Technical Data Sheet

V450 Mouse Anti-Bcl-6

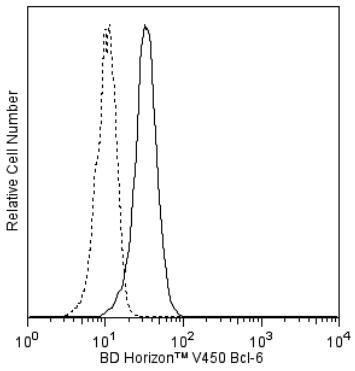
Product Information

Material Number:	**562204**
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 protein stabilizer and ≤0.09% sodium azide.

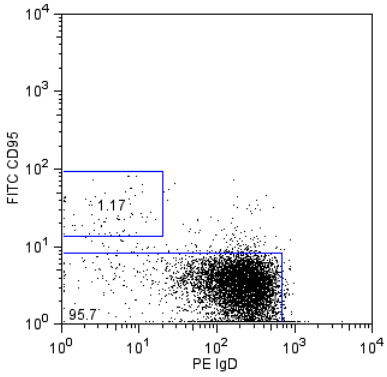
Description

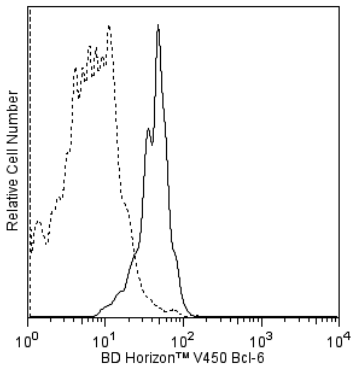
The K112-91 monoclonal antibody specifically binds to Bcl-6. Bcl-6 was first identified as a proto-oncogene frequently deregulated by chromosomal translocations in non-Hodgkin B-cell lymphomas. It is a nuclear transcriptional repressor of the BTB/POZ zinc-finger family of transcription factors. In addition 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.

The antibody is conjugated to BD Horizon™ V450, which has been developed for use in multicolor flow cytometry experiments and is available exclusively from BD Biosciences. It is excited by the Violet laser Ex max of 406 nm and has an Em Max at 450 nm. Conjugates with BD Horizon™ V450 can be used in place of Pacific Blue™ conjugates.



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™ V450 Mouse Anti-Human Bcl-6 antibody (Cat. No. 562204, solid line histogram) or a BD Horizon™ V450 mIgG1, κ Isotype Control (Cat. No. 560373; 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 Cytometer System.*





Multicolor flow cytometric analysis of Bcl-6 expression in mouse B lymphocytes (Middle and Right Panel). *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 PE Rat Anti-Mouse IgD (Cat. No. 558597) antibodies. 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™ V450 Mouse Anti-Bcl-6 antibody (Cat. No. 562204). 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 lymphocytes 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. Flow cytometric fluorescence histograms (Right Panel) derived from gated cell subpopulations show intracellular Bcl-6 staining levels for mouse GC B cells (solid line histogram) and non-GC B cells (dashed line histogram). 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™ V450 under optimum conditions, and unreacted BD Horizon™ V450 was removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

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)
560373	V450 Mouse IgG1, κ Isotype Control	0.1 mg	MOPC-21

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. 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.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
4. BD Horizon™ V450 has a maximum absorption of 406 nm and maximum emission of 450 nm. Before staining with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
5. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.
6. 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).
7. An isotype control should be used at the same concentration as the antibody of interest.
8. This product is sold under license to the following patent: US Patent No. 6,174,997.

References

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Ye BH, Lista F, Lo Coco F, Knowles DM, Offit K, Chaganti RS, Dalla-Favera R. Alterations of a zinc finger-encoding gene, BCL-6, in diffuse large-cell lymphoma. *Science.* 1993; 262(5134):747-750. (Biology)

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