Technical Data Sheet

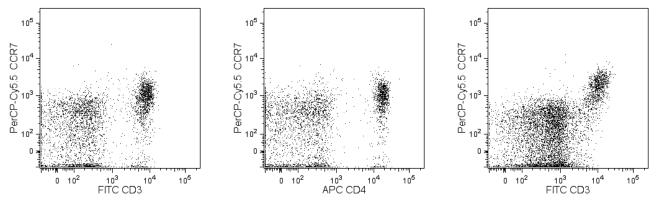
PerCP-Cy™5.5 Rat Anti-Mouse CD197 (CCR7)

Product Information

Material Number:	560812
Alternate Name:	CD197; C-C chemokine receptor type 7; EBI1; Ebi1h; CMKBR7
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	4B12
Isotype:	Rat (LOU) IgG2a
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing $\leq 0.09\%$ sodium azide.

Description

The monoclonal antibody 4B12/CCR7 reacts with the mouse C-C chemokine receptor type 7 (CCR7). CCR7 is also known as CD197 (previously known as EB11, Ebi1h and CMKBR7) and plays a central role in mediating homeostatic B and T lymphocyte trafficking to and within secondary lymphoid tissues. CD197 is a seven-transmembrane, G-protein-coupled, 43 kDa glycoprotein receptor that is specific for the CC chemokines, MIP3B/Exodus-3/ELC/CKb11/Scya19/CCL19 and 6Ckine/Exodus-2/SLC/TCA4/CKb9/Scya21/CCL21. The mouse *Ccr7* gene is located on chromosome 11. CD197 (CCR7) is differentially expressed by subsets of thymocytes. Positive CD197 expression appears to be involved in the cortex-to-medulla migration of positively-selected thymocytes wherein they complete functional maturation including the establishment of central tolerance. It is most highly expressed by some mature medullary single-positive thymocytes. CD197 is also expressed by subsets of mature peripheral CD4+ and CD8+ T lymphocytes including naïve and regulatory T cells and central memory T cells. In addition, it is differentially expressed by subsets of B lymphocytes, dendritic cells, and Langerhans cells. CD197 serves as a homing receptor that helps guide these various cell types to and within lymphoid tissues. In this way, CCR7 supports protective immunity while safeguarding self tolerance. Reportedly, the 4B12/CCR7 antibody is not agonistic, is not blocked by CCL21 nor by physiologic levels of CCL19, nor does the antibody block the binding of CCL21 to CCR7. The immunogen used to generate the 4B12 hybridoma was a mouse CCR7-transfected rat cell line.



Flow cytometric analysis of CCR7 (CD197) on mouse splenocytes and thymocytes. Freshly isolated mouse spleen or thymus cells were stained with PerCP-Cy™5.5 Rat Anti-Mouse CD197 (CCR7) (Cat. No. 560812), APC Rat Anti-Mouse CD4 (Cat. No.553051) and FITC Hamster Anti-Mouse CD3e (Cat. No. 553062). Two-color flow cytometric dot plots showing the correlated expression patterns of CD3 (left panel) or CD4 (middle panel) versus CCR7 (CD197) on splenocytes and CD3 versus CCR7 (right panel) on thymocytes are shown. The dot plots were derived from gated events with the forward and side light-scatter characteristics of viable lymphocytes or thymocytes. Flow cytometry was performed using a BD LSR™ II flow cytometry system.

Preparation and Storage

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. Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application Flow cytor		Routinely Tested					
BD Bioscie							
bdbiosciences. United States 877.232.8995	Com Canada 888.268.5430	Europe 32.53.720.550	Japan 0120.8555.90	Asia Pacific 65.6861.0633	Latin America/Caribbean 0800.771.7157		⊗ BD
Conditions: The ir of any patents. Bl	nformation disclose D Biosciences will ne	d herein is not to b ot be held responsi	ble for patent infring	ommendation to use gement or other vio	r/ e the above product in violation lations that may occur with the ct either as a stand-alone		•
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Suggested Companion Products

Catalog Number	Name	Size	Clone
553051	APC Rat Anti-Mouse CD4	0.1 mg	RM4-5
553062	FITC Hamster Anti-Mouse CD3e	0.5 mg	145-2C11
550765	PerCP-Cy [™] 5.5 Rat IgG2a, κ Isotype Control	0.1 mg	R35-95
554656	Stain Buffer (FBS)	500 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.

2. 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.

3. 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.

- 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.
- 5. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
- 6. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
- 7. An isotype control should be used at the same concentration as the antibody of interest.
- 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. 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.
- 10. 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.

References

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Ritter U, Wiede F, Mielenz D, Kiafard Z, Zwirner J, Korner H.. Analysis of the CCR7 expression on murine bone marrow-derived and spleen dendritic cells.. J Leukoc Biol. 2004; 76(2):472-476. (Clone-specific: Flow cytometry)

Schweickart VL, Raport CJ, Godiska R, et al. Cloning of human and mouse EBI1, a lymphoid-specific G-protein-coupled receptor encoded on human chromosome 17q12-q21.2. *Genomics.* 1994; 23(3):643-650. (Biology)