Technical Data Sheet

APC-H7 Mouse anti-Human CD8

Product Information

Material Number: Alternate Name: Size: Vol. per Test: Clone: Immunogen: Isotype: Reactivity:

Workshop: Storage Buffer: 560179 CD8α; CD8A; CD8 alpha; Leu2a; MAL; T8; p32 100 Tests 5 µl SK1 Human Peripheral Blood T Cells Mouse (BALB/c) IgG1, κ QC Testing: Human Tested in Development: Rhesus, Cynomolgus, Baboon I T51,74; III T118,152,571 Aqueous buffered solution containing BSA, protein stabilizer, and ≤0.09% sodium azide.

Description

CD8 recognizes the 32-kDa a-subunit of a disulfide-linked bimolecular complex. The majority of peripheral blood CD8+ T lymphocytes express an a/b heterodimer (Mr 32, 30 kDa), while CD8+CD16+ natural killer (NK) lymphocytes and CD8+ T-cell receptor (TCR)- γ/δ + lymphocytes express a/a homodimer (Mr 30 kDa). CD8+TCR- α/β + lymphocytes can express either an α/α homodimer or α/β heterodimer. The CD8 antigenic determinant binds to class I major histocompatibility (MHC) molecules resulting in increased adhesion between the CD8+ T lymphocytes and target cells. Binding of the CD8 antigen is coupled to a protein tyrosine kinase p56lck. The CD8:p56lck complex can play a role in T-lymphocyte activation through mediation of the interactions between the CD8 antigen and the CD3/TCR complex.



Flow cytometric analysis of APC-H7 anti-human CD8 on human lymphocytes. Whole blood was stained with APC-H7 anti-human CD8 (clone SK1, Cat. No. 560179) and compared to whole blood stained with a APC-H7 mouse IgG1 isotype control (clone MOPC-21, Cat. No. 560167). The isotype control is represented by a dashed line and the APC-H7 anti-human CD8 by the solid line. Lymphocytes were selected by light scatter profile. 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				
Suggested Cor	mpanion Product	S					
Catalog Number	Name	Name				Clone	
560167	APC-H7	APC-H7 Mouse IgG1, κ Isotype Control				MOPC-21	
554656 Stain Buffer (FBS)					500 mL	(none)	
BD Bioscience	es						
United States Can 877.232.8995 866	ada Europe .979.9408 32.2.400.98.9	Japan 5 0120.8555.90	Asia Pacific 65.6861.0633	Latin America/Caribbean 55.11.5185.9995			
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Product Notices

- 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 1. sample (a test).
- An isotype control should be used at the same concentration as the antibody of interest. 2
- Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before 3 discarding to avoid accumulation of potentially explosive deposits in plumbing.
- Source of all serum proteins is from USDA inspected abattoirs located in the United States. 4.
- 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.

- Although BD APC-H7 is engineered to minimize spillover to the APC channel and is more stable and less affected by light, temperature, and 6. 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. 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.
- 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

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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)

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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)

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