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

MONOCLONAL ANTIBODY

Mouse IgG3 (isotype control)

Code No.	Clone	Subclass	Quantity	Concentration
M078-3	6A3	Mouse IgG3	100 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: 100 µg IgG in 100 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:

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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 x 10^6 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 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 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 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
9) Resuspend the cells with 500 µL 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 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 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 minuites at room temperature.
7) Centrifuge at 500 x g for 1 minuite at room temperature.
8) Add 1 mL of washing buffer folled by centrifugation at 500 x g for 1 minuite at room temperature. Remove supernatant by careful aspiration.
9) Resuspend the cells with 500 µL of washing buffer and analyze by a flow cytometer.

Immunoprecipitation

1) Wash the cells (approximately 1x10^7 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.

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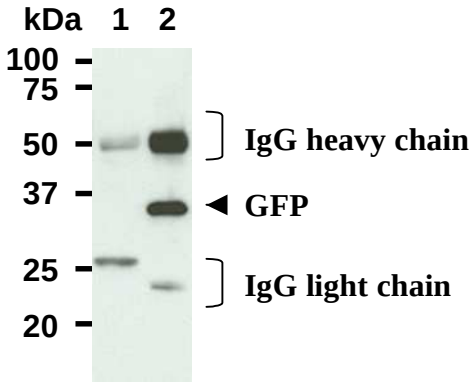
3) Add the isotype control antibody at the equal amount of the antibody for immunoprecipitation to the supernatant. Vortex briefly and incubate with gently agitation for 30-120 minutes at 4°C.
4) Add 20 µL of 50% protein A agarose beads into the tube. Mix well and incubate with gentle agitation for 30-60 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^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 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)
M078-3M2	Mouse IgG3 (isotype control) (6A3)
M079-3M2	Mouse IgM (isotype control) (7E10)
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-A48	Mouse IgG1 (isotype control)-Alexa Fluor® 488 (2E12)
M075-A64	Mouse IgG1 (isotype control)-Alexa Fluor® 647 (2E12)
M075-8	Mouse IgG1 (isotype control)-Agarose (2E12)
M075-11	Mouse IgG1 (isotype control)-Magnetic Beads (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-A48	Mouse IgG2a (isotype control)-Alexa Fluor® 488 (6H3)
M076-A64	Mouse IgG2a (isotype control)-Alexa Fluor® 647 (6H3)
M076-11	Mouse IgG2a (isotype control)-Magnetic Beads (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-A48	Mouse IgG2b (isotype control)-Alexa Fluor® 488 (3D12)
M077-A64	Mouse IgG2b (isotype control)-Alexa Fluor® 647 (3D12)
M077-11	Mouse IgG2b (isotype control)-Magnetic Beads (3D12)
M078-3	Mouse IgG3 (isotype control) (6A3)
M078-4	Mouse IgG3 (isotype control)-FITC (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® 488 (1H5)
M080-A64	Rat IgG1 (isotype control)-Alexa Fluor® 647 (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-A48	Rat IgG2a (isotype control)-Alexa Fluor® 488 (2H3)
M081-A64	Rat IgG2a (isotype control)-Alexa Fluor® 647 (2H3)
M081-8	Rat IgG2a (isotype control)-Agarose (2H3)
M081-11	Rat IgG2a (isotype control)-Magnetic Beads (2H3)
M082-3	Rat IgG2c (isotype control) (6E12)
M082-4	Rat IgG2c (isotype control)-FITC (6E12)
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® 488 (3G8)
M090-A64	Rat IgG2b (isotype control)-Alexa Fluor® 647 (3G8)
PM035	Normal Rabbit IgG (polyclonal)
M189-3	Syrian Hamster IgG (isotype control)
M199-3	Armenian Hamster IgG (isotype control)
PM084	Normal Chicken IgY (polyclonal)
PM089	Normal Sheep IgG (polyclonal)
PM094	Normal Goat IgG (polyclonal)