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

MONOCLONAL ANTIBODY

FITC labeled Mouse CD170/Siglec-5

Code No.	Clone	Subclass	Quantity	Concentration
M096-4	8D2	Rat IgG2b	1 mL	50 μg/mL

BACKGROUND: CD170 (Siglec-5/Sigle-E) is a putative adhesion molecule that mediates sialic-acid dependent binding to cells. CD170 is expressed on neutrophil and monocyte populations, both in the blood and bone marrow, as a dimeric, disulfide linked, 140 kDa type I membrane protein containing cytoplasmic immune receptor-based tyrosine signalling motifs. CD170 recruits the SH2 domain-containing protein tyrosine phosphatases SHP-1 and SHP-2, which block signal transduction through dephosphorylation of signaling molecules. Thus CD170 acts as an inhibitory receptor for ligand induced tyrosine phosphorylation.

SOURCE: This antibody was purified from hybridoma (clone 8D2) supernatant using protein G agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Wister rat lymphnode immunized with mouse CD170 transfected L cell.

FORMULATION: 50 μg IgG in 1 mL volume of PBS containing 1% BSA 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 azaide into drain.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at 4°C.

REACTIVITY: This antibody reacts with mouse CD170 antigen on Flow cytometry.

APPLICATION:
Flow cytometry; 25 μg/mL (final concentration)
*Please refer to the data sheet (MBL code no. M096-3) for other applications.

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	Not Tested	splenocyte	Not Tested
Reactivity on FCM		+	

INTENDED USE:
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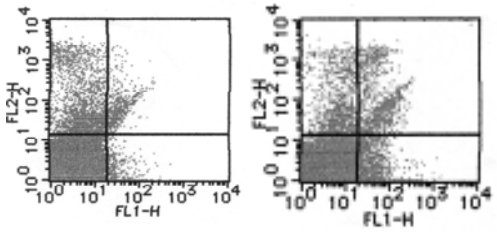
REFERENCE:
1) Zhang, J. Q., *et al., Eur. J. Immunol*. **34**, 1175-1184 (2004)

RELATED PRODUCT:
M096-3 mouse CD170/Siglec-5 (8D2)



Flow cytometric analysis mouse CD170 expression on mouse splenocytes. *The staining intensity of rat IgG2b at 25 μg/mL (left) or M096-4 at 25 μg/mL (right) are shown in the horizontal axis with CD11c staining on the vertical axis.*

PROTOCOL:
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 ($5x10^6$ 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 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 10 μL of FITC labeled anti-mouse CD170 monoclonal antibody (8D2). Add 20 μL of 1:50 PE conjugated anti-mouse CD11c (Pharmingen: code no. 557401) diluted with the washing buffer. Mix well and incubate for 30 minutes at room temperature.

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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 μL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; mouse splenocyte)