Technical Data Sheet

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

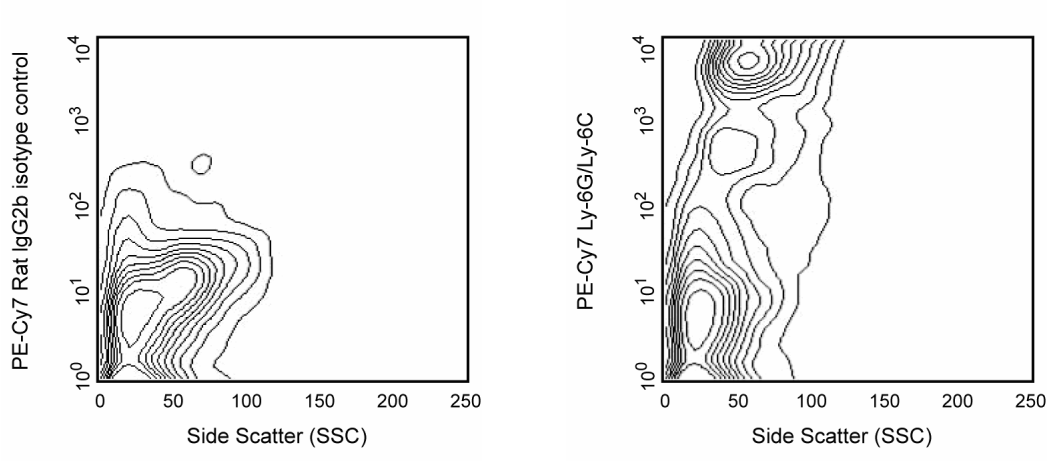
Product Information

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
Isotype:	Rat IgG2b, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing protein stabilizer and ≤0.09% sodium azide.

Description

The RB6-8C5 monoclonal antibody recognizes 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 antibody is a component of the "lineage cocktail" used in studies of hematopoietic cell lineages. The 1A8 antibody (Cat. No. 551461) specifically recognizes Ly-6G, but not Ly-6C.

Based on comparison of the staining patterns given by 1A8 versus RB6-8C5 antibodies on total blood leucocytes, it is evident that the 1A8 antibody 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 Technical Data Sheets for Cat. No. 551459 and 553128 for more detailed 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™7 Rat IgG2b, κ Isotype Control (Cat. No. 552849; left panel) or PE-Cy™7 Rat Anti-Mouse Ly-6G and Ly-6C (Cat. No. 552985/565033; 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 FACSCalibur™ 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

BD Biosciences

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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)
554657	Stain Buffer (BSA)	500 mL	(none)
565033	PE-Cy™7 Rat Anti-Mouse Ly-6G and Ly-6C	0.1 mg	RB6-8C5

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. An isotype control should be used at the same concentration as the antibody of interest.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
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. 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).
6. 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.
7. 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.
8. Cy is a trademark of GE Healthcare.
9. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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Jutila MA, Kroese FG, Jutila KL, et al. 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)

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