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

MONOCLONAL ANTIBODY

Rat IgG2a (isotype control)-PE

Code No.	Clone	Subclass	Quantity	Concentration
M081-5	2H3	Rat IgG2a κ	1 mL (50 tests)	10 μg/mL

SOURCE: This antibody was purified from hybridoma (clone 2H3) 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 tests 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 detected on mouse splenocyte.

APPLICATION:
Flow cytometry; 20 μL (ready for use)
This antibody can be used as negative isotypic control. The concentration of antibody will depend on condition.

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

INTENDED USE:
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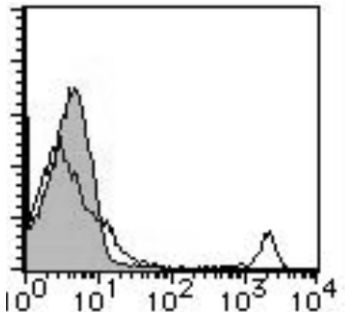

Flow cytometric analysis of PE labeled rat IgG2a on mouse splenocyte. Shaded histogram indicates the reaction of M081-5 to the cells. Open histogram indicates the reaction of mouse PE labeled CD8 to the cells.

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 0.1% 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 10 μL of normal goat serum containing 0.09% NaN_3 to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
5) Add 20 μL of the PE labeled Rat IgG2a Isotype control. Mix well and incubate for 20 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 Fisher tubes or equivalents as reaction tubes for all steps described below.
1) Add 20 μL of PE labeled Rat IgG2a Isotype control into each tube.
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 [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. 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 μL of the washing buffer and analyze by a flow cytometer.

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