Technical Data Sheet

V450 Rat anti-Mouse CD8a

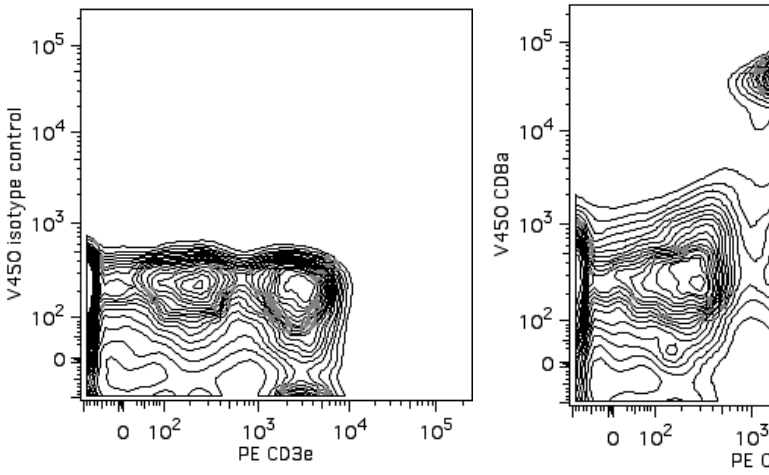
Product Information

Material Number:	**560469**
Alternate Name:	Cd8a; CD8 alpha chain; Ly-2; Lyt2; Lyt-2; Ly-35; Ly-B
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	53-6.7
Immunogen:	Mouse Spleen Cells or Thymocyte Membranes
Isotype:	Rat (LOU) IgG2a, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing protein stabilizer and ≤0.09% sodium azide.

Description

The 53-6.7 monoclonal antibody specifically binds to the 38 kDa α and 34 kDa α' chains of the CD8 differentiation antigen (Ly-2 or Lyt-2) of all mouse strains tested. The CD8 α and α' chains (CD8a) form heterodimers with the CD8 β 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 αβ heterodimer. Subsets of γδ TCR-bearing T cells, intestinal intrapithelial 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 α and α' chains arise from alternatively spliced messengers of a single *CD8a* gene. The longer α form associates with p56 [lck] via a CXCP motif in its cytoplasmic domain, which it shares with CD4, but not with CD8b. The truncated α' 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+ peripheral T lymphocytes. The 53-6.7 antibody has also been reported to cross-react with CD8 α- and α'-like polypeptides on subsets of thymic and peripheral lymphocytes in the Egyptian toad, *Bufo regularis*.

The antibody is conjugated to BD Horizon™ V450, which has been developed for use in multicolor flow cytometry experiments and is available exclusively from BD Biosciences. It is excited by the **V**iolet laser Ex max of 406 nm and has an Em Max at **450** nm. Conjugates with BD Horizon™ V450 can be used in place of Pacific Blue™ conjugates.



Analysis of CD8a on mouse splenocytes. *Splenocytes from BALB/c mice were stained simultaneously with BD Horizon™ V450 Rat anti-Mouse CD8a (Cat. No. 560469/560471, right panel) or BD Horizon™ V450 Rat IgG2a, κ Isotype Control (Cat. No. 560377, left panel), and PE Hamster Anti-Mouse CD3e (Cat. No. 553063/553064). The contour plots were derived from gated events based on light scattering characteristics of splenocytes. Flow cytometry was performed on a BD LSR™ II flow cytometry 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™ V450 under optimum conditions, and unreacted BD Horizon™ V450 was removed.

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	866.979.9408	32.2.400.98.95	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**

Application Notes

Application

Flow cytometry	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
560377	V450 Rat IgG2a, κ Isotype Control	0.1 mg	R35-95
553063	PE Hamster Anti-Mouse CD3e	0.1 mg	145-2C11
554657	Stain Buffer (BSA)	500 mL	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
560471	V450 Rat anti-Mouse CD8a	25 µg	53-6.7
553064	PE Hamster Anti-Mouse CD3e	0.2 mg	145-2C11

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. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
5. BD Horizon™ V450 has a maximum absorption of 406 nm and maximum emission of 450 nm. Before staining with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
6. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.
7. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Bierer BE, Sleckman BP, Ratnofsky SE, Burakoff SJ. The biologic roles of CD2, CD4, and CD8 in T-cell activation. *Annu Rev Immunol.* 1989; 7:579-599. (Biology)

Fujiura Y, Kawaguchi M, Kondo Y, et al. Development of CD8 alpha alpha+ intestinal intraepithelial T cells in beta 2-microglobulin- and/or TAP1-deficient mice. *J Immunol.* 1996; 156(8):2710-2715. (Biology)

Hathcock KS. T cell depletion by cytotoxic elimination. *Curr Protoc Immunol.* 1991; 1:3.4.1-3.4.3. (Biology)

