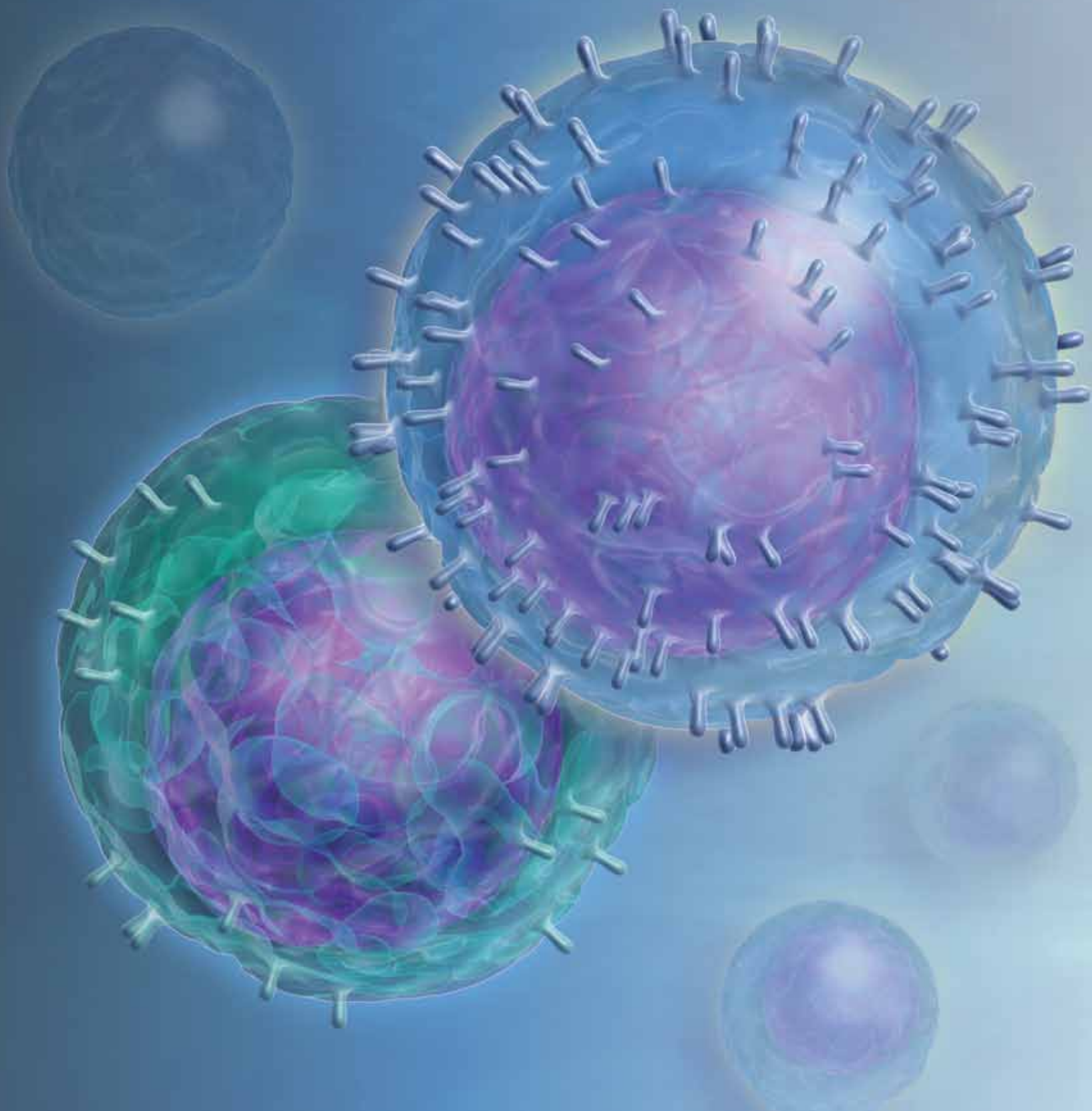# B-Cell Research

Flow cytometry tools for the study of B-cell biology

Supporting B-Cell Research:
Providing Innovative and Flexible Ways
to Study B-Cell Phenotypes and Functions

B cells remain an active area of research because they play a critical role in the immune system, and because perturbations in B-cell development or function are implicated in several disease states. Understanding the basis of B-cell function and dysfunction is of particular interest for the development of new vaccines and therapies.

