

Technical Data Sheet

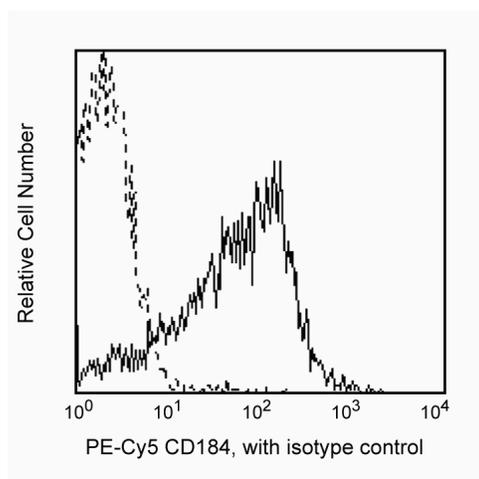
PE-Cy™5 Mouse Anti-Human CD184

Product Information

Material Number:	560937
Alternate Name:	CXCR4; Fusin; SDF-1 receptor; LAP3; LCR1; LESTR; NPY3R; NPY3R; WHIM; HM8
Size:	25 tests
Vol. per Test:	20 µl
Clone:	12G5
Isotype:	Mouse (BALB/c) IgG2a, κ
Reactivity:	QC Testing: Human
Workshop:	VII 70204, 70305
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The 12G5 monoclonal antibody specifically binds to CD184, also known as CXCR4 and Fusin. CD184/CXCR4 is a seven-transmembrane domain, G-protein-linked, glycoprotein chemokine receptor. CD184 serves as a receptor for the C-X-C chemokine, SDF-1. It is expressed on a wide variety of hematopoietic cells, vascular endothelial cells and cells of the nervous system. CD184 plays a variety of roles in hematopoiesis, vascularization and neural development. CD184 also functions as a coreceptor for infection with T-cell tropic strains of HIV-1 and as a receptor for CD4-independent infection by some HIV isolates. The 12G5 antibody has been reported to block CD4-independent infection by HIV-2 and CD4-dependent infection by some T-cell tropic isolates of HIV-1.



Profile of peripheral blood lymphocytes analyzed by flow cytometry.

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with PE-Cy5 (formerly known as BD Cy-Chrome™) under optimum conditions, and unconjugated antibody and free PE-Cy5 were removed.

Application Notes

Application

Flow cytometry	Routinely Tested
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Recommended Assay Procedure:

Flow cytometry: Chemokine receptors are known to internalize during manipulation resulting in low frequency expression. Investigators are advised to perform immunophenotyping studies of chemokine receptors on freshly collected samples (<24 Hrs). Incubation with the antibody should be done at 4°C in the dark. Cellular manipulation, such as Ficoll separation, freezing, or exposure to cold temperatures prior to staining should be minimized and have been shown to cause a decrease in staining intensity and/or inconsistent results.

Investigators should note that alternative staining procedures may be necessary. A multiple-step staining procedure is strongly recommended, in some instances, to amplify immunofluorescent signals for the flow cytometric analysis of human CXCR4 expression. Investigators may find the Purified Mouse Anti-Human CD184 antibody (MN 555972) to be useful in conjunction with appropriate secondary and tertiary reagents for

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detecting low frequency expression, such as with Biotin Goat Anti-Mouse Ig (MN 553999) and PE Streptavidin (MN 554061) or PE-CyTM5 Streptavidin (MN 554062).

Suggested Companion Products

Catalog Number	Name	Size	Clone
555575	PE-Cy TM 5 Mouse IgG2a, κ Isotype Control	100 tests	G155-178
555899	Lysing Buffer	100 ml	(none)
555972	Purified Mouse Anti-Human CD184	0.1 mg	12G5
553999	Biotin Goat Anti-Mouse Ig (Multiple Adsorption)	0.5 mg	Polyclonal
554061	PE Streptavidin	0.5 mg	(none)
554062	PE-Cy TM 5 Streptavidin	0.1 mg	(none)

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1×10^6 cells in a 100- μ l experimental sample (a test).
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. PE-Cy5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the PE-Cy5 tandem fluorochrome, extra care must be taken when using dual-laser cytometers which may directly excite both PE and Cy5TM.
6. PE-Cy5 is a tandem fluorochrome composed of R-phycoerythrin (PE), which is excited by the 488 nm light of an Argon ion laser and serves as an energy donor, coupled to the cyanine dye Cy5, which acts as an energy acceptor and fluoresces at 670 nm. BD Biosciences Pharmingen has maximized the fluorochrome energy transfer in PE-Cy5, thus maximizing its fluorescence emission intensity, minimizing residual emission from PE, and minimizing lot-to-lot variation.
7. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
8. PE-Cy5 tandem fluorochromes have been reported to bind some classes of human macrophages and granulocytes via Fc receptors, and PE has been reported to bind to mouse B lymphocytes via Fc receptors. Preincubation of mouse leukocytes with Mouse BD Fc BlockTM purified anti-mouse CD16/CD32 mAb 2.4G2 can reduce the non-specific binding of PE-Cy5-conjugated reagents to mouse B cells. However, PE-Cy5 conjugated reagents should not be used to stain splenocytes of SJL, NOD, and MRL mice as B lymphocytes and/or other leukocytes have been reported to non-specifically stain regardless of the use of Mouse BD Fc BlockTM (the CD72c complex has been implicated for PE-Cy5 binding in these strains). Reagents conjugated to PE, PerCP, PerCP-Cy5.5, APC, and APC-Cy7 tandem fluorochrome can be used on leukocytes from these mouse strains.
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10. Cy is a trademark of Amersham Biosciences Limited.
11. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
12. Please refer to www.bdbiosciences.com/pharminggen/protocols for technical protocols.

References

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