Technical Data Sheet

Purified Rat Anti-Human CD184

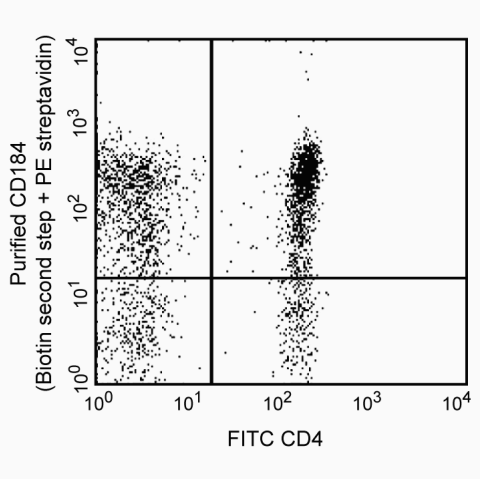
Product Information

Material Number:	**551413**
Alternate Name:	CXCR4, Fusin
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	1D9
Immunogen:	Human CXCR4 fusion protein
Isotype:	Rat IgG2a, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The monoclonal antibody 1D9 reacts with the human CD184 which is also known as CXC chemokine receptor, CXCR4. CXCR4 (previously known as fusin, LESTR and HUMSTR), a seven-transmembrane, G-protein-coupled receptor, is the specific receptor for CXC chemokines, SDF-1/CXCL12. CXCR4 was widely expressed by hematopoietic and non-hematopoietic cell types including PMN, monocytes, T cells, B cells, CD34+ progenitor cells, endothelial cells, neurons and astrocytes. The human CXCR4 is used by T-tropic HIV-1 as a co-receptor for viral entry. The human CXCR4 gene has been mapped to chromosome 2q21. The immunogen used to generate 1D9 hybridoma was human CXCR4 fusion protein.

This antibody is routinely tested by flow cytometric analysis. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.



Detection of CXCR4 expression on human peripheral lymphocytes by purified 1D9. *Human lysed whole blood were stained with 0.25 µg of purified 1D9 using 3-step staining protocol outlined below and anti-human CD4-FITC (Cat. No. 555346) The data reflects gating on lymphocytes, based on forward and side scattered light signals. The level of nonspecific staining was assessed by using purified rat IgG2a (Cat. No. 555841) as isotype control. The quadrant markers for the bivariate dot plots were set based on the isotype control. The samples were analyzed with a FACScan Flow Cytometer (BD Biosciences, San Jose, CA).*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at 4° C.

Application Notes

Application

Flow cytometry	Routinely Tested

Recommended Assay Procedure:

The purified 1D9 antibody can be used for the immunofluorescent staining and flow cytometric analyses of human leukocytes and cell lines that express CXCR4 (see figure).

BD Biosciences

www.bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.259.0187	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

For country-specific contact information, visit **www.bdbiosciences.com/how_to_order/**

A multistep step staining procedure is recommended to amplify immunofluorescent signals for the flow cytometric analysis of human CXCR4 expression:

Step 1: Incubate 10e6 cells with 0.06 - 0.5 µg of purified 1D9 antibody at 4°C for 15 - 20 minutes. Wash cells two times with staining medium containing sodium azide (e.g., Dulbecco's PBS or tissue culture medium [without phenol red and biotin] with 0.09% sodium azide and 2% heat-inactivated FCS or 0.2% BSA).
Step 2: Incubate the cells with biotinylated mouse anti-rat IgG2a (Cat. No. 553894) at 4°C for 20 minutes. Wash cells two times.
Step 3: Incubate the cells with ≤0.06 µg of streptavidin-phycoerythrin (Cat. No. 554061) at 4°C for 20 minutes. Wash two times. Resuspend cells in staining medium and analyze stained cells by flow cytometry.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554061	PE Streptavidin	0.5 mg	(none)
553894	Biotin Mouse Anti-Rat IgG2a	0.5 mg	RG7/1.30
555841	Purified Rat IgG2a, κ Isotype Control	0.1 mg	R35-95

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
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.

References

Bleul CC, Farzan M, Choe H, et al. The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. *Nature.* 1996; 382(6594):829-833.(Biology)
Bleul CC, Wu L, Hoxie JA, Springer TA, Mackay CR. The HIV coreceptors CXCR4 and CCR5 are differentially expressed and regulated on human T lymphocytes.. *Proc Natl Acad Sci U S A.* 1997; 94(5):1925-1930.(Biology)
Federsppiel B, Melhado IG, Duncan AM, et al. Molecular cloning of the cDNA and chromosomal localization of the gene for a putative seven-transmembrane segment (7-TMS) receptor isolated from human spleen. *Genomics.* 1993; 16(3):707-712.(Biology)
Feng Y, Broder CC, Kennedy PE, Berger EA. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science.* 1996; 272(5263):872-877.(Biology)
Forster R, Kremmer E, Schubel A, et al. Intracellular and surface expression of the HIV-1 coreceptor CXCR4/fusin on various leukocyte subsets: rapid internalization and recycling upon activation. *J Immunol.* 1998; 160(3):1522-1531.(Immunogen)
Gupta SK, Lysko PG, Pillarisetti K, Ohlstein E, Stadel JM. Chemokine receptors in human endothelial cells. Functional expression of CXCR4 and its transcriptional regulation by inflammatory cytokines. *J Biol Chem.* 1998; 273(7):4282-4287.(Biology)
He J, Chen Y, Farzan M, et al. CCR3 and CCR5 are co-receptors for HIV-1 infection of microglia. *Nature.* 1997; 385(6617):645-649.(Biology)
Hesselgesser J, Halks-Miller M, DelVecchio V, et al. CD4-independent association between HIV-1 gp120 and CXCR4: functional chemokine receptors are expressed in human neurons. *Curr Biol.* 1997; 7(2):112-121.(Biology)
Loetscher M, Geiser T, O'Reilly T, Zwahlen R, Baggiolini M, Moser B. Cloning of a human seven-transmembrane domain receptor, LESTR, that is highly expressed in leukocytes. *J Biol Chem.* 1994; 269(1):232-237.(Biology)
Nomura H, Nielsen BW, Matsushima K. Molecular cloning of cDNAs encoding a LD78 receptor and putative leukocyte chemotactic peptide receptors. *Int Immunol.* 1993; 5(10):1239-1249.(Biology)
Oberlin E, Amara A, Bachelerie F, et al. The CXC chemokine SDF-1 is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1. *Nature.* 1996; 382(6594):833-835.(Biology)

BD Biosciences

www.bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.259.0187	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

For country-specific contact information, visit **www.bdbiosciences.com/how_to_order/**