Technical Data Sheet

PE-Cy™7 Rat Anti-Human CD197 (CCR7)

Product Information

Material Number:	557648	
Alternate Name:	CCR7, BLR-2, EBI-1, CMKBR7	
Size:	100 tests	
Vol. per Test:	5 µl	
Clone:	3D12	
Immunogen:	Human CCR7 protein	
Isotype:	Rat IgG2a, ĸ	
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-3 β /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

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

Application Notes

Flow cytometry

Application

Routinely Tested

Recommended Assay Procedure:

The Purified-, PE-, Biotin-, and PE-Cy7-conjugated 3D12 antibody (Cat No. 552175, 552176, 552174 and 557648) can be used for the immunofluorescent staining and flow cytometric analyses of human leukocytes and cell lines that express CCR7.

Recommendation: 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 hours). 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 have been shown to cause a decrease in staining intensity and inconsistent results.

A multiple-step staining procedure is strongly recommended to amplify immunofluorescent signals in the flow cytometric analysis of human CCR7 expression. Purified anti-human CCR7, clone 3D12, Cat. No. 552175 and a 3-step staining procedure is recommended for detecting low frequency expression.

Suggested Companion Products

Catalog Number	Name	Size	Clone
552784	PE-Cy TM 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. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
- 3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
- 4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.

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- 5. 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.
- 6. 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.
- 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.
- 8. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
- 9. 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.
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