Technical Data Sheet

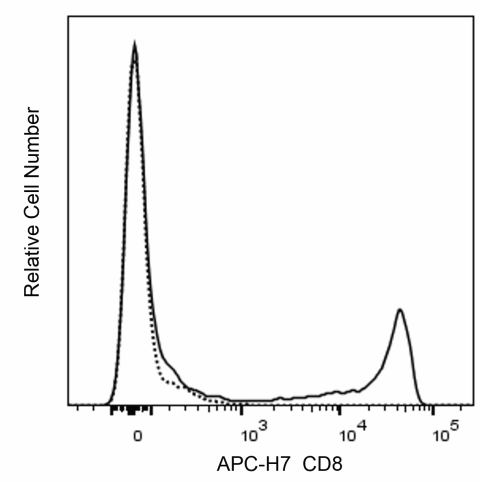
APC-H7 Mouse Anti-Human CD8

Product Information

Material Number:	**566856**
Alternate Name:	CD8α; CD8A; CD8 alpha; Leu2a; MAL; T8; p32
Size:	25 Tests
Vol. per Test:	5 µl
Clone:	HIT8a
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Workshop:	V 5T-CD08.10
Storage Buffer:	Aqueous buffered solution containing BSA, protein stabilizer, and ≤0.09% sodium azide.

Description

The HIT8a monoclonal antibody specifically binds to CD8a (CD8α). CD8α is a type I transmembrane glycoprotein and a member of the immunoglobulin superfamily. CD8α is expressed by the majority of thymocytes, by subpopulations of αβ T cells and γδ T cells and by some NK cells. Cell surface CD8α is expressed either as a disulfide-linked homodimer (CD8αα) or as a heterodimer (CD8αβ) when disulfide-bonded to a CD8 beta chain (CD8β). CD8-positive αβ T cells coexpress both CD8αα homodimers and CD8αβ heterodimers whereas some γδ T cells and NK cells express CD8αα homodimers. CD8 plays important roles in T cell activation and selection. The extracellular IgSF domain of CD8α binds to a non-polymorphic determinant on HLA class I molecules (α3 domain) and enables CD8 to function as a coreceptor with MHC class I-restricted TCR during T cell recognition of antigen. The cytoplasmic domain of CD8α associates with Lck, a Src family protein tyrosine kinase that is involved in intracellular signaling. Clones HIT8a and RPA-T8 are not cross-blocking.



Flow cytometric analysis of CD8 expression on human peripheral blood lymphocytes. *Whole blood was stained with either APC-H7 Mouse IgG1 κ Isotype Control (Cat. No. 561427; dashed line histogram) or APC-H7 Mouse Anti-Human CD8 antibody (Cat. No. 566855/566856; solid line histogram). The erythrocytes were lysed with BD FACS™ Lysing Solution (Cat. No. 349202). The histogram showing CD8 expression [or Ig Isotype control staining] was derived from gated events with the forward and side-light scatter characteristics of intact cells. Flow cytometry and data analysis were performed using a BD LSRFortessa™ X-20 Cell Analyzer System and FlowJo™ software.*

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

BD Biosciences

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Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
561427	APC-H7 Mouse IgG1, κ Isotype Control	0.1 mg	X40
349202	BD FACS™ Lysing Solution	100 mL	(none)
555899	Lysing Buffer	100 mL	(none)
566855	APC-H7 Mouse Anti-Human CD8	100 Tests	HIT8a

Product Notices

1. 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.
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. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
7. 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.
8. 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.
9. Cy is a trademark of GE Healthcare.
10. Please refer to http://regdocs.bd.com to access safety data sheets (SDS).
11. Please refer to www.bdbiosciences.com/us/s/resources for technical protocols.

References

Han L, Chen J, Ding K, et al. Efficient generation of bispecific IgG antibodies by split intein mediated protein trans -splicing system. *Sci Rep* . 2017; 7(1):8360. (Clone-specific: Flow cytometry)

Schlossman SF. Stuart F. Schlossman .. et al., ed. *Leucocyte typing V : white cell differentiation antigens : proceedings of the fifth international workshop and conference held in Boston, USA, 3-7 November, 1993.* Oxford: Oxford University Press; 1995(Clone-specific: Flow cytometry)