For more than two decades researchers have made significant discoveries about B-cell biology and phenotypes using BD Biosciences flow cytometry products, including instruments, reagents, and protocols.

BD continues to support researchers by introducing innovative tools to study multiple aspects of B-cell biology. Newly defined, key markers include the transcription factors Blimp-1 and XPB-1s, which allow analysis of these key regulators in different B-cell subsets. To support and simplify the detection of these critical intracellular molecules in B cells by flow cytometry, we offer antibodies validated for flow cytometry, as well as optimized buffer systems for staining.

To make multicolor flow cytometry more accessible, BD continually optimizes methods for panel design and offers markers in a wide selection of fluorochromes. Innovations in dyes further simplify doing more complex, multimarker analyses.

This brochure illustrates how the following innovative systems can be successfully applied to study B-cell development and function:

Cell Surface Markers to identify cells from heterogenous samples

Brighter Fluorochromes to detect even rare or dim subsets

BD™ Cytometric Bead Array (CBA) to measure secreted immunoglobulins and cytokines

Intracellular Markers and specialized buffers and protocols to detect transcription factors, phospho-signaling proteins, and cytokines within individual cells

From intracellular and surface markers to secreted proteins and functional assays, BD Biosciences offers the latest reagents and methods to study B-cell biology in health and disease.

B Cells: Subsets and Markers

B cells pass through a number of developmental stages both before and after exposure to antigens. As they mature and differentiate, they give rise to multiple functionally distinct subsets. Differential expression of cell surface and intracellular markers, as well as their distinct immunoglobulin and cytokine secretion profiles, provide valuable clues to the diverse nature and function of the different B-cell subsets.

For example, the expression of syndecan-1 (CD138) distinguishes circulating plasmablasts and plasma cells, the "professional" antibody-secreting B cells, from other developmental and functional subsets.

To support the use of multicolor flow cytometry for the study of B cells, BD offers a wide portfolio of reagents for B-cell phenotyping. They are available in multiple formats, to provide maximum flexibility in panel design. We continue to add new specificities as new markers emerge.

