

MONOCLONAL ANTIBODY

Mouse IgG3 (isotype control)

Code No.	Clone	Subclass	Quantity	Concentration
M078-3	6A3	Mouse IgG3	100 μ L	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: 100 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 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.

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

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 0.09% NaN_3].
*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.
- 2) Resuspend the cells with washing buffer (5×10^6 cells/mL).
- 3) Add 50 μ L of the cell suspension into each tube, and centrifuge at $500 \times g$ for 1 minute at room temperature ($20\sim 25^{\circ}\text{C}$). Remove supernatant by careful aspiration.

- 4) Add 20 μ 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 washing buffer followed by centrifugation at $500 \times g$ for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 7) Add 20 μ L of FITC conjugated anti-mouse IgG antibody diluted with washing buffer. Mix well and incubate for 20 minutes at room temperature.
- 8) Add 1 mL of the washing buffer followed by centrifugation at $500 \times g$ for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 9) Resuspend the cells with 500 μ L of 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.

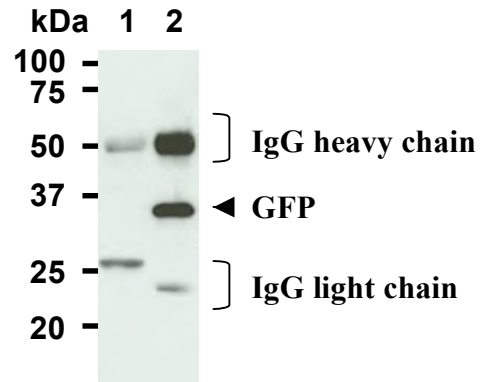
- 1) Add the isotype control antibody at the concentrations comparable to those of the specific antibody of interest.
- 2) Add 50 μ L of whole blood into each tube. Mix well and incubate for 30 minutes at room temperature ($20\sim 25^{\circ}\text{C}$).
- 3) Add 1 mL of the washing buffer followed by centrifugation at $500 \times g$ for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 4) Add 20 μ L of FITC conjugated secondary antibody diluted with 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_2O to each tube and incubate for 10 minutes at room temperature.
- 7) Centrifuge at $500 \times g$ for 1 minute at room temperature.
- 8) Add 1 mL of washing buffer followed by centrifugation at $500 \times g$ for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 9) Resuspend the cells with 500 μ L of washing buffer and analyze by a flow cytometer.

Immunoprecipitation

- 1) Wash the cells (approximately 1×10^7 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°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/cm² 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.

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