

MONOCLONAL ANTIBODY

Anti-CD184 (CXCR4) mAb-FITC

Code No.	Clone	Subclass	Quantity	Concentration
D123-4	A145	Rat IgG1	100 µL	500 µg/mL

BACKGROUND: CXCR4/CD184/LESTR/fusin/NPY3R is a G protein-coupled receptor for the CXC chemokine SDF-1. Binding of SDF-1 induces CXCR4 phosphorylation by Ser/Thr kinases, leading to CXCR4 internalization via clathrin-coated pits. CXCR4 functions include co-stimulation in pre-B cell proliferation, induction of apoptosis, and HIV entry, since CXCR4 is one of the 2 major HIV/SIV co-receptors. Early infection with HIV-1 is dominated by CCR5-tropic (R5) viruses. The evolution of CXCR4-tropic (X4) viruses occurs later in the infection and is associated with rapid disease progression. CXCR4 mediates chemotaxis in mature and progenitor blood cells and is essential for B lympho- and myelopoiesis, cardiogenesis, blood vessel formation and cerebellar development. Although ubiquitously expressed in blood and tissue cells, its role in blood and tissue homeostasis is not fully understood. CXCR4 is predominantly stored intracellularly, and may contribute to the inefficiency in transmission and propagation of X4-tropic viruses.

SOURCE: This antibody was purified from ascites fluid of C.B-17 SCID mice by ammonium sulfate precipitation followed by gel filtration through Superdex 200 in PBS. This hybridoma (clone A145) was established by fusion of mouse myeloma cell SP2/0 with WKA/H rat splenocyte immunized with CXCR4 transfected Cos-1 cells.

FORMULATION: 50 µg IgG in 100 µL volume of PBS containing 1% BSA and 0.09% NaN₃.

*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.

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

REACTIVITY: This antibody reacts with CD184 antigen on Flow cytometry. This clone A145 recognizes N-terminus extracellular domain of CXCR4.

APPLICATION:

Flow cytometry; 10-20 µg/mL (final concentration)

*Please refer to the data sheet (MBL code no. D123-3) for other applications.

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Jurkat, HPB-MLT	Not tested	Not tested
Reactivity on FCM	+		

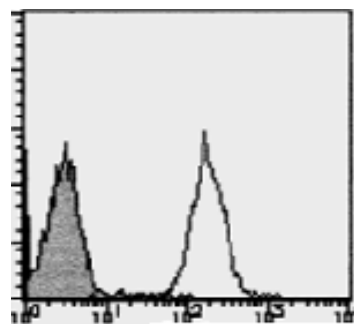
INTENDED USE:

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

REFERENCES:

- 1) Cesano, A., *et al.*, *Cytometry B Clin Cytom.* **82**, 158-172 (2012) [FCM]
- 2) Nimura, F., *et al.*, *Experimental Biology and Medicine* **231**, 431-443 (2006)
- 3) Navenot, J-M., *et al.*, *Cancer Res.* **65**, 10450-10456 (2005)
- 4) Ichiyama, K., *et al.*, *PNAS* **100**, 4185-4190 (2003)
- 5) Tanaka, R., *et al.*, *J. Virol.* **75**, 11534-11543 (2001)

Clone A145 is used in these references.



Flow cytometric analysis of CD184/CXCR4 expression on HPB-MLT cells. Shaded histogram indicates the reaction of isotypic control to the cells. Open histogram indicates the reaction of D123-4 to the cells.

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% NaN₃].
- 2) Resuspend the cells with washing buffer (5x10⁶ 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 suggested 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) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; HPB-MLT)

D124-3	Anti-CD195 (CCR5) (Human) mAb (T227)
D124-4	Anti-CD195 (CCR5) (Human) mAb-FITC (T227)
D074-3	Anti-CD197 (CCR7) (Human) mAb (6B3)
K0223-3	Anti-CXCR7 (RDC1) (Human) mAb (9C4)
K0223-5	Anti-CXCR7 (RDC1) (Human) mAb-PE (9C4)
M080-3	Rat IgG1 (isotype control) (1H5)
M080-4	Rat IgG1 (isotype control)-FITC (1H5)
M080-5	Rat IgG1 (isotype control)-PE (1H5)

Flow cytometric analysis for whole blood cells

We usually use Falcon tubes or equivalents as reaction tubes for all steps described below.

- 1) Add 50 µL of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted with the washing buffer 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) 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.
- 5) Add 1 mL of H₂O to each tube and incubate for 10 minutes at room temperature.
- 6) Centrifuge at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 7) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 8) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

RELATED PRODUCTS:

D123-3	Anti-CD184 (CXCR4) mAb (A145)
D123-4	Anti-CD184 (CXCR4) mAb-FITC (A145)
D070-3	Anti-CX ₃ CR1 (Human) mAb (2A9-1)
D070-4	Anti-CX ₃ CR1 (Human) mAb-FITC (2A9-1)
D070-5	Anti-CX ₃ CR1 (Human) mAb-PE (2A9-1)
D070-A48	Anti-CX ₃ CR1 (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)