## **Technical Data Sheet**

# APC-H7 Mouse Anti-Human CD8

#### **Product Information**

Material Number:	561423
Alternate Name:	CD8α; CD8A; CD8 alpha; Leu2a; MAL; T8; p32
Size:	50 Tests
Vol. per Test:	5 μl
Clone:	SK1
Immunogen:	Human Peripheral Blood T Cells
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Testing: Rhesus, Cynomolgus, Baboon
	Tested in Development: Human
Storage Buffer:	Aqueous buffered solution containing BSA, protein stabilizer, and $\leq 0.09\%$ sodium azide.

#### Description

The SK1 monoclonal antibody specifically binds to CD8 alpha (CD8 $\alpha$ ). CD8 $\alpha$  is a type I transmembrane glycoprotein and a member of the immunoglobulin superfamily. CD8 $\alpha$  is expressed by the majority of thymocytes, by subpopulations of  $\alpha\beta$  T cells and  $\gamma\delta$  T cells and by some NK cells. Cell surface CD8 $\alpha$  is expressed either as a disulfide-linked homodimer (CD8 $\alpha\alpha$ ) or as a heterodimer (CD8 $\alpha\beta$ ) when disulfide-bonded to a CD8 beta chain (CD8 $\beta$ ). CD8-positive  $\alpha\beta$  T cells coexpress both CD8 $\alpha\alpha$  homodimers and CD8 $\alpha\beta$  heterodimers whereas some  $\gamma\delta$  T cells and NK cells express CD8 $\alpha\alpha$  homodimers. CD8 plays important roles in T cell activation and selection. The extracellular IgSF domain of CD8 $\alpha$  binds to a non-polymorphic determinant on HLA class I molecules ( $\alpha$ 3 domain) and enables CD8 to function as a co-receptor with MHC class I-restricted TCR during T cell recognition of antigen. The cytoplasmic domain of CD8 $\alpha$  associates with Lck, a Src family protein tyrosine kinase that is involved in intracellular signaling.



Flow cytometric analysis of CD8 expression on Rhesus macaque peripheral blood lymphocytes. Rhesus macaque whole blood was stained with APC-H7 Mouse anti-Human CD8 antibody (Cat. No. 561423; solid line histogram) or with an APC-H7 Mouse IgG1, κ Isotype Control (Cat. No. 560167; dashed line histogram). The erythrocytes were lysed with BD PharmLyse™ Lysing Buffer (Cat. No. 555899). The fluorescence histograms were derived from events with the forward and side light-scatter characteristics of viable lymphocytes. Flow cytometry was performed using a BD™ LSR II Flow Cytometer 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 cytom	etry				`ested			
Suggested	Companio	on Products	;					
Catalog Num	ber	Name					Size	Clone
560167	0167 APC-H7 Mouse IgG1, κ Isotype Control					0.1 mg	MOPC-21	
555899		Lysing Bu	ıffer				100 mL	(none)
554656		Stain Buff	fer (FBS)				500 mL	(none)
BD Biosci	ences							
bdbiosciences.	com							
United States 877.232.8995	<b>Canada</b> 866.979.9408	Europe 32.2.400.98.95	<b>Japan</b> 0120.8555.90	Asia Pacific 65.6861.0633	Latin America/Caribbean 55.11.5185.9995			

For country contact information, visit bdbiosciences.com/contact

Conditions: The information disclosed herein is not to be construed as a recommendation to use the above product in violation of any patents. BD Biosciences will not be held responsible for patent infringement or other violations that may occur with the use of our products. Purchase does not include or carry any right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of Becton, Dickinson and Company is stictly prohibited.

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for resale. Unless otherwise noted, BD, BD Logo and all other trademarks are property of Becton, Dickinson and Company. © 2015 BD

#### **Product Notices**

- 1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use  $1 \times 10^{6}$  cells in a 100-µl experimental sample (a test).
- 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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
- 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.
- 6. 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.

