

MONOCLONAL ANTIBODY

# Mouse IgG2b (isotype control)

Code No.	Clone	Subclass	Quantity	Concentration
M077-3	3D12	Mouse IgG2b $\kappa$	100 $\mu$ L	1 mg/mL

**SOURCE:** This antibody was purified from hybridoma (clone 3D12) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse lymphnodes immunized with KLH.

**FORMULATION:** 100  $\mu$ 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 binding is detected on human peripheral blood leukocytes.

## 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.

## PROTOCOLS:

### Flow cytometric analysis for floating cells

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

- 1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1%  $\text{NaN}_3$ ].
- 2) Resuspend the cells with washing buffer ( $5 \times 10^6$  cells/mL).
- 3) Add 50  $\mu$ L of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature ( $20\text{--}25^{\circ}\text{C}$ ). Remove supernatant by careful aspiration.
- 4) Add 20  $\mu$ L 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.
- 5) 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.

- 6) 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.
- 7) Add 20  $\mu$ L of 1:100 FITC conjugated anti-mouse IgG (MBL; code no. IM-0819) diluted with the washing buffer. Mix well and incubate for 20 minutes at room temperature.
- 8) 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.
- 9) Resuspend the cells with 500  $\mu$ L of the washing buffer and analyze by a flow cytometer.

### Flow cytometric analysis for whole blood cells

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

- 1) Add the isotype control antibody into each tube at the concentrations comparable to those of the specific antibody of interest.
- 2) Add 50  $\mu$ L of whole blood into each tube. Mix well and incubate for 30 minutes at room temperature ( $20\text{--}25^{\circ}\text{C}$ ).
- 3) Add 2 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 4) Add 20  $\mu$ L of secondary antibody 1:100 FITC conjugated anti-mouse IgG (MBL; code no. IM-0819) diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
- 5) Lyse with OptiLyse C (for analysis on Beckman Coulter instruments, MBL; code no. A11895) or OptiLyse B (for analysis on BD instruments, MBL; code no. IM-1400), using the procedure recommended in the respective package inserts.
- 6) Add 1 mL of  $\text{H}_2\text{O}$  to each tube and incubate for 10 minutes at room temperature.
- 7) Centrifuge at 500 x g for 1 minute at room temperature.
- 8) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 9) Resuspend the cells with 500  $\mu$ L of the washing buffer and analyze by a flow cytometer.

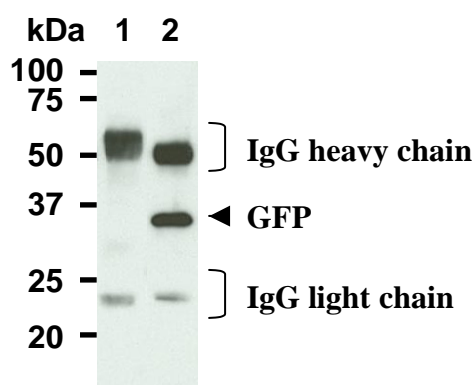
### Immunoprecipitation

- 1) Wash the cells (approximately  $1 \times 10^7$  cells) 3 times with PBS and suspend with 2 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 x 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 30-120 minutes at 4°C.
- 4) Add 20 µL 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 x g for 10 seconds).
- 6) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
- 7) Load 10 µL 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<sup>2</sup> 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 6 times).
- 12) Incubate the membrane with 1:10,000 anti-IgG (Mouse) pab-HRP (MBL; code no. 330) 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 6 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 3 minutes. Develop the film as usual. The condition for exposure and development may vary.

#### **RELATED PRODUCTS:**

Other related antibodies and kits are also available.  
Please visit our web site at <https://ruo.mbl.co.jp>.



***Immunoprecipitation from GFP expressed in 293T with mouse IgG2b isotype control, M077-3 (1) or anti-GFP, M048-3 (2). After immunoprecipitated with the antibody, immunocomplexes were resolved on SDS-PAGE and immunoblotted with M048-3.***