The schematic summarizes the main developmental and differentiation stages, as well as some of the key markers associated with each B-cell subset in mouse and human.
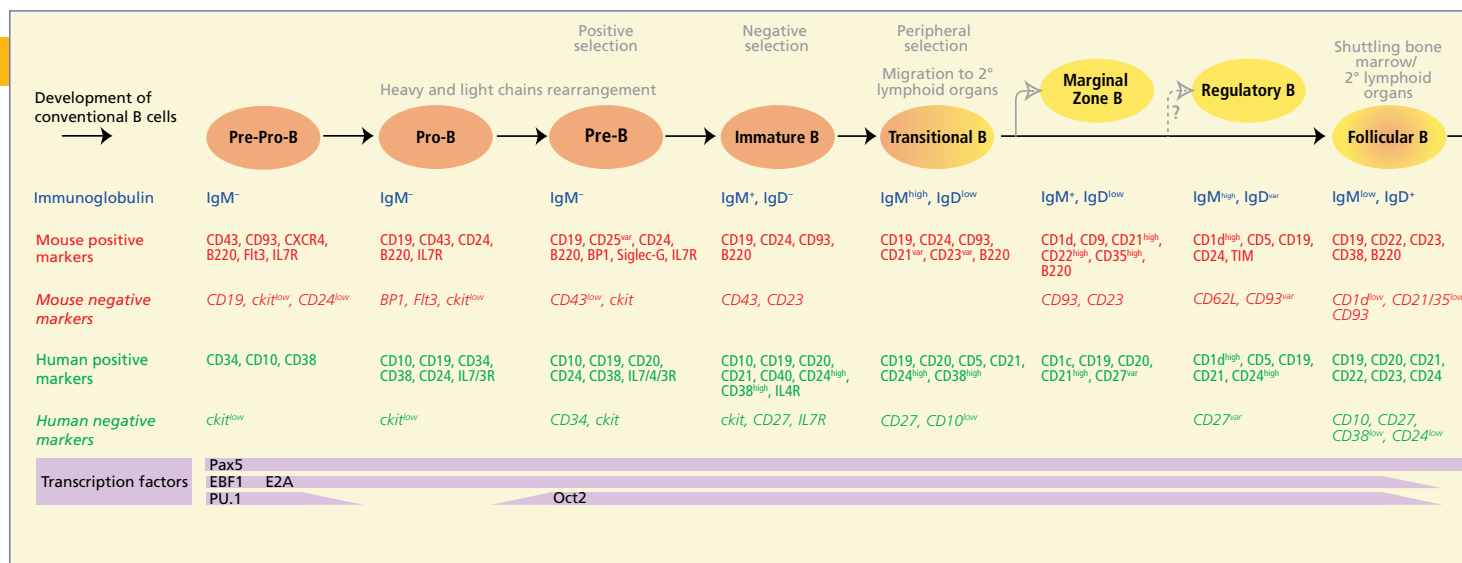
Conventional B-Cell Maturation

B cells originate from hematopoietic stem cells (HSCs) located in bone marrow, where they pass through the first stages of development. The still immature B cells then migrate to secondary lymphoid tissues, where most of them continue their development into mature follicular B cells.[1] When the B-cell receptor (BCR) complex of a mature B cell, consisting of the membrane-bound (m) forms of IgM and IgD, binds its cognate foreign antigen, the cell becomes activated and differentiates into an antibody-secreting plasma cell.[2]

B-Cell Subsets with Different Functions

B cells also follow alternative differentiation pathways from those of conventional B cells, resulting in subsets that have distinct functions and marker expression patterns. For example, marginal-zone (MZ) B cells function as innate-like cells. Unlike conventional B cells, they can be activated through Toll-like receptor (TLR)-ligation, bypassing the BCR. They also tend to express CD1d and CD21, but not CD23.[1,2] B-cell subsets with regulatory function have been identified and are distinguished by their ability to secrete IL-10 or TGF-β-1.[1–4]

