

## Technical Data Sheet

## V500 Rat anti-Mouse CD8a

## Product Information

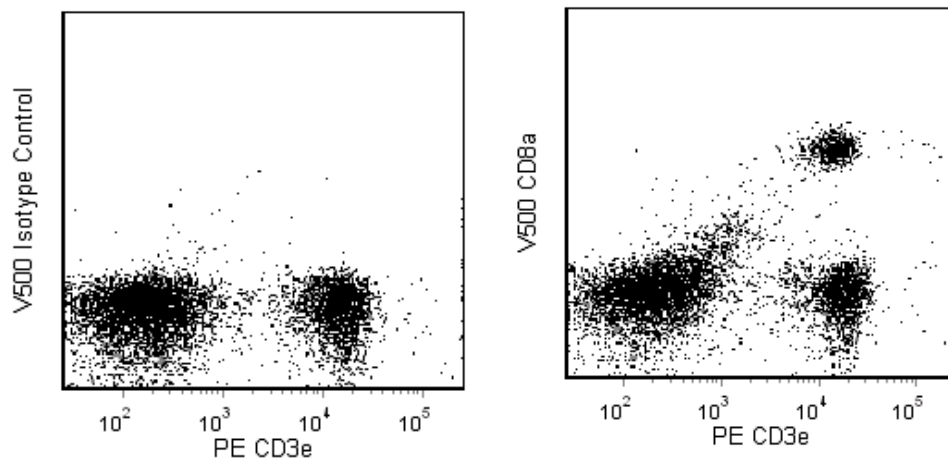
<b>Material Number:</b>	560776
<b>Alternate Name:</b>	CD8a; CD8 alpha chain; Ly-2; Lyt2; Lyt-2; Ly-35; Ly-B
<b>Size:</b>	0.1 mg
<b>Concentration:</b>	0.2 mg/ml
<b>Clone:</b>	53-6.7
<b>Immunogen:</b>	Mouse Spleen Cells or Thymocyte Membranes
<b>Isotype:</b>	Rat (LOU) IgG2a, $\kappa$
<b>Reactivity:</b>	QC Testing: Mouse
<b>Storage Buffer:</b>	Aqueous buffered solution containing protein stabilizer, glycerol and $\leq 0.09\%$ sodium azide.

## Description

The 53-6.7 monoclonal antibody specifically binds to the 38 kDa  $\alpha$  and 34 kDa  $\alpha'$  chains of the CD8 differentiation antigen (Ly-2 or Lyt-2) of all mouse strains tested. The CD8  $\alpha$  and  $\alpha'$  chains (CD8a) form heterodimers with the CD8  $\beta$  chain (CD8b, Ly-3, or Lyt-3) on the surface of most thymocytes. A subpopulation of mature T lymphocytes (i.e., MHC class I-restricted T cells, including most T suppressor/cytotoxic cells) expresses almost exclusively the CD8  $\alpha\beta$  heterodimer. Subsets of  $\gamma\delta$  TCR-bearing T cells, intestinal intraepithelial lymphocytes, and dendritic cells express CD8a without CD8b. It has been suggested that the expression of the CD8a/CD8b heterodimer is restricted to T lymphocytes which matured in the thymus or in an extrathymic environment that had been influenced by thymus-initiated neuroendocrine signals. CD8 is an antigen coreceptor on the T-cell surface which interacts with MHC class I molecules on antigen-presenting cells or epithelial cells. It participates in T-cell activation through its association with the T-cell receptor complex and protein tyrosine kinase lck (p56 [lck]). The CD8  $\alpha$  and  $\alpha'$  chains arise from alternatively spliced messengers of a single *CD8a* gene. The longer  $\alpha$  form associates with p56 [lck] via a CXCP motif in its cytoplasmic domain, which it shares with CD4, but not with CD8b. The truncated  $\alpha'$  chain is unable to associate with p56 [lck], and it may function to attenuate the CD8-mediated costimulatory signal during intrathymic T-cell maturation. In vivo and in vitro treatment with 53-6.7 mAb has reportedly been effective at depleting CD8<sup>+</sup> peripheral T lymphocytes. The 53-6.7 antibody has also been reported to cross-react with CD8  $\alpha$ - and  $\alpha'$ -like polypeptides on subsets of thymic and peripheral lymphocytes in the Egyptian toad, *Bufo regularis*.

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.



**Analysis of CD8a on mouse splenocytes.** Splenocytes from BALB/c mice were stained simultaneously with BD Horizon™ V500 Rat anti-Mouse CD8a (Cat. No. 560776/560778, right panel) or BD Horizon™ V500 Rat IgG2a,  $\kappa$  Isotype Control (Cat. No. 560786, left panel), and PE Hamster Anti-Mouse CD3e (Cat. No. 553063/553064). The two-color analysis plots were derived from gated events based on light scattering characteristics of splenocytes. Flow cytometry was performed on a BD FACSCanto™ II flow cytometry system.

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560776 Rev. 2



## 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™ V500 under optimum conditions, and unreacted BD Horizon™ V500 was removed.

## Application Notes

### Application

Flow cytometry	Routinely Tested
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## Suggested Companion Products

Catalog Number	Name	Size	Clone
553063	PE Hamster Anti-Mouse CD3e	0.1 mg	145-2C11
560786	V500 Rat IgG2a, κ Isotype Control	0.1 mg	R35-95
553064	PE Hamster Anti-Mouse CD3e	0.2 mg	145-2C11
560778	V500 Rat anti-Mouse CD8a	25 µg	53-6.7
554656	Stain Buffer (FBS)	500 mL	(none)

## Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. An isotype control should be used at the same concentration as the antibody of interest.
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.
4. 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.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at [www.bdbiosciences.com/colors](http://www.bdbiosciences.com/colors).
6. BD Horizon™ V500 is covered by one or more of the following US patents: 8,431,416.
7. Please refer to [www.bdbiosciences.com/pharming/protocols](http://www.bdbiosciences.com/pharming/protocols) for technical protocols.

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