

MONOCLONAL ANTIBODY

FITC labeled Rat IgG2c Isotype control

Code No.	Clone	Subclass	Quantity	Concentration
M082-4	6E12	Rat IgG2c	50 µg	50 µg/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: 50 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 detected on human peripheral blood leukocytes.

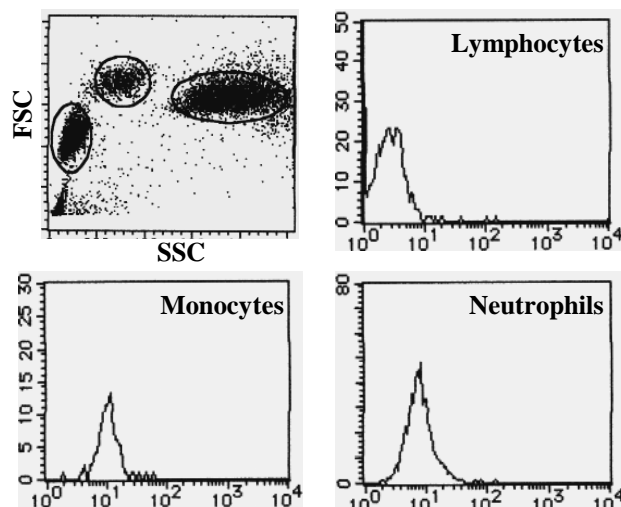
APPLICATION:

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 FITC labeled Rat IgG2c isotype control (M082-4) reactivity on human peripheral blood leukocytes.

PROTOCOLS:

Flow cytometric analysis for floating cells

We usually use Fisher tubes or equivalents as reaction tubes for all step 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 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 normal goat serum containing 1 mg/mL normal human IgG and 0.1% NaN₃ or 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 30 µL of the FITC labeled Rat IgG2c Isotype control diluted with the washing buffer. (The concentration will depend on condition.) 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 for whole blood cells

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

- 1) Add 20 µL of the FITC Labeled Rat IgG2c Isotype control diluted with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN₃] into each tube. (The concentration will depend on condition.)
- 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 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 H₂O to each tube and incubate for 10 minutes at room temperature.
- 6) Centrifuge at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 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:

- M075-3 Mouse IgG1 Isotype control (2E12)
- M075-4 FITC labeled Mouse IgG1 Isotype control (2E12)
- M075-8 Agarose conjugated Mouse IgG1 Isotype control (2E12)
- M076-3 Mouse IgG2a Isotype control (6H3)
- M076-4 FITC labeled Mouse IgG2a Isotype control (6H3)
- M077-3 Mouse IgG2b Isotype control (3D12)
- M077-4 FITC labeled Mouse IgG2b Isotype control (3D12)
- M078-3 Mouse IgG3 Isotype control (6A3)
- M078-4 FITC labeled Mouse IgG3 Isotype control (6A3)
- M079-3 Mouse IgM Isotype control (7E10)
- M080-3 Rat IgG1 Isotype control (1H5)
- M080-4 FITC labeled Rat IgG1 Isotype control (1H5)
- M081-3 Rat IgG2a Isotype control (2H3)
- M081-4 FITC labeled Rat IgG2a Isotype control (2H3)
- M081-8 Agarose conjugated Rat IgG2a Isotype control (2H3)
- M090-3 Rat IgG2b Isotype control (3G8)
- M090-4 FITC labeled Rat IgG2b Isotype control (3G8)
- M082-3 Rat IgG2c Isotype control (6E12)
- PM035-8 Agarose conjugated Normal Rabbit IgG (polyclonal)