

## Technical Data Sheet

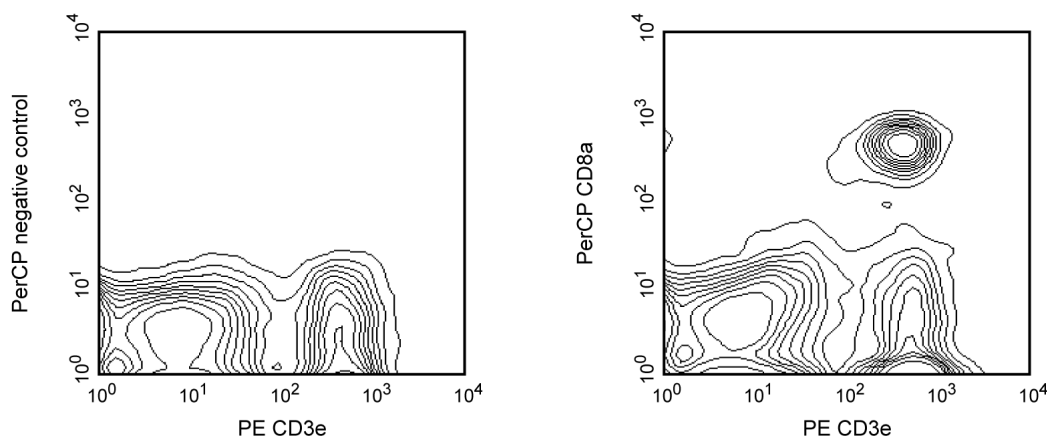
## PerCP Rat Anti-Mouse CD8a

## Product Information

<b>Material Number:</b>	<b>561092</b>
<b>Alternate Name:</b>	Cd8a; CD8 alpha chain; Ly-2; Lyt2; Lyt-2; Ly-35; Ly-B
<b>Size:</b>	25 µg
<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, κ
<b>Reactivity:</b>	QC Testing: Mouse
<b>Storage Buffer:</b>	Aqueous buffered solution containing ≤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*.



**Two-color analysis of the expression of CD8a on mouse spleen T lymphocytes.** BALB/c splenocytes were stained with PE Hamster Anti-Mouse CD3e (Cat. No. 553064/553063; both panels) plus the PerCP Rat Anti-Mouse CD8a (Cat. No. 561092, right panel). Flow cytometry was performed on a BD FACScan™ 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 PerCP under optimum conditions, and unconjugated antibody and free PerCP were removed. Storage of PerCP conjugates in unoptimized diluent is not recommended and may result in loss of signal intensity.

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## Application Notes

### Application

Flow cytometry

Routinely Tested

### Suggested Companion Products

Catalog Number	Name	Size	Clone
553933	PerCP Rat IgG2a, $\kappa$ Isotype Control	0.1 mg	R35-95
553064	PE Hamster Anti-Mouse CD3e	0.2 mg	145-2C11
553063	PE Hamster Anti-Mouse CD3e	0.1 mg	145-2C11
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	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. 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).
5. PerCP is a photosynthetic accessory pigment from *Glenodinium* species of dinoflagellates, which is excited by the 488-nm light of an Argon ion laser and fluoresces at 675 nm. Therefore, PerCP-labelled antibodies can be used with FITC- and R-PE-labelled reagents in most single-laser flow cytometers with no significant spectral overlap of PerCP fluorescence with that of FITC or R-PE. PerCP has been reported to undergo significant photobleaching, the magnitude of which increases as laser power is increased or beam focus is narrowed. For third-color flow cytometric analysis using  $\geq 25$ -mW laser power, we recommend PE-Cy5-, PE-Cy7-, or PerCP-Cy5.5-conjugated reagents.
6. Cy is a trademark of GE Healthcare.
7. Please refer to [www.bdbiosciences.com/pharming/protocols](http://www.bdbiosciences.com/pharming/protocols) for technical protocols.

### References

Afar B, Merrill J, Clark EA. Detection of lymphocyte subsets using three-color/single-laser flow cytometry and the fluorescent dye peridinin chlorophyll-alpha protein. *J Clin Immunol*. 1991; 11(5):254-261. (Methodology: Flow cytometry)

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)

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)