Technical Data Sheet

PE-Cy™7 Rat Anti-Mouse CD8a

Product Information

Material Number:	**552877**
Alternate Name:	Ly-2, Lyt-2
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	53-6.7
Immunogen:	Mouse thymus / spleen Cells
Isotype:	Rat (LOU) IgG2a, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The 53-6.7 antibody has been reported to react with 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 (the α' chain is absent). 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-reaact with CD8 α- and α'-like polypeptides on subsets of thymic and peripheral lymphocytes in the Egyptian toad, *Bufo regularis*.

Preparation and Storage

The antibody was conjugated with PE-Cy7 under optimum conditions, and unconjugated antibody and free PE-Cy7 were removed.
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.

Application Notes

Application

Flow cytometry	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
552784	PE-Cy™7 Rat IgG2a, κ Isotype Control	0.1 mg	R35-95

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
4. PE-Cy7 is a tandem fluorochrome composed of R-phycoerythrin (PE), which is excited by 488-nm light and serves as an energy donor, coupled to the cyanine dye Cy7, which acts as an energy acceptor and fluoresces maximally at 780 nm. PE-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher. Although every effort is made to minimize the lot-to-lot variation in the efficiency of the fluorochrome energy transfer, differences in the residual emission from PE may be observed. Therefore, we recommend that individual compensation controls be performed for every PE-Cy7 conjugate. PE-Cy7 is optimized for use with a single argon ion laser emitting 488-nm light, and there is no significant overlap between PE-Cy7 and FITC emission spectra. When using dual-laser cytometers, which may directly excite both PE and Cy7, we recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
5. 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.

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6. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
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8. 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.

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. In: Coligan JE, Kruisbeek AM, Margulies DH, Shevach EM, Strober W, ed. *Current Protocols in Immunology.* New York: John Wiley and Sons; 1991:3.4.1-3.4.3. (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. In: Coligan J, Kruisbeek AM, Margulies D, Shevach EM, Strober W, ed. *Current Protocols in Immunology.* New York: John Wiley and Sons; 1991:3.12.1-3.12.14. (Biology)

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

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)

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)

Roederer M, Kantor AB, Parks DR, Herzenberg LA. Cy7PE and Cy7APC: bright new probes for immunofluorescence. *Cytometry.* 1996; 24(3):191-197. (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. The CD8 coreceptor revisited: one chain good, two chains better. *Immunity.* 1994; 1(4):243-246. (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)