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

MONOCLONAL ANTIBODY

Mouse CD170/Siglec-5

Code No.	Clone	Subclass	Quantity	Concentration
M096-3	8D2	Rat IgG2b	100 µg	1 mg/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: 100 µg IgG 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: This antibody reacts with mouse CD170 antigen on Flow cytometry.

APPLICATIONS:

Western blotting; Not recommended
Immunoprecipitation; 2 µg/300 µL of cell extract from $5x10^6$ cells
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 5-10 µg/mL (final concentration)

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

SPECIES CROSS REACTIVITY:

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

INTENDED USE:

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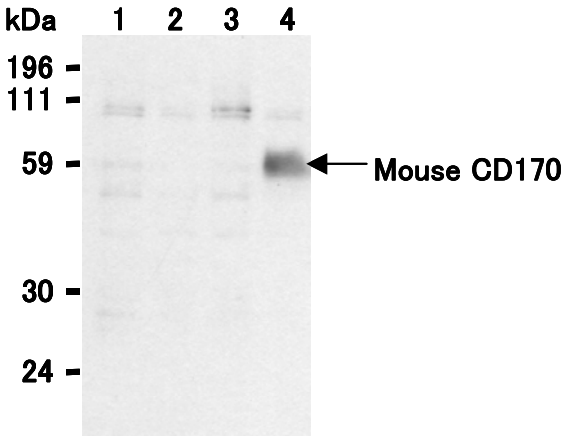
REFERENCE:

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

RELATED PRODUCT:

M096-4 FITC labeled mouse CD170/Siglec-5 (8D2)



Immunoprecipitation of mouse CD170 from 293T cells (1 and 3) and mouse CD170 transfected cells (2 and 4) with rat IgG2b (1 and 2) or M096-3 (3 and 4). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with HRP-Streptavidin.*

PROTOCOLS:

Immunoprecipitation

1) Wash the Biotin labeled transfectant cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (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 tube.
3) Add primary antibody as suggest in the **APPLICATIONS** into 300 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 30 µL of 50% protein G agarose beads resuspended in the cold Lysis buffer. Mix well and

incubate with gentle agitation for 60 minutes at 4°C.

4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
5) Resuspend the beads in 20 μL of Laemmli's sample buffer and boil the samples for 3 minutes and centrifuge. Load 10 μL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
6) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
7) 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.
8) Incubate the membrane with the 1:10,000 HRP-conjugated streptavidin (MBL; code no. IM-0309) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
9) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 6 times).
10) 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.
11) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

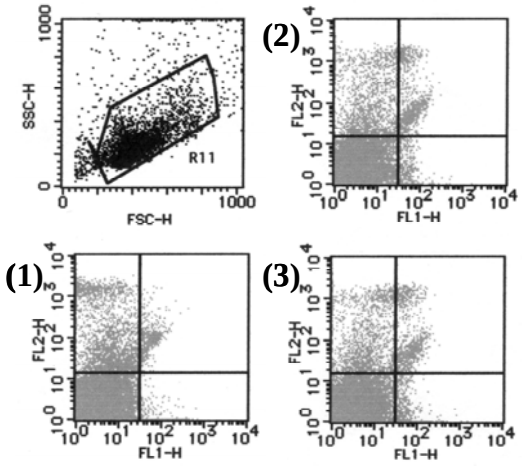
(Positive control for Immunoprecipitation; transfectant)

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 40 μL of the mouse CD170 monoclonal antibody (8D2) as suggest in **APPLICATIONS** 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-rat IgG (MBL; code no. IM-0827) diluted with the washing buffer. Mix well and incubate for 15 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) 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 15 minutes at room temperature.
10) 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.
11) Resuspend the cells with 500 μL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; mouse splenocyte)



Flow cytometric analysis mouse CD170 expression on mouse splenocytes. *The staining intensity of rat IgG2b at 10 μg/mL (1) or M096-3 at 5 μg/mL (2) and 10 μg/mL (3) are shown in the horizontal axis with CD11c staining on the vertical axis.*