Technical Data Sheet

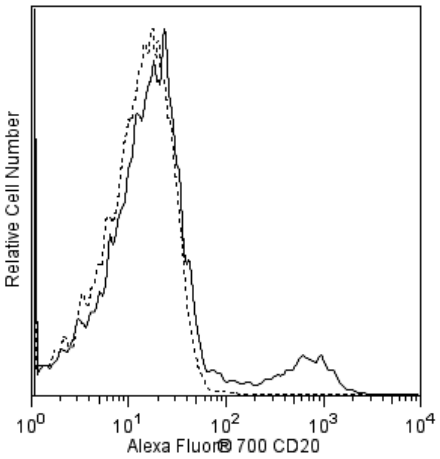
Alexa Fluor® 700 Mouse Anti-Human CD20

Product Information

Material Number:	**561171**
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 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) 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 Alexa Fluor® 700 Mouse IgG2a, κ Isotype Control MOPC-173 (Cat. No. 560894; dashed line histogram) or Alexa Fluor® 700 Mouse Anti-Human CD20 (cytoplasmic) antibody (Cat. No. 561171; solid line histogram). The fluorescence histograms were 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 to Alexa Fluor® 700 under optimum conditions, and unreacted Alexa Fluor® 700 was 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)
560894	Alexa Fluor® 700 Mouse IgG2a, κ Isotype Control	0.1 mg	MOPC-173
554656	Stain Buffer (FBS)	500 mL	(none)
557885	Perm/Wash Buffer I	125 mL	(none)
554657	Stain Buffer (BSA)	500 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. An isotype control should be used at the same concentration as the antibody of interest.
3. 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.
4. Alexa Fluor® 700 has an adsorption maximum of ~700nm and a peak fluorescence emission of ~720nm. Before staining cells with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
5. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
6. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
8. 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. Stuart F. Schlossman .. et al., ed. *Leucocyte typing V : white cell differentiation antigens : proceedings of the fifth international workshop and conference held in Boston, USA, 3-7 November, 1993.* Oxford: Oxford University Press; 1995:705-706. (Immunogen)

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