

MONOCLONAL ANTIBODY

Mouse IgG2a (isotype control)-PE

Code No.	Clone	Subclass	Quantity	Concentration
M076-5	6H3	Mouse IgG2a κ	1 mL (50 tests)	10 μ g/mL

SOURCE: This antibody was purified from hybridoma (clone 6H3) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse lymph nodes immunized with KLH.

FORMULATION: 10 μ g IgG in 1 mL volume of PBS containing 1% BSA and 0.09% NaN₃.

*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.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at 4°C.

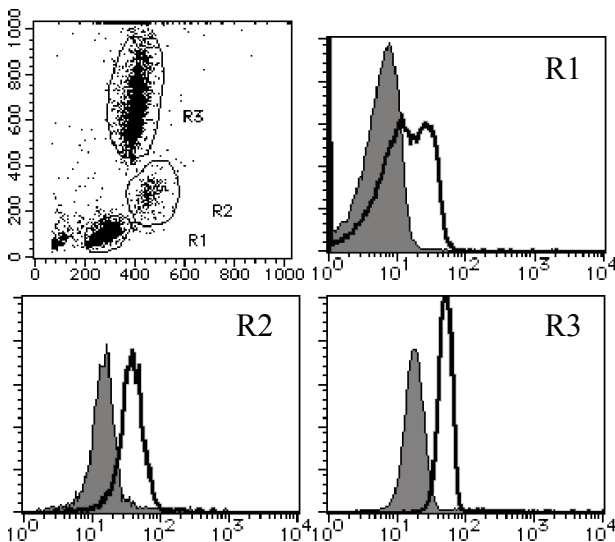
REACTIVITY: No specific binding is detected on human peripheral blood leukocytes.

APPLICATION:

Flow cytometry: 20 μ L (ready for use)

This antibody can be used as a negative isotypic control. The concentration is dependent on condition.

Detailed procedure is provided in the following **PROTOCOLS.**



Flow cytometric analysis of mouse IgG2a reactivity on lymphocyte (R1), monocyte (R2) and granulocyte (R3). Shaded histograms indicate the reaction of M076-5 to the cells. Open histograms indicate the reaction of PE labeled CD43 (D056-5) 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% NaN₃].
- 2) Resuspend the cells with washing buffer (5x10⁶ cells/mL).
- 3) Add 50 μ L 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 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 20 μ L of Mouse IgG2a (isotype control)-PE (M076-5) to the cell suspension. 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) Resuspend the cells with 500 μ 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 20 μ L of Mouse IgG2a (isotype control)-PE (M076-5) into each tube.
- 2) Add 100 μ L 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 [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN₃] followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 4) Lyse with OptiLyse C (for analysis on Beckman Coulter instruments) or OptiLyse B (for analysis on BD instruments), using the procedure recommended in the respective package inserts.
- 5) Add 1 mL of H₂O to each tube and incubate for 10 minutes at room temperature.
- 6) Centrifuge at 500 x g for 1 minute at room temperature.
- 7) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 8) Resuspend the cells with 500 μ L of the washing buffer and analyze by a flow cytometer.

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)
- 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)
- M075-8 Mouse IgG1 (isotype control)-Agarose (2E12)
- M075-9 Mouse IgG1 (isotype control)-Magnetic beads (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)
- M076-9 Mouse IgG2a (isotype control)-Magnetic beads (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)
- M077-9 Mouse IgG2b (isotype control)-Magnetic beads (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)
- 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[®] 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[®] 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 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>