Summary of the key developmental stages and markers of B cells

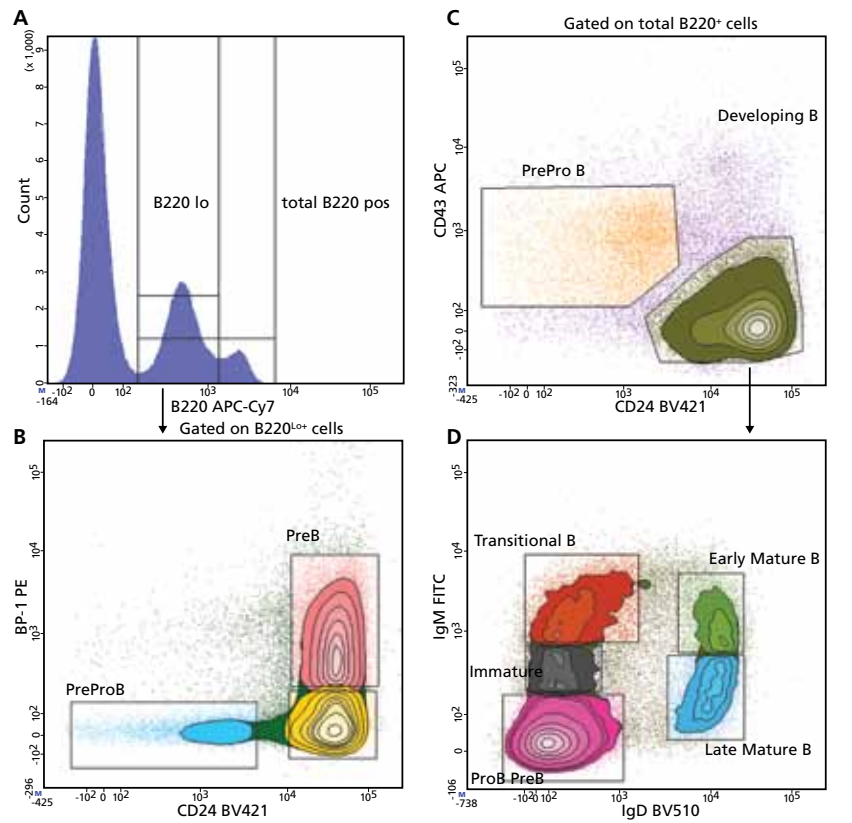|	Pre-Pro-B	Pro-B	Pre-B	Immature B	Transitional B	Marginal Zone B	Regulatory B	Follicular B
Immunoglobulin	IgM⁻	IgM⁻	IgM⁻	IgM$^+$, IgD⁻	IgM^{high}, IgD^{low}	IgM$^+$, IgD^{low}	IgM^{high}, IgD^{var}	IgM^{low}, IgD$^+$
Mouse positive markers	CD43, CD93, CXCR4, B220, Flt3, IL7R	CD19, CD43, CD24, B220, IL7R	CD19, CD25^{var}, CD24, B220, BP1, Siglec-G, IL7R	CD19, CD24, CD93, B220	CD19, CD24, CD93, CD21^{var}, CD23^{var}, B220	CD1d, CD9, CD21^{high}, CD22^{high}, CD35^{high}, B220	CD1d^{high}, CD5, CD19, CD24, TIM	CD19, CD22, CD23, CD38, B220
Mouse negative markers	*CD19, ckit^{low}, CD24^{low}*	*BP1, Flt3, ckit^{low}*	*CD43^{low}, ckit*	*CD43, CD23*		*CD93, CD23*	*CD62L, CD93^{var}*	*CD1d^{low}, CD21/35^{low} CD93*
Human positive markers	CD34, CD10, CD38	CD10, CD19, CD34, CD38, CD24, IL7/3R	CD10, CD19, CD20, CD24, CD38, IL7/4/3R	CD10, CD19, CD20, CD21, CD40, CD24^{high}, CD38^{high}, IL4R	CD19, CD20, CD5, CD21, CD24^{high}, CD38^{high}	CD1c, CD19, CD20, CD21^{high}, CD27^{var}	CD1d^{high}, CD5, CD19, CD21, CD24^{high}	CD19, CD20, CD21, CD22, CD23, CD24
Human negative markers	*ckit^{low}*	*ckit^{low}*	*CD34, ckit*	*ckit, CD27, IL7R*	*CD27, CD10^{low}*		*CD27^{var}*	*CD10, CD27, CD38^{low}, CD24^{low}*
Transcription factors	Pax5 / EBF1 E2A / PU.1	Oct2						

This schematic is not intended to be comprehensive, and markers can be altered as a result of cellular environment, differentiation state, and other factors.

Analysis of B-Cell Maturation

With a comprehensive selection of antibodies to mouse and human markers, BD can support a wide variety of phenotyping panels for the study of B cells across all developmental stages.

