Technical Data Sheet

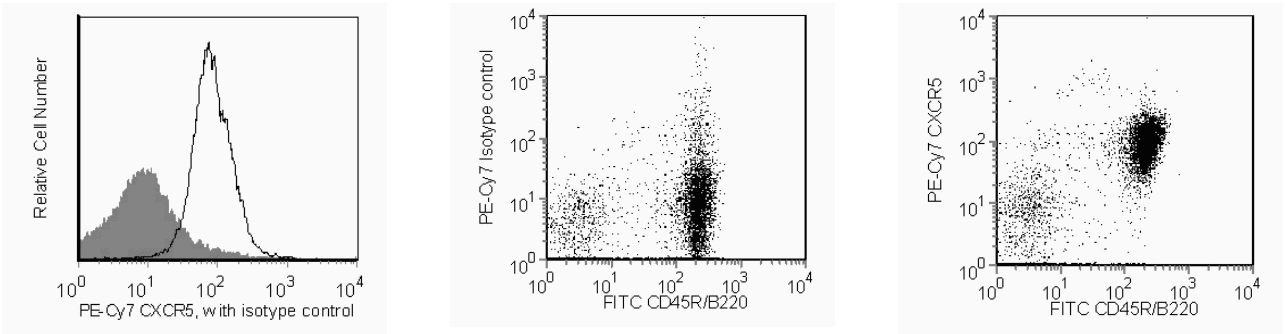
PE-Cy™7 Rat Anti-Mouse CXCR5

Product Information

Material Number:	**560617**
Size:	50 µg
Concentration:	0.2 mg/ml
Clone:	2G8
Immunogen:	Mouse CXCR5
Isotype:	Rat IgG2a, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The monoclonal antibody 2G8 reacts with the mouse CXC chemokine receptor, CXCR5. CXCR5 (*a.k.a.* BLR1, NLR and MDR15), a seven-transmembrane, G-protein-coupled receptor, is the specific receptor for CXC chemokines, 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, hippcampus and pituitary. In mouse spleen, CXCR5 was strictly expressed by mature B cells and a small subset of T lymphocytes. 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 PE-Cy™7 Rat IgG2a, κ isotype control (shaded) or with the PE-Cy™7 Rat Anti-Mouse CXCR5 antibody (unshaded). 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 FITC Rat Anti-Mouse CD45R/B220 antibody (Cat.No. 553088) and either a PE-Cy™7 Rat IgG2a, κ isotype control (middle panel) or the PE-Cy™7 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 PE-Cy7 under optimum conditions, and unconjugated antibody and free PE-Cy7 were removed.

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.

Investigators should note that alternative staining procedures may be neccessary. A multiple-step staining procedure is strongly recommended, in some instances, to amplify immunofluorescent signals for the flow cytometric analysis of mouse CXCR5 expression. Investigators may find the Purified Rat Anti-Mouse CXCR5 antibody (MN 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 (MN 553894) and PE Streptavidin (MN 554061) or PE-Cy™7 Streptavidin (MN 557598).

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Suggested Companion Products

Catalog Number	Name	Size	Clone
552784	PE-Cy™7 Rat IgG2a, κ Isotype Control	0.1 mg	R35-95
553088	FITC Rat Anti-Mouse CD45R/B220	0.5 mg	RA3-6B2
551961	Purified Rat Anti-Mouse CXCR5	0.1 mg	2G8
553894	Biotin Mouse Anti-Rat IgG2a	0.5 mg	RG7/1.30
554061	PE Streptavidin	0.5 mg	(none)
557598	PE-Cy™7 Streptavidin	0.1 mg	(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. Warning: Some APC-Cy7 and PE-Cy7 conjugates show changes in their emission spectrum with prolonged exposure to formaldehyde. If you are unable to analyze fixed samples within four hours, we recommend that you use BD™ Stabilizing Fixative (Cat. No. 338036).
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. 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.
6. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under license from Amersham Biosciences Corp. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one return this material, unopened to BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121 and any money paid for the material will be refunded.
7. 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.
8. 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.
9. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
10. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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Kouba M, Vanetti M, Wang X, Schafer M, Hollt V. Cloning of a novel putative G-protein-coupled receptor (NLR) which is expressed in neuronal and lymphatic tissue. *FEBS Lett.* 1993; 321(2-3):173-178. (Biology)

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