Technical Data Sheet

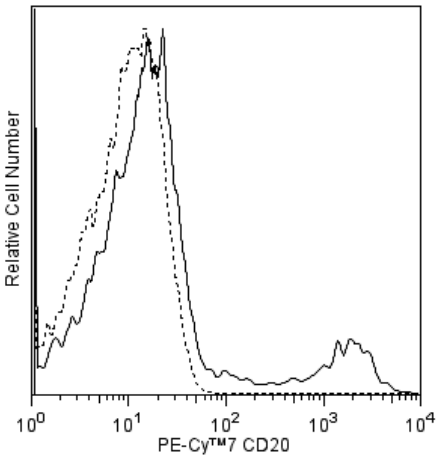
PE-Cy™7 Mouse Anti-Human CD20

Product Information

Material Number:	**561175**
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 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) expression by 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 either PE-Cy™7 Mouse IgG2a, κ Isotype Control MOPC-173 (Cat. No. 560906; dashed line histogram) or PE-Cy™7 Mouse anti-human CD20 (cytoplasmic) antibody (Cat. No. 561175; solid line histogram). The fluorescence histograms were derived from gated 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 PE-Cy7 under optimum conditions, and unconjugated antibody and free PE-Cy7 were removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
560906	PE-Cy™7 Mouse IgG2a, κ Isotype Control	0.1 mg	MOPC-173
558049	Lyse/Fix Buffer 5X	250 mL	(none)
557885	Perm/Wash Buffer I	125 mL	(none)

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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-µl experimental sample (a test).
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
5. PE-Cy7 is a tandem fluorochrome composed of R-phycoerythrin (PE), which is excited by 488-nm light and serves as an energy donor, coupled to the cyanine dye Cy7, which acts as an energy acceptor and fluoresces maximally at 780 nm. PE-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher. Although every effort is made to minimize the lot-to-lot variation in the efficiency of the fluorochrome energy transfer, differences in the residual emission from PE may be observed. Therefore, we recommend that individual compensation controls be performed for every PE-Cy7 conjugate. PE-Cy7 is optimized for use with a single argon ion laser emitting 488-nm light, and there is no significant overlap between PE-Cy7 and FITC emission spectra. When using dual-laser cytometers, which may directly excite both PE and Cy7, we recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
6. Cy is a trademark of Amersham Biosciences Limited.
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. Warning: Some APC-Cy7 and PE-Cy7 conjugates show changes in their emission spectrum with prolonged exposure to formaldehyde. If you are unable to analyze fixed samples within four hours, we recommend that you use BD™ Stabilizing Fixative (Cat. No. 338036).
9. 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.
10. An isotype control should be used at the same concentration as the antibody of interest.

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.* Oxford: Oxford University Press; 1995:705-706. (Immunogen)

Schlossman SF, Boumsell L, Gilks W, et al, ed. *Leukocyte Typing V: White Cell Differentiation Antigens.* Oxford: Oxford University Press; 1995. (Biology)

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