

MONOCLONAL ANTIBODY

Mouse IgM (isotype control)

Code No.	Clone	Subclass	Quantity	Concentration
M079-3	7E10	Mouse IgM	100 μ L	1 mg/mL

SOURCE: This antibody was purified from hybridoma (clone 7E10) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse lymphnodes immunized with KLH.

FORMULATION: 100 μ g IgM in 100 μ 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°C .

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

APPLICATIONS:

Flow cytometry; This antibody can be used as a negative isotypic control. The concentration to be used will be dependent on condition.

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

INTENDED USE:

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

PROTOCOLS:

Flow cytometric analysis for floating cells

We usually use Fisher tubes or equivalents as reaction tubes for all step 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 (5×10^6 cells/mL).
- 3) Add 50 μ L of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature ($20\sim 25^{\circ}\text{C}$). Remove supernatant by careful aspiration.
- 4) Add 10 μ L of normal goat serum containing 1 mg/mL normal human IgG and 0.1% NaN_3 to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
- 5) Add 40 μ L of the mouse IgM isotype control (25 μ g/mL) diluted with the washing buffer. 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) Add 20 μ L of 1:100 FITC conjugated anti-mouse IgM (MBL; code no. IM-0821) 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 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 9) Resuspend the cells with 500 μ 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 step described below.

- 1) Add 50 μ L of the mouse IgM isotype control (20 μ g/mL) diluted with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN_3] into each tube.
- 2) Add 50 μ 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 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 4) Add 20 μ L of secondary antibody 1:100 FITC conjugated anti-mouse IgM (MBL; code no. IM-0821) 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) or OptiLyse B (for analysis on BD instruments), using the procedure recommended in the respective package inserts.
- 6) Add 1 mL of H_2O 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 μ L of the washing buffer and analyze by a flow cytometer.

RELATED PRODUCTS:

Functional grade antibodies

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

Purified antibodies

- 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)
- M075-9 Mouse IgG1 (isotype control)-Magnetic beads (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)
- M076-9 Mouse IgG2a (isotype control)-Magnetic beads (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)
- M077-9 Mouse IgG2b (isotype control)-Magnetic beads (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)
- M194-3 Human IgG1 isotype control chimeric mAb
- M195-3 Human IgG2 isotype control chimeric mAb
- 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)
- M081-9 Rat IgG2a (isotype control)-Magnetic beads (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 Normal Rabbit IgG (polyclonal)
- PM035-8 Normal Rabbit IgG-Agarose (polyclonal)
- PM067 Normal Guinea Pig IgG (polyclonal)
- M189-3 Syrian Hamster IgG (isotype control)
- M199-3 Armenian Hamster IgG (isotype control)

Other related antibodies and kits are also available.

Please visit our web site at <http://ruo.mbl.co.jp>