Technical Data Sheet

BB515 Mouse Anti-Human CD195

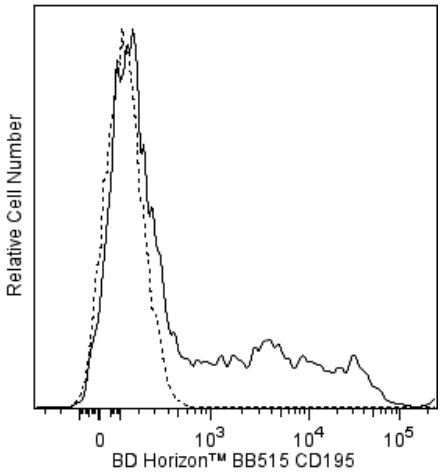
Product Information

Material Number:	**564512**
Alternate Name:	CCR-5; Chemokine (C-C motif) receptor 5; CMKBR5; CKR5; CKR-5; CHEMR13
Size:	100 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 ≤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.

The antibody was conjugated to BD Horizon BB515 which is part of the BD Horizon Brilliant™ Blue family of dyes. With an Ex Max near 490 nm and an Em Max near 515 nm, BD Horizon BB515 can be excited by the blue laser (488 nm) laser and detected with a 530/30 nm filter. This dye has been exclusively developed by BD Biosciences and is up to seven times brighter than FITC with less spillover into the PE channel. Due to similar excitation and emission properties, BB515, FITC, and Alexa Fluor® 488 cannot be used simultaneously. It is not recommended to use BB515 in cocktails that include Streptavidin conjugates as it may cause high background.



Flow cytometric analysis of CD195 expression on human peripheral blood lymphocytes. *Whole blood was stained with either BD Horizon™ BB515 Mouse IgG2a, κ Isotype Control (Cat. No. 564515; dashed line histogram) or BD Horizon BB515 Mouse Anti-Human CD195 antibody (Cat. No. 564512; solid line histogram). Erythrocytes were lysed with BD FACS Lysing Solution (Cat. No. 349202). The fluorescence histograms were derived from gated events with the forward and side light-scatter characteristics of intact lymphocytes. Flow cytometric analysis was performed using a BD™ LSR II Flow Cytometer 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 BD Horizon™ BB515 under optimum conditions and unconjugated antibody was removed.

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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.

BD™ CompBeads can be used as surrogates to assess fluorescence spillover (Compensation). When fluorochrome conjugated antibodies are bound to CompBeads, they have spectral properties very similar to cells. However, for some fluorochromes there can be small differences in spectral emissions compared to cells, resulting in spillover values that differ when compared to biological controls. It is strongly recommended that when using a reagent for the first time, users compare the spillover on cells and CompBead to ensure that BD Comp beads are appropriate for your specific cellular application.

For optimal results, it is recommended to perform 2 washes after staining with antibodies. Cells may be prepared, stained with antibodies and washed twice with wash buffer per established protocols for immunofluorescence staining, prior to acquisition on a flow cytometer. Performing fewer than the recommended wash steps may lead to increased spread of the negative population.

For optimal and reproducible results, BD Horizon Brilliant Stain Buffer should be used anytime two or more BD Horizon Brilliant dyes are used in the same experiment. Fluorescent dye interactions may cause staining artifacts which may affect data interpretation. The BD Horizon Brilliant Stain Buffer was designed to minimize these interactions. More information can be found in the Technical Data Sheet of the BD Horizon Brilliant Stain Buffer (Cat. No. 563794/566349) or the BD Horizon Brilliant Stain Buffer Plus (Cat. No. 566385).

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
563794	Brilliant Stain Buffer	100 Tests	(none)
564515	BB515 Mouse IgG2a, κ Isotype Control	50 µg	G155-178
349202	BD FACS™ Lysing Solution	100 mL	(none)
555899	Lysing Buffer	100 mL	(none)
566349	Brilliant Stain Buffer	1000 Tests	(none)
566385	Brilliant Stain Buffer Plus	1000 Tests	(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. 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. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
5. BD Horizon Brilliant Stain Buffer is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,575,303; 8,354,239.
6. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
7. Ficoll-Paque is a trademark of Amersham Biosciences Limited.
8. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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Hancock WW. Chemokines and the pathogenesis of T cell-dependent immune responses. *Am J Pathol.* 1996; 148(3):681-684. (Biology)

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Rottman JB, Ganley KP, Williams K, Wu L, Mackay CR, Ringler DJ. Cellular localization of the chemokine receptor CCR5. Correlation to cellular targets of HIV-1 infection. *Am J Pathol.* 1997; 151(5):1341-1351. (Biology)

Wu L, Paxton WA, Kassam N, et al. CCR5 levels and expression pattern correlate with infectability by macrophage-tropic HIV-1, in vitro. *J Exp Med.* 1997; 185(9):1681-1689. (Immunogen: Flow cytometry, Functional assay, Inhibition, Neutralization)