

POLYCLONAL ANTIBODY

# Normal Rabbit IgG

Code No.	Quantity	Concentration	Form
PM035	100 $\mu$ L	5 mg/mL	Purified IgG

**SOURCE:** This antibody was purified from rabbit serum using protein A agarose.

**FORMULATION:** 500  $\mu$ g IgG in 100  $\mu$ L 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  $-20^{\circ}\text{C}$ .

**REACTIVITY:** No specific reaction was detected on Immunoprecipitation and Flow cytometry.

**APPLICATIONS:**

Immunoprecipitation:

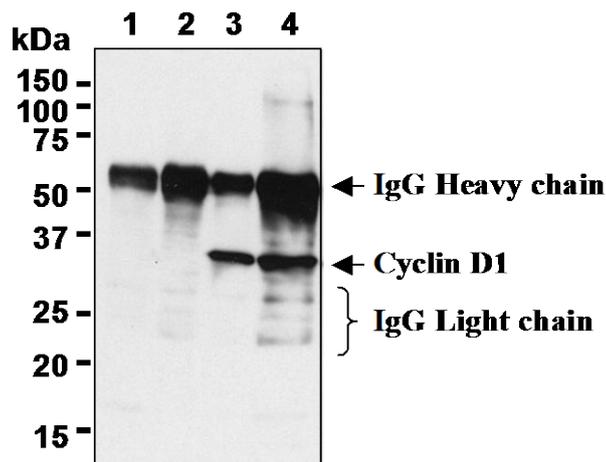
Flow cytometry:

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

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

**INTENDED USE:**

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

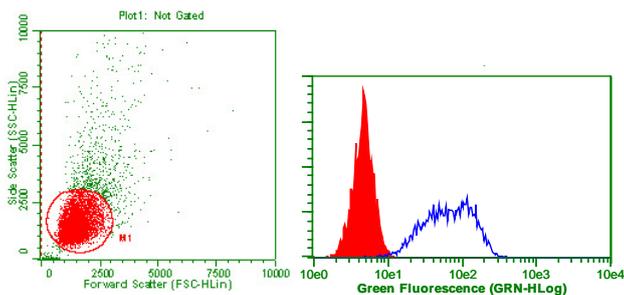


**Immunoprecipitation of Cyclin D1 from ZR-75-1 with PM035 (1  $\mu$ g: 1, 5  $\mu$ g:2) or anti-Cyclin D1 (Code no. 553, 1  $\mu$ g: 3, 5  $\mu$ g: 4). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with 553.**

**PROTOCOLS:**

**Immunoprecipitation**

- 1) Wash the cells (approximately  $1 \times 10^7$  cells) 3 times with PBS and suspend with 1 mL of cold Lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.05% NP-40) containing protease inhibitors at appropriate concentrations. Incubate it at  $4^{\circ}\text{C}$  with rotating for 30 minutes, thereafter, briefly sonicate the mixture (up to 10 seconds).
- 2) Centrifuge the tube at  $12,000 \times g$  for 10 minutes at  $4^{\circ}\text{C}$  and transfer the supernatant to another fresh 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  $4^{\circ}\text{C}$ .
- 4) Add 20  $\mu$ L of 50% protein A agarose beads into the tube. Mix well and incubate with gentle agitation for 30-60 minutes at  $4^{\circ}\text{C}$ .
- 5) Wash the beads 3-5 times with cold Lysis buffer (centrifuge the tube at  $2,500 \times g$  for 10 seconds).
- 6) Resuspend the beads in 30  $\mu$ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
- 7) Load 15  $\mu$ L of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
- 8) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at  $1 \text{ mA/cm}^2$  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.
- 9) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at  $4^{\circ}\text{C}$ .
- 10) 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.)
- 11) Wash the membrane with PBS (5 minutes  $\times$  3 times).
- 12) Incubate the membrane with HRP-conjugated secondary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 13) Wash the membrane with PBS (5 minutes  $\times$  3 times).
- 14) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 15) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.



**Flow cytometric analysis of Cyclin D1 (Code no.553) expression on ZR-75-1.** Open histograms indicate the reaction of 553 to the cells. Shaded histograms indicate the reaction of PM035 to the cells.

### Flow cytometric analysis for adherent cells

We usually use Fisher tubes or equivalents as reaction tubes for all steps described below.

- 1) Detach the cells from culture dish.
- 2) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.09% NaN<sub>3</sub>].  
\*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.
- 3) Resuspend the cells with washing buffer (5x10<sup>6</sup> cells/mL).
- 4) 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.
- 5) 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.
- 6) 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.
- 7) 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.
- 8) Add FITC conjugated anti-rabbit IgG antibody diluted with the washing buffer. Mix well and incubate for 30 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) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

### **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)

- M080-3M2 Rat IgG1 (isotype control) FG (1H5)
- M081-3M2 Rat IgG2a (isotype control) FG (2H3)
- M090-3M2 Rat IgG2b (isotype control) FG (3G8)

#### 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)
- M075-6 Mouse IgG1 (isotype control)-Biotin (2E12)
- M075-8 Mouse IgG1 (isotype control)-Agarose (2E12)
- M075-11 Mouse IgG1 (isotype control)-Magnetic Beads (2E12)
- M075-A48 Mouse IgG1 (isotype control)-Alexa Fluor<sup>®</sup> 488 (2E12)
- M075-A64 Mouse IgG1 (isotype control)-Alexa Fluor<sup>®</sup> 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)
- M076-6 Mouse IgG2a (isotype control)-Biotin (6H3)
- M076-11 Mouse IgG2a (isotype control)-Magnetic Beads (6H3)
- M076-A48 Mouse IgG2a (isotype control)-Alexa Fluor<sup>®</sup> 488 (6H3)
- M076-A64 Mouse IgG2a (isotype control)-Alexa Fluor<sup>®</sup> 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)
- M077-6 Mouse IgG2b (isotype control)-Biotin (3D12)
- M077-11 Mouse IgG2b (isotype control)-Magnetic Beads (3D12)
- M077-A48 Mouse IgG2b (isotype control)-Alexa Fluor<sup>®</sup> 488 (3D12)
- M077-A64 Mouse IgG2b (isotype control)-Alexa Fluor<sup>®</sup> 647 (3D12)
- M078-3 Mouse IgG3 (isotype control) (6A3)
- M078-4 Mouse IgG3 (isotype control)-FITC (6A3)
- M078-6 Mouse IgG3 (isotype control)-Biotin (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<sup>®</sup> 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)
- M081-11 Rat IgG2a (isotype control)-Magnetic Beads (2H3)
- M081-A48 Rat IgG2a (isotype control)-Alexa Fluor<sup>®</sup> 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<sup>®</sup> 488 (3G8)
- M090-A64 Rat IgG2b (isotype control)-Alexa Fluor<sup>®</sup> 647 (3G8)
- M082-3 Rat IgG2c (isotype control) (6E12)
- M082-4 Rat IgG2c (isotype control)-FITC (6E12)
- PM035 Normal Rabbit IgG (polyclonal)
- PM035-8 Normal Rabbit IgG-Agarose (polyclonal)
- PM067 Normal Guinea Pig IgG (polyclonal)
- M189-3 Syrian Hamster IgG (isotype control)
- M199-3 Armenian Hamster IgG (isotype control)
- PM084 Normal Chicken IgY (polyclonal)
- PM084-4 Normal Chicken IgY-FITC (polyclonal)
- PM089 Normal Sheep IgG
- PM094 Normal Goat IgG