

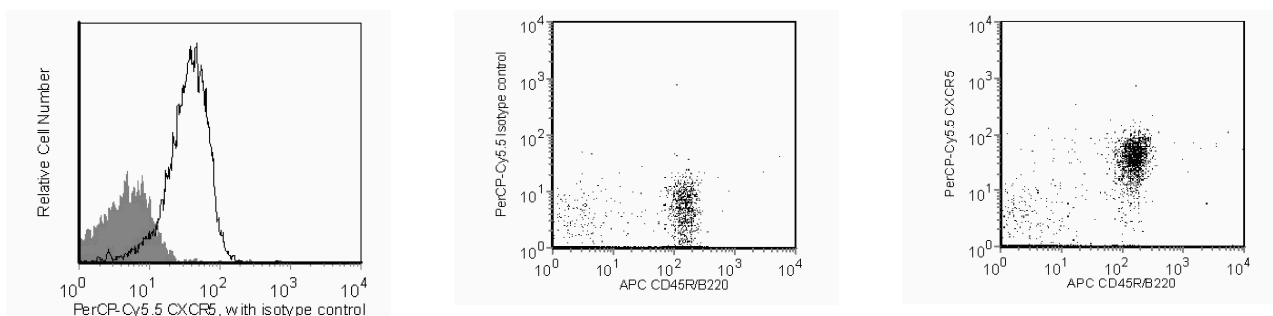
Technical Data Sheet

PerCP-Cy™ 5.5 Rat Anti-Mouse CD185 (CXCR5)**Product Information**

Material Number:	560528
Alternate Name:	Blr1; C-X-C chemokine receptor type 5; CXC-R5; CXCR-5; Gpcr6; MDR15
Size:	50 µg
Concentration:	0.2 mg/ml
Clone:	2G8
Immunogen:	Mouse CXCR5
Isotype:	Rat (LOU) IgG2a, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The 2G8 monoclonal antibody specifically binds to the mouse C-X-C Chemokine Receptor type 5, CXCR5. CXCR5 is also known as CD185, BLR1, NLR and MDR15. CXCR5 is a seven-transmembrane, G-protein-coupled receptor that is specific for the CXC chemokine, CXCL13/BLC/BCA-1. The expression of CXCR5 has been detected in spleen, lymph nodes, tonsils, brain, bone marrow, T cells, B cells, cerebrum, cerebellum, hippocampus and pituitary. In mouse spleen, CXCR5 was strictly expressed by mature B cells and a small subset of T lymphocytes. CXCR5 plays a role in directing the migration of B and T cells to B cell follicles with the spleen and certain other lymphoid tissues. The immunogen used to generate 2G8 hybridoma was a recombinant protein containing N-terminal amino acids of mouse CXCR5 (GST-NmBLR1).



Flow cytometric analysis of CXCR5 on mouse splenocytes. **Left Panel:** Splenocytes from C57BL/6 mice were stained either with a PerCP-Cy™ 5.5 Rat IgG2a, κ isotype control (Cat. No. 550765; shaded histogram) or with the PerCP-Cy™ 5.5 Rat Anti-Mouse CXCR5 antibody (Cat. No. 50528; unshaded histogram). Histograms were derived from gated events based on light scattering characteristics for CD45R/B220+ cells. **Middle and Right Panels:** Splenocytes from C57BL/6 mice were stained with both a APC Rat Anti-Mouse CD45R/B220 antibody (Cat.No. 553092) and either a PerCP-Cy™ 5.5 Rat IgG2a, κ isotype control (middle panel) or the PerCP-Cy™ 5.5 Rat Anti-Mouse CXCR5 antibody (right panel). Dot plots were derived from gated events based on light scattering characteristics for splenocytes. Flow cytometry was performed on a BD™ LSR II flow cytometry system.

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 PerCP-Cy5.5 under optimum conditions, and unconjugated antibody and free PerCP-Cy5.5 were removed. Storage of PerCP-Cy5.5 conjugates in unoptimized diluent is not recommended and may result in loss of signal intensity.

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. Investigators are advised to perform immunophenotyping studies of chemokine receptors on freshly collected samples (<24 Hrs). 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 should be minimized and have been shown to cause a decrease in staining intensity and/or inconsistent results.

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Investigators should note that alternative staining procedures may be necessary. A multiple-step staining procedure is strongly recommended, in some instances, to amplify immunofluorescent signals for the flow cytometric analysis of mouse CD185 (CXCR5) expression. Investigators may find the Purified Rat Anti-Mouse CD185 (CXCR5) antibody (Cat. No. 551961) to be useful in conjunction with appropriate secondary and tertiary reagents for detecting low frequency expression, such as with Biotin Mouse Anti-Rat IgG2a (Cat. No. 553894) and PE Streptavidin (Cat. No. 554061) or PerCP-CyTM5.5 Streptavidin (Cat. No. 551419).

Suggested Companion Products

<u>Catalog Number</u>	<u>Name</u>	<u>Size</u>	<u>Clone</u>
550765	PerCP-Cy TM 5.5 Rat IgG2a, κ Isotype Control	0.1 mg	R35-95
551961	Purified Rat Anti-Mouse CD185 (CXCR5)	0.1 mg	2G8
553894	Biotin Mouse Anti-Rat IgG2a	0.5 mg	RG7/1.30
553092	APC Rat Anti-Mouse CD45R/B220	0.1 mg	RA3-6B2
551419	PerCP-Cy TM 5.5 Streptavidin	0.1 mg	(none)
554061	PE Streptavidin	0.5 mg	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. An isotype control should be used at the same concentration as the antibody of interest.
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.
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. This PerCP-conjugated product is sold under license to the following patent: US Patent No. 4,876,190.
6. PerCP-Cy5.5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using dual-laser cytometers, which may directly excite both PerCP and Cy5.5TM. We recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
7. PerCP-Cy5.5-labelled antibodies can be used with FITC- and R-PE-labelled reagents in single-laser flow cytometers with no significant spectral overlap of PerCP-Cy5.5, FITC, and R-PE fluorescence.
8. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
9. Cy is a trademark of GE Healthcare.
10. Please refer to <http://regdocs.bd.com> to access safety data sheets (SDS).
11. Please refer to www.bdbiosciences.com/us/s/resources for technical protocols.

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