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

MONOCLONAL ANTIBODY

Rat IgG2c Isotype control

Code No.	Clone	Subclass	Quantity	Concentration
M082-3	6E12	Rat IgG2c	100 µL	1 mg/mL

SOURCE: This antibody was purified from hybridoma (clone 6E12) supernatant using protein G agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with rat lymph nodes 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 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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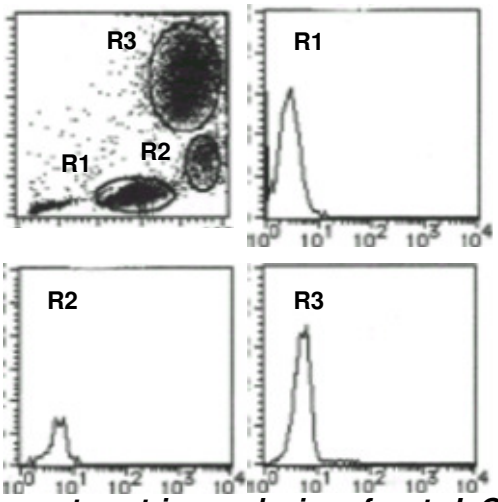

Flow cytometric analysis of rat IgG2c isotype control reactivity on lymphocyte (R1), monocyte (R2) and granulocyte (R3). *Open histograms indicate the reaction of M082-3 to the cells.*

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_3].
2) Resuspend the cells with washing buffer ($5x10^6$ 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 the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
7) Add 30 µL of 1:40 FITC conjugated anti-rat IgG (MBL; code no. IM-0827) 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 30 µL of secondary antibody 1:100 FITC conjugated anti-rat IgG (MBL; code no. IM-0827) 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 1ml of H_2O to each tube and incubate for 10 minuites at room temperature.

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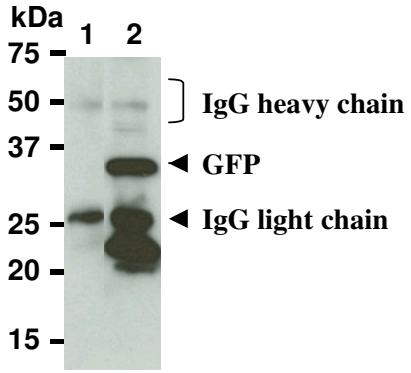
7) Centrifuge at 500 x g for 1 minuite at room temperature.
8) Add 1 mL of washing buffer folled by centrifugation at 500 x g for 1 minuite at room temperature. Remove supernatant by careful aspiration.
9) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.



Immunoprecipitation from GFP expressed in 293T with rat IgG2c isotype control, M082-3 (1) or anti-GFP, D153-3 (2). *After immunoprecipitated with the antibody, immunocomplexes were resolved on SDS-PAGE and immunoblotted with M048-3.*

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.
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 G 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.

RELATED PRODUCTS:

Functional grade antibody

M075-3M2	Mouse IgG1 isotype control FG (2E12)
M076-3M2	Mouse IgG2a isotype control FG (6H3)
M077-3M2	Mouse IgG2b isotype control FG (3D12)
M080-3M2	Rat IgG1 isotype control FG (1H5)
M081-3M2	Rat IgG2a isotype control FG (2H3)
M090-3M2	Rat IgG2b isotype control FG (3G8)

Purified antibody

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-A48	Mouse IgG1 isotype control-Alexa Fluor® 488 (2E12)
M075-A64	Mouse IgG1 isotype control-Alexa Fluor® 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-A48	Mouse IgG2a isotype control-Alexa Fluor® 488 (6H3)
M076-A64	Mouse IgG2a isotype control-Alexa Fluor® 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-A48	Mouse IgG2b isotype control-Alexa Fluor® 488 (3D12)
M077-A64	Mouse IgG2b isotype control-Alexa Fluor® 647 (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)
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-A48	Rat IgG2a isotype control-Alexa Fluor® 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® 488 (3G8)
M082-3	Rat IgG2c isotype control (6E12)
M082-4	Rat IgG2c isotype control-FITC (6E12)
PM035-8	Normal Rabbit IgG-Agarose (polyclonal)