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

MONOCLONAL ANTIBODY

Anti-CX_3CR1 (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D070-3	2A9-1	Rat IgG2b κ	100 µL	1 mg/mL

BACKGROUND: There are several subfamilies in the chemokine superfamily. In addition to CXC, CC, and C subfamily, Fractalkine (FKN), which has the novel CX_3C chemokine motif and the mucin-like domain, has recently been identified and reported. This mucin-chemokine hybrid type of protein can exist in two forms; either membrane-bound form or soluble secreted form. The membrane-bound form of FKN protein is markedly induced on primary endothelial cells by inflammatory cytokines, and it promotes strong adhesion of NK cells and $CD8^+$ T cells. The soluble secreted form of FKN can be released, presumably by proteolysis at a membrane-proximal dibasic cleavage site, and has chemotactic activity for these leukocytes.

CX_3CR1, which is recently identified FKN receptor, is also G-protein-coupled seven-transmembrane receptor as another chemokine receptor families, and is expressed on the cell surface of NK cells and $CD8^+$ T cells. It is also reported that CX_3CR1 and FKN mediate both leukocytes migration and adhesion.

SOURCE: This antibody was purified from hybridoma (clone 2A9-1) supernatant using protein G agarose. This hybridoma was established by fusion of mouse myeloma P3U1 with WKY/NCrj lymph nodes immunized with non-mammalian cells expressing human CX_3CR1 protein.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol and 0.5 M NaCl, 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 human CX_3CR1 on Flow cytometry.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Transfectant, Peripheral blood lymphocyte	Not tested	Not tested
Reactivity on FCM	+		

INTENDED USE:
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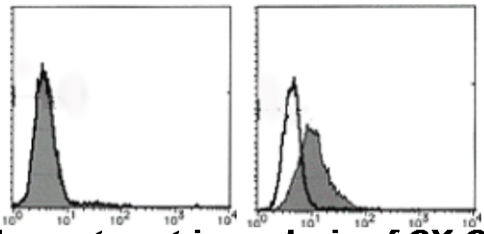
APPLICATIONS:
Western blotting; Not tested
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunofluorescence; Not tested*
*It is reported that this antibody can be used in this application in the reference number 2).
Immunocytochemistry; Not tested
Flow cytometry; 5 µg/mL (final concentration)
Function; Not tested*
*It is reported that this antibody can be used as a blocking antibody in the reference number 1), 3), 5) and 6).

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

REFERENCES:
1) Johnson, S. M., *et al.*, *PLoS Pathog.* **11**, e1005318 (2015) [Function]
2) Stolla, M., *et al.*, *PLoS One* **7**, e43572 (2012) [IF]
3) Aspinall, A. I., *et al.*, *Hepatology* **51**, 2030-2039 (2010) [Function]
4) Harcourt, J., *et al.*, *J. Immunol.* **176**, 1600-1608 (2006) [FCM]
5) Lee, R. H., *et al.*, *Blood* **107**, 2153-2161 (2006) [Function]
6) Tripp, R. A., *et al.*, *J. Virol.* **77**, 6580-6584 (2003) [Function]
7) Imai, T., *et al.*, *Cell* **91**, 521-530 (1997)
8) Bazan, J. F., *et al.*, *Nature* **385**, 640-644 (1997)
9) Rollins, B. J., *Blood* **90**, 909-928 (1997)
10) Premack, B. A., *et al.*, *Nat. Med.* **2**, 1174-1178 (1996)
11) Baggiolini, M., *et al.*, *Adv. Immunol.* **55**: 97-179 (1994)
12) Miller, M. D., *et al.*, *Crit. Rev. Immunol.* **12**, 17-46 (1992)
13) Oppenheim, J. J., *et al.*, *Annu. Rev. Immunol.* **9**, 617-648 (1991)

Clone 2A9-1 is used in reference 1)-7).



Flow cytometric analysis of CX_3CR1 expression on K562 (left) and CX_3CR1 transfected K562 (right). Open histogram indicates the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D070-3 to the cells.

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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 ($5x10^6$ 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 0.09% NaN_3 to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
5) Add the primary antibody at the concentration as suggested in the **APPLICATION** 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 FITC conjugated anti-rat IgG antibody 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) Resuspend the cells with 500 μL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; transfectant)

Flow cytometric analysis for whole blood cells

We usually use Fisher tubes or equivalents as reaction tubes for all step described below.

1) Add 20 μL of the primary antibody diluted with the 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 washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
4) Add FITC conjugated anti-rat IgG antibody diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
5) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
6) 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.
7) Add 1 mL of H_2O to each tube and incubate for 10 minutes at room temperature.
8) Centrifuge at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
9) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
10) Resuspend the cells with 500 μL of the washing buffer and analyze by a flow cytometer.

RELATED PRODUCTS:

D070-4	Anti-CX_3CR1 (Human) mAb-FITC (2A9-1)
D070-5	Anti-CX_3CR1 (Human) mAb-PE (2A9-1)
D070-A48	Anti-CX_3CR1 (Human) mAb-Alexa Fluor® 488 (2A9-1)
D063-3	Anti-CD191 (CCR1) (Human) mAb (#141-2)
D063-5	Anti-CD191 (CCR1) (Human) mAb-PE (#141-2)
D085-3	Anti-CD193 (CCR3) (Human) mAb (444-11)
D085-4	Anti-CD193 (CCR3) (Human) mAb-FITC (444-11)
D085-5	Anti-CD193 (CCR3) (Human) mAb-PE (444-11)
D074-3	Anti-CD197 (CCR7) (Human) mAb (6B3)
D124-3	Anti-CD195 (CCR5) (Human) mAb (T227)
D124-4	Anti-CD195 (CCR5) (Human) mAb-FITC (T227)
D123-3	Anti-CD184 (CXCR4) mAb (A145)
D123-4	Anti-CD184 (CXCR4) mAb-FITC (A145)
K0223-3	Anti-CXCR7 (RDC1) (Human) mAb (9C4)
K0223-5	Anti-CXCR7 (RDC1) (Human) mAb-PE (9C4)