

POLYCLONAL ANTIBODY

Normal Rabbit IgG

Code No.	Quantity	Concentration	Form
PM035	100 μ L	5 mg/mL	Purified IgG

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

FORMULATION: 500 μ g IgG in 100 μ 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°C .

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

APPLICATIONS:

Immunoprecipitation:

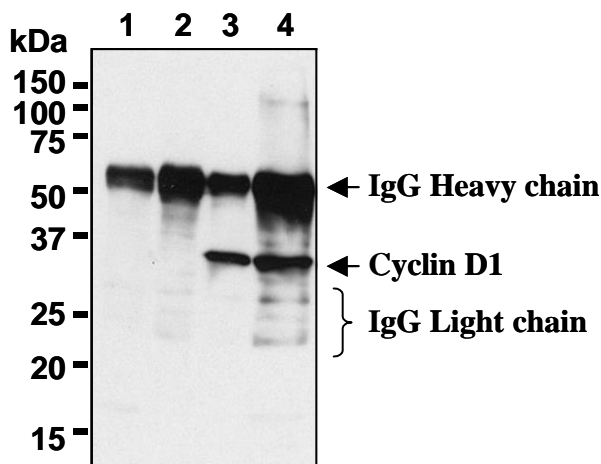
Flow cytometry:

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 μ g: 1, 5 μ g:2) or anti-Cyclin D1 (Code no. 553, 1 μ g: 3, 5 μ g: 4). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with 553.

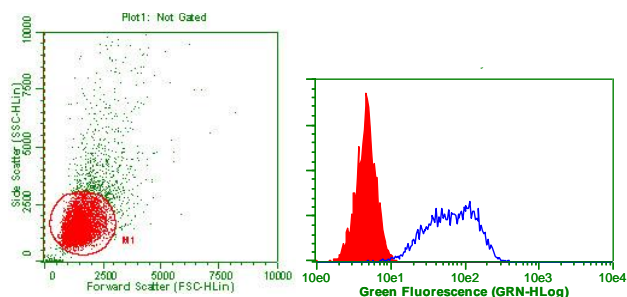
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOLS:

Immunoprecipitation

- 1) Wash the cells (approximately 1×10^7 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°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°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°C .
- 4) Add 20 μ L of 50% protein A agarose beads into the tube. Mix well and incubate with gentle agitation for 30-60 minutes at 4°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 μ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
- 7) Load 15 μ 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 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°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 x 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 x 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% NaN₃].
*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 (5x10⁶ 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.

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