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

MONOCLONAL ANTIBODY

CD191/CCR1

Code No.	Clone	Subclass	Quantity	Concentration
D063-3	#141-2	Mouse IgG1 κ	100 µg	1 mg/mL

BACKGROUND: Chemokines, which are a group of 70 to 90 amino acids structurally related polypeptides, were first isolated as the regulatory factors of leukocyte recirculation and homing in inflammatory and immunological responses. Several studies have shown that chemokines are crucially involved not only in such events but also in certain physiological and pathogenic processes, including hematopoiesis, angiogenesis, allergy, autoimmune diseases, and viral infectious diseases, therefore, it is now one of the most interesting molecules. There are two major groups in the chemokine super family; the CXC subfamily, in which the two NH_2-terminal cysteines of well-conserved four cysteines among chemokine superfamily are separated by a single amino acid, the CC subfamily, in which they are adjacent. Another subfamilies are designated as C subfamily and CX_3C subfamily. CCR is the G-protein-coupled seven-transmembrane receptor, to which CC chemokines bind specifically. It is expressed on the cell surface of various types of blood cells, including monocytes, acidocytes, basocytes, and T cells.

SOURCE: This antibody was purified from hybridoma (clone #141-2) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3XAg8 with Balb/c mouse splenocyte immunized with stable transfectant expressed human CD191/CCR1.

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 human CD191/CCR1 on Flow cytometry.

SPECIES CROSS REACTIVITY:

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

INTENDED USE:
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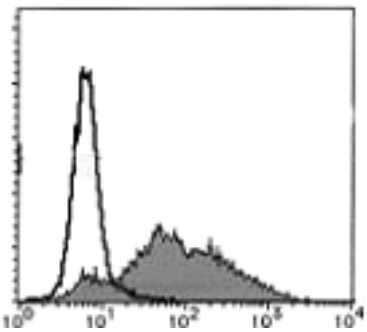
APPLICATIONS:
Western blotting; Not tested
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 5-10 µg/mL (final concentration)
Other; *This antibody has been shown to suppress Dendritic cell (DC) chemotactic migration, capacity of monocyte derived DCs to stimulates allogeneic T cells proliferation and IFN-γ secretion.

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

REFERENCES:
1) Cappello, P., *et al*., *J. Immunol*. **177**, 6143-6151 (2006)
2) Sato, Y., *et al*., *Blood* **106**, 428-435 (2005)
3) Sato, Y., *et al*., *Development* **130**, 5519-5532 (2003)
4) Sato. K., *et al*., *J. Immunol*. **168**, 6263-6272 (2002)
5) Sato. K., *et al*., *J. Immunol*. **166**, 1659-1666 (2001)
6) Sato. K., *et al*., *Int. Immunol*. **13**, 167-179 (2001)
7) Sato. K., *et al*., *Blood* **93**, 34-42 (1999)
8) Rollins, B. J., *et al*., *Blood* **90**, 909-928 (1997)
9) Premack, B. A., *et al*., *Nat. Med*. **2**, 1174-1178 (1996)
10) Kelner G. S., *et al*., *Science* **266**, 1395-1399 (1994)
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)



Flow cytometric analysis of CD191 expression on transfectant. *Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of D063-3 to the cells.*

Clone #141-2 is used in reference number 1) - 7).

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PROTOCOLS:

Flow cytometric analysis for floating cells

We usually use Fisher tubes or equivalents as reaction tubes for all steps 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 20 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 primary antibody at the concentration as suggest in the **APPLICATIONS** diluted in 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 30 μL of 1:40 FITC conjugated anti-mouse IgG (MBL; code no. 238) 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.

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 CD191 monoclonal antibody (#141-2) at the concentration of as suggest in the **APPLICATIONS** diluted with the washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% 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 30 μL of 1:100 FITC conjugated anti-mouse IgG (MBL; code no. 238) diluted with 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:

D063-5	PE labeled CD191/CCR1 (#141-2)
D085-3	CD193/CCR3 (444-11)
D085-4	FITC labeled CD193/CCR3 (444-11)
D085-5	PE labeled CD193/CCR3 (444-11)
D123-3	CD184/CXCR4 (A145)
D123-4	FITC labeled CD184/CXCR4 (A145)
D124-3	CD195/CCR5 (T227)
D124-4	FITC labeled CD195/CCR5 (T227)
D074-3	CD197/CCR7 (6B3)
D070-3	anti-Human CX_3CR1 (2A9-1)
D070-4	FITC labeled anti-Human CX_3CR1 (2A9-1)
D070-5	PE labeled anti-Human CX_3CR1 (2A9-1)