

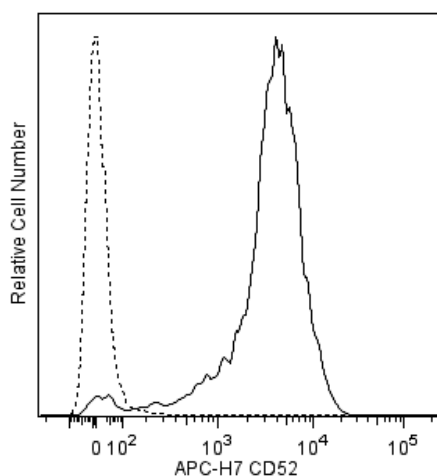
Technical Data Sheet

APC-H7 Mouse Anti-Human CD52**Product Information**

Material Number:	563611
Alternate Name:	Cambridge pathology 1 Ag; CAMPATH-1; Epididymal secretory protein E5; HE5
Size:	50 Tests
Vol. per Test:	5 µl
Clone:	4C8
Immunogen:	Human T Lymphocytes
Isotype:	Mouse (BALB/c) IgG3, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA, protein stabilizer, and ≤0.09% sodium azide.

Description

The 4C8 monoclonal antibody specifically binds to CD52 which is also known as Cambridge pathology 1 antigen (CAMPATH-1) or Human epididymis-specific protein 5 (HE5). CD52 is a highly N-glycosylated, 25-29 kDa protein whose C-terminus is glycoposphatidylinositol anchored in the membrane. It is highly expressed on the surface of thymocytes and mature lymphocytes but not on their stem cell precursors. It is also expressed on monocytes, dendritic cells, eosinophils and epithelial cells of the epididymis and seminal vesicles but not on neutrophils, plasma cells, platelets or erythrocytes. Although its functional role is not well characterized, the CD52 antigen serves as an exquisitely sensitive target antigen for antibody and complement-mediated lysis of CD52-positive cells. Anti-CD52 antibodies are being used clinically to remove lymphocytes from transplanted bone marrow cell preparations and in the treatment of some malignant diseases.



Flow cytometric analysis of human CD52 expression on human peripheral blood lymphocytes. Whole blood was stained with either APC-H7 Mouse IgG3, κ Isotype Control (Cat. No. 563627; dashed line histogram) or APC-H7 Mouse Anti-Human CD52 antibody (Cat. No. 563611; solid line histogram). Erythrocytes were lysed with BD Pharm Lyse™ Lysing Buffer (Cat. No. 555899). The fluorescence histograms were derived from gated events with the forward and side light-scatter characteristics of viable lymphocytes. Flow cytometric analysis 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 cytometry	Routinely Tested
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563611 Rev. 2



Suggested Companion Products

<u>Catalog Number</u>	<u>Name</u>	<u>Size</u>	<u>Clone</u>
554656	Stain Buffer (FBS)	500 mL	(none)
563627	APC-H7 Mouse IgG3, κ Isotype Control	50 μ g	J606
555899	Lysing Buffer	100 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
349202	BD FACST [™] Lysing Solution	100 mL	(none)

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1×10^6 cells in a 100- μ l experimental sample (a test).
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. An isotype control should be used at the same concentration as the antibody of interest.
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. 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.
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 www.bdbiosciences.com/pharming/protocols for technical protocols.

References

Masuyama J, Yoshio T, Suzuki K, et al. Characterization of the 4C8 antigen involved in transendothelial migration of CD26(hi) T cells after tight adhesion to human umbilical vein endothelial cell monolayers. *J Exp Med*. 1999; 189(6):979-990. (Immunogen: Blocking, Flow cytometry, Stimulation, Western blot)

Zola H. *Leukocyte and stromal cell molecules : the CD markers*. Hoboken, N.J.: Wiley-Liss; 2007(Biology)