Technical Data Sheet

PE-CF594 Mouse Anti-Human CD20

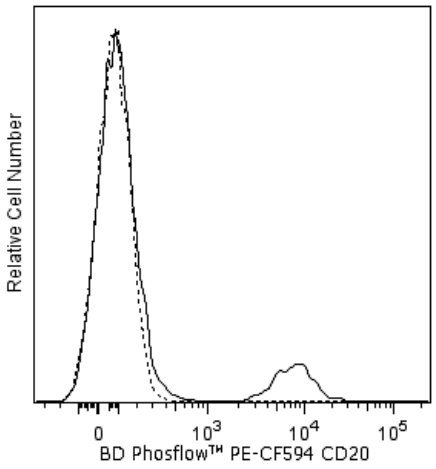
Product Information

Material Number:	**563430**
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.

This antibody is conjugated to BD Horizon™ PE-CF594, which has been developed exclusively by BD Biosciences as a better alternative to PE-Texas Red®. PE-CF594 excites and emits at similar wavelengths to PE-Texas Red® yet exhibits improved brightness and spectral characteristics. Due to PE having maximal absorption peaks at 496 nm and 564 nm, PE-CF594 can be excited by the blue (488-nm), green (532-nm) and yellow-green (561-nm) lasers and can be detected with the same filter set as PE-Texas Red® (eg 610/20-nm filter).



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 leucocytes in one step. The leucocytes were permeabilized with BD Phosflow™ Perm/Wash Buffer I (Cat. No. 557885) for 20 minutes. The cells were then stained with either BD Horizon™ PE-CF594 Mouse IgG2a, κ Isotype Control MOPC-173 (Cat. No. 563484; dashed line histogram) or BD Phosflow™ PE-CF594 Mouse Anti-Human CD20 (cytoplasmic) antibody (Cat. No. 563430; solid line histogram). The fluorescence histograms were derived from gated events with the forward and side light-scatter characteristics of intact 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 BD Horizon™ PE-CF594 under optimum conditions, and unconjugated antibody and free PE-CF594 were removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

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Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
563484	PE-CF594 Mouse IgG2a, κ Isotype Control	50 µg	MOPC-173
558049	Lyse/Fix Buffer 5X	250 ml	(none)
557885	Perm/Wash Buffer I	125 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-µl 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 refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
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. 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. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
8. Texas Red is a registered trademark of Molecular Probes, Inc., Eugene, OR.
9. CF™ is a trademark of Biotium, Inc.
10. When excited by the yellow-green (561-nm) laser, the fluorescence may be brighter than when excited by the blue (488-nm) laser.
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12. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using multi-laser cytometers, which may directly excite both PE and CF™594.

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: Immunohistochemistry, Immunoprecipitation)

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: Immunofluorescence, Immunohistochemistry, Immunoprecipitation)

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