Technical Data Sheet

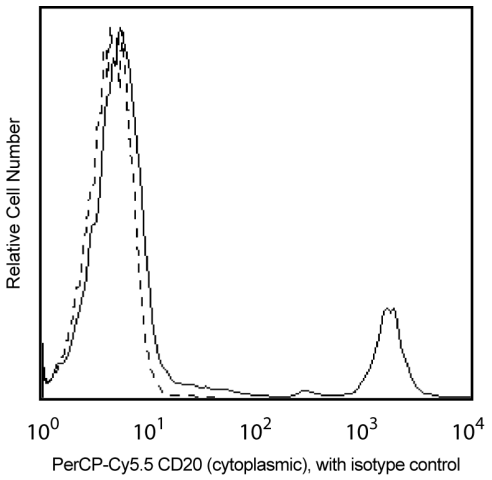
PerCP-Cy™5.5 Mouse Anti-Human CD20

Product Information

Material Number:	**558021**
Alternate Name:	MS4A1; membrane-spanning 4-domains subfamily A member 1; B1; Bp35; LEU-16
Size:	50 Tests
Vol. per Test:	20 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.



Analysis of CD20 (cytoplasmic) in human peripheral blood lymphocytes. *Human peripheral blood mononuclear cells were fixed with pre-warmed BD Cytofix™ buffer (Cat. No. 554655) for 10 minutes at 37ºC and permeabilized with BD Phosflow™ Perm Buffer II (Cat. No. 558052) on ice for 30 minutes. The cells were then stained with either PerCP-Cy5.5 Mouse IgG2a, κ, isotype control (Cat. No. 558020, dashed line) or PerCP-Cy5.5 Mouse Anti-Human CD20 (cytoplasmic) (solid line). For data analysis, lymphocytes were selected by their scatter profile. CD20 (cytoplasmic) was not detected in the monocytes (not shown). Flow cytometry was performed on a BD FACSCalibur™ 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 PerCP-Cy5.5 under optimum conditions, and unconjugated antibody and free PerCP-Cy5.5 were removed. Storage of PerCP-Cy5.5 conjugates in unoptimized diluent is not recommended and may result in loss of signal intensity.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

This antibody conjugate is suitable for intracellular staining of human whole blood (using BD Phosflow™ Lyse/Fix Buffer) and peripheral blood mononuclear cells (using BD Cytofix™ Fixation Buffer or BD Phosflow™ Fix Buffer I). Any of the three BD Phosflow™ permeabilization buffers may be used.

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Suggested Companion Products

Catalog Number	Name	Size	Clone
557870	Fix Buffer I	250 mL	(none)
554655	Fixation Buffer	100 mL	(none)
558049	Lyse/Fix Buffer 5X	250 mL	(none)
557885	Perm/Wash Buffer I	125 mL	(none)
558052	Perm Buffer II	125 mL	(none)
558050	Perm Buffer III	125 mL	(none)
558020	PerCP-Cy™5.5 Mouse IgG2a, κ Isotype Control	50 Tests	MOPC-173

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. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
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. PerCP-Cy5.5–labelled antibodies can be used with FITC- and R-PE–labelled reagents in single-laser flow cytometers with no significant spectral overlap of PerCP-Cy5.5, FITC, and R-PE fluorescence.
6. PerCP-Cy5.5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using dual-laser cytometers, which may directly excite both PerCP and Cy5.5™. We recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
7. 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.
8. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
9. 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)

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