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

MBL
A JSR Life Sciences Company

MONOCLONAL ANTIBODY

Mouse IgG1 (isotype control)-PE

Code No.	Clone	Subclass	Quantity	Concentration
M075-5	2E12	Mouse IgG1 κ	1 mL (50 tests)	10 μg/mL

SOURCE: This antibody was purified from hybridoma (clone 2E12) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse lymphocyte immunized with KLH.

FORMULATION: 10 μg 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 μL (ready for use)
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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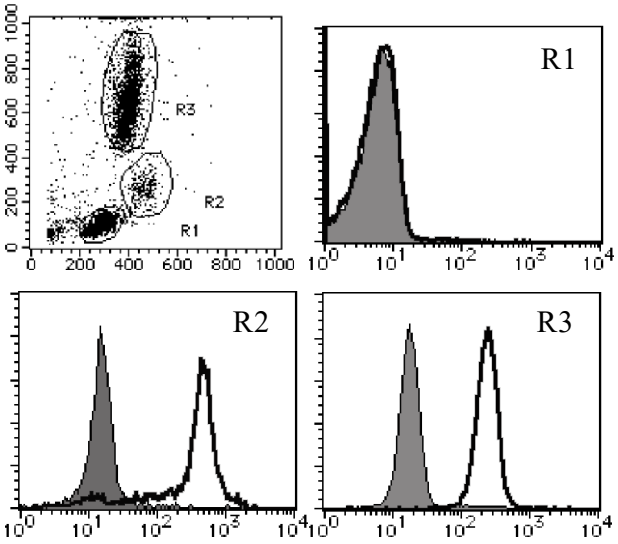
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

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 ($5x10^6$ 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 Mouse IgG1 (isotype control)-PE (MBL; code no. M075-5) as suggested in the **APPLICATION**. 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 μL of the washing buffer and analyze by a flow cytometer.



Flow cytometric analysis of mouse IgG1 reactivity on lymphocyte (R1), monocyte (R2) and granulocyte (R3). *Shaded histograms indicate the reaction of M075-5 to the cells. Open histograms indicate the reaction of PE labeled CD157/BST-1 (D036-5) to the cells.*

Flow cytometric analysis for whole blood cells

We usually use Falcon tubes or equivalents as reaction tubes for all steps described below.

1) Add Mouse IgG1 (isotype control)-PE (MBL; code no. M075-5) as suggested in the **APPLICATION** into each tube.
2) Add 100 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 [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.
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 µL of the washing buffer and analyze by a flow cytometer.

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