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Product Specification Sheet

Anti-Human Monoclonal Anti-Human CCR7 (CD197) PE-Cy7 conjugate

Cat. # CCR7-PC-100 Rat monoclonal anti-Human Monoclonal Anti-Human CCR7 (CD197) PE-Cy7 conjugate **SIZE**: 100 Tests

CC chemokine receptor, CCR7. CCR7 (previously known as BLR-2, EBI-1 and CMKBR7), a seven-transmembrane, G-protein-coupled receptor, is the specific receptor for CC chemokines, MIP-3β/Exodus 3/ELC/ CCL19 and 6Ckine/Exodus 2/SLC/TCA4/CCL21. It has been shown that CCR7 mRNA is expressed mainly in lymphoid tissues including spleen, lymph nodes and tonsil. CCR7 mRNA was also detected in peripheral T and B lymphocytes, in bone marrow and cord blood CD34-positive cells and mature dendritic cells. The human CCR7 gene, unlike other CC chemokine receptor genes, has been mapped to chromosome 17q12. Human CCR7 is 378 aa.

Expression of CCR7 divides human memory T cells into 2 functionally distinct subsets. CCR7- memory cells express receptors for migration to inflamed tissues and display immediate effector function. In contrast, CCR7+ memory cells express lymph node homing receptors and lack immediate effector function, but efficiently stimulate dendritic cells and differentiate into CCR7- effector cells upon secondary stimulation. The CCR7+ and CCR7- T cells, which Sallusto et al. (1999) named central memory (T-CM) and effector memory (T-EM), differentiate in a step-wise fashion from naive T cells, persist for years after immunization, and allow a division of labor in the memory response.

Source of Antigen and Antibodies

Antigen	Human CCR7 N-terminus and 2nd Extracellular domain; epitope location ~ N-terminus
Ab Host/type	Rat monoclonal, IgG2a,k Protein A/G pure. Purified antibody was coupled to PE-Cy7 and free unconjugated antibody and free PE-Cy7 were removed by gel filtration chromatography. Anti-CCR7-PE-Cy7 conjugate Cat # CCR7-PC-100) is supplied in PBS, 0.1% BSA, 0.005% azide, store at 4oC or in frozen form in small aliquots.
-ve control IgG	Cat # 20102-102, **Mouse IgG2a** (non-immune) purified, suitable for ELISA, Western, IHC as –ve control

Recommended Assay Procedure:

Anti-CCR7-PE-Cy7 conjugate can be used for the immunofluorescent staining and flow cytometric analyses of human leukocytes and cell lines that express CCR7.

Recommendation: Chemokine receptors are known to internalize during manipulation resulting in low frequency expression. Immunophenotyping studies of chemokine receptors need to be performed on freshly collected whole blood (<24 hours). 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 have been shown to cause a decrease in staining intensity and inconsistent results.

A multiple-step staining procedure is strongly recommended to amplify immunofluorescent signals in the flow cytometric analysis of human CCR7 expression.

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. **We recommend 5 ul/test** (use 1 X 10^6 cells in a 100-µl experimental sample or a test). **Note**: Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. PE-Cy7 is a tandem fluorochrome composed of R-phycoerythrin (PE), which is excited by the 488-nm line of a laser and serves as an energy donor, coupled to the cyanine dye Cy7., which acts as an energy acceptor and fluoresces at 780 nm. The product has maximal fluorochrome energy transfer in PE-Cy7, thus maximizing its fluorescence emission intensity and minimizing residual emission from PE. **Note**: The lot-to-lot variation in residual emission from PE is minimized, it is strongly recommended that every lot be tested for differences in the amount of compensation required and that individual compensation controls are run for each PE-Cy7 conjugate.
3. PE-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher. PE-Cy7-labeled antibodies can be used with FITC- and R-PE-labeled reagents in single-laser flow cytometers with no significant spectral overlap between PE-Cy7 and FITC.
4. PE-Cy7 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the PE-Cy7 tandem fluorochrome, extra care must be taken 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.
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. Cy. is a trademark of Amersham Biosciences Limited. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University.

Species Crossreactivity
Anjti-human CCR7 antibody and the conjugates have been tested with human samples. Antibody crossreactivity in various other species is not established.

General References: Birkenbach M, (1993) Nature. 1993; 67:2209-2220; Burgstahler R (1995) Biochem Biophys Res Commun. 1995; 215:737-743; Kim CH (1998) 161:2580-2585; Lipp M (2000) Curr Top Microbiol Immunol. 251:173-179; Sallusto F (1999) Nature 401:708-712; Schweickart VL (1994 Genomics 23:643-650; Yanagihara S (1998) J Immunol.161:3096-3102; Yoshida R (1997) J Biol Chem. 272:13803-13809; Yoshida R (1998) JBC 273; 7118-7122

*This product is for In vitro research use only.

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