

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% 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.

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

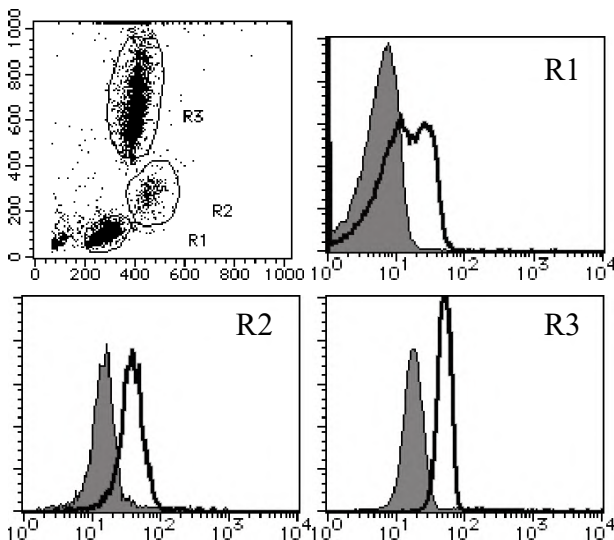
REACTIVITY: No specific binding is detected on human peripheral blood leukocytes.

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.

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^*].
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- 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 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 0.09% NaN_3^*] followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
*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.
- 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 H_2O 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.

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