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

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.
FORMURATION	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.

For more information, please visit our web site **https://ruo.mbl.co.jp/**

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
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RELATED PRODUCTS

Functional grade antibodies

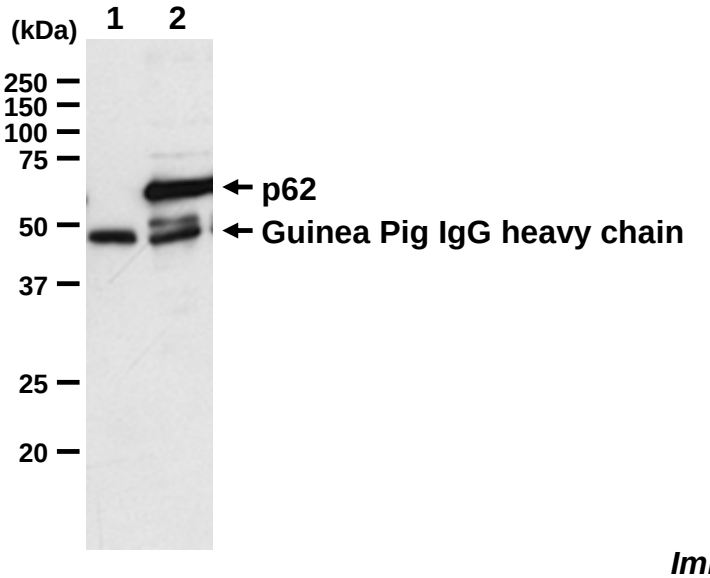
M075-3M2	Mouse IgG1 isotype control FG (2E12)
M076-3M2	Mouse IgG2a isotype control FG (6H3)
M077-3M2	Mouse IgG2b isotype control FG (3D12)
M078-3M2	Mouse IgG3 isotype control FG (6A3)
M080-3M2	Rat IgG1 isotype control FG (1H5)
M081-3M2	Rat IgG2a isotype control FG (2H3)
M090-3M2	Rat IgG2b isotype control FG (3G8)
M082-3M2	Rat IgG2c isotype control FG (6E12)

Purified antibodies

M075-3	Mouse IgG1 isotype control (2E12)
M075-4	Mouse IgG1 isotype control-FITC (2E12)
M075-5	Mouse IgG1 isotype control-PE (2E12)
M075-8	Mouse IgG1 isotype control-Agarose (2E12)
M075-A48	Mouse IgG1 isotype control-Alexa Fluor® 488 (2E12)
M075-A64	Mouse IgG1 isotype control-Alexa Fluor® 647 (2E12)
M076-3	Mouse IgG2a isotype control (6H3)
M076-4	Mouse IgG2a isotype control-FITC (6H3)
M076-5	Mouse IgG2a isotype control-PE (6H3)
M076-A48	Mouse IgG2a isotype control-Alexa Fluor® 488 (6H3)
M076-A64	Mouse IgG2a isotype control-Alexa Fluor® 647 (6H3)
M077-3	Mouse IgG2b isotype control (3D12)
M077-4	Mouse IgG2b isotype control-FITC (3D12)
M077-5	Mouse IgG2b isotype control-PE (3D12)
M077-A48	Mouse IgG2b isotype control-Alexa Fluor® 488 (3D12)
M077-A64	Mouse IgG2b isotype control-Alexa Fluor® 647 (3D12)
M078-3	Mouse IgG3 isotype control (6A3)
M078-4	Mouse IgG3 isotype control-FITC (6A3)
M079-3	Mouse IgM isotype control (7E10)
M080-3	Rat IgG1 isotype control (1H5)
M080-4	Rat IgG1 isotype control-FITC (1H5)
M080-5	Rat IgG1 isotype control-PE (1H5)
M080-A48	Rat IgG1 isotype control-Alexa Fluor® 488 (1H5)
M081-3	Rat IgG2a isotype control (2H3)
M081-4	Rat IgG2a isotype control-FITC (2H3)
M081-5	Rat IgG2a isotype control-PE (2H3)
M081-8	Rat IgG2a isotype control-Agarose (2H3)
M081-A48	Rat IgG2a isotype control-Alexa Fluor® 488 (2H3)
M090-3	Rat IgG2b isotype control (3G8)
M090-4	Rat IgG2b isotype control-FITC (3G8)
M090-5	Rat IgG2b isotype control-PE (3G8)
M090-A48	Rat IgG2b isotype control-Alexa Fluor® 488 (3G8)
M082-3	Rat IgG2c isotype control (6E12)
M082-4	Rat IgG2c isotype control-FITC (6E12)
M189-3	Hamster IgG isotype control (ttko)
PM035	Normal Rabbit IgG (polyclonal)
PM035-8	Normal Rabbit IgG-Agarose (polyclonal)

