

Technical Data Sheet

PE-CF594 Mouse Anti-Bcl-6

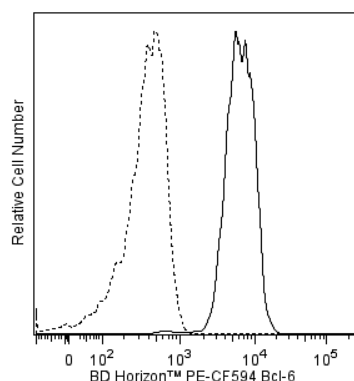
Product Information

Material Number:	562401
Alternate Name:	BCL6; B-cell lymphoma 6 protein; LAZ3; Laz-3, ZBTB27, ZNF51
Size:	50 tests
Vol. per Test:	5 µl
Clone:	K112-91
Immunogen:	Human Bcl-6 Recombinant Protein
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human Tested in Development: Mouse
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

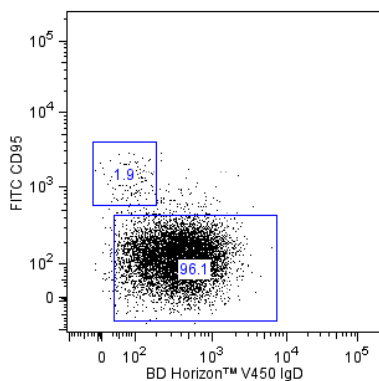
Description

The K112-91 monoclonal antibody specifically binds to Bcl-6. Bcl-6 was first identified as a proto-oncogene frequently deregulated by chromosomal translocations in non-Hodgkin B-cell lymphomas. It is a nuclear transcriptional repressor of the BTB/POZ zinc-finger family of transcription factors. In addition to its roles in cancer, Bcl-6 plays important roles in the differentiation of normal cells including B cells, thymocytes, CD4+ or CD8+ T cells. Bcl-6 is highly expressed in germinal center B cells, where it promotes the germinal center reaction by inducing proliferation and inhibiting the DNA-damage response. Bcl-6 has been identified as a key factor in promoting the differentiation of CD4+ follicular T helper (Tfh) cells that are involved in promoting germinal center formation and providing help to B cells. The interplay of Bcl-6 and another transcriptional repressor, Blimp-1, is thought to be critical in defining the results of both B-cell and T-cell differentiation.

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 Bcl-6 expression in Ramos cells (Left Panel). Human Ramos cells were fixed with BD Cytofix™ Fixation Buffer (Cat. No. 554655) and permeabilized with BD Phosflow™ Perm Buffer III (Cat. No. 558050) followed by intracellular staining with either BD Horizon™ PE-CF594 Mouse Anti-Human Bcl-6 antibody (Cat. No. 562401, solid line histogram) or a BD Horizon™ PE-CF594 mIgG1, κ Isotype Control (Cat. No. 562292; dashed line histogram). Flow cytometric fluorescence histograms were derived from gated events with the forward and side light-scatter characteristics of intact cells. Flow cytometry was performed using a BD™ LSR II Flow Cytometer System.



Multicolor flow cytometric analysis of Bcl-6 expression in mouse B lymphocytes (Middle and Right Panels). BALB/c mouse mesenteric lymph node cells were stained with APC Rat Anti-Mouse B220 (Cat. No. 553092/561880), PerCP-Cy™5.5 Rat Anti-Mouse CD4 (Cat. No. 550954/561115), FITC Hamster Anti-Mouse Fas/CD95 (Cat. No. 554257), and BD Horizon™ V450 Rat Anti-Mouse IgD (Cat. No. 560869) Abs. Cells were washed, resuspended in RPMI with 10% FBS, and fixed with BD Phosflow™ Lyse/Fix Buffer (Cat. No. 558049). Cells were permeabilized with BD Phosflow™ Perm/Wash Buffer I (Cat. No. 557885), followed by intracellular staining with BD Horizon™ PE-CF594 Mouse Anti-Bcl-6 antibody (Cat. No. 562401). A two-color flow cytometric dot plot shows the expression of IgD versus Fas/CD95 by B cells identified as CD4-B220+ from gated events with the forward and side light-scatter characteristics of intact lymphocytes (Middle Panel). Germinal center (GC) B cells were identified as Fas/CD95-positive B cells that expressed low levels of IgD (IgDloCD95/Fas+) whereas non-GC B cells primarily expressed intermediate to high levels of IgD and little or no CD95/Fas. Histograms (Right Panel) derived from gated cell subsets show intracellular Bcl-6 staining levels for mouse GC B cells (solid line) and non-GC B cells (dashed line). Flow cytometry was performed using a BD™ LSR II Flow Cytometer System.

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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

Suggested Companion Products

Catalog Number	Name	Size	Clone
562292	PE-CF594 Mouse IgG1, κ Isotype Control	0.1 mg	X40
554656	Stain Buffer (FBS)	500 ml	(none)
554655	Fixation Buffer	100 ml	(none)
557885	Perm/Wash Buffer I	125 ml	(none)
558052	Perm Buffer II	125 ml	(none)
558050	Perm Buffer III	125 ml	(none)

Product Notices

1. Please refer to www.bdbiosciences.com/pharming/protocols for technical protocols.
2. 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).
3. An isotype control should be used at the same concentration as the antibody of interest.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
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.
11. This product is provided under an Agreement between BIOTIUM and BD Biosciences. The manufacture, use, sale, offer for sale, or import of this product is subject to one or more patents or pending applications owned or licensed by Biotium, Inc. This product, and only in the amount purchased by buyer, may be used solely for buyer's own internal research, in a manner consistent with the accompanying product literature. No other right to use, sell or otherwise transfer (a) this product, or (b) its components is hereby granted expressly, by implication or by estoppel. This product is for research use only. Diagnostic uses require a separate license from Biotium, Inc. For information on purchasing a license to this product including for purposes other than research, contact Biotium, Inc., 3159 Corporate Place, Hayward, CA 94545, Tel: (510) 265-1027. Fax: (510) 265-1352. Email: btinfo@biotium.com.
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.
13. This product is sold under license to the following patent: US Patent No. 6,174,997.
14. Cy is a trademark of Amersham Biosciences Limited.

References

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