGE & Western Blotting

1) Wash the 1 x 10^7 cells 3 times with PBS and suspend with 1 mL of Laemmli's sample buffer.
2) Boil the samples for 2 minutes and centrifuge. Load 20 μL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour 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, place the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
5) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
6) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
7) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
8) Incubate the membrane with 1:10,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
9) Wash the membrane with PBS-T (5 minutes x 3 times).
10) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
11) 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 minutes.
13) Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; HeLa, Raji, HepG2, A549 and 293T)

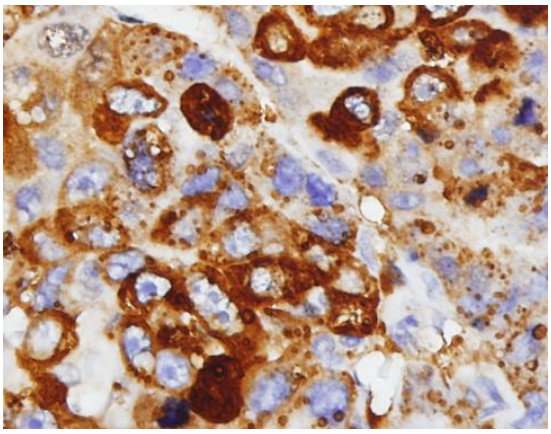


Immunoprecipitation of p62 from HeLa with Mouse IgG1 (1) or M162-3 (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with PM045.*

Immunoprecipitation

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40] containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another fresh tube.
3) Add primary antibody as suggested in the **APPLICATIONS** into 250 μL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C.
4) Add 20 μL of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
5) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
6) Resuspend the beads in 20 μL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 μL/lane for the SDS-PAGE analysis.
   (See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation; HeLa)



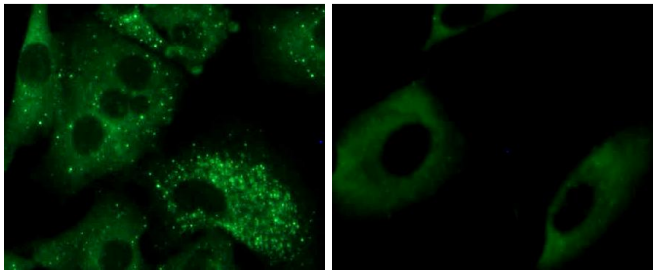
Immunohistochemical detection of p62 on paraffin embedded section of human liver carcinoma with M162-3.

Immunohistochemical staining for paraffin-embedded sections

1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes each.
4) Heat treatment
   Heat treatment by Microwave:
   Place the slides put on staining basket in 500 mL beaker with 500 mL of 10 mM citrate buffer (pH 6.0). Cover the beaker with plastic wrap, then process the slides 2 times for 10 minutes each at 500 W with microwave oven. Let the slides cool down in the beaker at room temperature for about 40 minutes.
5) Remove the slides from the citrate buffer and cover each section with 3% H_2O_2 for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.

6) Remove the slides from PBS, wipe gently around each section and cover tissues with blocking buffer (20 mM HEPES, 1% BSA, 135 mM NaCl) for 5 minutes to block non-specific staining. Do not wash.
7) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with blocking buffer as suggested in the **APPLICATIONS**.
8) Incubate the sections for 1 hour at room temperature.
9) Wash the slides 3 times in PBS for 5 minutes each.
10) Wipe gently around each section and cover tissues with ENVISION+Dual Link (DAKO; code no. K4063). Incubate for 15 minutes at room temperature. Wash as in step 9).
11) Visualize by reacting for 5 minutes with DAB substrate solution (DAKO; code no. K3465). *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
12) Wash the slides in water for 5 minutes.
13) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
14) Now ready for mounting.

(Positive control for Immunohistochemistry; Human liver carcinoma)

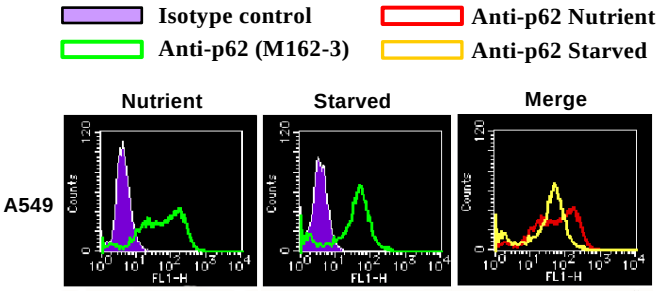


Immunocytochemical detection of p62 on starved A549 cells (left) and nutrient A549 cells (right) with M162-3.

Immunocytochemistry

1) Spread the cells in the nutrient condition on a glass slide, then incubate in a CO_2 incubator for one night.
2) Remove the culture supernatant by careful aspiration.
3) To obtain serum-starved conditions, culture the cells with Hank's solution or DMEM for 2-4 hours at 37°C.
4) Fix the cells by immersing the slide in 4% paraformaldehyde (PFA)/PBS for 10 minutes at room temperature (20~25°C).
5) Prepare a wash container such as a 500 mL beaker with a magnetic stirrer. Then wash the fixed cells on the glass slide by soaking the slide with a plenty of PBS in the wash container for 5 minutes. Take care not to touch the cells. Repeat another wash once more.
6) Immerse the slide in 100 µg/mL of Digitonin for 10 minutes at room temperature.
7) Wash the slide in a plenty of PBS as in the step 5).
8) Add the primary antibody diluted with PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 1 hour at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
9) Wash the slide in a plenty of PBS as in the step 5).
10) Add 200 µL of 1:500 Goat anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor® 488 conjugate (Invitrogen; code no. A-11001) diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
11) Wash the slide in a plenty of PBS as in the step 5).
12) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
13) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; A549)



Flow cytometric analysis of p62 expression in nutrient or starved of A549. *Fluorescence intensity of p62 on starved cells was reduced.*

References
・Shvets, E., and Elazar, Z., *Methods Enzymol*, **452**, 131-141 (2009)
・Shvets, E., *et al.*, *Autophagy* **4**, 621-628 (2008)

Flow cytometric analysis for adherent cells

We usually use Fisher tubes or equivalents as reaction tubes for all steps after 4).

1) To obtain serum-starved conditions, culture the cells with Hank's solution or DMEM for 3 hours at 37°C.
2) Detach the cells from culture dish by trypsinization. *Excessive trypsinization may reduce the antigenicity.
3) Wash the cells with PBS.
4) Fix the cells with 4% paraformaldehyde (PFA)/PBS for 10 minutes at room temperature (20~25°C). Wash the cells 2 times with PBS.
5) Permeabilize the cells with 100 µg/mL of Digitonin for 10 minutes at room temperature. Wash the cells 2 times with PBS.
6) Resuspend the cells with PBS ($5x10^6$ cells/mL).
7) Add 50 µL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful aspiration.
8) Add 40 µL of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted in the washing buffer. Mix well and incubate for 20 minutes at room temperature.
9) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.

10) Add 40 µL of FITC conjugated anti-mouse IgG antibody diluted with the washing buffer. Mix well and incubate for 20 minutes at room temperature.
11) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
12) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

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