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Not for use in diagnostic procedures.

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MONOCLONAL ANTIBODY

Rat IgG2a (isotype control)-Alexa Fluor® 488

Code No.	Clone	Subclass	Quantity	Concentration
M081-A48	2H3	Rat IgG2a	100 µg	1 mg/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: 100 µg IgG in 100 µL 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 mouse splenocytes.

APPLICATION:

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

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

INTENDED USE:

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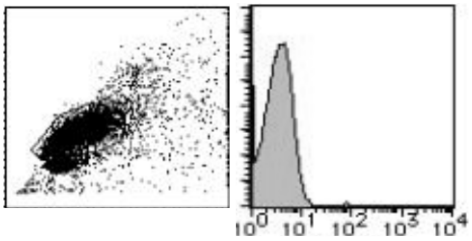

Flow cytometric analysis of rat IgG2a reactivity on mouse splenocytes.

The descriptions of the following protocols are examples. Each user should determine the appropriate condition

PROTOCOL:

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 ($6x10^6$ 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
4) (20~25°C). Remove supernatant by careful aspiration.
5) Add 10 µL of normal goat serum to the cell pellet after tapping. Mix well and incubate for 10 minutes at room temperature.
6) 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.
7) 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.
8) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

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