Technical Data Sheet

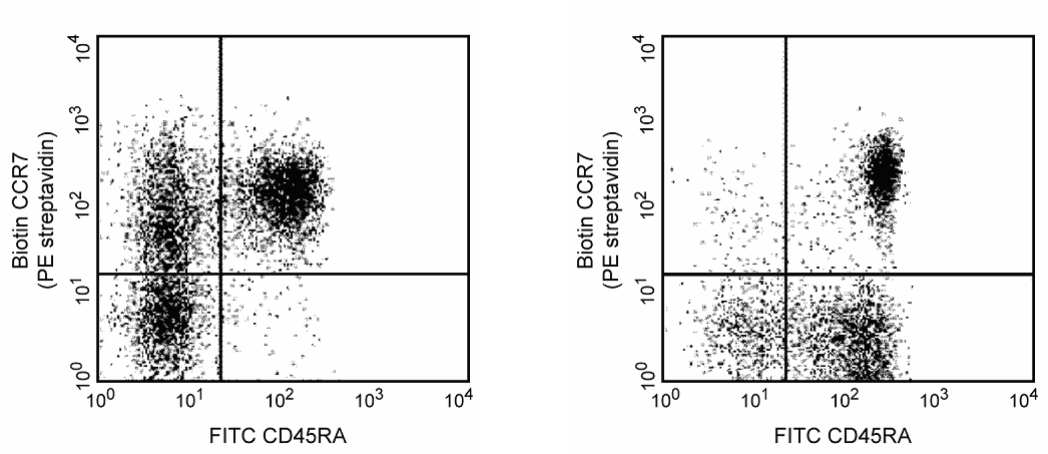
Biotin Rat Anti-Human CD197 (CCR7)

Product Information

Material Number:	**552174**
Alternate Name:	CCR7, BLR-2, EBI-1, CMKBR7
Size:	100 tests
Vol. per Test:	20 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 epitode mapping to the N-terminus of human CCR7.



Detection of CCR7 expression on CD4 and CD8-positive human peripheral lymphocytes by biotinylated anti-human CCR7 antibody 3D12. *Human PBMC were stained with 20 µl/test of biotinylated 3D12 and anti-human CD45RA-FITC (Cat. No. 555488). The data shown are derived from the CD4-positive (based on staining with anti-human CD4-APC, Cat. No. 555349, left panel) and CD8-positive (based on staining with anti-human CD8-APC, Cat. No. 555369, right panel) lymphocyte gated populations and displayed as bivariate dot plots.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with biotin under optimum conditions, and unreacted biotin was removed.
Store undiluted at 4° C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Flow cytometry	Routinely Tested

BD Biosciences

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Recommended Assay Procedure:
The biotinylated 3D12 antibody can be used with a very bright second-step reagent, such as Streptavidin-phycoerythrin (Cat. No. 554061) for the immunofluorescent staining and flow cytometric analyses of human leukocytes and cell lines that express CCR7 (see Figure). We are unable to generate reproducible results using less bright reagent such as Streptavidin-FITC or -PerCP with this antibody. The use of Streptavidin-FITC or -PerCP is not recommended.

Recommendation: Chemokine receptors are know 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 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
554061	PE Streptavidin	0.5 mg	(none)
555488	FITC Mouse Anti-Human CD45RA	100 tests	HI100
555349	APC Mouse Anti-Human CD4	100 tests	RPA-T4
555369	APC Mouse Anti-Human CD8	100 tests	RPA-T8
555842	Biotin Rat IgG2a, κ Isotype Control	100 tests	R35-95

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 X 10e6 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. 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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

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