Immunoprecipitation

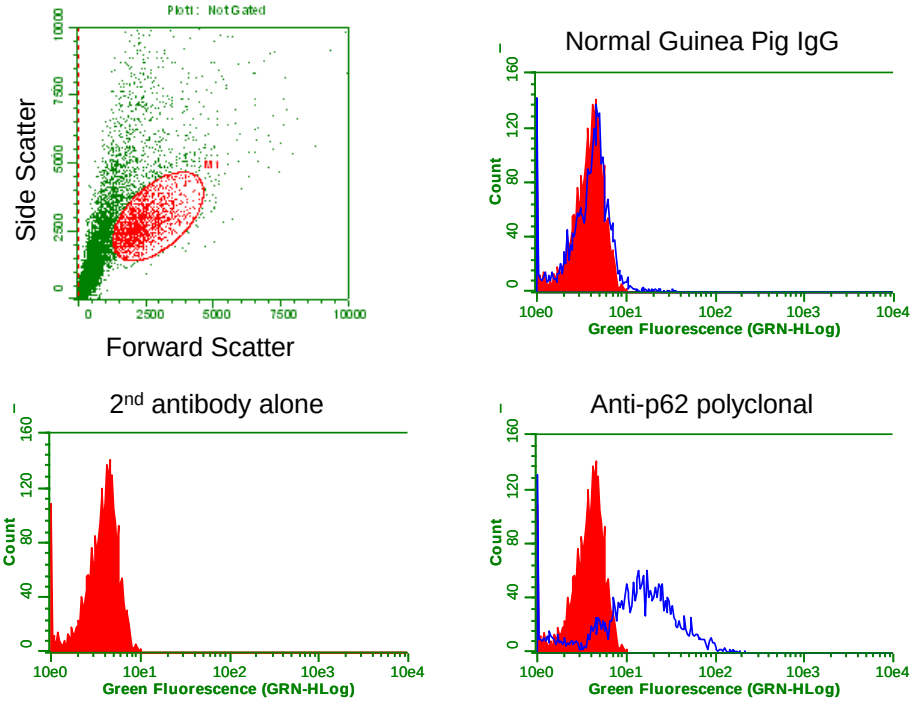
1) Wash 1 x 10^7 cells 2 times with PBS and resuspend them with 1 mL of ice-cold Lysis buffer (50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 0.05% NP-40) containing appropriate protease inhibitors. Incubate it on ice for 15 minutes, thereafter, sonicate briefly (up to 15 seconds).
2) Centrifuge the tube at 12,000 x g for 5 minutes 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 gently agitation for 60-120 minutes 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 gently agitation for 1 hour 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 hour at room temperature.
7) Wash the beads 6 times with 1 mL of Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
8) Resuspend the beads in 20 μL of Laemmli's sample buffer, boil for 3 minutes and centrifuge for 5 minutes.
9) Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel for electrophoresis.
10) 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.
11) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
12) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
13) Wash the membrane with PBS (5 minutes x 3 times).
14) Incubate the membrane with HRP-conjugated anti-rabbit IgG (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
15) Wash the membrane with PBS (5 minutes x 3 times).
16) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 minute.
17) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
18) Expose to an X-ray film in a dark room for 30 seconds. Develop the film as usual. The condition for exposure and development may vary.



Immunoprecipitation from NIH/3T3
Lane 1: IP with Normal Guinea Pig IgG (PM067)
Lane 2: IP with anti-p62 (PM066)
Immunoblotted with anti-p62 (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 minutes 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 minutes 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 minute at room temperature (20~25°C). Remove 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 minutes 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 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) Add 40 µL of 1:200 FITC conjugated anti-Guinea Pig IgG (Beckman coulter; code no. 732866) diluted with the washing buffer. Mix well and incubate for 30 minutes at room temperature.
13) 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.
14) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.



Flow cytometry on HeLa
Closed: Secondary antibody alone
Open: Normal Guinea Pig IgG (PM067) or anti-p62 (PM066)