To illustrate the use of differential marker expression for B-cell analysis, seven cell surface markers were used to analyze B-cell subsets in mouse bone marrow, allowing discrimination of seven different developmental phases in this tissue.
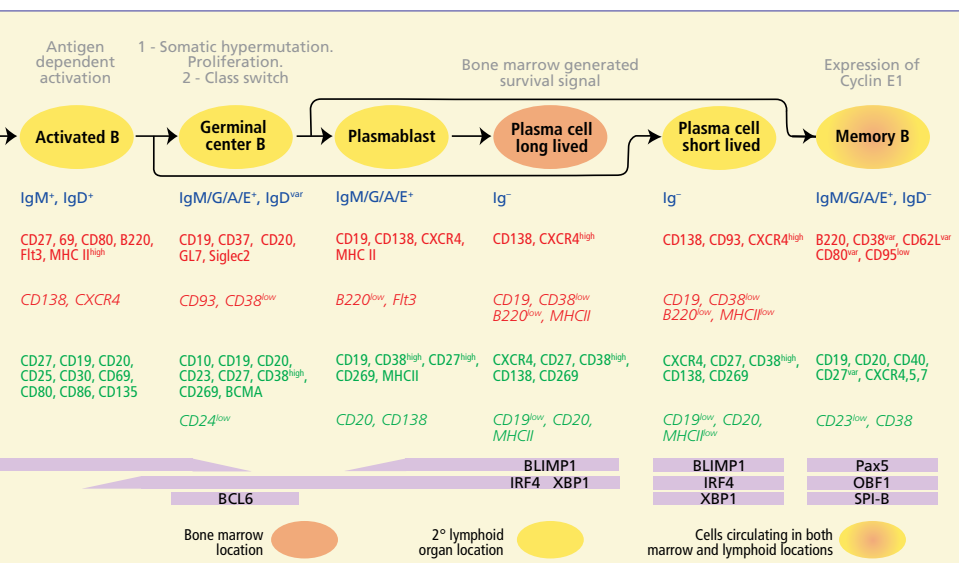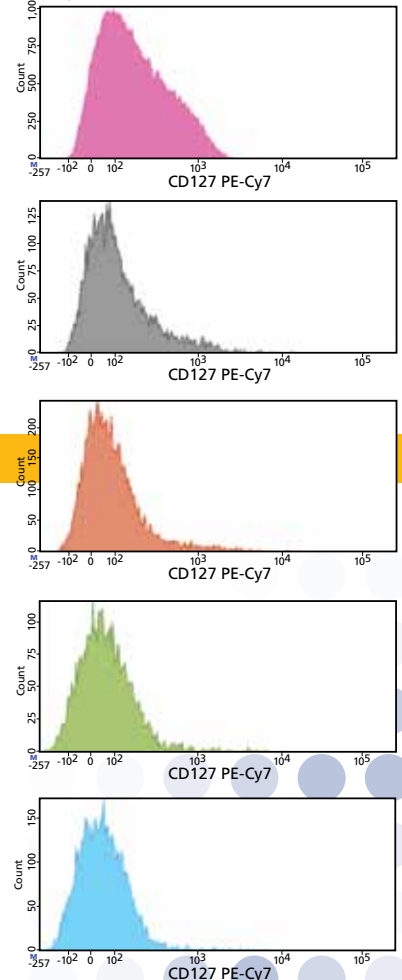
Pre-pro-B, Pro-B, and Pre-B cells could be distinguished within the low positive CD45R/B220 population based on their differential expression of BP1 and CD24. Immature, transitional, and early and late mature B cells could be segregated based on differential expression of IgM and IgD. The expression of the IL-7 receptor, CD127, was analyzed in these different subsets, and was shown to decrease as B cells matured.

A

B220 lo   total B220 pos

B220 APC-Cy7

B

Gated on B220^Lo+ cells

PreB

PreProB

CD24 BV421

C

Gated on total B220⁺ cells

Developing B

PrePro B

CD24 BV421

D

Transitional B

Early Mature B

Immature

ProB PreB

Late Mature B

IgD BV510

Analysis of B-cell developmental stages in mouse bone marrow

C57BL/6 mouse bone marrow cells were stained with the following fluorescent antibodies: IgM FITC, CD43 APC, BP-1 PE, CD127 PE-Cy™7, CD45R/B220 APC-Cy7, CD24 BD Horizon Brilliant™ Violet 421 (BV421), and IgD BD Horizon Brilliant™ Violet 510 (BV510), and analyzed using a BD FACSCanto™ II flow cytometer.

