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Normal Guinea Pig IgG

CODE No.	PM067
CLONALITY	Polyclonal
QUANTITY	100 µL, 1 mg/mL
SOURCE	Purified IgG from normal guinea pig serum using protein A agarose.
REACTIVITY	No specific reaction was detected on immunoprecipitation and flow cytometry.
FORMULATION	1 mg/mL in PBS containing 50% glycerol. 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

Immunoprecipitation

Flow cytometry

This antibody can be used as a negative isotypic control.
The concentration will depend on the conditions.

APPLICATION-REPORTED

Immunohistochemistry Reference 1) and 2)

REFERENCES

1) Murai, N., *et al*., *PLoS One* **12**, e0186637 (2017) [IHC]
2) Yamane, T., *et al*., *PLoS One* **12**, e0176809 (2017) [WB, IHC]

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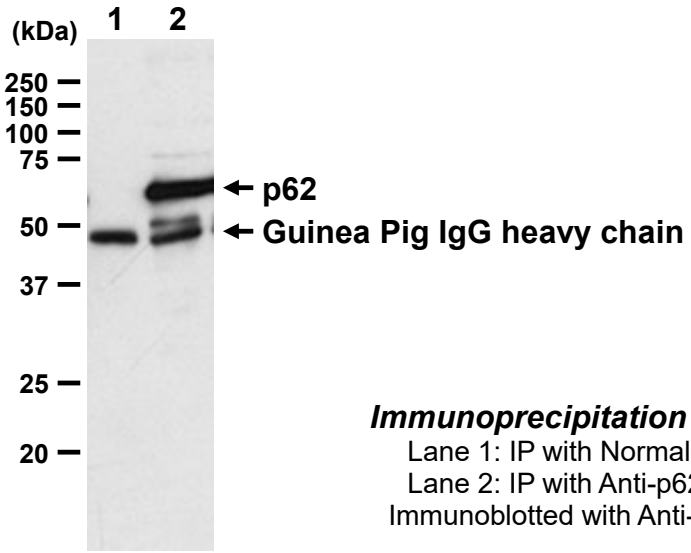
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

