

Technical Data Sheet

PE Rat Anti-Mouse Ly-6G and Ly-6C

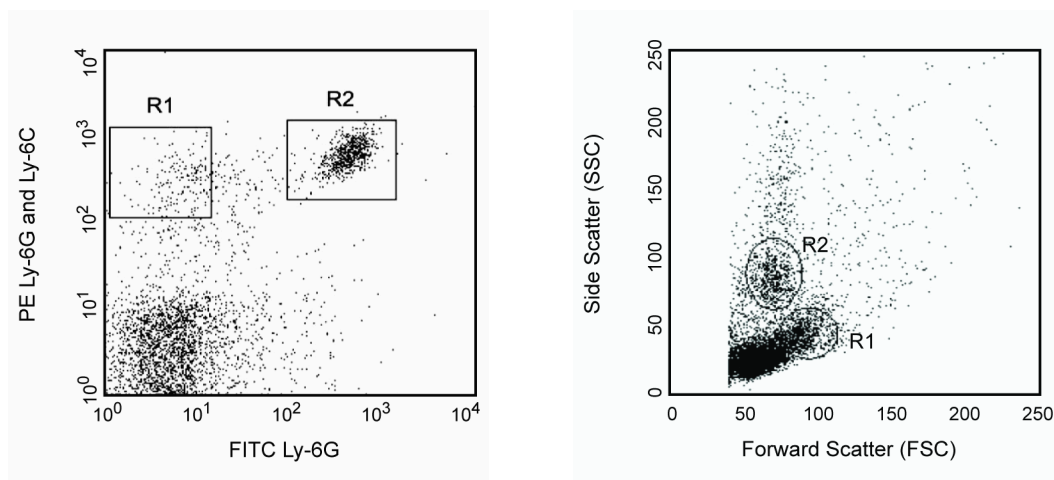
Product Information

Material Number:	553128
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.



Representative staining of peripheral blood leukocytes with PE-conjugated antibody RB6-8C5. C57BL/6 whole blood was stained with PE-conjugated RB6-8C5 (anti-Ly-6G and Ly-6C) and FITC-conjugated 1A8 (anti-mouse Ly-6G, Cat. No. 551460) monoclonal antibodies in the presence of Mouse BD Fc Block™ purified anti-mouse CD16/CD32 mAb 2.4G2 (Cat. No. 553141/553142, left panel). Erythrocytes were lysed (BD Pharm Lyse™ lysis buffer, Cat. No. 555899) and non-viable leukocytes were excluded by staining with propidium iodide. The left panel demonstrates that mAb 1A8 stains the RB6-8C5^{hi} population, corresponding to Ly-6G-expressing granulocytes; whereas, the RB6-8C5^{lo} population is 1A8-negative and corresponds to Ly-6C-expressing lymphocytes and monocytes. Backgating of the RB6-8C5^{hi}/1A8⁻ population (R1) onto the light-scatter profile (right panel) indicates that this population falls within the monocyte region of the light-scatter profile. The RB6-8C5^{hi}/1A8⁺ population (R2) falls within the granulocyte region of the light-scatter profile (right panel). 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 R-PE under optimum conditions, and unconjugated antibody and free PE were removed.

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Application Notes

Application

Flow cytometry

Routinely Tested

Recommended Assay Procedure:

Mouse BD Fc Block™ purified anti-mouse CD16/CD32 mAb 2.4G2 (Cat. No. 553141/553142) may help to reduce non-specific binding to cells bearing Fcγ-receptors.

Suggested Companion Products

<u>Catalog Number</u>	<u>Name</u>	<u>Size</u>	<u>Clone</u>
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2
553989	PE 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. 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.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at wwwbdbiosciences.com/colors.
4. Please refer to wwwbdbiosciences.com/pharming/protocols for technical protocols.

References

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)

Fleming TJ, Fleming ML, Malek TR. Selective expression of Ly-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)

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, 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)

Lagasse E, Weissman IL. Flow cytometric identification of murine neutrophils and monocytes. *J Immunol Methods.* 1996; 197(1-2):139-150. (Biology)

Osawa M, Tokumoto Y, Nakauchi H. Hematopoietic stem cells. In: Herzenberg LA, Weir DM, Blackwell C, ed. *Weir's Handbook of Experimental Immunology, 5th Edition.* Cambridge: Blackwell Science; 1996:66.1-66.5. (Biology)

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)