E CD127 expression on Pro-B/Pre-B, Immature Transitional, Early Mature, and Late Mature B cells

CD127 PE-Cy7

CD127 PE-Cy7

CD127 PE-Cy7

CD127 PE-Cy7

CD127 PE-Cy7

Antigen dependent activation	1 - Somatic hypermutation. Proliferation. 2 - Class switch		Bone marrow generated survival signal		Expression of Cyclin E1
Activated B	**Germinal center B**	**Plasmablast**	**Plasma cell long lived**	**Plasma cell short lived**	**Memory B**
IgM⁺, IgD⁺	IgM/G/A/E⁺, IgDᵛᵃʳ	IgM/G/A/E⁺	Ig⁻	Ig⁻	IgM/G/A/E⁺, IgD⁻
CD27, 69, CD80, B220, Flt3, MHC IIʰⁱᵍʰ	CD19, CD37, CD20, GL7, Siglec2	CD19, CD138, CXCR4, MHC II	CD138, CXCR4ʰⁱᵍʰ	CD138, CD93, CXCR4ʰⁱᵍʰ	B220, CD38ᵛᵃʳ, CD62Lᵛᵃʳ CD80ᵛᵃʳ, CD95ˡᵒʷ
CD138, CXCR4	CD93, CD38ˡᵒʷ	B220ˡᵒʷ, Flt3	CD19, CD38ˡᵒʷ B220ˡᵒʷ, MHCII	CD19, CD38ˡᵒʷ B220ˡᵒʷ, MHCIIˡᵒʷ	
CD27, CD19, CD20, CD25, CD30, CD69, CD80, CD86, CD135	CD10, CD19, CD20, CD23, CD27, CD38ʰⁱᵍʰ, CD269, BCMA	CD19, CD38ʰⁱᵍʰ, CD27ʰⁱᵍʰ, CD269, MHCII	CXCR4, CD27, CD38ʰⁱᵍʰ, CD138, CD269	CXCR4, CD27, CD38ʰⁱᵍʰ, CD138, CD269	CD19, CD20, CD40, CD27ᵛᵃʳ, CXCR4,5,7
	CD24ˡᵒʷ	CD20, CD138	CD19ˡᵒʷ, CD20, MHCII	CD19ˡᵒʷ, CD20, MHCIIˡᵒʷ	CD23ˡᵒʷ, CD38
			BLIMP1 IRF4 XBP1	BLIMP1 IRF4 XBP1	Pax5 OBF1 SPI-B
	BCL6				

Bone marrow location

2° lymphoid organ location

Cells circulating in both marrow and lymphoid locations

Tools to Study B Cells:
Surface Immunophenotyping

A multicolor immunophenotyping approach is well suited to the study of B cells, since B cells by nature require the use of more markers than, for example, T cells, to define the basic subsets present in most samples.

Backbone Markers
A wide variety of B-cell studies employs cell surface markers that define the major B-cell subsets, and these form the "backbone," or core, for panels to do more detailed analysis.

The key marker for B-cell panels is the lineage marker CD19, which is expressed by almost all cells belonging to the B-cell lineage. In the mouse, CD45R/B220 is traditionally used. Most panels also include surface-expressed IgD and IgM, since the BCR isotype provides information about the differentiation stage of the B cells.

To provide flexibility in panel design, BD offers all of these markers in a wide selection of formats.

