

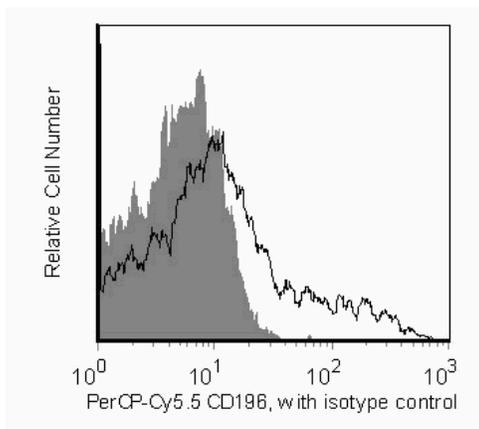
Technical Data Sheet

PerCP-Cy™ 5.5 Mouse Anti-Human CD196 (CCR6)**Product Information**

Material Number:	560621
Alternate Name:	BN-1; C-C chemokine receptor type 6; C-C CKR-6; CC-CKR-6; CCR-6; CD196
Size:	50 Tests
Vol. per Test:	5 µl
Clone:	11A9
Immunogen:	Human CD196/CCR6 Peptide
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human Tested in Development: Rhesus, Cynomolgus, Baboon
Workshop:	IX 48
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The 11A9 monoclonal antibody specifically binds to CD196, which is also known as CCR6. CCR6 is a seven-transmembrane, G-protein-coupled, glycoprotein receptor that is a member of the beta chemokine receptor family. The human *CCR6* gene has been mapped to chromosome 6q27. CCR6 is a receptor for the CC chemokine CCL20/MIP-3alpha/LARC/Exodus and also binds with lower affinity to and mediates responses to beta-defensin2/hBD-2. CCR6 is predominantly expressed by B lymphocytes, certain subsets of effector and memory T cells and by immature dendritic cells but not by monocytes, NK cells, or granulocytes. Skin-homing CLA (Cutaneous Lymphocyte Antigen)-positive memory T cells, Th1 cells, regulatory T cells and IL-17A-producing Th17 cells predominantly express high levels of CCR6. CCR6 mediates the trafficking of T, B, and dendritic cells to epithelial sites near the skin and mucosal surfaces during inflammatory and immunological responses. An N-terminal peptide of human CCR6 was used as an immunogen to generate the 11A9 hybridoma. The 11A9 antibody does not cross-react with human CCR1, CCR2, CCR3, CCR4, CCR5, CCR7, CCR8, CCR9, CXCR1, CXCR2, CXCR3, CXCR4 and CXCR5 receptors. This antibody is NOT a neutralizing antibody.



Flow cytometric analysis of CD196 expression on Rhesus macaque lysed whole blood. Whole blood was lysed with BD Pharm Lyse™ Lysing Buffer (Cat. No. 555899), then stained with either PerCP-Cy™ 5.5 Mouse Anti-Human CD196 (CCR6) antibody (Cat. No. 560621/560467/561752; empty histogram) or PerCP-Cy™ 5.5 Mouse IgG1, κ isotype control (Cat. No. 552834; shaded histogram). Fluorescent histograms depicting CD196 (or Ig isotype control) expression were derived from gated events based on forward and side light scatter characteristics of viable 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

BD Biosciences

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560621 Rev. 3



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 FicolI 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 necessary. A multiple-step staining procedure may be helpful, in some instances, to amplify immunofluorescent signals for the flow cytometric analysis of CD196 expression. Investigators may find the Purified Mouse Anti-Human CD196 antibody (Cat. No. 559560) to be useful in conjunction with appropriate secondary and tertiary reagents for detecting low frequency expression, such as with Biotin Goat Anti-Mouse Ig (Cat. No. 553999) and PE Streptavidin (Cat. No. 554061) or PerCP-CyTM5.5 Streptavidin (Cat. No. 551419).

Suggested Companion Products

Catalog Number	Name	Size	Clone
552834	PerCP-Cy TM 5.5 Mouse IgG1 κ Isotype Control	50 Tests	MOPC-21
555899	Lysing Buffer	100 mL	(none)
559560	Purified Mouse Anti-Human CD196 (CCR6)	0.5 mg	11A9
553999	Biotin Goat Anti-Mouse Ig (Multiple Adsorption)	0.5 mg	Polyclonal
554061	PE Streptavidin	0.5 mg	(none)
551419	PerCP-Cy TM 5.5 Streptavidin	0.1 mg	(none)
349202	BD FACST TM Lysing Solution	100 mL	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
560467	PerCP-Cy TM 5.5 Mouse anti-Human CD196 (CCR6)	100 Tests	11A9
561752	PerCP-Cy TM 5.5 Mouse Anti-Human CD196 (CCR6)	25 Tests	11A9

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- μ l 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 Cy5.5TM. 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. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
10. Cy is a trademark of GE Healthcare.
11. Please refer to www.bdbiosciences.com/pharming/protocols for technical protocols.

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