

Technical Data Sheet

PE-CF594 Rat Anti-Mouse CD8a

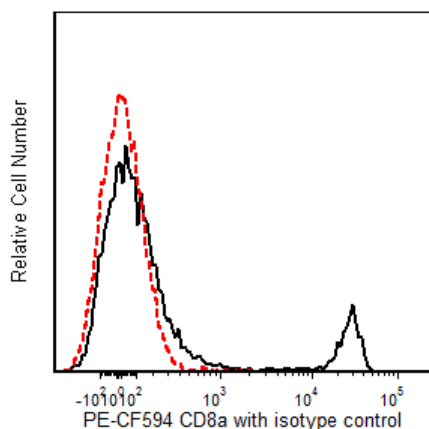
Product Information

Material Number:	562315
Alternate Name:	Cd8a; CD8 alpha chain; Ly-2; Lyt2; Lyt-2; Ly-35; Ly-B
Size:	25 µg
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 BSA 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 $\alpha\beta$ 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 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*.

This antibody is conjugated to BD Horizon™ 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 CD8a expressed on mouse splenocytes. Splenocytes from a BALB/c mouse were stained with either BD Horizon™ PE-CF594 Rat IgG2a, κ Isotype Control (Cat. No. 562302; dashed line histogram) or BD Horizon™ PE-CF594 Rat Anti-Mouse CD8a antibody (Cat. No. 562283/562315; solid line histogram). The fluorescence histograms were derived from events with the forward and side light-scatter characteristics of viable splenocytes. Flow cytometry was performed using a BD™ LSR II Flow Cytometer System.

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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.

Application Notes

Application

Flow cytometry

Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 mL	(none)
562302	PE-CF594 Rat IgG2a, κ Isotype Control	0.1 mg	R35-95
554657	Stain Buffer (BSA)	500 mL	(none)
562283	PE-CF594 Rat Anti-Mouse CD8a	0.1 mg	53-6.7

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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. 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.
5. When excited by the yellow-green (561-nm) laser, the fluorescence may be brighter than when excited by the blue (488-nm) laser.
6. 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.
7. 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 CF™594.
8. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
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11. Texas Red is a registered trademark of Molecular Probes, Inc., Eugene, OR.
12. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

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