

 My select sampler set

## Mouse IgG3 (isotype control)

| Code No. | Clone | Subclass   | Quantity | Concentration |
|----------|-------|------------|----------|---------------|
| M078-3MS | 6A3   | Mouse IgG3 | 20 µL    | 1 mg/mL       |

**SOURCE:** This antibody was purified from hybridoma (clone 6A3) 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:** 20 µg IgG in 20 µL 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 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% NaN<sub>3</sub>].
- 2) Resuspend the cells with washing buffer (5x10<sup>6</sup> cells/mL).
- 3) Add 50 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.
- 4) 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.
- 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 µL 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 µL 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.

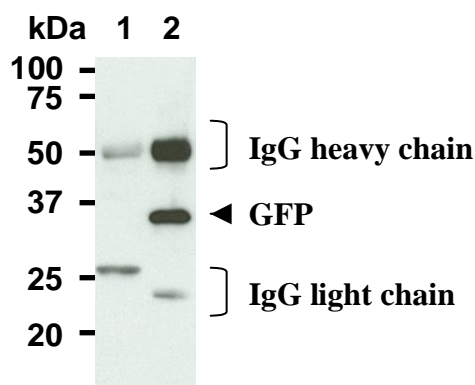
- 1) Add the isotype control antibody at the concentrations comparable to those of the specific antibody of interest.
- 2) Add 50 µL of whole blood into each tube. Mix well and incubate for 30 minutes at room temperature (20~25°C).
- 3) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 4) Add 20 µL 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 H<sub>2</sub>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 µL of the washing buffer and analyze by a flow cytometer.

#### Immunoprecipitation

- 1) Wash the cells (approximately 1x10<sup>7</sup> 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°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°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 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 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.



**Immunoprecipitation from GFP expressed in 293T with mouse IgG3 isotype control, M078-3 (1) or anti-GFP, M048-3 (2). After immunoprecipitated with the antibody, immunocomplexes were resolved on SDS-PAGE and immunoblotted with M048-3.**

## RELATED PRODUCTS:

### Functional grade antibodies

|          |                                      |
|----------|--------------------------------------|
| M075-3M2 | Mouse IgG1 (isotype control) (2E12)  |
| M076-3M2 | Mouse IgG2a (isotype control) (6H3)  |
| M077-3M2 | Mouse IgG2b (isotype control) (3D12) |
| M080-3M2 | Rat IgG1 (isotype control) (1H5)     |
| M081-3M2 | Rat IgG2a (isotype control) (2H3)    |
| M090-3M2 | Rat IgG2b (isotype control) (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)                            |
| M075-8   | Mouse IgG1 (isotype control)-Agarose (2E12)                       |
| M075-9   | 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)                            |
| M076-9   | 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)                             |
| M077-9   | 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)                           |
| M079-3   | Mouse IgM (isotype control) (7E10)                                |
| M194-3   | Human IgG1 isotype control chimeric mAb                           |
| M195-3   | Human IgG2 isotype control chimeric mAb                           |
| 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)                         |
| M081-9   | 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)    |
| 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)                            |

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