

Bcl-6

An important transcription factor for B and T follicular helper (Tfh) cell differentiation

Features

Useful marker for the study of germinal center reactions and Tfh cell differentiation

Tested by several leading immunology laboratories

Suitable for use in several applications including flow cytometry, immunoblotting, and immunohistochemistry

Available in several formats including purified, Alexa Fluor® 488, Alexa Fluor® 647, and PE

Compatible with several BD Phosflow™ buffer systems including BD Phosflow Lyse/Fix Buffer, BD Cytotfix™ Fixation Buffer, and BD Phosflow Permeabilization Buffers I-IV.

BD Biosciences now offers a monoclonal antibody to human and mouse Bcl-6, a key transcription factor expressed in germinal center B cells and CD4⁺ T follicular helper (Tfh) cells.¹

Bcl-6 (clone K112-91) purified and fluorochrome-conjugated antibodies

The new BD Pharmingen™ Bcl-6 monoclonal antibody reacts with human and mouse Bcl-6, a transcriptional repressor and member of the BTB/POZ zinc-finger family of transcription factors.²

Available conjugates include Alexa Fluor® 488, Alexa Fluor® 647, and PE formats to enable maximum flexibility for design of multicolor fluorescent antibody staining panels in combination with any of our family of BD FACST™ brand flow cytometers.

Bcl-6 expression and function

Bcl-6 expression is induced by IL-21 and can lead to the expression of CXCR5, a homing molecule for the germinal center.¹ Germinal centers are regions within the lymph nodes where antigen-specific B cells mature into memory B cells or plasma cells. Bcl-6 is believed to facilitate B cell proliferation and somatic hypermutation within the germinal center. Bcl-6 is hypothesized to support somatic hypermutation through the repression of the DNA damage sensor ATR. In contrast, as B cells mature into plasma cells, Bcl-6 levels and cell proliferative capacity decrease while levels of Blimp-1 and secreted effectors increase.²⁻⁴

In addition to its role in B cell maturation, Bcl-6 is considered to be the master transcription factor for Tfh cells. Tfh cells help B cells proliferate and undergo affinity maturation. Bcl-6 facilitates the differentiation of CD4⁺ T cells into Tfh cells through the inhibition of other key T helper cell transcription factors such as T-bet (Th1), GATA-3 (Th2), and RORγT (Th17).^{1,4,5}

Visit bdbiosciences.com/tcells for more information.

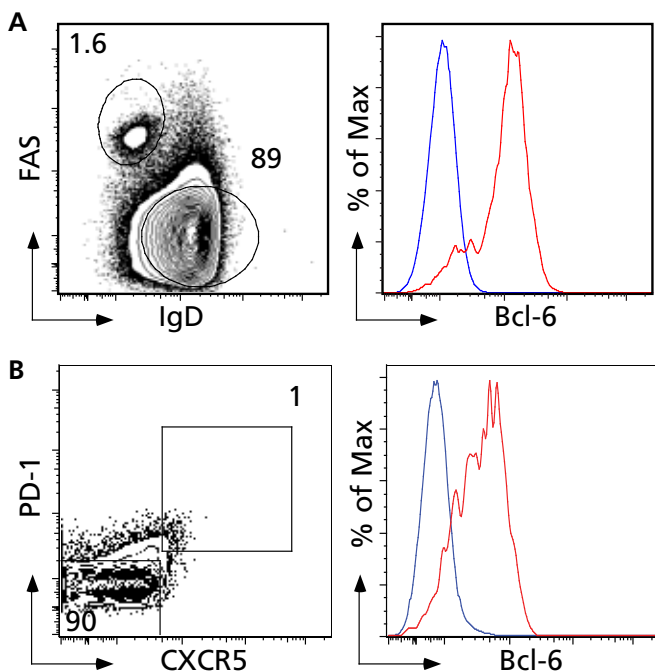


Figure 1. Bcl-6 expression in mouse germinal center B cells and Tfh cells.

A. Draining murine lymph node cells were stained 7 days after subcutaneous NP-OVA/alum immunization. Contour plot (left) shows surface staining of CD19⁺ B cells followed by intracellular Bcl-6 staining (right). Germinal center (GC) B cells were identified as IgD^{low}FAS^{high} cells expressing high levels of Bcl-6 (red line). Non-GC B cells (IgD^{high}FAS^{low}) served as a control population (blue line).

B. Draining murine lymph node cells were stained 7 days after subcutaneous NP-OVA/alum immunization. Contour plot (left) shows surface staining of CD4⁺ T cells with PD-1-FITC and CXCR5-biotin antibodies followed by streptavidin-APC. CD19⁺ B cells were excluded from the analysis. Intracellular staining was performed with BD Phosflow Lyse/Fix buffer, BD Phosflow Perm/Wash buffer, and Bcl-6-PE antibody according to the manufacturer's instructions. Tfh cells were gated as CXCR5^{high}PD-1^{high} cells and Bcl-6 expression (red line) is shown in the histogram (right). CXCR5^{low}PD-1^{low} non-Tfh cells served as a control population (blue line).

