

Technical Data Sheet

Alexa Fluor® 488 Mouse anti-Bcl-6

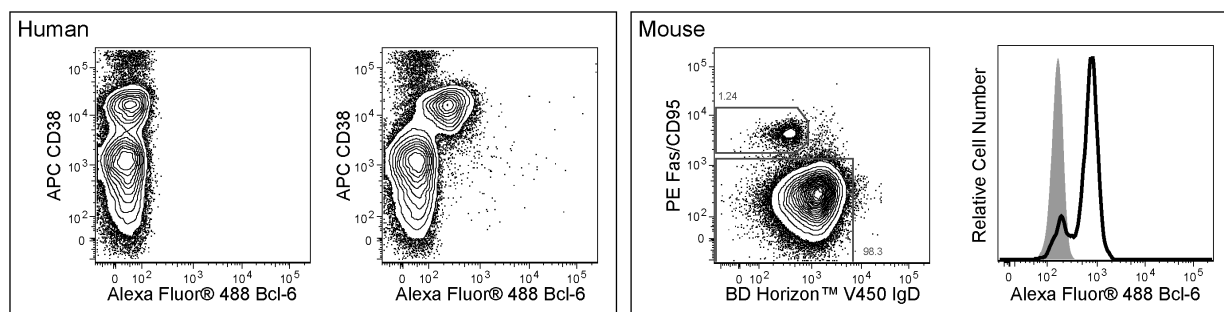
Product Information

Material Number:	561524
Alternate Name:	BCL6; B-cell lymphoma 6 protein; LAZ3; Laz-3, ZBTB27, ZNF51
Entrez Gene ID:	604,12053
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 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 (T_{fh}) 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 K112-91 antibody is compatible with multiple cellular fixation and permeabilization treatments as listed in the *Recommended Assay Procedure* section (please see below).



Bcl-6 expression in Human B lymphocytes (Left Panel). Tonsil cells were stained with BD Horizon™ V450 Anti-Human CD19 (Cat. No. 560353), PE-Cy™7 Anti-Human CD4 (Cat. No. 560649) and APC Anti-Human CD38 (Cat. No. 555462). Cells were washed, fixed with BD Cytotfix™ Fixation Buffer (Cat. No. 554655) and permeabilized with BD Phosflow™ Perm/Wash Buffer I (Cat. No. 557885), followed by no staining (Left Figure) or intracellular staining with Alexa Fluor® 488 Anti-Bcl-6 (Right Figure; Cat. No. 561524). Contour plots show expression of Bcl-6 (or background fluorescence) versus CD38 by B cells identified as CD4-CD19+ events with the light-scatter characteristics of intact lymphocytes. Bcl-6 staining is observed in CD38+ germinal center B cells but not in CD38- naive B cells nor CD38++ plasma cells. Cytometry was performed with a BD FACSCanto™ II Flow Cytometer. Data courtesy of Mark Kroenke & Shane Crotty, La Jolla Institute for Allergy & Immunology.

Bcl-6 expression in Mouse B lymphocytes (Right Panel). BALB/c mesenteric lymph node cells were stained with APC Anti-Mouse B220 (Cat. No. 553092), PerCP-Cy™5.5 Anti-Mouse CD4 (Cat. No. 550954), PE Anti-Mouse Fas/CD95 (Cat. No. 554258), and BD Horizon™ V450 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 Alexa Fluor® 488 Anti-Bcl-6 (Cat. No. 561524). A contour plot shows the expression of IgD versus Fas/CD95 by B cells identified as CD4-B220+ events with the light-scatter characteristics of intact lymphocytes (Left Figure). Germinal center B cells were identified as IgDloFas+ B lymphocytes. Flow histograms (Right Figure) show intracellular Bcl-6 staining of mouse germinal center B cells (open histogram) and non-GC B cells (shaded histogram). Cytometry was performed with a BD LSRFortessa™ Flow Cytometer.

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® 488 under optimum conditions, and unreacted Alexa Fluor® 488 was removed.

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Application Notes

Application

Intracellular staining (flow cytometry)

Routinely Tested

Recommended Assay Procedure:

This fluorescent antibody conjugate is suitable for intracellular staining of cell lines and primary cells fixed using BD Cytofix™ Fixation Buffer (Cat. No. 554655) or BD Phosflow™ Lyse/Fix Buffer (Cat. No. 558049) and permeabilized using BD Phosflow™ Perm/Wash Buffer I (Cat. No. 557885) or BD Phosflow™ Perm Buffers II (Cat. No. 558052), III (Cat. No. 558050) or IV (Cat. No. 560746).

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)
557782	Alexa Fluor® 488 Mouse IgG1 κ Isotype Control	50 tests	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. Alexa Fluor® 488 fluorochrome emission is collected at the same instrument settings as for fluorescein isothiocyanate (FITC).
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
4. 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.
5. 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.
6. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
7. Cy is a trademark of Amersham Biosciences Limited.
8. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
9. This product is sold under license to the following patent: US Patent No. 6,174,997.
10. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

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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