

POLYCLONAL ANTIBODY

Anti-p62 (SQSTM1) pAb

Code No.	Quantity	Form
PM045	100 µL	Affinity Purified

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 degraded by autophagic-lysosome pathway. p62 regulates ubiquitin-positive protein aggregates caused by autophagy deficiency.

SOURCE: This antibody was purified from rabbit serum using affinity column. The rabbit was immunized with the recombinant human p62 protein corresponding to amino acids 120-440.

FORMULATION: 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 p62 on Western blotting, Immunoprecipitation, Immunohistochemistry and Immunocytochemistry.

APPLICATIONS:

Western blotting; 1:1,000

Immunoprecipitation; 2 µL/300 µL of cell extract from 1 x 10⁷ cells

Immunohistochemistry; 1:1,000

Heat treatment is necessary for paraffin embedded sections.

Microwave oven; twice for 10 minutes each in 10 mM citrate buffer (pH 6.3)

Immunocytochemistry; 1:500

Flow cytometry; Not tested

Immuno-EM; Not tested*

*It is reported that this antibody can be used in this application in the reference. Please visit our website at <https://ruo.mbl.co.jp/>.

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

INTENDED USE:

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster	Zebrafish
Cells	HeLa, 293T	NIH/3T3, MEF	PC12, NRK	CHO	Not tested*
Reactivity on WB	+	+	+	+	

*Reactivity of this antibody to zebrafish is not confirmed in our laboratory. However, it is reported that this antibody reacts with p62 expressed in the liver of type 2 diabetes-like zebrafish.

Meng, X. H., *et al.*, *Int. J. Bio. Sci.* **13**, 985-995 (2017)

REFERENCES:

Please visit our website at <https://ruo.mbl.co.jp/>.

As this antibody is widely used, many researchers have been reported. These references are a part of such reports.

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

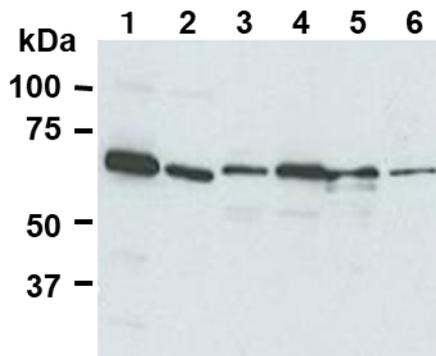
PROTOCOLS:

SDS-PAGE & Western blotting

- 1) Wash the 1 x 10⁷ 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 10 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/cm² for 1 hour 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.
- 4) To reduce nonspecific binding, place the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
- 5) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
- 6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
- 7) 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 hour at room temperature.
- 8) Wash the membrane with PBS-T (5 minutes x 3 times).
- 9) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.

- 10) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 11) Expose to an X-ray film in a dark room for 3 minutes.
- 12) Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; 293T, HeLa, NIH/3T3, MEF, PC12, NRK and CHO)

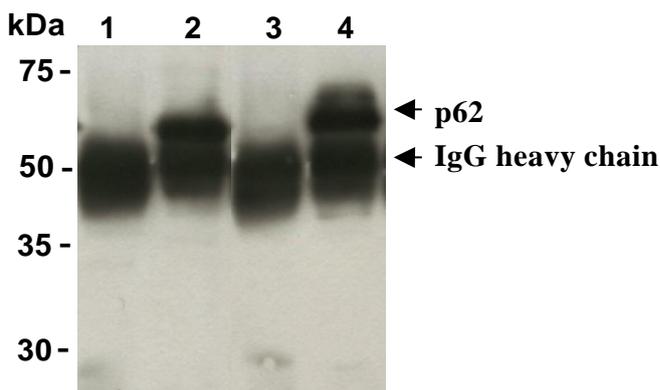


Western blotting analysis of p62 expression in HeLa (1), 293T (2), MEF (3), NIH/3T3 (4), PC12 (5) and CHO (6) using PM045.

