Technical Data Sheet

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

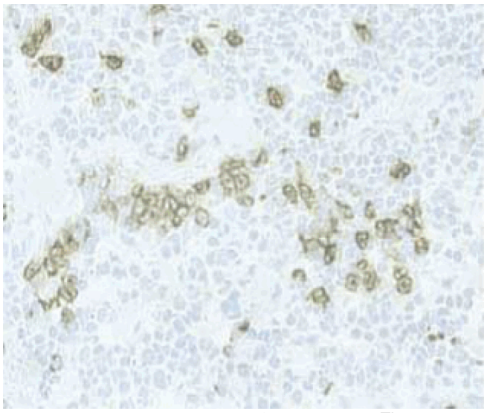
Product Information

Material Number:	**550291**
Alternate Name:	Gr-1
Size:	1.0 ml
Concentration:	62.5 µg/ml
Clone:	RB6-8C5
Isotype:	Rat IgG2b, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing BSA, goat serum, 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.



Immunohistochemical staining of granulocytes. *The frozen section of normal mouse spleen was reacted with RB6-8C5 mAb. Granulocytes are identified by the brown labeling of their cell surface membranes.*

Rat IgG2bκ isotype control. *The frozen section of normal mouse spleen was reacted with rat IgG2b isotype control (Cat. No. 559478).*

Preparation and Storage

Store undiluted at 4°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Application Notes

Application

Flow cytometry	Routinely Tested
Immunohistochemistry-frozen	Tested During Development
Immunohistochemistry-zinc-fixed	Tested During Development
Immunohistochemistry-formalin (antigen retrieval required)	Not Recommended

Recommended Assay Procedure:

For optimal indirect immunohistochemical staining, the RB6-8C5 antibody should be titrated (1:10 to 1:50 diluent) and visualized via a three-step staining procedure in combination with Biotin Anti-Rat IgG2b (Cat. No. 550327) as the secondary antibody and Streptavidin-HRP (Cat. No. 550946) together with the DAB detection system (Cat. No. 550880). Alternatively, Anti-Rat Ig HRP Detection Kit (Cat. No. 551013) containing

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all secondary reagents, may be used. An appropriate isotype control is Purified Rat IgG2b κ Isotype Control, (Cat. No. 559478).

Suggested Companion Products

Catalog Number	Name	Size	Clone
559478	Purified Rat IgG2b, κ Isotype Control	0.25 mg	A95-1
550327	Biotin Mouse Anti-Rat IgG2b	1.0 ml	G15-337
550946	Streptavidin HRP	50 ml	(none)
550880	DAB Substrate Kit	500 tests	(none)
551013	Anti-Rat Ig HRP Detection Kit	200 tests	(none)

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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. This antibody has been developed for the immunohistochemistry application. However, a routine immunohistochemistry test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.
5. An isotype control should be used at the same concentration as the antibody of interest.
6. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

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, Western blot)

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)

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)

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)

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)

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. (Biology)

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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