

Technical Data Sheet

APC-Cy7™7 Rat Anti-Mouse Ly-6G and Ly-6C

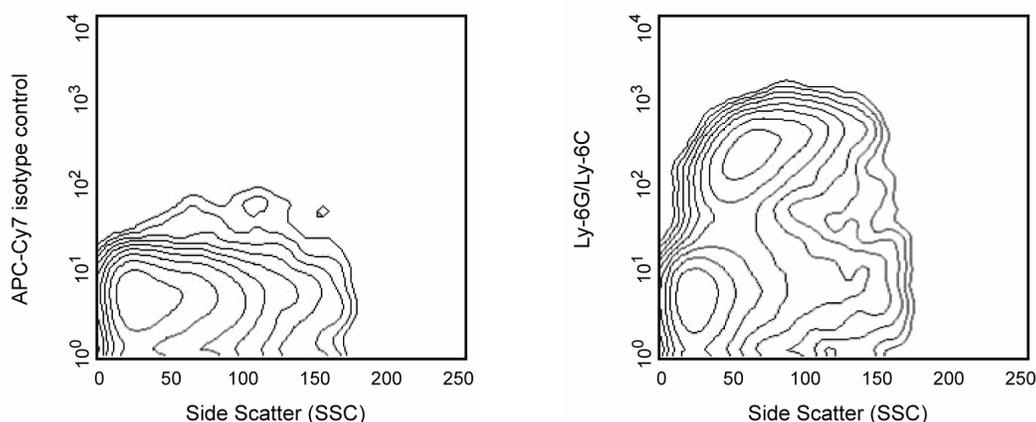
Product Information

Material Number:	557661
Alternate Name:	Gr-1
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	RB6-8C5
Immunogen:	Not Reported
Isotype:	Rat IgG2b, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing protein stabilizer and $\leq 0.09\%$ 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. C57BL/6 bone marrow cells were stained with either APC-Cy7-conjugated rat IgG2b κ isotype control mAb A95-1 (Cat. No. 552773, left panel) or APC-Cy7 conjugated mAb RB6-8C5 (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 FACSVantage SE™ 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 APC-Cy7 under optimum conditions, and unconjugated antibody and free APC-Cy7 were removed.

Application Notes

Application

Flow cytometry	Routinely Tested
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Suggested Companion Products

<u>Catalog Number</u>	<u>Name</u>	<u>Size</u>	<u>Clone</u>
552773	APC-Cy TM 7 Rat IgG2b κ Isotype Control	0.1 mg	A95-1

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to wwwbdbiosciences.com/pharmingen/protocols for technical protocols.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at wwwbdbiosciences.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 BDTM Stabilizing Fixative (Cat. No. 338036).
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. APC-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher.
7. APC-Cy7 is a tandem fluorochrome composed of Allophycocyanin (APC), which is excited by laser lines between 595 and 647 nm and serves as an energy donor, coupled to the cyanine dye Cy7TM, which acts as an energy acceptor and fluoresces at 780 nm. BD Biosciences Pharmingen has maximized the fluorochrome energy transfer in APC-Cy7, thus maximizing its fluorescence emission intensity, minimizing residual emission from APC, and minimizing required electronic compensation in multilaser-laser flow cytometry systems. Note: Although every effort is made to minimize the lot-to-lot variation in residual emission from APC, 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 APC-Cy7 conjugate.
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. 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.
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