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Anti-p62 C-terminal pAb

CODE No. PM066

CLONALITY Polyclonal
ISOTYPE Guinea pig Ig, affinity purified
QUANTITY 100 µL

SOURCE Purified Ig from guinea pig serum
IMMUNOGEN KLH conjugated human p62 C-terminus peptide
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 1:1,000
Immunoprecipitation 5 µL/300 µL of cell extract from 3 x 10^6 cells
Immunohistochemistry 1:100
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
Samples	HeLa, 293T	MEF, NIH/3T3, brain, liver, spleen, kidney	PC12	CHO
Reactivity	+	+	+	+

Entrez Gene ID 8878 (Human), 18412 (Mouse), 113894 (Rat), 100768352 (Hamster)

REFERENCES

1) Matsumoto, G., *et al.*, *Sci. Rep.* **8**, 9585 (2018) [IC]
2) De Pace, R., *et al.*, *PLoS Genet.* **14**, e1007363 (2018) [WB]
3) Takahashi, K., *et al.*, *Sci. Rep.* **7**, 2817 (2017) [WB, IHC]
4) Kimura, H., *et al.*, *JCI Insight* **2**, e89462 (2017) [IHC]
5) Komatsu, M., *et al.*, *Cell* **131**, 1149-1163 (2007)
6) Moscat, J., *et al.*, *Mol. Cell* **23**, 631-640 (2006)
7) Seibenhener, M. L., *et al.*, *Mol. Cell Biol.* **24**, 8055-8068 (2004)

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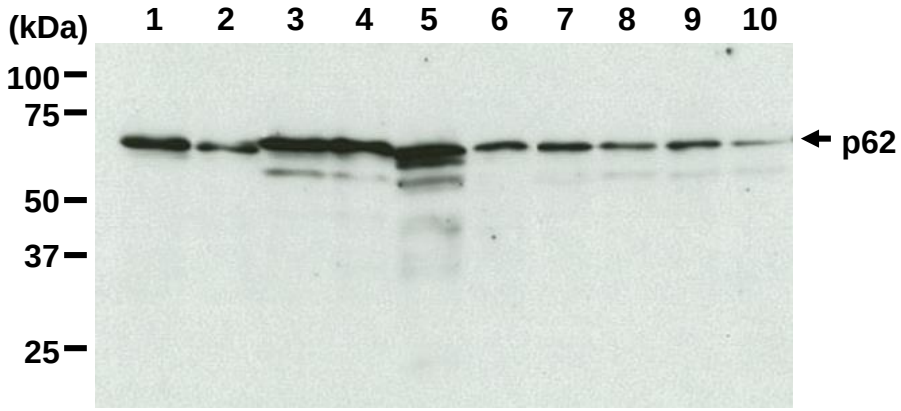
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

SDS-PAGE & Western blotting

1) Wash 1 x 10^7 cells 3 times with PBS and suspends them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 20 sec.).
2) Boil the samples for 3 min. and centrifuge. Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
3) 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.
4) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
5) Incubate the membrane with primary antibody diluted 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.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
7) Incubate the membrane with the 1:5,000 HRP-conjugated anti-guinea Pig IgG (Invitrogen; code no. 61-4620) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
8) Wash the membrane with PBS-T (5 min. x 3 times).
9) Wipe excess buffer on the membrane, and 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.
10) Expose to an X-ray film in a dark room for 1 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; HeLa, 293T, MEF, NIH/3T3, PC12, CHO, mouse brain, liver, spleen and kidney)



Western blot analysis of p62

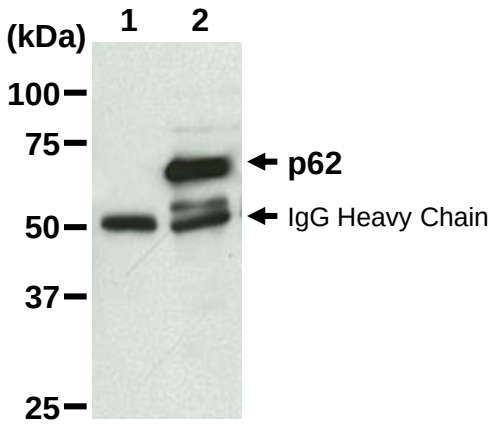
Lane 1: HeLa
Lane 2: 293T
Lane 3: MEF
Lane 4: NIH/3T3
Lane 5: PC12
Lane 6: CHO
Lane 7: Mouse brain
Lane 8: Mouse liver
Lane 9: Mouse spleen
Lane 10: Mouse kidney

Immunoblotted with Anti-p62 C-terminal pAb (PM066)

Immunoprecipitation

1) Wash 1 x 10^7 cells 2 times with PBS and resuspend them with 1 mL of ice-cold Lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40] containing appropriate protease inhibitors, 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) Mix 20 µL of 50% protein A agarose beads slurry resuspended in 300 µL of IP buffer [10 mM Tris-HCl (pH 8.0), 500 mM NaCl, 0.1% NP-40] with primary antibody as suggested in the **APPLICATIONS**. Incubate with gentle agitation for 1 hr. at room temperature.
4) Wash the beads 3 times with 1 mL of IP buffer.
5) Add 300 µL of cell lysate (prepared sample of step 2), then incubate with gentle agitation for 1 hr. at room temperature.
6) Wash the beads 5 times with 1 mL of Lysis buffer.
7) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3 min. and centrifuge.
8) Load 10 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) Incubate the membrane with 1:1,000 Anti-p62 (SQSTM1) pAb (MBL; code no. PM045) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
12) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
13) 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.
14) Wash the membrane with PBS-T (5 min. x 3 times).
15) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min.
16) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
17) Expose to an X-ray film in a dark room for 30 sec. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; NIH/3T3)



