

MONOCLONAL ANTIBODY

# Rat IgG2a (isotype control)

Code No.	Clone	Subclass	Quantity	Concentration
M081-3	2H3	Rat IgG2a	100 $\mu$ L	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  $\mu$ g IgG in 100  $\mu$ L volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

**STORAGE:** This antibody solution is stable for one year from the date of purchase when stored at  $-20^{\circ}\text{C}$ .

**REACTIVITY:** No specific binding detected on human peripheral blood leukocytes.

## APPLICATIONS:

Immunoprecipitation;

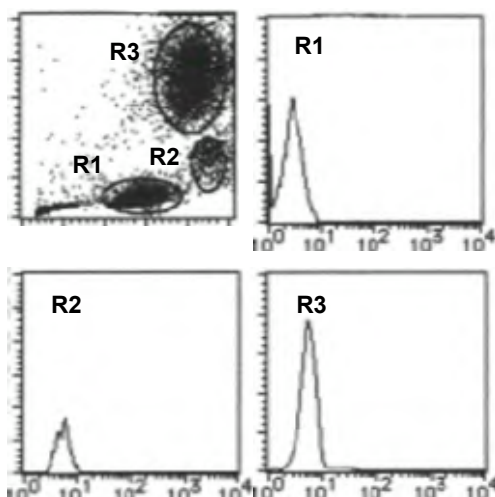
Flow cytometry;

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

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

## INTENDED USE:

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



**Flow cytometric analysis of rat IgG2a isotype control reactivity on lymphocyte (R1), monocyte (R2) and granulocyte (R3). Open histograms indicate the reaction of M081-3 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 0.09%  $\text{NaN}_3$ ].  
\*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.
- 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  $500 \times g$  for 1 minute at room temperature ( $20 \sim 25^{\circ}\text{C}$ ). 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  $500 \times g$  for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 7) Add FITC conjugated anti-rat IgG antibody diluted with the washing buffer. Mix well and incubate for 20 minutes at room temperature.
- 8) Add 1 mL of the washing buffer followed by centrifugation at  $500 \times g$  for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 9) Resuspend the cells with 500  $\mu$ L of the washing buffer and analyze by a flow cytometer.

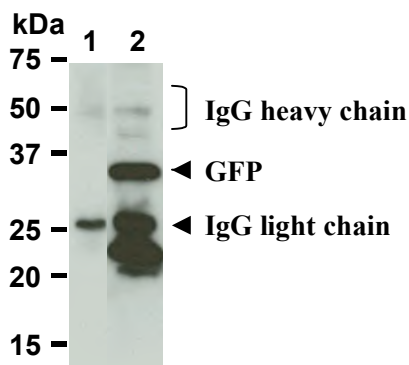
### Flow cytometric analysis for whole blood cells

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

- 1) Add the isotype control antibody at the concentrations comparable to those of the specific antibody of interest.
- 2) Add 50  $\mu$ L of whole blood into each tube. Mix well and incubate for 30 minutes at room temperature ( $20 \sim 25^{\circ}\text{C}$ ).
- 3) Add 1 mL of the washing buffer followed by centrifugation at  $500 \times g$  for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 4) Add secondary antibody diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
- 5) Lyse with OptiLyse C (for analysis on Beckman Coulter instruments, MBL; code no. A11895) or OptiLyse B (for analysis on BD instruments, MBL; code no. IM-1400),

using the procedure recommended in the respective package inserts.

- 6) Add 1 mL of H<sub>2</sub>O to each tube and incubate for 10 minutes at room temperature.
- 7) Centrifuge at 500 x g for 1 minute at room temperature.
- 8) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 9) Resuspend the cells with 500  $\mu$ L of the washing buffer and analyze by a flow cytometer.



**Immunoprecipitation from GFP expressed in 293T with rat IgG2a isotype control, M081-3 (1) or anti-GFP, D153-3 (2). After immunoprecipitated with the antibody, immunocomplexes were resolved on SDS-PAGE and immunoblotted with M048-3.**

### **Immunoprecipitation**

- 1) Wash the cells (approximately  $1 \times 10^7$  cells) 3 times with PBS and suspend with 2 mL of cold Lysis buffer [50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 0.05% NP-40] containing protease inhibitors at appropriate concentrations. Incubate it at 4°C with rotating for 30 minutes, thereafter, briefly sonicate the mixture (up to 10 seconds).
- 2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another fresh tube.
- 3) Add the isotype control antibody at the equal amount of the antibody for immunoprecipitation to the supernatant. Vortex briefly and incubate with gentle agitation for 30-120 minutes at 4°C.
- 4) Add 20  $\mu$ L of 50% protein G agarose beads into the tube. Mix well and incubate with gentle agitation for 30-60 minutes at 4°C.
- 5) Wash the beads 3-5 times with cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
- 6) Resuspend the beads in 20  $\mu$ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
- 7) Load 10  $\mu$ L of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
- 8) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm<sup>2</sup> for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer's manual for precise transfer procedure.
- 9) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room

temperature, or overnight at 4°C.

- 10) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
- 11) Wash the membrane with PBS (5 minutes x 6 times).
- 12) Incubate the membrane with HRP-conjugated secondary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 13) Wash the membrane with PBS (5 minutes x 6 times).
- 14) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 15) Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The condition for exposure and development may vary.

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