Data courtesy of Dirk Baumjohann and Mark Ansel, University of California San Francisco.

Bcl-6

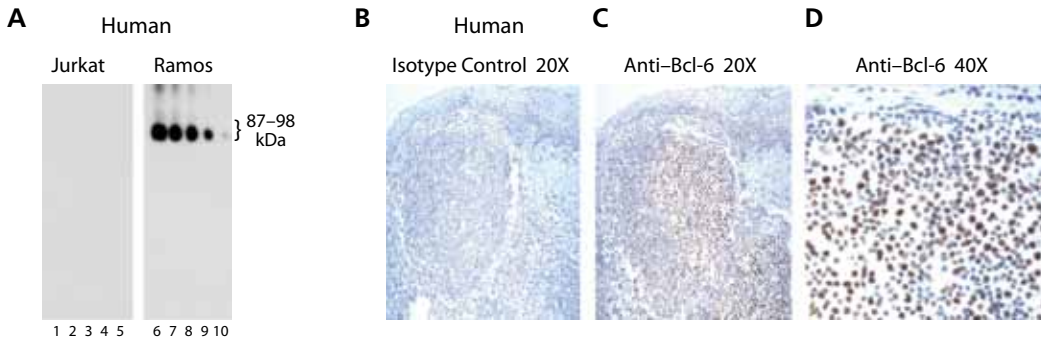


Figure 2. Analysis of Bcl-6 expression by Western blot and immunohistochemistry.

A: Western blot analysis of Bcl-6 expressed by Human Jurkat (Cat. No. 611451) and Ramos cell lines. Cell lysates from untreated Jurkat (lanes 1–5) and Ramos (lanes 6–10) cells (15 µg total cellular protein/lane) were electrophoresed (SDS-PAGE), transferred to membranes, and then probed with Purified Mouse Anti-Bcl-6 antibody (Clone K112-91; Cat. No. 561520) at concentrations of 2 (lanes 1, 6), 0.667 (lanes 2, 7), 0.222 (lanes 3, 8), 0.074 (lanes 4, 9), and 0.025 (lanes 5, 10) µg/mL. Bcl-6 is identified as a band of ~87–98 kDa in the Ramos cell lysate.

B-D: Bcl-6 staining of human tonsil. Following antigen retrieval with BD Pharmingen Retrieval A buffer (Cat. No. 550524), the formalin-fixed paraffin-embedded sections were stained with either Purified Mouse IgG₁, κ Isotype Control (Cat. No. 550878; B) or Purified Mouse Anti-Bcl-6 antibody (Clone K112-91; Cat. No. 561520; C and D), with Hematoxylin counterstaining. Bcl-6 is detected in the nuclei of the lymphocytes within the lymphoid follicles of the tonsil. Original magnifications: 20X and 40X as indicated.

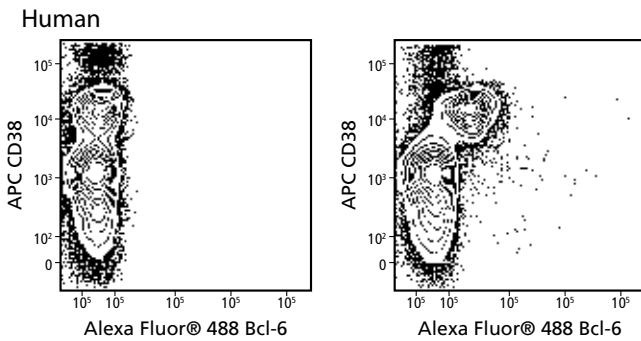


Figure 3. Bcl-6 expression in Human B lymphocytes.

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 Cytofix Fixation Buffer (Cat. No. 554655) and permeabilized with BD Phosflow Perm/Wash Buffer I (Cat. No. 557885), followed by no staining (left) or intracellular staining with Alexa Fluor® 488 Anti-Bcl-6 (Cat. No. 561524)(right). Contour plots show expression of Bcl-6 (or background fluorescence) vs 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 or CD38⁺⁺ plasma cells. Cytometry was performed with a BD FACSCanto™ II flow cytometer.

Data courtesy of Mark Kroenke and Shane Crotty, La Jolla Institute for Allergy & Immunology.

Key Markers of Germinal Center B and Tfh Cells

Germinal Center B Cells	
Human	CD19 ⁺ , CD38 ⁺
Mouse	B220 ⁺ , GL7 ⁺ , Fas/CD95 ⁺ , IgD ⁻
Tfh Cells ⁵	
Surface markers	CD4 ⁺ , CD57 ⁺ , CXCR5 ⁺ , PD-1 ⁺ , ICOS ⁺
Cytokines	IL-21
Transcription factor	Bcl-6

References

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Ordering Information

Description	Clone	Isotype	Format	Size	Cat.No.
Human and Mouse Bcl-6	K112-91	Ms IgG ₁ , κ	Purified	0.1 mg	561520
			Alexa Fluor® 488	50 tests	561524
			Alexa Fluor® 647	50 tests	561525
			PE	50 tests	561522



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