Technical Data Sheet

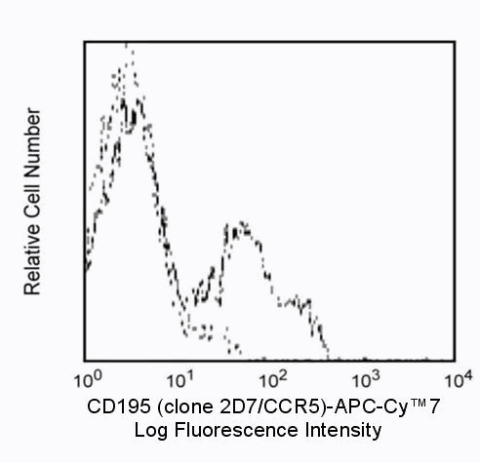
APC-Cy™7 Mouse Anti-Human CD195

Product Information

Material Number:	**560913**
Alternate Name:	CCR5; C-C chemokine receptor type 5; CC-CKR-5; CKR5; CHEMR13
Size:	25 Tests
Vol. per Test:	5 µl
Clone:	2D7/CCR5
Immunogen:	Human CCR5 Transfected Cell Line
Isotype:	Mouse (C57BL/6) IgG2a, κ
Reactivity:	QC Testing: Human
Workshop:	VII 70307
Storage Buffer:	Aqueous buffered solution containing BSA, protein stabilizer, and ≤0.09% sodium azide.

Description

The 2D7/CCR5 monoclonal antibody specifically binds to the human chemokine receptor CCR5, also known as CD195. CCR5 is a seven transmembrane-spanning G protein-associated molecule that belongs to the beta chemokine receptor family and expressed on a subset of T lymphocytes (CD3+CD45RO+CD95+). CCR5 regulates lymphocyte chemotaxis activation and transendothelial migration during inflammation by signaling a response to at least three chemokines: Regulated upon Activation Normal T-cell Expressed and Secreted (RANTES), Macrophage Inflammatory Protein-1 (MIP-1), and Monocyte Chemoattractant Protein 2 (MCP-2). Additionally, CCR5 has been found to be a coreceptor for macrophage-tropic HIV-1 on CD4+ cells, a characteristic that is important in viral transmission. Reports indicate that individuals who have partial (heterozygous) or complete (homozygous) deletion of the CCR5 allele demonstrate resistance to HIV infection. This antibody has been shown to block ligand and gp120 binding. It is also able to neutralize HIV infection.



Flow cytometric analysis of CD195 expression on human peripheral blood lymphocytes. *Whole blood was stained with either APC-Cy™7 Mouse IgG2a, κ Isotype Control (Cat. No. 557751; dashed line histogram) or APC-Cy™7 Mouse Anti-Human CD195 antibody (Cat. No. 560913 ; solid line histogram). Erythrocytes were lysed with BD FACS Lysing Solution (Cat. No. 349202). The fluorescence histograms were derived from events with the forward and side light-scatter characteristics of intact lymphocytes.*

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 APC-Cy7 under optimum conditions, and unconjugated antibody and free APC-Cy7 were removed.

Application Notes

Application

Flow cytometry	Routinely Tested
Functional assay	Tested During Development
Immunofluorescence	Tested During Development

Recommended Assay Procedure:

BD Biosciences
bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	866.979.9408	32.2.400.98.95	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**

Immunophenotyping studies of chemokine receptors need to be performed on freshly collected whole blood (<24 Hrs). Incubation with the antibody should be done at room temperature in the dark. Cellular manipulation, such as Ficoll-Paque™ separation, freezing, or exposure to cold temperatures prior to staining have been shown to cause a decrease in staining intensity and inconsistent results.

Suggested Companion Products

Catalog Number	Name	Size	Clone
557751	APC-Cy™7 Mouse IgG2a, κ Isotype Control	100 Tests	G155-178
554656	Stain Buffer (FBS)	500 mL	(none)
555899	Lysing Buffer	100 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
349202	BD FACS™ Lysing Solution	100 mL	(none)
557755	APC-Cy™7 Mouse Anti-Human CD195	100 Tests	2D7/CCR5

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-µl experimental sample (a test).
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. 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.
5. 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.
6. APC-Cy7 is a tandem fluorochrome composed of Allophycocyanin (APC), which is excited by laser lines between 595 and 647 nm and serves as an energy donor, coupled to the cyanine dye Cy7™, which acts as an energy acceptor and fluoresces at 780 nm. BD Biosciences Pharmingen has maximized the fluorochrome energy transfer in APC-Cy7, thus maximizing its fluorescence emission intensity, minimizing residual emission from APC, and minimizing required electronic compensation in multilaser-laser flow cytometry systems. Note: Although every effort is made to minimize the lot-to-lot variation in residual emission from APC, it is strongly recommended that every lot be tested for differences in the amount of compensation required and that individual compensation controls are run for each APC-Cy7 conjugate.
7. APC-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher.
8. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
9. Ficoll-Paque is a trademark of Amersham Biosciences Limited.
10. Warning: Some APC-Cy7 and PE-Cy7 conjugates show changes in their emission spectrum with prolonged exposure to formaldehyde. If you are unable to analyze fixed samples within four hours, we recommend that you use BD™ Stabilizing Fixative (Cat. No. 338036).
11. Cy is a trademark of GE Healthcare.
12. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Beavis AJ, Pennline KJ. Allo-7: a new fluorescent tandem dye for use in flow cytometry. *Cytometry.* 1996; 24(4):390-395. (Biology)
Deng H, Liu R, Ellmeier W, et al. Identification of a major co-receptor for primary isolates of HIV-1. *Nature.* 1996; 381(6584):661-666. (Biology)
Doranz BJ, Rucker J, Yi Y, et al. A dual-tropic primary HIV-1 isolate that uses fusin and the beta-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. *Cell.* 1996; 85(7):1149-1158. (Biology)
Dragic T, Litwin V, Allaway GP, et al. HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5. *Nature.* 1996; 381(6584):667-673. (Biology)
Raport CJ, Gosling J, Schweickart VL, Gray PW, Charo IF. Molecular cloning and functional characterization of a novel human CC chemokine receptor (CCR5) for RANTES, MIP-1beta, and MIP-1alpha. *J Biol Chem.* 1996; 271(29):17161-17166. (Biology)
Roederer M, Kantor AB, Parks DR, Herzenberg LA. Cy7PE and Cy7APC: bright new probes for immunofluorescence. *Cytometry.* 1996; 24(3):191-197. (Biology)
Wu L, Paxton WA, Kassam N, et al. CCR5 levels and expression pattern correlate with infectability by macrophage-tropic HIV-1, in vitro. *J Exp Med.* 1997; 185(9):1681-1689. (Biology)

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	866.979.9408	32.2.400.98.95	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**