- 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.
- 8. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
- 9. Cy is a trademark of Amersham Biosciences Limited.
- 10. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
- 11. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

#### References

Barclay NA, Brown MH, Birkeland ML, et al, ed. The Leukocyte Antigen FactsBook. San Diego, CA: Academic Press; 1997. (Biology)

Bernard A, Boumsell L, Hill C. Joint report of the first international workshop on human leucocyte differentiation antigens by the investigators of the participating laboratories: T2 protocol. In: Bernard A, Boumsell L, Dausset J, Milstein C, Schlossman SF, ed. *Leucocyte Typing*. New York, NY: 1984:25-60. (Clone-specific) Dongworth DW, Gotch FM, Carter NP, Hildreth PDK, McMichael AJ. Inhibition of virus-specific, HLA-restricted, T cell-mediated lysis by monoclonal anti-T cell antibodies. In: Bernard A, Boumsell L, Dausset J, Milstein C, Schlossman SF, ed. *Leucocyte Typing*. New York, NY: 1984:320-328. (Clone-specific: Functional assay, Inhibition)

Engleman EG, Benike CJ, Glickman E, Evans RL. Antibodies to membrane structures that distinguish suppressor/cytotoxic and helper T lymphocyte subpopulations block the mixed leukocyte reaction in man. *J Exp Med.* 1981; 154(1):193-198. (Clone-specific: Cell separation, Flow cytometry, Functional assay, Inhibition)

Engleman EG, Benike CJ, Grumet FC, Evans RL. Activation of human T lymphocyte subsets: helper and suppressor/cytotoxic T cells recognize and respond to distinct histocompatibility antigens. *J Immunol.* 1981; 127(5):2124-2129. (Clone-specific: Cell separation, Flow cytometry, Fluorescence activated cell sorting) Evans RL, Wall DW, Platsoucas CD, et al. Thymus-dependent membrane antigens in man: inhibition of cell-mediated lympholysis by monoclonal antibodies to TH2 antigen. *Proc Natl Acad Sci U S A.* 1981; 78(1):544-548. (Immunogen: Flow cytometry, Functional assay, Inhibition)

Jonker M, Meurs G. Monoclonal antibodies specific for B cells, cytotoxic/suppressor T cells, and a subset of cytotoxic/suppressor T cells in the Rhesus monkey. In: Bernard A, Boumsell L, Dausset J, Milstein C, Schlossman SF, ed. *Leucocyte Typing*. New York, NY: 1984:328-336. (Clone-specific: Flow cytometry) Knapp W, Dörken B, Gilks WR, et al, ed. *Leucocyte Typing IV*. New York, NY: Oxford University Press; 1989:1-1182. (Biology)

Ledbetter JA, Evans RL, Lipinski M, Cunningham-Rundles C, Good RA, Herzenberg LA. Evolutionary conservation of surface molecules that distinguish T lymphocyte helper/inducer and cytotoxic/suppressor subpopulations in mouse and man. *J Exp Med.* 1981; 153(2):310-323. (Clone-specific: Flow cytometry, Immunoprecipitation)

McMichael AJ, Beverly PCL, Gilks W, et al, ed. Leukocyte Typing III: White Cell Differentiation Antigens. New York: Oxford University Press; 1987. (Clone-specific: Flow cytometry, Immunoprecipitation)

Reichert T, DeBruyere M, Deneys V, et al. Lymphocyte subset reference ranges in adult Caucasians. *Clin Immunol Immunopathol.* 1991; 60(2):190-208. (Biology) Schlossman SF, Boumsell L, Gilks W, et al, ed. *Leukocyte Typing V: White Cell Differentiation Antigens*. Oxford: Oxford University Press; 1995. (Biology) Warner NL, Lanier LL, Jackson A, Babcock G, Evans R. Multiparameter approaches to FACS analysis of human leucocyte cell surface antigens. In: Bernard A, Boumsell L, Dausset J, Milstein C, Schlossman SF, ed. *Leucocyte Typing*. New York, NY: 1984:621-630. (Clone-specific: Flow cytometry)

#### **BD Biosciences**

bdbiosciences.com

	babiosciences.com								
	United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean			
	877.232.8995	866.979.9408	32.2.400.98.95	0120.8555.90	65.6861.0633	55.11.5185.9995			
For country contact information, visit bdbiosciences.com/contact									

Conditions: The information disclosed herein is not to be construed as a recommendation to use the above product in violation of any patents. BD Biosciences will not be held responsible for patent infringement or other violations that may occur with the use of our products. Purchase does not include or carry any right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of Becton, Dickinson and Company is stictly prohibited. For Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for resale.

Unless otherwise noted, BD, BD Logo and all other trademarks are property of Becton, Dickinson and Company. © 2015 BD

