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Product datasheet for AM26473FC-N

CXCR4 (Extracell. Dom)(N-term) Rat Monoclonal Antibody [Clone ID: A145]

Product data:

Product Type:	Primary Antibodies
Clone Name:	A145
Applications:	FC
Recommended Dilution:	**Flow Cytometry:** 10-20 µg/ml (final concentration). For details see ***Protocol*** below.
Reactivity:	Human
Host:	Rat
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	CXCR4 transfected Cos-1 cells
Specificity:	This clone A145 recognizes N-terminus extracellular domain of CXCR4.
Formulation:	PBS Label: FITC State: Liquid purified Ig fraction Stabilizer: 1% BSA Preservative: 0.09% Sodium Azide
Concentration:	lot specific
Purification:	Ammonium sulfate precipitation followed by gel filtration through Superdex 200 in PBS
Conjugation:	FITC
Storage:	Store undiluted at 2-8°C. This product is photosensitive and should be protected from light. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	C-X-C motif chemokine receptor 4
Database Link:	Entrez Gene 7852 Human P61073

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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.

Synonyms: CXC-R4, CXCR-4, Fusin, LCR1, FB22, NPYRL, HM89, SDF1 receptor, LESTR

Note: This product was originally produced by MBL International.

Protocol:

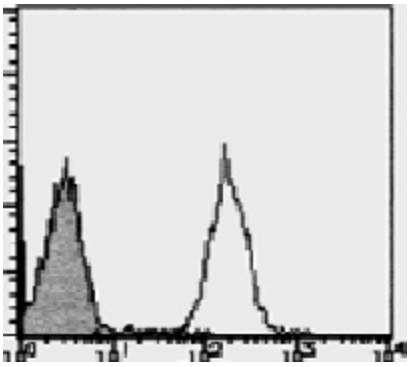
Flow cytometric analysis for floating cells

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 0.09% NaN3].
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) 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)

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.
Add 1 mL of washing buffer followed by centrifugation 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.

Product images:



Flow cytometric analysis ofCD184/CXCR4 expression on HPB-MLT cells. Shaded histogram indicates the reaction of isotypic control to the cells. Open histogram indicates the reaction of AM26473FC-N to the cells.