

Technical Data Sheet

Purified Rat Anti-Mouse Ly-6G

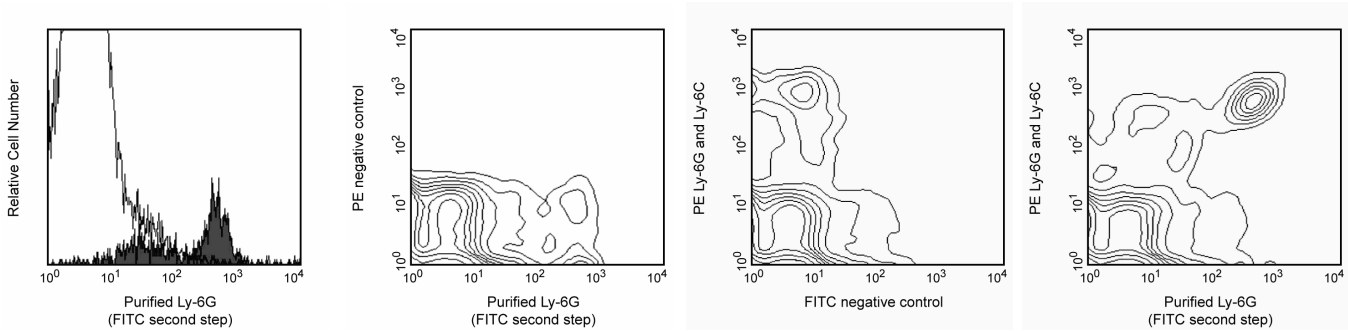
Product Information

Material Number:	551459
Size:	0.5 mg
Concentration:	0.5 mg/ml
Clone:	1A8
Immunogen:	Ly-6G-transfected EL4J cell line
Isotype:	Rat (LEW) IgG2a, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The 1A8 antibody reacts with Ly-6G, a 21-25-kDa GPI-anchored protein. In the bone marrow, Ly-6G is expressed on the majority of the largest cells, which are predominantly granulocytes, and not on lymphoid or erythroid cells. In the periphery, it is expressed on granulocytes. The mAb RB6-8C5 (Cat. No. 557445/553123) recognizes both Ly-6G and Ly-6C and blocks the binding of mAb 1A8 to Ly-6G.

This antibody is routinely tested by flow cytometric analysis. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.



Expression of Ly-6G on peripheral-blood leukocytes. C57BL/6 whole blood was stained with purified 1A8 mAb in the presence of Mouse Fc Block^a (purified anti-mouse CD16/CD32, Cat. No. 553141/553142), followed by FITC-conjugated anti-rat IgG2a mAb RG7/1.30 (Cat. No. 553896, Far and middle left panels, right panel), then PE-conjugated anti-mouse Ly-6G and Ly-6C mAb RB6-8C5 (Cat. No. 553128, far and middle right panels). Erythrocytes were lysed (PharmLyse™, Cat. No. 555899), non-viable leukocytes were excluded by staining with propidium iodide, and leukocyte subsets were distinguished by their light-scatter profiles. Far left panel displays the expression of Ly-6G on granulocytes (filled histogram) and lymphocytes/monocytes (open histogram). Note that Ly-6G expression is almost exclusively on granulocytes. Middle left and far and middle right panels compare the staining patterns of mAbs 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. Flow cytometry was performed on a FACSCalibur™ (BDIS, San Jose, CA).

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography. Store undiluted at 4° C.

Application Notes

Application

Flow cytometry	Routinely Tested
Immunoprecipitation	Reported
Immunohistochemistry-paraffin	Reported
Immunohistochemistry-frozen	Reported
Induction	Reported

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Recommended Assay Procedure:

For flow cytometry of leukocyte suspensions, we recommend the use of Mouse Fc Block™ (purified anti-mouse CD16/CD32 mAb 2.4G2, Cat. No. 553141/553142). If Mouse Fc Block™ is used, it is important that the second-step antibody does not react with the 2.4G2 mAb (rat IgG2b, κ); we recommend FITC-conjugated anti-rat IgG2a mAb RG7/1.30 (Cat. No. 553896). Other reported applications include immunoprecipitation, immunohistochemical staining (IHC) of paraffin-embedded and acetone fixed frozen sections, and induction of T-cell inhibitory signalling. For IHC, we recommend the use of purified RB6-8C5 mAb in our special formulation for immunohistochemistry, Cat. No. 550291.

Suggested Companion Products

Catalog Number	Name	Size	Clone
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2
553896	FITC Mouse Anti-Rat IgG2a	0.5 mg	RG7/1.30
553128	PE Rat Anti-Mouse Ly-6G and Ly-6C	0.1 mg	RB6-8C5
555899	Lysing Buffer	100 ml	(none)
553927	Purified Rat IgG2a, κ Isotype Control	0.5 mg	R35-95

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharming/en/protocols for technical protocols.
3. 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.

References

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.(Immunogen: Immunoprecipitation)
Fleming TJ, Malek TR. Multiple glycosylphosphatidylinositol-anchored Ly-6 molecules and transmembrane Ly-6E mediate inhibition of IL-2 production. *J Immunol.* 1994; 153(5):1955-1962.(Clone-specific: Induction)