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MONOCLONAL ANTIBODY

Mouse IgM (isotype control)

Code No.	Clone	Subclass	Quantity	Concentration
M079-3	7E10	Mouse IgM	100 µL	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 µg IgM in 100 µL 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.

APPLICATION:

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:

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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 0.09% 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.
2) Resuspend the cells with washing buffer (5×10^6 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 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 40 µL of the mouse IgM isotype control (25 µg/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 µL of 1:100 FITC conjugated anti-mouse IgM (Beckman coulter; code no. IM0821) 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 µL 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 µL of the mouse IgM isotype control (20 µg/mL) diluted with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.09% NaN_3] 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 the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
4) Add 20 µL of secondary antibody 1:100 FITC conjugated anti-mouse IgM (Beckman coulter; code no. IM0821) 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 µL of the washing buffer and analyze by a flow cytometer.

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