Technical Data Sheet

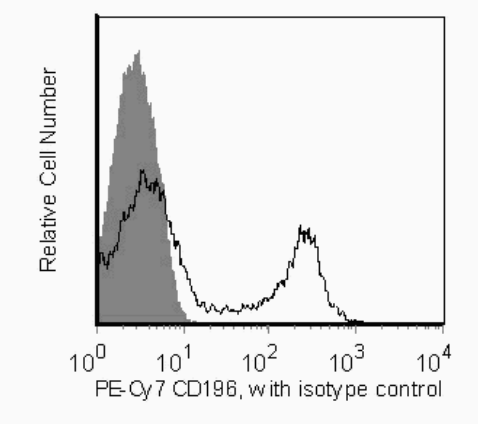
PE-Cy™7 Mouse Anti-Human CD196 (CCR6)

Product Information

Material Number:	**560620**
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
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Workshop:	HLDA VII
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

CCR6 is a seven-transmembrane, G-protein-coupled, glycoprotein receptor that is a member of the beta chemokine receptor family. CCR6 was clustered as CD196 in the 7th HLDA Workshop. 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 granulocytyes. 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.
The monoclonal antibody 11A9 reacts with the human CC chemokine receptor, CCR6. 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 on lysed whole blood. *Human (left panel lysed whole blood was stained with the PE-Cy™7 Mouse Anti-Human CD196 antibody (unshaded) or with a PE-Cy™7 Mouse IgG1, κ isotype control (shaded). 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 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.

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Investigators should note that alternative staining procedures may be neccessary. 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 (MN 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 (MN 553999) and PE Streptavidin (MN 554061) or PE-Cy™7 Streptavidin (MN 557598).

Suggested Companion Products

Catalog Number	Name	Size	Clone
557872	PE-Cy™7 Mouse IgG1 κ Isotype Control	100 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)
557598	PE-Cy™7 Streptavidin	0.1 mg	(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. 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. 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.
5. 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.
6. 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.
7. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
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. 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.
10. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
11. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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