

## Technical Data Sheet

## V500 Rat anti-Mouse Ly-6A/E

## Product Information

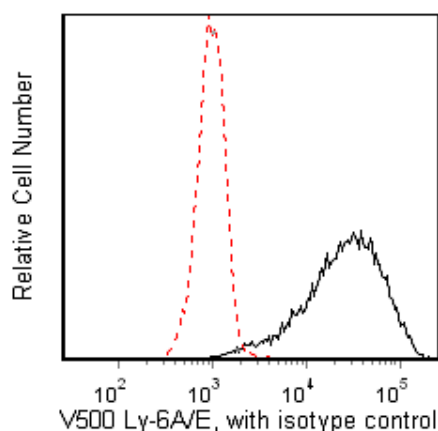
<b>Material Number:</b>	<b>561229</b>
<b>Alternate Name:</b>	Ly-6A/E; Lymphocyte antigen 6A-2/6E-1; Ly-6A.2/Ly-6E.1; Sca-1; TAP
<b>Size:</b>	25 µg
<b>Concentration:</b>	0.2 mg/ml
<b>Clone:</b>	D7
<b>Immunogen:</b>	IL-2-dependent mouse T-cell line CTL-L
<b>Isotype:</b>	Rat (LEW) IgG2a, κ
<b>Reactivity:</b>	QC Testing: Mouse
<b>Storage Buffer:</b>	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](http://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 Concanavalin 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
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## 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 [www.bdbiosciences.com/pharmingen/protocols](http://www.bdbiosciences.com/pharmingen/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 [www.bdbiosciences.com/colors](http://www.bdbiosciences.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.

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