Technical Data Sheet

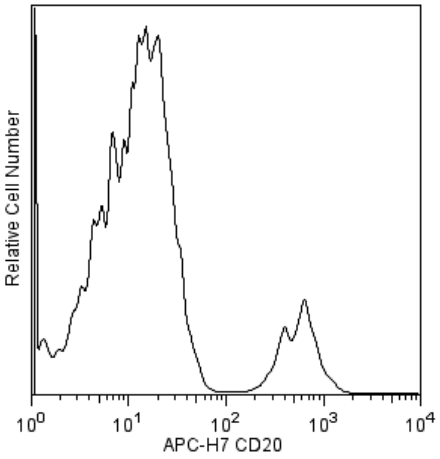
APC-H7 Mouse Anti-Human CD20

Product Information

Material Number:	**561172**
Alternate Name:	MS4A1; membrane-spanning 4-domains subfamily A member 1; B1; Bp35; LEU-16
Size:	50 tests
Vol. per Test:	5 µl
Clone:	H1 (also known as FB1)
Immunogen:	Human B lymphoma cell line
Isotype:	Mouse (BALB/c) IgG2a, κ
Reactivity:	QC Testing: Human
Workshop:	V cB010
Storage Buffer:	Aqueous buffered solution containing BSA, protein stabilizer, and ≤0.09% sodium azide.

Description

The H1 (FB1) antibody specificially binds to a cytoplasmic domain of CD20. CD20 is a 33-37-kDa four transmembrane phosphoprotein that is expressed by B lymphocytes from the pre-B stage and most malignant B cells and is lost during plasma cell differentiation. Low level CD20 expression is observed on a subset of normal circulating T lymphocytes, and CD20-positive T-cell lymphomas have been reported. The CD20 molecule is associated with membrane lipid raft domains, acts as a channel for calcium ions, and is involved in the regulation of B cell activation and survival. The cytoplasmic domain regions are serine and threonine rich and contain multiple phosphorylation consensus sequences.



Flow cytometric analysis of CD20 (cytoplasmic domain) in human peripheral blood lymphocytes. *Human whole blood was treated with BD™ Phosflow Lyse/Fix Buffer (Cat. No. 558049) for 10 min at 37°C to lyse erythrocytes and fix the leukocytes in one step. The leukocytes were permeabilized with BD™ Phosflow Perm Buffer I (Cat. No. 557885) for 20 minutes. The cells were then stained with APC-H7 Mouse anti-human CD20 (cytoplasmic) (Cat. No. 561172). The fluorescence histogram was derived from gated events with the forward and side light-scatter characteristics of 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

Intracellular staining (flow cytometry)	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
558049	Lyse/Fix Buffer 5X	250 ml	(none)
557885	Perm/Wash Buffer I	125 ml	(none)

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Product Notices

1. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
2. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. 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.
4. 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.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
6. 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.
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. Cy is a trademark of Amersham Biosciences Limited.
9. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Cragg MS, Walshe CA, Ivanov AO, Glennie MJ. The biology of CD20 and its potential as a target for mAb therapy. *Curr Dir Autoimmun.* 2005; 8:140-174. (Biology)

Kitamura A, Yamashita Y, Mori N. CD20-positive cytotoxic T cell lymphoma: report of two cases and review of the literature. *J Clin Exp Hematop.* 2005; 45(1):45-50. (Biology)

Nozawa Y, Abe M, Ohno H, Fukuhara S, Wakasa H. Production of two monoclonal antibodies (FB1 and FB21) useful for the identification of human B lymphocytes in formalin-fixed, paraffin-embedded tissues. *J Pathol.* 1994; 173:347-354. (Immunogen)

Nozawa Y, Abe M, Wakasa H. Three mAb, FUN-1, FB1, and FB21, that recognize B-cell antigens in frozen or paraffin-embedded tissue sections. In: Schlossman SF, Boumsell L, Gilks W, et al, ed. *Leukocyte Typing V: White Cell Differentiation Antigens.* New York: Oxford University Press; 1995:705-706. (Immunogen)