Technical Data Sheet

Purifed Rat Anti-Human CXCR5

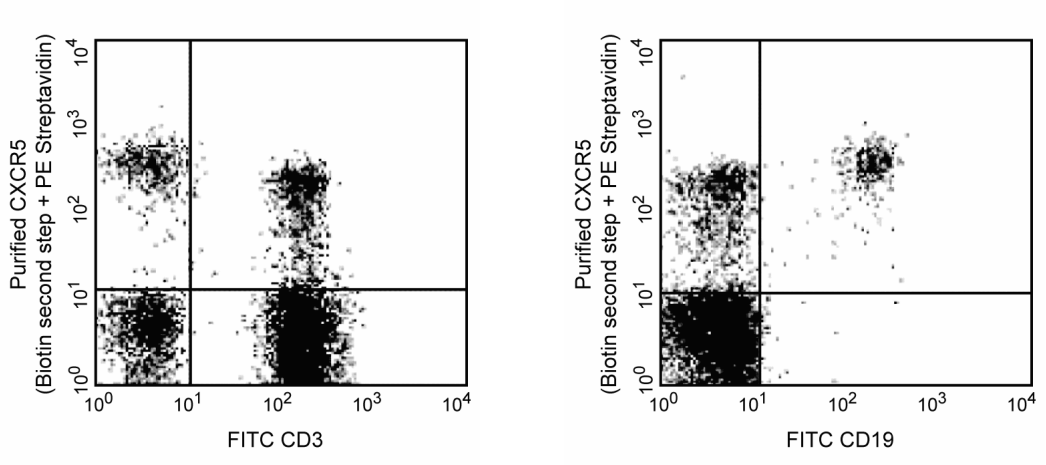
Product Information

Material Number:	**552032**
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	RF8B2
Immunogen:	Human CXCR5
Isotype:	Rat IgG2b, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The monoclonal antibody RF8B2 reacts with the human CXC chemokine receptor, CXCR5. CXCR5 (aka BLR-1 NLR and MDR15), a seven transmembrane, G-protein-coupled receptor, is the specific receptor for CXC chemokine, CXCL13/BLC/BCA-1. In peripheral blood, CXCR5 expression is restricted to B lymphocytes and a small subset of CD4+ and CD8+ lymphocytes. The restricted expression pattern of CXCR5 on B cells suggested that this receptor might function as a regulator of B cell migration. Stimulation of T cells with anti-CD3 monoclonal antibody leads to the down-regulation of CXCR5.

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 CXCR5 expression on human peripheral lymphocytes by purified RF8B2. *Human peripheral blood mononuclear cells were stained with 0.25 µg/test of purified RF8B2 using 3-step staining protocol outlined above and anti-human CD3-FITC (Cat. No. 555339, left panel) or anti-human CD19 FITC (Cat. No. 555412, right panel). The data reflects gating on lymphocytes, based on forward and side scattered light signals. The level of nonspecific staining was assessed by using purified rat IgG2b (Cat. No. 555846 and FITC-conjugated mouse IgG2a (Cat. No. 555573) or FITC-conjugated mouse IgG1 (Cat. No. 555748) as isotype controls. The quadrant markers for the bivariate dot plots were set based on the isotype controls. Flow cytometry was performed using a BD 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.

Application Notes

Application

Flow cytometry	Routinely Tested

BD Biosciences
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Recommended Assay Procedure:
The purified RF8B2 antibody can be used for the immunofluorescent staining and flow cytometric analyses of human leukocytes and cell lines that express CXCR5 (see Figure).

A multistep step staining procedure is recommended to amplify immunofluorescent signals for the flow cytometric analysis of human CXCR5 expression:

Step 1: Incubate 10e6 cells with 0.06 - 0.5 mg of purified RF8B2 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 biotinylated mouse anti-rat IgG2b (Cat. No. 553898) at 4°C for 20 minutes. Wash cells two times.

Step 3: Incubate the cells with $\leq$ 0.06 mg of streptavidin-phycoerythrin (Cat. No. 554061) at 4°C for 20 minutes. Wash two times. Resuspend cells in staining medium and analyze stained cells by flow cyotmetry, using appropriate specificity and compensation controls.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554061	PE Streptavidin	0.5 mg	(none)
555748	FITC Mouse IgG1, κ Isotype Control	100 tests	MOPC-21
553898	Biotin Mouse Anti-Rat IgG2b	0.5 mg	RG7/11.1
555846	Purified Rat IgG2b, κ Isotype Control	0.1 mg	R35-38
555573	FITC Mouse IgG2a, κ Isotype Control	100 tests	G155-178
555339	FITC Mouse Anti-Human CD3	100 tests	HIT3a
555412	FITC Mouse Anti-Human CD19	100 tests	HIB19

Product Notices

1. 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.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. Since applications vary, each investigator should titrate the reagent to obtain optimal results.

References

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Forster R, Emrich T, Kremmer E, Lipp M. Expression of the G-protein--coupled receptor BLR1 defines mature, recirculating B cells and a subset of T-helper memory cells. *Blood.* 1994; 84(3):830-840.(Immunogen)

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Legler DF, Loetscher M, Roos RS, Clark-Lewis I, Baggiolini M, Moser B. B cell-attracting chemokine 1, a human CXC chemokine expressed in lymphoid tissues, selectively attracts B lymphocytes via BLR1/CXCR5. *J Exp Med.* 1998; 187(4):655-660.(Biology)