Technical Data Sheet

PE-Cy™7 Rat Anti-Human CD197 (CCR7)

Product Information

Material Number:	**560922**
Alternate Name:	CCR7, BLR-2, EBI-1, CMKBR7
Size:	25 tests
Vol. per Test:	5 µl
Clone:	3D12
Immunogen:	Human CCR7 protein
Isotype:	Rat IgG2a, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The monoclonal antibody 3D12 reacts with the human CC chemokine receptor, CCR7. CCR7 (previously known as BLR-2, EBI-1 and CMKBR7), a seven-transmembrane, G-protein-coupled receptor, is the specific receptor for CC chemokines, MIP-3β/Exodus 3/ELC/ CCL19 and 6Ckine/Exodus 2/SLC/TCA4/CCL21. It has been shown that CCR7 mRNA is expressed mainly in lymphoid tissues including spleen, lymph nodes and tonsil. CCR7 mRNA was also detected in peripheral T and B lymphocytes, in bone marrow and cord blood CD34-positive cells and mature dendritic cells. The human CCR7 gene, unlike other CC chemokine receptor genes, has been mapped to chromosome 17q12. The immunogen used to generate 3D12 hybridoma was the N-terminus as well as parts of the second extracellular loop of human CCR7 protein. The monoclonal antibody 3D12 recognizes an epitope mapping to the N-terminus of human CCR7.

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

Application Notes

Application

Flow cytometry	Routinely Tested

Recommended Assay Procedure:

Flow Cytometry: Chemokine receptors are known to internalize during manipulation resulting in low frequency expression. Immunophenotyping studies of chemokine receptors need to be performed on freshly collected whole blood (<24 Hrs). Incubation with the antibody should be done in the dark. Cellular manipulation, such as Ficoll separation, freezing, or exposure to cold temperatures prior to staining have been shown to cause a decrease in staining intensity and provide for inconsistent results. Investigators may also want to consider using a multiple-step staining procedure for detecting low frequency CCR7 expression using Purified Rat Anti-Human CCR7 (Cat. No. 552175).

Suggested Companion Products

Catalog Number	Name	Size	Clone
552784	PE-Cy™7 Rat IgG2a, κ Isotype Control	0.1 mg	R35-95

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. 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).
4. 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.
5. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
6. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under license from Amersham Biosciences Corp. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one return this material, unopened to BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121 and any money paid for the material will be refunded.

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7. PE-Cy7 is a tandem fluorochrome composed of R-phycoerythrin (PE), which is excited by 488-nm light and serves as an energy donor, coupled to the cyanine dye Cy7, which acts as an energy acceptor and fluoresces maximally at 780 nm. PE-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher. Although every effort is made to minimize the lot-to-lot variation in the efficiency of the fluorochrome energy transfer, differences in the residual emission from PE may be observed. Therefore, we recommend that individual compensation controls be performed for every PE-Cy7 conjugate. PE-Cy7 is optimized for use with a single argon ion laser emitting 488-nm light, and there is no significant overlap between PE-Cy7 and FITC emission spectra. When using dual-laser cytometers, which may directly excite both PE and Cy7, we recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
8. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
9. 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.
10. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
11. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.

References

Birkenbach M, Josefsen K, Yalamanchili R, Lenoir G, Kieff E. Epstein-Barr virus-induced genes: first lymphocyte-specific G protein-coupled peptide receptors. *Nature.* 1993; 67(4):2209-2220. (Biology)

Burgstahler R, Kempkes B, Steube K, Lipp M. Expression of the chemokine receptor BLR2/EBI1 is specifically transactivated by Epstein-Barr virus nuclear antigen 2. *Biochem Biophys Res Commun.* 1995; 215(2):737-743. (Biology)

Kim CH, Pelus LM, White JR, Broxmeyer HE. Macrophage-inflammatory protein-3 beta/EBI1-ligand chemokine/CK beta-11, a CC chemokine, is a chemoattractant with a specificity for macrophage progenitors among myeloid progenitor cells. *J Immunol.* 1998; 161(5):2580-2585. (Biology)

Lipp M, Burgstahler R, Muller G, et al. Functional organization of secondary lymphoid organs by the chemokine system. *Curr Top Microbiol Immunol.* 2000; 251:173-179. (Immunogen)

Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature.* 1999; 401(6754):708-712. (Biology)

Schweickart VL, Raport CJ, Godiska R, et al. Cloning of human and mouse EBI1, a lymphoid-specific G-protein-coupled receptor encoded on human chromosome 17q12-q21.2. *Genomics.* 1994; 23(3):643-650. (Biology)

Yanagihara S, Komura E, Nagafune J, Watarai H, Yamaguchi Y. EBI1/CCR7 is a new member of dendritic cell chemokine receptor that is up-regulated upon maturation. *J Immunol.* 1998; 161(6):3096-3102. (Biology)

Yoshida R, Imai T, Hieshima K, et al. Molecular cloning of a novel human CC chemokine EBI1-ligand chemokine that is a specific functional ligand for EBI1, CCR7. *J Biol Chem.* 1997; 272(21):13803-13809. (Biology)

Yoshida R, Nagira M, Imai T, et al. EBI1-ligand chemokine (ELC) attracts a broad spectrum of lymphocytes: activated T cells strongly up-regulate CCR7 and efficiently migrate toward ELC. *Int Immunol.* 1998; 10(7):901-910. (Biology)

Yoshida R, Nagira M, Kitaura M, Imagawa N, Imai T, Yoshie O. Secondary lymphoid-tissue chemokine is a functional ligand for the CC chemokine receptor CCR7. *J Biol Chem.* 1998; 273(12):7118-7122. (Biology)