Technical Data Sheet

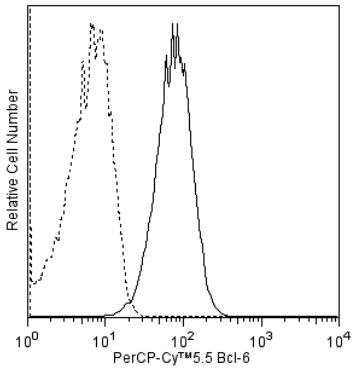
PerCP-Cy™5.5 Mouse Anti-Bcl-6

Product Information

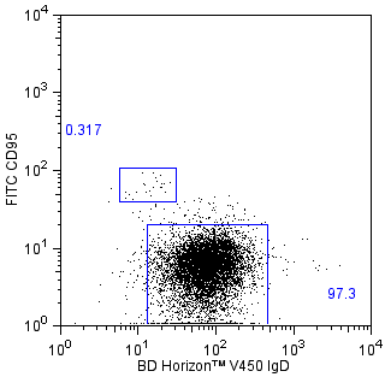
Material Number:	**562198**
Alternate Name:	BCL6; B-cell lymphoma 6 protein; LAZ3; Laz-3, ZBTB27, ZNF51
Size:	50 tests
Vol. per Test:	5 µl
Clone:	K112-91
Immunogen:	Human Bcl-6 Recombinant Protein
Isotype:	Mouse IgG1, κ
Reactivity:	QC Tested: Human Tested in Development: Mouse
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

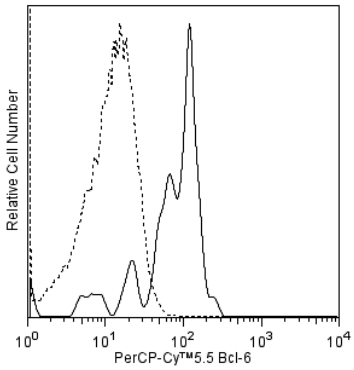
Description

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 role in cancer, Bcl-6 plays an important role in normal lymphocyte differentiation. 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. Additionally, Bcl-6 has been identified as a key factor in promoting the differentiation of CD4+ follicular T helper (Tfh) cells, which 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.



Flow cytometric analysis of Bcl-6 expression on Human Ramos (Left Panel). *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 PerCP-Cy™5.5 Mouse anti-Human Bcl-6 antibody (Cat. No. 562198, solid line histogram) or a PerCP-Cy™5.5 mIgG1, κ isotype control (Cat. No. 550795; dashed line histogram). Flow cytometric fluorescence histograms were derived from gated events with the forward and side light-scatter characteristics of viable cells. Flow cytometry was performed using a BD LSR™ II Flow Cytometry System.*





Multicolor flow cytometric analysis of Bcl-6 expression on Mouse B lymphocytes (Middle and Right Panel). *BALB/c mesenteric lymph node cells were stained with APC Rat Anti-Mouse B220 (Cat. No. 553092/561880), Alexa Fluor® 647 Rat Anti-Mouse CD4 (Cat. No. 557956/561025), FITC Hamster Anti-Mouse Fas/CD95 (Cat. No. 554257), and BD Horizon™ V450 Rat Anti-Mouse IgD (Cat. No. 560869). 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 PerCP-Cy™5.5 Mouse Anti-Human Bcl-6 (Cat. No. 562198). The 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 B cells were identified as IgDloFas+ B lymphocytes. Flow cytometric fluorescence histograms (Right Panel) show intracellular Bcl-6 staining of mouse germinal center B cells (solid line histogram) and non-GC B cells (dashed line histogram). Flow cytometry was performed using a BD LSR™ II 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

BD Biosciences

bdbiosciences.com

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877.232.8995	888.268.5430	32.53.720.550	0120.8555.90	65.6861.0633	0800.771.7157

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Recommended Assay Procedure:

We validate the quality of each batch of the K112-91 antibody conjugate by flow cytometry on human cell lines. Investigators may use the same cell lines as controls for their staining procedure, namely Ramos (Positive; ATCC CRL-1596) and Jurkat (Negative; ATCC TIB-152) human cell lines actively growing in log phase (do not overgrow). Cells are fixed with BD Cytofix™ Fixation Buffer (Cat. No. 554655; 10 minutes at 37°C), permeabilized with BD Phosflow™ Perm Buffer III (Cat. No. 558050; 30 minutes on ice), and washed using BD Pharmingen™ Stain Buffer (Cat. No. 554656), followed by intracellular staining with Mouse anti-Bcl-6 for 45 minutes at room temperature.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
554655	Fixation Buffer	100 ml	(none)
557885	Perm/Wash Buffer I	125 ml	(none)
558049	Lyse/Fix Buffer 5X	250 ml	(none)
558052	Perm Buffer II	125 ml	(none)
558050	Perm Buffer III	125 ml	(none)
560746	Perm Buffer IV 10×	50 ml	(none)
550795	PerCP-Cy™5.5 Mouse IgG1 κ Isotype Control	0.1 mg	MOPC-21

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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
5. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
6. 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.
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. 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.
9. 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.
10. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
11. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under license from Amersham Biosciences Corp. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one return this material, unopened to BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121 and any money paid for the material will be refunded.
12. This product is sold under license to the following patent: US Patent No. 6,174,997.

References

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