

Technical Data Sheet

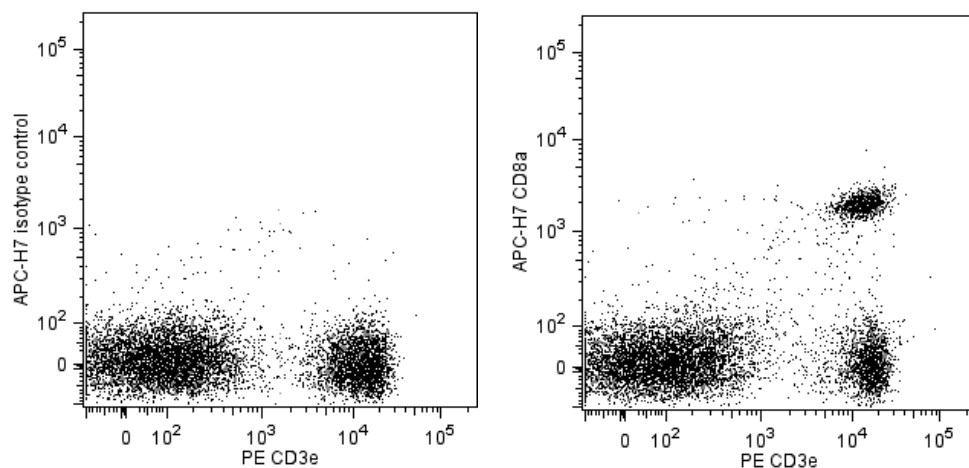
APC-H7 Rat anti-Mouse CD8a

Product Information

Material Number:	560247
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 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 $\alpha\beta$ 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*.



Two-color analysis of the expression of CD8a on mouse T cells. C57BL/6 splenocytes were stained with APC-H7 Rat anti-mouse CD8a (Cat. No. 560182/560247, right panel) or APC-H7 Rat IgG2a, κ isotype control (Cat. No. 560197, left panel) and PE Hamster anti-mouse CD3e (Cat. No. 553063/553064). Nonviable leukocytes were excluded from the analysis by staining with 7-AAD (Cat. No. 559925). 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 APC-H7 under optimum conditions, and unconjugated antibody and APC-H7 were removed.

Application Notes

Application

Flow cytometry	Routinely Tested
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560247 Rev. 3



Suggested Companion Products

Catalog Number	Name	Size	Clone
560197	APC-H7 Rat IgG2a, κ Isotype Control	0.1 mg	R35-95
553063	PE Hamster Anti-Mouse CD3e	0.1 mg	145-2C11
560182	APC-H7 Rat anti-Mouse CD8a	0.1 mg	53-6.7
553064	PE Hamster Anti-Mouse CD3e	0.2 mg	145-2C11
559925	7-AAD	2 mL	(none)
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. 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.
5. BD APC-H7 is a tandem conjugate and an analog of APC-Cy7 with the same spectral properties. It has decreased intensity but it is engineered for greater stability and less spillover in the APC channel and consequently offers better performance than APC-Cy7. It has an absorption maximum of approximately 650 nm. When excited by light from a red laser, the APC fluorochrome can transfer energy to the cyanine dye, which then emits at a longer wavelength. The resulting fluorescent emission maximum is approximately 767 nm. BD recommends that a 750-nm longpass filter be used along with a red-sensitive detector such as the Hamamatsu R3896 PMT. As with APC-Cy7 special filters are required when using APC-H7 in conjunction with APC.
Note: Although our APC-H7 products demonstrate higher lot-to-lot consistency than other APC tandem conjugate products, and every effort is made to minimize the lot-to-lot variation in residual emission from APC, it is strongly recommended that every lot be tested for differences in the amount of compensation required and that individual compensation controls are run for each APC-H7 conjugate.
Note: Cy is a trademark of Amersham Biosciences Limited.
6. Although BD APC-H7 is engineered to minimize spillover to the APC channel and is more stable and less affected by light, temperature, and formaldehyde-based fixatives, compared to other APC-cyanine tandem dyes, it is still good practice to minimize as much as possible, any light, temperature and fixative exposure when working with all fluorescent conjugates.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
8. Cy is a trademark of GE Healthcare.
9. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

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