Immunoprecipitation

- 1) Wash the cells 3 times with PBS and suspend with 10 volumes of cold Lysis buffer [10 mM Tris-HCl (pH 8.0), 500 mM NaCl, 0.1% 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 tube.
- 3) Add primary antibody as suggested in the **APPLICATIONS** into 300 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) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
- 6) Resuspend the beads with the cold Lysis buffer.
- 7) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
- 8) Repeat steps 6)-7) 3-5 times.
- 9) 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 controls for Immunoprecipitation; HeLa and MEF)



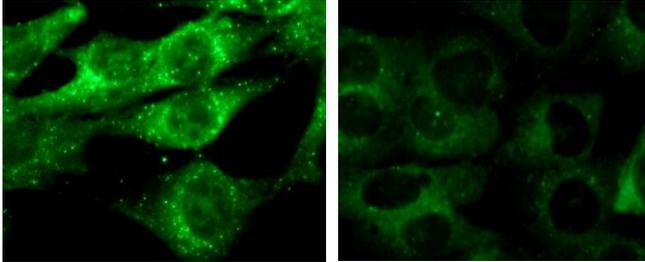
Immunoprecipitation of p62 from HeLa (1, 2) and MEF (3, 4) with PM045 (2, 4) or normal rabbit IgG (1, 3). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with PM045.

Immunohistochemical staining for paraffin-embedded sections

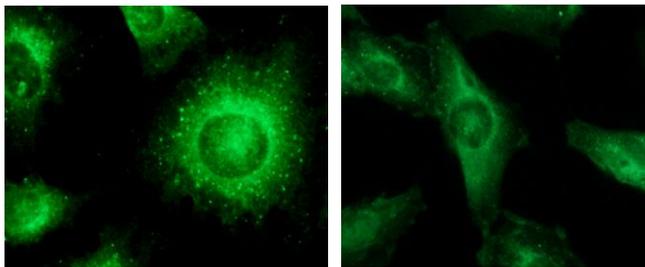
- 1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
- 2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
- 3) Wash the slides with PBS 3 times for 3-5 minutes each.
- 4) Heat treatment
 Heat treatment by Microwave:
 Place the slides put on staining basket in 500 mL beaker with 500 mL of 10 mM citrate buffer (pH 6.3). Cover the beaker with plastic wrap, then process the slides twice 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% H₂O₂ 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 4% PFA fixed starved MEF cells (left) and nutrient MEF cells with PM045.



Immunocytochemical detection of p62 on 4% PFA fixed starved normal rat kidney (NRK) cells (left) and nutrient NRK cells with PM045.

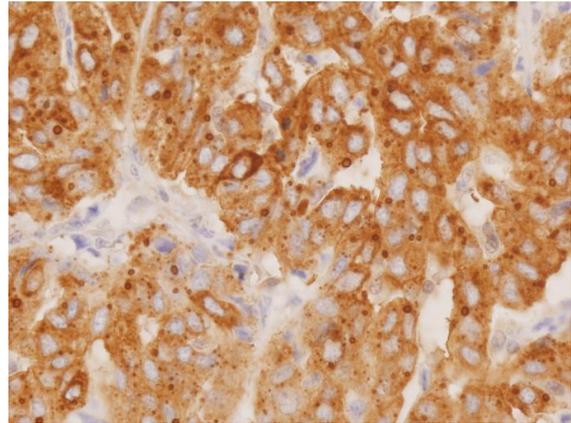
Immunocytochemistry

- 1) Spread the cells in the nutrient condition on a glass slide, then incubate in a CO₂ incubator overnight.
- 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).

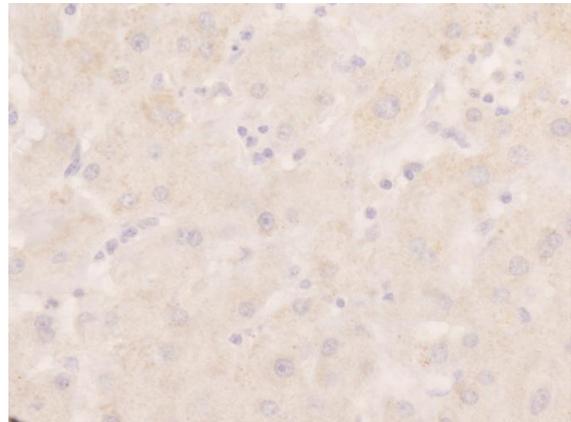
- 10) Add FITC-conjugated anti-rabbit IgG antibody diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
- 11) Wash the slide in a plenty of PBS as in the step 5).
- 12) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
- 13) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive controls for Immunocytochemistry; MEF and NRK)

liver carcinoma



normal liver



Immunohistochemical detection of p62 on paraffin embedded section of human liver carcinoma and normal liver with PM045.

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