

Technical Data Sheet

V500 Rat anti-Mouse Ly-6A/E

Product Information

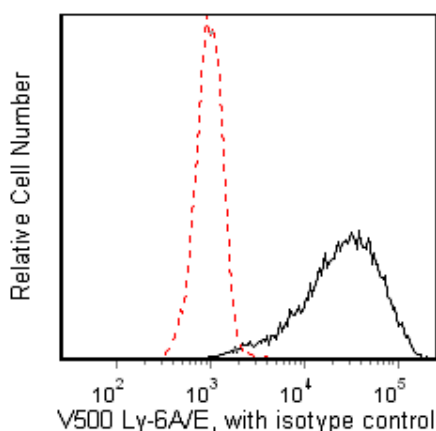
Material Number:	561229
Alternate Name:	Ly-6A/E; Lymphocyte antigen 6A-2/6E-1; Ly-6A.2/Ly-6E.1; Sca-1; TAP
Size:	25 µg
Concentration:	0.2 mg/ml
Clone:	D7
Immunogen:	IL-2-dependent mouse T-cell line CTL-L
Isotype:	Rat (LEW) IgG2a, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing protein stabilizer, glycerol 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.

The antibody is conjugated to BD Horizon™ V500, which has been developed for use in multicolor flow cytometry experiments and is available exclusively from BD Biosciences. It is excited by the Violet laser with an Ex max of 415 nm and Em Max at 500 nm. BD Horizon V500 conjugates emit at a similar wavelength to Amcyan yet exhibit reduced spillover into the FITC channel. For more information on BD Horizon V500, visit bdbiosciences.com/colors.

When compensating dyes in this spectral range (such as Horizon™ V500 and AmCyan), the most accurate compensation can be obtained using single stained cellular controls. Due to spectral differences between cells and beads in this channel, using BD CompBeads can result in spillover errors for V500 and AmCyan reagents. Therefore, the use of BD CompBeads or BD CompBeads Plus to determine spillover values for these reagents is not recommended. Different V500 reagents (e.g. CD4 vs. CD45) can have slightly different fluorescence spillover therefore, it may also be necessary to use clone specific compensation controls when using these reagents.



Flow cytometric analysis of Ly-6A/E expression on stimulated mouse splenocytes. BALB/c splenocytes were stimulated with 2.5-5.0 µg/mL Concanavilin A (Sigma-Aldrich cat. no. C2010) for 48 hours and were subsequently stained with either BD Horizon™ V500 Rat anti-Mouse Ly-6A/E (Cat. No. 561229; solid line histogram) or a BD Horizon™ V500 Rat IgG2a, κ Isotype Control (Cat. No. 560786 used at the same concentration; dashed line histogram). The fluorescence histograms were derived from events with the forward and side light-scatter characteristics of viable lymphocytes. Flow cytometry was performed using a BD FACSCanto™ II Flow Cytometer System.

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Preparation and Storage

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

The antibody was conjugated with BD Horizon™ V500 under optimum conditions, and unreacted BD Horizon™ V500 was removed.

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

Application Notes

Application

Flow cytometry

Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
560786	V500 Rat IgG2a, κ Isotype Control	0.1 mg	R35-95
554656	Stain Buffer (FBS)	500 ml	(none)
559971	Biotin Mouse Lineage Panel	1000 tests	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to wwwbdbiosciences.com/pharming/protocols for technical protocols.
3. BD Horizon™ V500 has a maximum absorption of 415 nm and maximum emission of 500 nm. Before staining with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at wwwbdbiosciences.com/colors.
5. 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

- Auerbach R, Huang H, Lu L. Hematopoietic stem cells in the mouse embryonic yolk sac. *Stem Cells*. 1996; 14(3):269-280. (Biology)
- 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. (Biology)
- 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. (Biology)
- 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. (Biology)
- 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. (Biology)
- 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. (Biology)
- Jurecic R, Van NT, Belmont JW. Enrichment and functional characterization of Sca-1+WGA+, Lin-WGA+, Lin-Sca-1+, and Lin-Sca-1+WGA+ bone marrow cells from mice with an Ly-6a haplotype. *Blood*. 1993; 82(9):2673-2683. (Biology)
- 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, 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. (Biology)
- Morrison SJ, Hemmati HD, Wandycz AM, Weissman IL. The purification and characterization of fetal liver hematopoietic stem cells. *Proc Natl Acad Sci U S A*. 1995; 92(22):10302-10306. (Biology)
- Morrison SJ, Wandycz AM, Hemmati HD, Wright DE, Weissman IL. Identification of a lineage of multipotent hematopoietic progenitors. *Development*. 1997; 124(10):1929-1939. (Biology)
- Morrison SJ, Wright DE, Weissman IL. Cyclophosphamide/granulocyte colony-stimulating factor induces hematopoietic stem cells to proliferate prior to mobilization. *Proc Natl Acad Sci U S A*. 1997; 94(5):1908-1913. (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)
- Osawa M, Nakamura K, Nishi N, et al. In vivo self-renewal of c-Kit+ Sca-1+ Lin(low/-) hemopoietic stem cells. *J Immunol*. 1996; 156(9):3207-3214. (Biology)
- 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. (Biology)
- 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)
- Spangrude GJ, Aihara Y, Weissman IL, Klein J. The stem cell antigens Sca-1 and Sca-2 subdivide thymic and peripheral T lymphocytes into unique subsets. *J Immunol*. 1988; 141(11):3697-3707. (Biology)
- Spangrude GJ, Brooks DM. Mouse strain variability in the expression of the hematopoietic stem cell antigen Ly-6A/E by bone marrow cells. *Blood*. 1993; 82(11):3327-3332. (Biology)
- Spangrude GJ, Heimfeld S, Weissman IL. Purification and characterization of mouse hematopoietic stem cells. *Science*. 1988; 241(4861):58-62. (Biology)
- Spangrude GJ, Klein J, Heimfeld S, Aihara Y, Weissman IL. Two monoclonal antibodies identify thymic-repopulating cells in mouse bone marrow. *J Immunol*. 1989; 142(2):425-430. (Biology)
- van de Rijn M, Heimfeld S, Spangrude GJ, Weissman IL. Mouse hematopoietic stem-cell antigen Sca-1 is a member of the Ly-6 antigen family. *Proc Natl Acad Sci U S A*. 1989; 86(12):4634-4638. (Biology)
- Yamamoto Y, Yasumizu R, Amou Y, et al. Characterization of peripheral blood stem cells in mice. *Blood*. 1996; 88(2):445-454. (Biology)
- 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)