Technical Data Sheet

PE-CF594 Rat Anti-Mouse Ly-6A/E

Product Information

Material Number: 562730

Alternate Name: Ly-6A/E; Lymphocyte antigen 6A-2/6E-1; Ly-6A.2/Ly-6E.1; Sca-1; TAP

 Size:
 50 µg

 Concentration:
 0.2 mg/ml

 Clone:
 D7

Immunogen: IL-2-dependent mouse T-cell line CTL-L

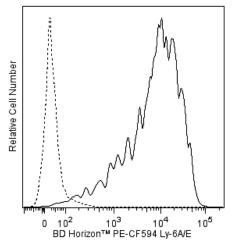
 $\begin{array}{ll} \textbf{Isotype:} & \text{Rat (LEW) IgG2a, } \kappa \\ \textbf{Reactivity:} & \text{QC Testing: Mouse} \end{array}$

Storage Buffer: Aqueous buffered solution containing BSA and ≤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.

This antibody is conjugated to BD HorizonTM PE-CF594, which has been developed exclusively by BD Biosciences as a better alternative to PE-Texas Red®. PE-CF594 excites and emits at similar wavelengths to PE-Texas Red® yet exhibits improved brightness and spectral characteristics. Due to PE having maximal absorption peaks at 496 nm and 564 nm, PE-CF594 can be excited by the blue (488-nm), green (532-nm) and yellow-green (561-nm) lasers and can be detected with the same filter set as PE-Texas Red® (eg 610/20-nm filter).



Flow cytometric analysis of Ly-6A/E expression on BALB/c mouse splenocytes. Concanavlin A-activated mouse splenocytes were stained with either BD Horizon™ PE-CF594 Rat Anti-Mouse Ly-6A/E antibody (Cat. No. 562730, solid line histogram) or BD Horizon™ PE-CF594 Rat IgG2a, κ Isotype Control (Cat. No. 562302, dashed line histogram). Flow cytometric fluorescence histograms were derived from gated events with the forward and side light-scatter characteristics of viable splenocytes. Flow cytometry was performed using a BD™ LSR II Flow Cytometer 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 BD Horizon™ PE-CF594 under optimum conditions, and unconjugated antibody and free PE-CF594 were removed.

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Application Notes

Application

ı	Flow cytometry	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
562302	PE-CF594 Rat IgG2a, κ Isotype Control	0.1 mg	R35-95
554656	Stain Buffer (FBS)	500 ml	(none)

Product Notices

- 1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
- 2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
- 3. An isotype control should be used at the same concentration as the antibody of interest.
- 4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
- 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.
- 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.
- For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
- 8. Texas Red is a registered trademark of Molecular Probes, Inc., Eugene, OR.
- 9. CFTM is a trademark of Biotium, Inc.
- 10. When excited by the yellow-green (561-nm) laser, the fluorescence may be brighter than when excited by the blue (488-nm) laser.
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- Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using multi-laser cytometers, which
 may directly excite both PE and CFTM594.

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)

Flood PM, Dougherty JP, Ron Y. Inhibition of Ly-6A antigen expression prevents T cell activation. *J Exp Med.* 1990; 172(1):115-120. (Biology)

Ito M, Anan K, Misawa M, Kai S, Hara H. In vitro differentiation of murine Sca-1+Lin- cells into myeloid, B cell and T cell lineages. Stem Cells. 1996; 14(4):412-418. (Biology)

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, Danis KM, Codias EK. Tumor necrosis factor synergistically acts with IFN-gamma to regulate Ly-6A/E expression in T lymphocytes, thymocytes and bone marrow cells. *J Immunol.* 1989; 142(6):1929-1936. (Biology)

Malek TR, Ortega G, Chan C, Kroczek 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)

Moore T, Bennett M, Kumar V. Transplantable NK cell progenitors in murine bone marrow. J Immunol. 1995; 154(4):1653-1663. (Biology)

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)

Rock KL, Reiser H, Bamezai A, McGrew J, Benacerraf B. The LY-6 locus: a multigene family encoding phosphatidylinositol-anchored membrane proteins concerned with T-cell activation. *Immunol Rev.* 1989; 111:195-224. (Biology: Flow cytometry)

Yonemura Y, Ku H, Lyman SD, Ogawa M. In vitro expansion of hematopoietic progenitors and maintenance of stem cells: comparison between FLT3/FLK-2 ligand and KIT ligand. *Blood*. 1997; 89(6):1915-1921. (Biology)

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