Basic Panel
Depending on the type of sample that is being analyzed, different markers can be selected to define the specific B-cell subsets of interest. The example shown analyzes human peripheral blood using CD19, CD20, IgD, CD27, CD38, and CD24. With this six-color panel it is possible to identify transitional B cells (CD24^{high}, CD38^{high}), to discriminate between naïve and memory cells (naïve cells are CD27$^-$IgD$^+$, memory cells CD27$^+$IgD$^-$), and identify plasmablasts (CD38$^+$).[5]

Deeper Phenotyping
Because the phenotype of some subsets is very similar to others, often a more extensive phenotyping panel is required. For example, a panel might distinguish the many B-cell developmental stages present in bone marrow B cells.

Adding markers can also provide a more fine-detail analysis, for example, defining memory cells or different types of plasma cells. Other molecules offer insight into the potential for cells to home and localize within the body (chemokine receptors, CD62L). Adding activation markers (CD69, CD25, CD80, CD86) can inform about which subsets are activated, and provide important clues about an individual's immune response.

Tools for Building Panels
Antigen Density. When adding markers to a panel, researchers can take advantage of antigen-density information to optimize antigen-fluor selection. In general, we recommend choosing bright fluorochromes for markers with low antigen density, and the less bright fluorochromes for highly expressed markers. To support researchers, BD is generating information about the antigen density of a number of markers on peripheral blood cells.

Six-color analysis of human peripheral blood cells
Human peripheral blood mononuclear cells (PBMCs) were stained with the following fluorescent antibodies: CD19 PerCP-Cy™5.5, CD20 APC-H7, IgD FITC, CD27 PE-Cy7, CD38 APC, and CD24 PE, and analyzed using a BD FACSVerse™ flow cytometer.

Legend:
- Non-B lymphocytes (CD19⁻CD3⁺/⁻)
- Naïve B cells (CD19⁺CD27⁻IgD⁺)
- Transitional B cells (CD19⁺CD24⁺⁺CD38⁺⁺)
- Non–class-switched memory B cells (CD19⁺CD27⁺IgD⁺)
- Class-switched memory B cells (CD19⁺CD27⁺IgD⁻IgM⁻CD20⁺CD38⁺/⁻)
- Plasmablasts and plasma cells (CD19⁺CD27⁺IgD⁻IgM⁻CD20⁻CD38⁺⁺)
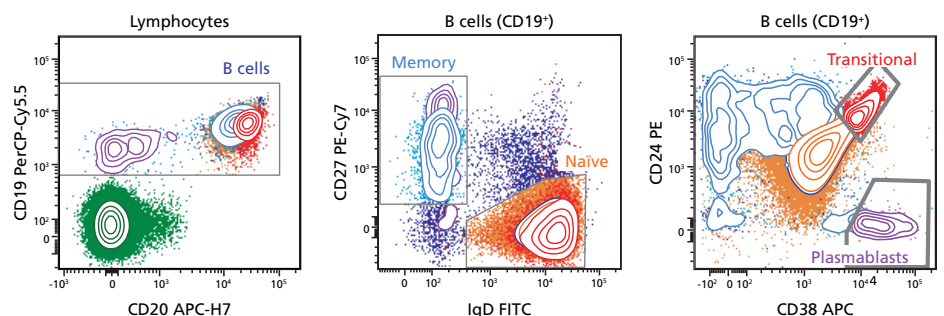
Bright Conjugates for Dim Markers. Certain B-cell populations are so rare, or defined by dimly expressed antigens, that achieving sufficient resolution is critical to obtaining good data in staining experiments. In particular for such rare or dim populations, the use of very bright fluorochromes helps improve resolution. A number of B-cell marker antibodies are available conjugated to very bright BD Horizon Brilliant™ Violet (BV) dyes, such as BV421, BV510, and BD Horizon Brilliant™ Violet 605 (BV605).

An Extended Panel for Defining Additional Subsets
In designing the panel used for the 10-color analysis experiment shown, information about antigen density helped optimize the fluorochrome distribution. Bright fluorochromes were chosen for the low-density antigen CD138 and to allow resolution of populations expressing different levels of IgD and CD38, and the extremely bright BV dyes were used to obtain optimal detection of CD27 and IgM.

