

MONOCLONAL ANTIBODY

CD184/CXCR4

Code No.	Clone	Subclass	Quantity	Concentration
D123-3	A145	Rat IgG1	100 µg	1 mg/mL

BACKGROUND: CD184/CXCR4/LESTR/fusin/NPY3R is a G protein-coupled receptor for the CXCR4 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: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol. 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 CD184 antigen on Flow cytometry. This clone A145 recognizes N-terminus extracellular domain of CXCR4.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	PM1, HPB-MLT	Not Tested	Not Tested
Reactivity on FCM	+		

INTENDED USE:

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

APPLICATIONS:

Western blotting; Not tested*

Immunoprecipitation; Not tested*

*It is reported that this monoclonal antibody can be used in Western blotting and Immunoprecipitation in the reference number 3).

Immunohistochemistry; Not tested

Immunocytochemistry; Not tested

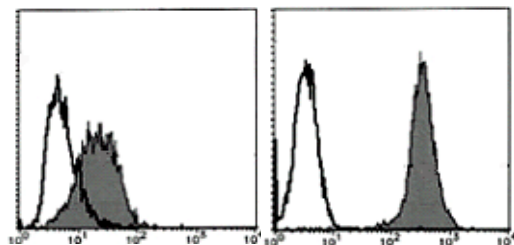
Flow cytometry; 10-20 µg/mL (final concentration)

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

REFERENCES:

- 1) Navenot, J-M., *et al.*, *Cancer Res.* **65**, 10450-10456 (2005)
- 2) Ichiyama, K., *et al.*, *PNAS* **100**, 4185-4190 (2003)
- 3) 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 PM1 (left) and HPB-MLT (right). Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D123-3 to the cells.

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 0.1% 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 10 µL of normal goat serum containing 1 mg/mL normal human IgG and 0.1% NaN₃ to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
 - 5) Add 40 µL of CD184 (A145) (25-50 µg/mL) 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 30 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) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive controls for Flow cytometry; PM1, HPB-MLT)

- D085-4 FITC labeled CD193/CCR3 (444-11)
- D124-3 CD195/CCR5 (T227)
- D124-4 FITC labeled CD195/CCR5 (T227)
- D074-3 CD197/ CCR7 (6B3)
- D123-4 FITC labeled CD184/CXCR4 (A145)
- D070-3 Anti-Human CX₃CR1 (2A9-1)
- D070-4 FITC labeled Anti-Human CX₃CR1 (2A9-1)
- D070-5 PE labeled Anti-Human CX₃CR1 (2A9-1)

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 CD184 monoclonal antibody (A145) (20 µg/mL) diluted with the washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN₃] 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-rat IgG (MBL; code no. IM-0827) 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 H₂O 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:

- D063-3 CD191/CCR1 (#141-2)
- D063-5 PE labeled CD191/CCR1 (#141-2)
- D085-3 CD193/CCR3 (444-11)