Immunoprecipitation

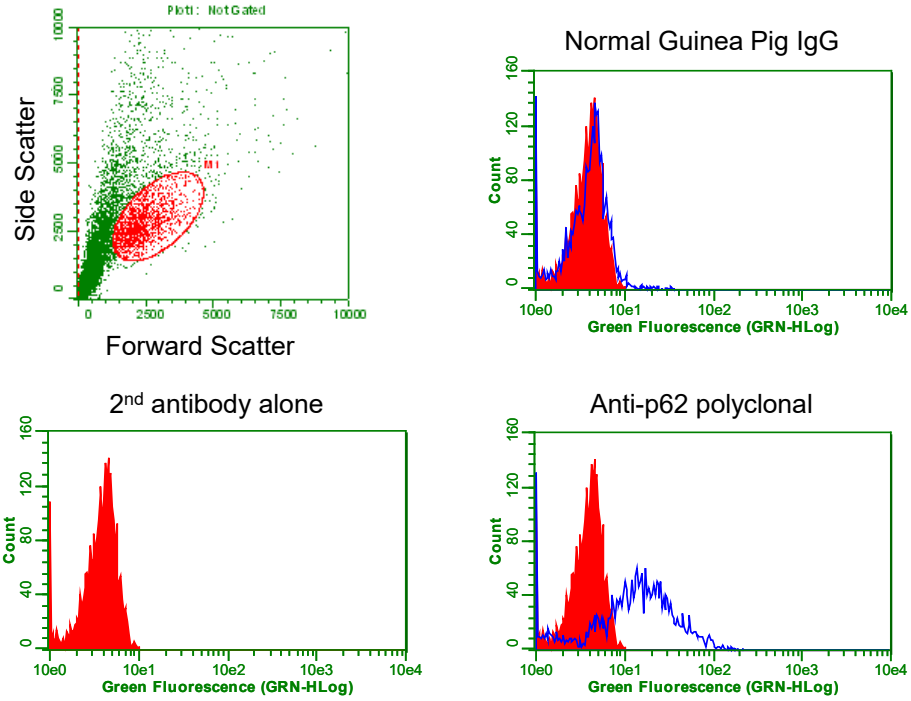
1) Wash 1 x 10^7 cells 2 times with PBS and resuspend them with 1 mL of ice-cold Lysis buffer [50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 0.05% NP-40] containing appropriate protease inhibitors. Incubate it on ice for 15 min., thereafter, sonicate briefly (up to 15 sec.).
2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
3) Add the isotype control antibody at the equal amount of the antibody for immunoprecipitation to the supernatant. Vortex briefly and incubate with gentle agitation for 60-120 min. at 4°C.
4) Mix 20 µL of 50% protein A agarose beads slurry resuspended in 400 µL of IP buffer [10 mM Tris-HCl (pH 8.0), 500 mM NaCl, 0.1% NP-40] with primary antibody. Incubate with gentle agitation for 1 hr. at room temperature.
5) Wash the beads 3 times with 1 mL of IP buffer.
6) Add 300 µL of cell lysate (prepared sample of step 2), then incubate with gentle agitation for 1 hr. at room temperature.
7) Centrifuge the tube at 2,500 x g for 10 sec. Carefully remove and discard the supernatant.
8) Resuspend the beads with 1 mL of Lysis buffer.
9) Centrifuge the tube at 2,500 x g for 10 sec. Carefully remove and discard the supernatant.
10) Repeat Steps 8)-9) 5 times.
11) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3 min. and centrifuge for 5 min.
12) Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel for electrophoresis.
13) 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.
14) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
15) Incubate the membrane with primary antibody 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.)
16) Wash the membrane with PBS (5 min. x 3 times).
17) Incubate the membrane with 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.
18) Wash the membrane with PBS (5 min. x 3 times).
19) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min.
20) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
21) 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.



Immunoprecipitation of mouse p62 from NIH/3T3 cells
Lane 1: IP with Normal Guinea Pig IgG (PM067)
Lane 2: IP with Anti-p62 C-terminal pAb (MBL; code no. PM066)
Immunoblotted with Anti-p62 (SQSTM1) pAb (MBL; code no. PM045)

Flow cytometric analysis for adherent cells

1) Detach the cells from culture dish.
2) Wash the cells 3 times with 1 mL of washing buffer [PBS containing 2% fetal calf serum (FCS)].
3) Add 200 µL of 4% paraformaldehyde (PFA) to the cell pellet after tapping. Mix well, then fix the cells for 10 min. at room temperature.
4) Wash the cells 2 times with 1 mL of washing buffer.
5) Add 200 µL of PBS containing 100 µg/mL Digitonin to the cell pellet after tapping. Mix well, then permeabilize the cells for 10 min. at room temperature.
6) Wash the cells 2 times with 1 mL of washing buffer.
7) Resuspend the cells with washing buffer (5 x 10^6 cells/mL).
8) Add 100 µL of the cell suspension into each tube, and centrifuge at 500 x g for 1 min. at room temperature (20~25°C). Remove the supernatant by careful aspiration.
9) Add 20 µL of Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 5 min. at room temperature.
10) Add the isotype control antibody at the concentrations comparable to those of the specific antibody of interest. Mix well and incubate for 30 min. at room temperature.
11) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 min. at room temperature. Remove supernatant by careful aspiration.
12) Add FITC-conjugated anti-Guinea Pig IgG antibody diluted with the washing buffer. Mix well and incubate for 30 min. at room temperature.
13) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 min. at room temperature. Remove supernatant by careful aspiration.
14) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.



Flow cytometric analysis of p62 in HeLa

Closed: Secondary antibody alone
Open: Normal Guinea Pig IgG (PM067) or Anti-p62 C-terminal pAb (MBL; code no. PM066)