

Technical Data Sheet

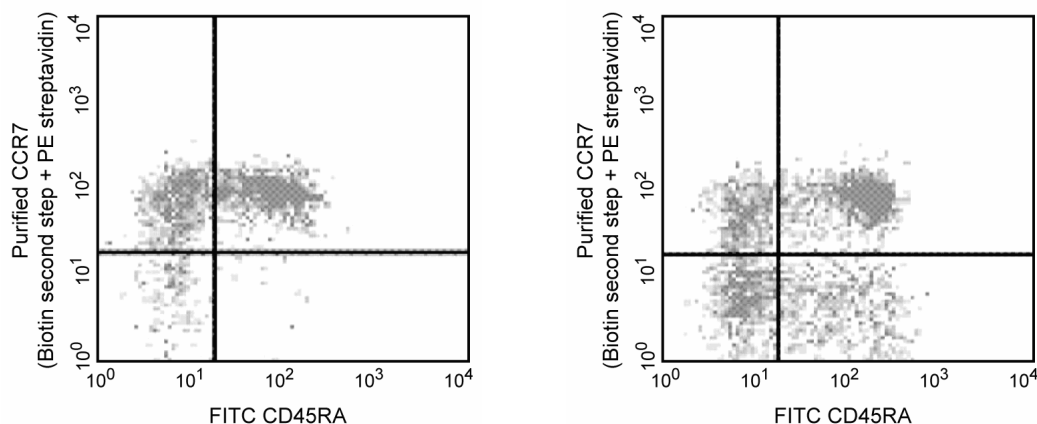
Purified Mouse Anti-Human CD197 (CCR7)**Product Information**

Material Number:	550937
Alternate Name:	BLR-2, EBI-1 and CMKBR7
Size:	0.25 mg
Concentration:	0.5 mg/ml
Clone:	2H4
Immunogen:	Human CCR7 transfected L1.2 mouse lymphoma cells
Isotype:	Mouse IgM, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing $\leq 0.09\%$ sodium azide.

Description

The monoclonal antibody 2H4 reacts with the human CC chemokine receptor, CCR7/CD197. 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•/Exodus 3/ELC/CCL19 and 6CKine/Exodus 2/SLC/TCA4/CCL214. 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 BM 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 2H4 hybridoma was human CCR7 transfected L1.2 mouse lymphoma cells. This antibody is not a neutralizing antibody.

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 CCR7 expression on CD4 and CD8-positive human peripheral lymphocytes by purified anti-human CCR7 antibody 2H4 (Cat. No. 550937). Human PBMC were stained with 4.0 $\mu\text{g}/10^6$ of purified 2H4 using 3-step staining protocol outlined below 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. Cells 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.

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Application Notes

Application

Flow cytometry	Routinely Tested
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Recommended Assay Procedure:

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 inconsistent results.

The purified 2H4 antibody can be used for the immunofluorescent staining and flow cytometric analyses of human leukocytes and cell lines that express CCR7 (see Figure). A multistep staining procedure is recommended to amplify immunofluorescent signals for the flow cytometric analysis of human CCR7 expression:

Step 1: Incubate 10⁶ cells with 2 - 4 µg of purified 2H4 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 0.25 µg of biotinylated rat anti-mouse IgM (Cat. No. 553406) 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 by flow cytometry using appropriate specificity and compensation controls.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554061	PE Streptavidin	0.5 mg	(none)
555581	Purified Mouse IgM, κ Isotype Control	0.1 mg	G155-228
553406	Biotin Rat Anti-Mouse IgM	0.5 mg	R6-60.2

Product Notices

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

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