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

MONOCLONAL ANTIBODY

Alexa Fluor® 647 labeled Mouse IgG2b isotype control

Code No.	Clone	Subclass	Quantity	Concentration
M077-A64	3D12	Mouse IgG2b κ	100 µL	1 mg/mL

SOURCE: This antibody was purified from hybridoma (clone 3D12) 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: 100 µg IgG in 100 µL 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 lymphocyte, monocyte and granulocyte.

APPLICATION:

Flow cytometry; This antibody can be used as a negative isotypic control. The concentration of antibody will depend on the conditions.

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

INTENDED USE:

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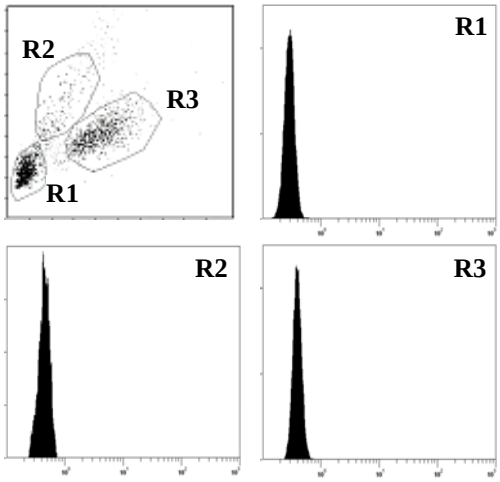

Flow cytometric analysis of mouse IgG2b reactivity on lymphocyte (R1), monocyte (R2) and granulocyte (R3).

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% NaN_3].
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 the isotype control antibody at the concentrations comparable to those of the specific antibody of interest. 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 steps described below.

1) Add the isotype control antibody into each tube at the concentrations comparable to those of the specific antibody of interest.
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 0.1% 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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e-mail support@mbl.co.jp, TEL 052-238-1904

RELATED PRODUCTS:

[Functional grade antibody]

M075-3M2	Mouse IgG1 isotype control FG (2E12)
M076-3M2	Mouse IgG2a isotype control FG (6H3)
M077-3M2	Mouse IgG2b isotype control FG (3D12)
M080-3M2	Rat IgG1 isotype control FG (1H5)
M081-3M2	Rat IgG2a isotype control FG (2H3)
M090-3M2	Rat IgG2b isotype control FG (3G8)

[Purified antibody]

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-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-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-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)
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-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)
PM035-8	Normal Rabbit IgG-Agarose (polyclonal)

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