Technical Data Sheet

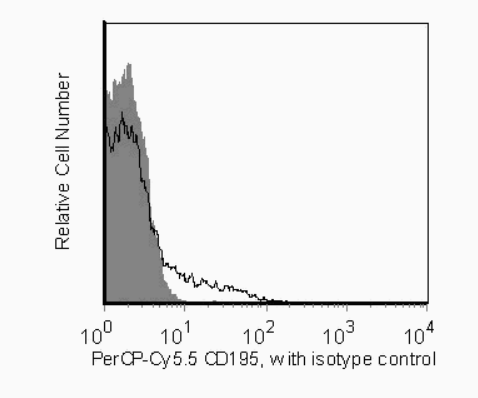
PerCP-Cy™5.5 Mouse Anti-Human CD195

Product Information

Material Number:	**560635**
Alternate Name:	CCR-5; Chemokine (C-C motif) receptor 5; CMKBR5; CKR5; CKR-5; CHEMR13
Size:	50 Tests
Vol. per Test:	5 µl
Clone:	3A9
Immunogen:	Human CCR5 Transfected Cell Line
Isotype:	Mouse (C57BL/6) IgG2a, κ
Reactivity:	QC Testing: Human Tested in Development: Rhesus, Cynomolgus
Workshop:	VII 70309
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The 3A9 monoclonal antibody recognizes CD195, which is also known as the chemokine receptor, CCR5, a seven transmembrane-spanning G protein-associated molecule. The 3A9 antibody also reportedly cross-reacts with human CCR8. Results of epitope mapping and sequence comparison between CCR5 and CCR8 reveals that the first three amino acid residues for these two receptors are identical: MDY (Met-Asp-Tyr). CCR5 belongs to the β-chemokine receptor family. It is expressed on subsets of T lymphocytes, NK cells, monocytes, macrophages, and dendritic cells. CCR5 regulates lymphocyte chemotaxis activation and transendothelial migration during inflammation. It signals a response to at least three chemokines: RANTES and macrophage inflammatory protein-1 (MIP-1) α and β. Additionally, CCR5 has been found to be a co-receptor for macrophage-tropic HIV-1 on CD4+ cells, a characteristic that is important in viral transmission. Reports indicate that individuals who have partial (heterozygous) or complete (homozygous) deletion of the CCR5 allele, demonstrate resistance to HIV infection. CCR5 has been clustered as CD195 in the VIIth HLDA workshop.



Flow cytometric analysis of CD195 on human peripheral lymphocytes. *Whole blood was stained with the PerCP-Cy™5.5 Mouse Anti-Human CD195 antibody (Cat. No. 560635; unshaded histogram) or with a PerCP-Cy™5.5 Mouse IgG2a, κ isotype control (Cat. No. 552577; shaded histogram). Erythrocytes were lysed with BD FACS™ Lysing Solution (Cat. No. 349202). Histograms were derived from gated events based on light scattering characteristics for lymphocytes. 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:

Immunophenotyping studies of chemokine receptors need to be performed on freshly collected whole blood (<24 Hrs). Incubation with the antibody should be done at room temperature 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.

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Suggested Companion Products

Catalog Number	Name	Size	Clone
552577	PerCP-Cy™5.5 Mouse IgG2a, κ Isotype Control	50 Tests	G155-178
555899	Lysing Buffer	100 mL	(none)
349202	BD FACS™ Lysing Solution	100 mL	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
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. 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.
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 Cy5.5™. 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. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
11. Ficoll-Paque is a trademark of Amersham Biosciences Limited.
12. Please refer to http://regdocs.bd.com to access safety data sheets (SDS).
13. Please refer to www.bdbiosciences.com/us/s/resources for technical protocols.

References

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