

MONOCLONAL ANTIBODY

# Mouse IgG1 (isotype control)-FITC

Code No.	Clone	Subclass	Quantity	Concentration
M075-4	2E12	Mouse IgG1 $\kappa$	1 mL	50 $\mu\text{g}/\text{mL}$

**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:** 50  $\mu\text{g}$  IgG in 1 mL volume of PBS containing 1% BSA and 0.1% ProClin 150.

**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 lymphocytes.

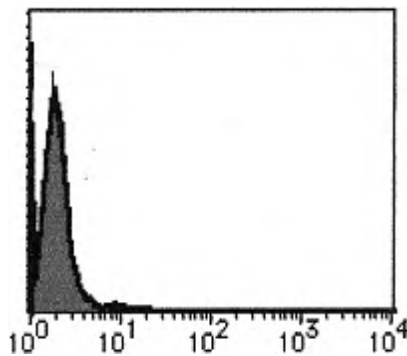
**APPLICATION:**

Flow cytometry: This antibody can be used as a negative isotypic control. The concentration will depend on condition.

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

**INTENDED USE:**

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



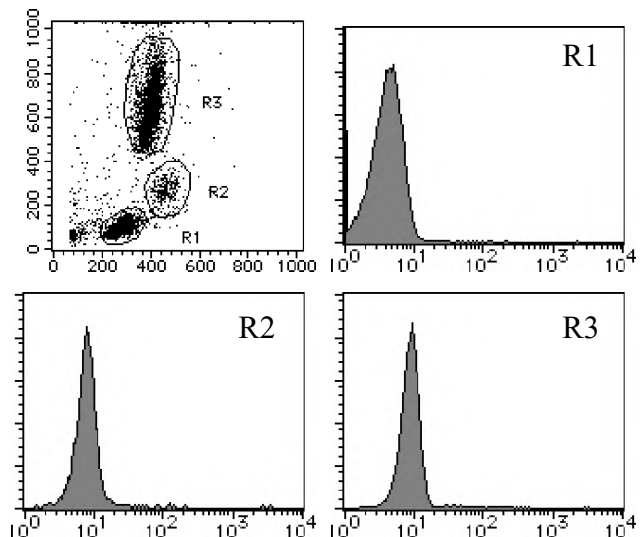
**Flow cytometric analysis of mouse IgG1 reactivity on THP-1.**

**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%  $\text{NaN}_3$ ].  
\*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 ( $5 \times 10^6$  cells/mL).
- 3) Add 50  $\mu\text{L}$  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  $\mu\text{L}$  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  $\mu\text{L}$  of Mouse IgG1 (isotype control)-FITC (M075-4) diluted with the washing buffer. 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  $\mu\text{L}$  of the washing buffer and analyze by a flow cytometer.



**Flow cytometric analysis of mouse IgG1 isotype control reactivity on lymphocyte (R1), monocyte (R2) and granulocyte (R3). Shaded histograms indicate the reaction of M075-4 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 20  $\mu$ L of Mouse IgG1 (isotype control)-FITC (M075-4) diluted with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN<sub>3</sub>] into each tube.
- 2) Add 100  $\mu$ 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 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<sub>2</sub>O 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  $\mu$ L of the washing buffer and analyze by a flow cytometer.

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