Janeway CA Jr. The T cell receptor as a multicomponent signalling machine: CD4/CD8 coreceptors and CD45 in T cell activation. *Annu Rev Immunol.* 1992; 10:645-674. (Biology)

Kruisbeek AM, Shevach EM. Proliferative assays for T cell function. *Curr Protoc Immunol.* 2004; 3:3.12.1-3.12.14. (Biology)

Ledbetter JA, Herzenberg LA. Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. *Immunol Rev.* 1979; 47:63-90. (Immunogen)

Ledbetter JA, Rouse RV, Micklem HS, Herzenberg LA. T cell subsets defined by expression of Lyt-1,2,3 and Thy-1 antigens. Two-parameter immunofluorescence and cytotoxicity analysis with monoclonal antibodies modifies current views. *J Exp Med.* 1980; 152(2):280-295. (Biology)

Ledbetter JA, Seaman WE, Tsu TT, Herzenberg LA. Lyt-2 and lyt-3 antigens are on two different polypeptide subunits linked by disulfide bonds. Relationship of subunits to T cell cytolytic activity. *J Exp Med.* 1981; 153(6):1503-1516. (Biology)

LeFrancois L. Extrathymic differentiation of intraepithelial lymphocytes: generation of a separate and unequal T-cell repertoire. *Immunol Today.* 1991; 12(12):436-438. (Biology)

Leishman AJ, Naidenko OV, Attinger A, et al. T cell responses modulated through interaction between CD8alphaalpha and the nonclassical MHC class I molecule, TL. *Science.* 2001; 294(5548):1848-1849. (Biology)

MacDonald HR, Schreyer M, Howe RC, Bron C. Selective expression of CD8 alpha (Ly-2) subunit on activated thymic gamma/delta cells. *Eur J Immunol.* 1990; 20(4):927-930. (Biology)

Mitnacht R, Bischof A, Torres-Nagel N, Hunig T. Opposite CD4/CD8 lineage decisions of CD4+8+ mouse and rat thymocytes to equivalent triggering signals: correlation with thymic expression of a truncated CD8 alpha chain in mice but not rats. *J Immunol.* 1998; 160(2):700-707. (Biology)

Murosaki S, Yoshikai Y, Ishida A, et al. Failure of T cell receptor V beta negative selection in murine intestinal intra-epithelial lymphocytes. *Int Immunol.* 1991; 3(10):1005-1013. (Biology)

Nakayama K, Nakayama K, Negishi I, et al. Requirement for CD8 beta chain in positive selection of CD8-lineage T cells. *Science.* 1994; 263(5150):1131-1133. (Biology)

Negm HI, Mansour MH, Saad AH, Abdel Halim RS. Structural characterization of an Lyt-2/3 homolog expressed on Bufo regularis lymphocytes. *Comp Biochem Physiol B Biochem Mol Biol.* 1996; 113(1):79-87. (Biology)

O'Rourke AM, Mescher MF. The roles of CD8 in cytotoxic T lymphocyte function. *Immunol Today.* 1993; 14(4):183-188. (Biology)

Rocha B, Vassalli P, Guy-Grand D. The extrathymic T-cell development pathway. *Immunol Today.* 1992; 14(3):140-141. (Biology)

Sydora BC, Brossay L, Hagenbaugh A, Kronenberg M, Cheroutre H. TAP-independent selection of CD8+ intestinal intraepithelial lymphocytes. *J Immunol.* 1996; 156(11):4209-4216. (Biology)

Traver D, Akashi K, Manz M, et al. Development of CD8alpha-positive dendritic cells from a common myeloid progenitor. *Science.* 2000; 290(5499):2152-2154. (Biology)

van Ewijk W, van Soest PL, van den Engh GJ. Fluorescence analysis and anatomic distribution of mouse T lymphocyte subsets defined by monoclonal antibodies to the antigens Thy-1, Lyt-1, Lyt-2, and T-200. *J Immunol.* 1981; 127(6):2594-2604. (Biology)

Walker ID, Murray BJ, Hogarth PM, Kelso A, McKenzie IF. Comparison of thymic and peripheral T cell Ly-2/3 antigens. *Eur J Immunol.* 1984; 14(10):906-910. (Biology)

Wang J, Klein JR. Thymus-neuroendocrine interactions in extrathymic T cell development. *Science.* 1994; 265(5180):1860-1862. (Biology)

Zamoyska R, Derham P, Gorman SD, et al. Inability of CD8 alpha' polypeptides to associate with p56lck correlates with impaired function in vitro and lack of expression in vivo. *Nature.* 1989; 342(6247):278-281. (Biology)

Zamoyska R, Vollmer AC, Sizer KC, Liaw CW, Parnes JR. Two Lyt-2 polypeptides arise from a single gene by alternative splicing patterns of mRNA. *Cell.* 1985; 43(1):153-163. (Biology)

Zamoyska R. The CD8 coreceptor revisited: one chain good, two chains better. *Immunity.* 1994; 1(4):243-246. (Biology)