

MONOCLONAL ANTIBODY

# Mouse IgG2b (isotype control)-Alexa Fluor™ 647

Code No.	Clone	Subclass	Quantity	Concentration
M077-A64	3D12	Mouse IgG2b $\kappa$	100 $\mu$ L	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  $\mu$ g IgG in 100  $\mu$ L volume of PBS containing 1% BSA and 0.09%  $\text{NaN}_3$ .

\*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.

**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.

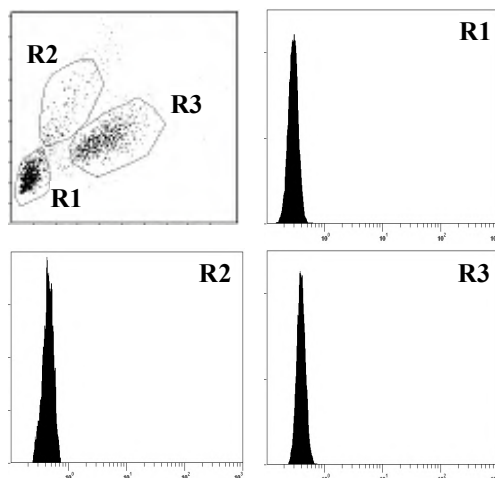
### APPLICATION:

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:

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



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

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%  $\text{NaN}_3$ ].
- 2) Resuspend the cells with washing buffer ( $5 \times 10^6$  cells/mL).
- 3) Add 50  $\mu$ 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  $\mu$ 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 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  $\mu$ L 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  $\mu$ 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 [PBS containing 2% fetal calf serum (FCS) and 0.1%  $\text{NaN}_3$ ] 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  $\text{H}_2\text{O}$  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.

**M077-A64**

**Lot 002~**

**Page 2**

8) Resuspend the cells with 500  $\mu$ L of the washing buffer and analyze by a flow cytometer.

**RELATED PRODUCTS:**

Please visit our website at <https://ruo.mbl.co.jp/>.

***LABEL LICENSES:***

*This product is provided under an agreement between LIFE TECHNOLOGIES Corporation, and Medical & Biological Laboratories Co., LTD.*

*Alexa Fluor™ is a trademark of LIFE TECHNOLOGIES Corporation.*