Technical Data Sheet

PE-Cy™7 Rat Anti-Mouse Ly-6G and Ly-6C

Product Information

Material Number:	552985
Alternate Name:	Ly6c, Lymphocyte antigen 6C2; Lymphocyte antigen 6G, Ly6g, Gr-1
Size:	20 µg
Concentration:	0.2 mg/ml
Clone:	RB6-8C5
Immunogen:	Not Reported
Isotype:	Rat IgG2b, ĸ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing protein stabilizer and ≤0.09% sodium
	azide.

Description

The RB6-8C5 antibody reacts with a common epitope on Ly-6G and Ly-6C, previously known as the myeloid differentiation antigen Gr-1. In the bone marrow, the level of antigen expression is directly correlated with granulocyte differentiation and maturation. The antigen is also expressed on the monocyte lineage in the bone marrow, but not on erythroid cells. In the periphery, RB6-8C5 antibody recognizes granulocytes (neutrophils and eosinophils) and monocytes. The RB6-8C5 mAb is a component of the "lineage cocktail" used in studies of hematopoietic lineages. The mAb 1A8 (Cat. No. 551461) specifically recognizes Ly-6G, but not Ly-6C.

Based on the comparison of the staining patterns of mAbs clones 1A8 and RB6-8C5 on total blood leukocytes, it is evident that mAb 1A8 stains the RB6-8C5-bright population, corresponding to Ly-6G-expressing granulocytes; whereas, the RB6-8C5-dim population is 1A8-negative and corresponds to Ly-6C-expressing lymphocytes and monocytes. Please refer to the TDS Cat. No. 551459 and 553128 for more detail information.



Two-parameter analysis of the expression of Ly-6G and/or Ly-6C on bone-marrow myeloid cells. BALB/c bone-marrow leukocytes were stained with either $PE-Cy^{TW7}$ R at IgG2b, κ Isotype Control (Cat. No. 552849; left panel) or $PE-Cy^{TW7}$ R at Anti-Mouse Ly-6G and Ly-6C (Cat. No. 552985; right panel). Please note that the population of cells having the lowest SSC (erythroid and lymphoid cells) shows little expression of Ly-6G/Ly-6C, while most of the leukocytes with moderate-to-high SSC (myeloid cells) are Ly-6G/Ly-6C-positive. Flow cytometry was performed on a BD FACSCaliburTM 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				ed			
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Suggested Companion Products

Catalog Number	Name	Size	Clone
552849	PE-Cy™7 Rat IgG2b, κ Isotype Control	0.1 mg	A95-1
554656	Stain Buffer (FBS)	500 ml	(none)

Product Notices

- Since applications vary, each investigator should titrate the reagent to obtain optimal results. 1.
- 2 Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
- For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at 3. www.bdbiosciences.com/colors
- 4 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).
- Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem 5. 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.
- PE-Cy7 is a tandem fluorochrome composed of R-phycoerythrin (PE), which is excited by 488-nm light and serves as an energy donor, 6. 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. 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.
- This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under 8. 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.
- 9 Caution: Sodium azide vields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
- An isotype control should be used at the same concentration as the antibody of interest. 10.

References

Brummer E, Sugar AM, Stevens DA. Immunological activation of polymorphonuclear neutrophils for fungal killing: studies with murine cells and blastomyces dermatitidis in vitro. J Leukoc Biol. 1984; 36(4):505-520. (Clone-specific: Cytotoxicity)

Conlan JW, North RJ. Neutrophils are essential for early anti-Listeria defense in the liver, but not in the spleen or peritoneal cavity, as revealed by a granulocyte-depleting monoclonal antibody. J Exp Med. 1994; 179(1):259-268. (Clone-specific: Depletion)

Czuprynski CJ, Brown JF, Maroushek N, Wagner RD, Steinberg H. Administration of anti-granulocyte mAb RB6-8C5 impairs the resistance of mice to Listeria monocytogenes infection. J Immunol. 1994; 152(4):1836-1846. (Clone-specific: Depletion, Western blot)

Fleming TJ, Fleming ML, Malek TR. Selective expression of Lv-6G on myeloid lineage cells in mouse bone marrow. RB6-8C5 mAb to granulocyte-differentiation antigen (Gr-1) detects members of the Ly-6 family. J Immunol. 1993; 151(5):2399-2408. (Clone-specific: Immunoprecipitation, Inhibition)

Gumley TP, McKenzie IF, Sandrin MS. Tissue expression, structure and function of the murine Ly-6 family of molecules. Immunol Cell Biol. 1995; 73(4):277-296. (Biology)

Hestdal K, Ruscetti FW, Ihle JN, et al. Characterization and regulation of RB6-8C5 antigen expression on murine bone marrow cells. J Immunol. 1991; 147(1):22-28. (Biology)

Jutila MA, Kroese FG, Jutila KL, Stall AM, Fiering S, Herzenberg LA, Berg EL, Butcher EC. Ly-6C is a monocyte/macrophage and endothelial cell differentiation antigen regulated by interferon-gamma. Eur J Immunol. 1988; 18(11):1819-1826. (Clone-specific: Western blot)

Lagasse E, Weissman IL. Flow cytometric identification of murine neutrophils and monocytes. J Immunol Methods. 1996; 197(1-2):139-150. (Biology) Lewinsohn DM, Bargatze RF, Butcher EC. Leukocyte-endothelial cell recognition: evidence of a common molecular mechanism shared by neutrophils, lymphocytes, and other leukocytes. J Immunol. 1987; 138(12):4313-4321. (Biology)

Roederer M, Kantor AB, Parks DR, Herzenberg LA. Cy7PE and Cy7APC: bright new probes for immunofluorescence. Cytometry. 1996; 24(3):191-197. (Methodology)

Stoppacciaro A, Melani C, Parenza M, et al. Regression of an established tumor genetically modified to release granulocyte colony-stimulating factor requires granulocyte-T cell cooperation and T cell-produced interferon gamma. J Exp Med. 1993; 178(1):151-161. (Clone-specific: Depletion, Immunohistochemistry) Tepper RI, Coffman RL, Leder P. An eosinophil-dependent mechanism for the antitumor effect of interleukin-4. Science. 1992; 257(5069):548-551. (Clone-specific: Depletion)

Tumpey TM, Chen SH, Oakes JE, Lausch RN. Neutrophil-mediated suppression of virus replication after herpes simplex virus type 1 infection of the murine cornea. J Virol. 1996; 70(2):898-904. (Clone-specific: Depletion)

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