

Technical Data Sheet

PE-Cy™7 Rat Anti-Mouse Ly-6A/E

Product Information

Material Number:	561021
Alternate Name:	Ly-6A/E; Lymphocyte antigen 6A-2/6E-1; Ly-6A.2/Ly-6E.1; Sca-1; TAP
Size:	25 µg
Concentration:	0.2 mg/ml
Clone:	D7
Immunogen:	IL-2-dependent mouse T-cell line CTL-L
Isotype:	Rat (LEW) IgG2a, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The D7 antibody reacts with Ly-6A.2 and Ly-6E.1, which are allelic members of the Ly-6 multigene family. Sca-1 (Ly6A/E), a phosphatidylinositol-anchored protein of about 18 kDa, is expressed on the multipotent hematopoietic stem cells (HSC) in the bone marrow of mice with both Ly-6 haplotypes. In mice expressing the Ly-6.2 haplotype (e.g., AKR, C57BL, C57BR, C57L, C58, DBA/2, PL, SJL, SWR, 129), Ly-6A/E is also expressed on distinct subpopulations of bone marrow and peripheral B lymphocytes and thymic and peripheral T lymphocytes. Strains with the Ly-6.1 haplotype (e.g., A, BALB/c, CBA, C3H/He, DBA/1, NZB) have few Ly-6A/E+ resting peripheral lymphocytes, whereas activation of lymphocytes from mice of both Ly-6 haplotypes leads to strong expression of the Sca-1 antigen. Studies with the D7 antibody have demonstrated that Ly-6A/E may be involved in the regulation of B and T lymphocyte responses, and it appears to be required for T-cell receptor-mediated T-cell activation. Purified E13-161.7 mAb (anti-Ly-6A/E, Cat. No. 553333) can block binding of FITC-conjugated D7 antibody (Cat. No. 557405) to mouse splenocytes, but purified mAb D7 is unable to block binding of FITC-conjugated E13-161.7 antibody (Cat. No. 553335). Anti-Ly-6A/E (Sca-1) mAb may be used in combination with the Mouse Lineage Panel (Cat. No. 559971) to identify HSC.

Preparation and Storage

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.

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Flow cytometry	Routinely Tested
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Suggested Companion Products

Catalog Number	Name	Size	Clone
552784	PE-Cy™7 Rat IgG2a, κ Isotype Control	0.1 mg	R35-95

Product Notices

- Since applications vary, each investigator should titrate the reagent to obtain optimal results.
- Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
- For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
- 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.
- 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).
- 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.

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7. 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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9. 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

- Codias EK, Cray C, Baler RD, Levy RB, Malek TR. Expression of Ly-6A/E alloantigens in thymocyte and T-lymphocyte subsets: variability related to the Ly-6a and Ly-6b haplotypes. *Immunogenetics*. 1989; 29(2):98-107. (Clone-specific: Immunohistochemistry)
- Codias EK, Malek TR. Regulation of B lymphocyte responses to IL-4 and IFN-gamma by activation through Ly-6A/E molecules. *J Immunol*. 1990; 144(6):2197-2204. (Clone-specific: Activation)
- Codias EK, Rutter JE, Fleming TJ, Malek TR. Down-regulation of IL-2 production by activation of T cells through Ly-6A/E. *J Immunol*. 1990; 145(5):1407-1414. (Clone-specific: Activation)
- Ivanov V, Fleming TJ, Malek TR. Regulation of nuclear factor-kappa B and activator protein-1 activities after stimulation of T cells via glycosylphosphatidylinositol-anchored Ly-6A/E. *J Immunol*. 1994; 153(6):2394-2406. (Clone-specific: Activation)
- Malek TR, Ortega G, Chan C, Kroczeck RA, Shevach EM. Role of Ly-6 in lymphocyte activation. II. Induction of T cell activation by monoclonal anti-Ly-6 antibodies. *J Exp Med*. 1986; 164(3):709-722. (Clone-specific: Activation)
- Ortega G, Korty PE, Shevach EM, Malek TR. Role of Ly-6 in lymphocyte activation. I. Characterization of a monoclonal antibody to a nonpolymorphic Ly-6 specificity. *J Immunol*. 1986; 137(10):3240-3246. (Immunogen: Flow cytometry, Immunoprecipitation, Western blot)
- Palfree RG, Dumont FJ, Hammerling U. Ly-6A.2 and Ly-6E.1 molecules are antithetical and identical to MALA-1. *Immunogenetics*. 1986; 23(3):197-207. (Clone-specific: Flow cytometry, Western blot)