Immunoprecipitation of p62 from NIH/3T3

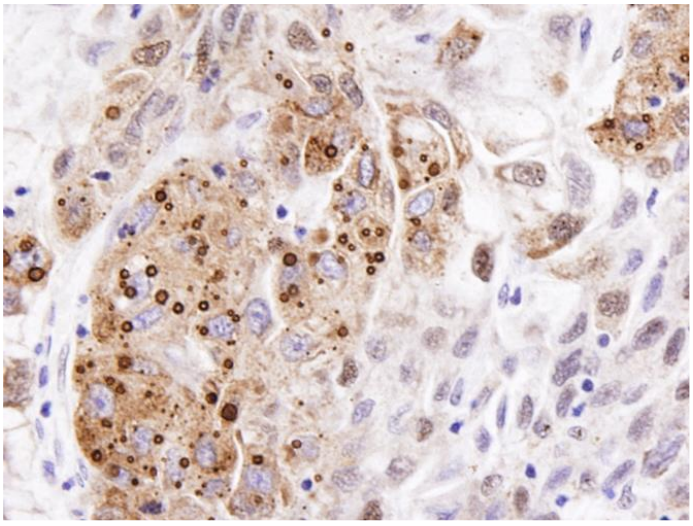
Lane 1: IP with isotype control (PM067)
Lane 2: IP with PM066

Immunoblotted with Anti-p62 (SQSTM1) pAb (MBL; code no. PM045)

Immunohistochemical staining for paraffin-embedded sections

1) Deparaffinize the sections with Xylene 3 times for 3-5 min. each.
2) Wash the slides with Ethanol 3 times for 3-5 min. each.
3) Wash the slides with PBS 3 times for 3-5 min. each.
4) Heat treatment
   Heat treatment by Microwave:
   Place the slides put on staining basket in 500 mL beaker with 500 mL of 100 mM citrate buffer (pH 6.3). Cover the beaker with plastic wrap, then process the slides 2 times for 10 min. each at 500 W with microwave oven. Let the slides cool down in the beaker at room temperature for about 40 min.
5) Remove the slides from the citrate buffer and cover each section with 3% H_2O_2 in PBS for 10 min. at room temperature to block endogenous peroxidase activity. Wash 2 times in PBS for 5 min. 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 min. at room temperature 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 hr. at room temperature.
9) Wash the slides 2 times in PBS for 5 min. each.
10) Wipe gently around each section and cover tissues with the 1:100 HRP-labeled anti-guinea pig IgG (DAKO; code no. P0141) diluted with blocking buffer. Incubate for 1 hour at room temperature. Wash as in step 9).
11) Visualize by reacting for 8 min. with DAB substrate solution (MBL; code no. 8469). *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
12) Wash the slides in water for 5 min.
13) 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. Dehydrate by immersing in Ethanol 3 times for 3 min. each, followed by immersing in Xylene 3 times for 3 min. each.
14) Now ready for mounting.

(Positive control for Immunohistochemistry; Human liver carcinoma)



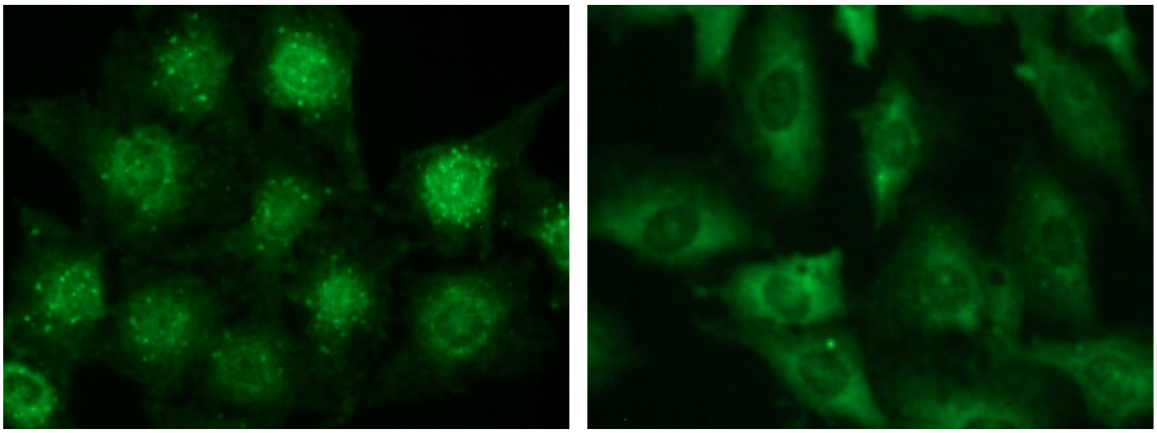
Immunohistochemical detection of p62 in human liver carcinoma

Brown: Anti-p62 C-terminal pAb (PM066)
Blue: Hematoxylin

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 hr. at 37°C.
4) Fix the cells by immersing the slide in 4% paraformaldehyde (PFA)/PBS for 10 min. 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 min. Take care not to touch the cells. Repeat another wash once more.
6) Immerse the slide in 100 μg/mL of Digitonin in PBS for 10 min. 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 hr. 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 Alexa Fluor®488 conjugated anti-guinea pig IgG (Invitrogen; code no. A11073) diluted with PBS onto the cells. Incubate for 30 min. 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; NRK)



Immunocytochemical detection of p62 in NRK

Left: Starved NRK
Right: Nutrient NRK

Green: Anti-p62 C-terminal pAb (PM066)