Analysis using this panel provided the additional information about Ig subclass expression, allowing identification of both IgG⁺ and IgG⁻ post-class-switched memory cells (IgM⁻IgD⁻CD38⁻CD27⁺/dim), and the clear discrimination of plasma cells (IgM⁻IgD⁻CD20⁻CD38⁺⁺).
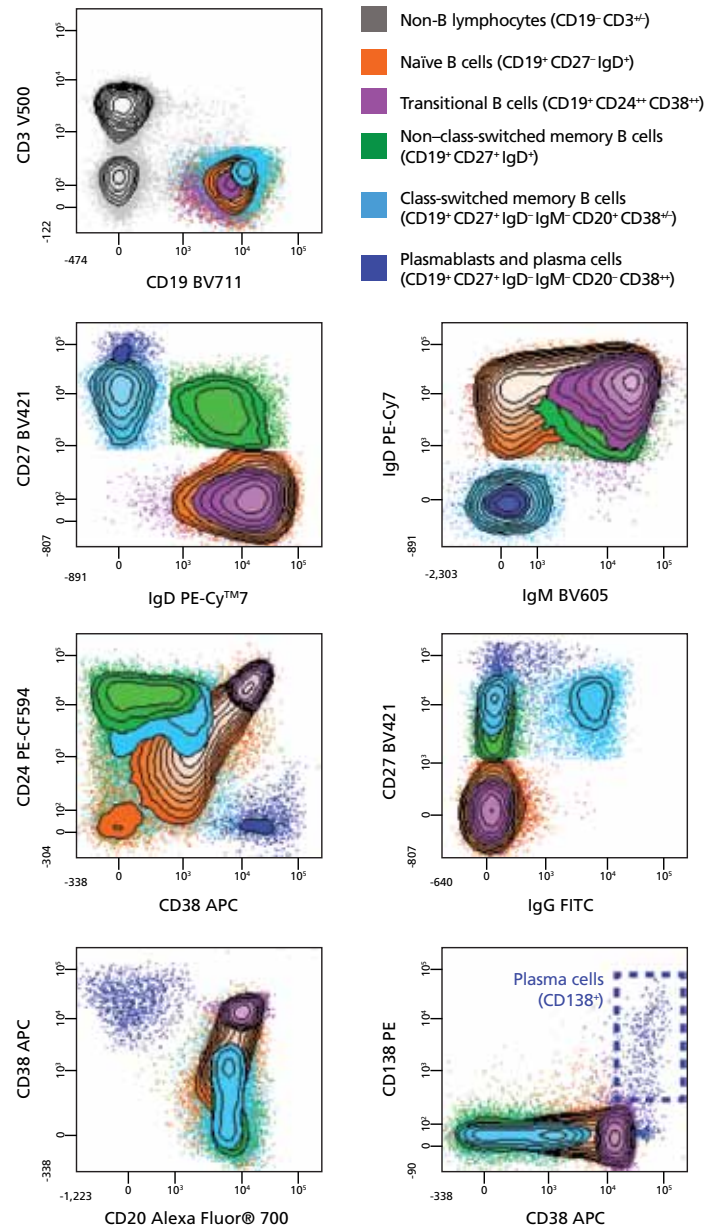
Ten-color analysis of human peripheral blood cells
Human PBMCs were stained with the following fluorescent antibodies: CD19 BD Horizon Brilliant™ Violet 711 (BV711), CD20 Alexa Fluor® 700, IgD PE-Cy7, CD27 BV421, CD38 APC, CD24 BD Horizon™ PE-CF594, CD3 BD Horizon™ V500, CD138 PE, IgM BV605, and IgG FITC, and analyzed using a BD LSRFortessa™ flow cytometer.

Antigen density of B-cell markers on human PBMCs
The antigen density of B-cell markers on human PBMCs from three healthy donors was calculated using the BD Quantibrite™ bead method, and analysis on a BD LSRFortessa and a BD FACSVerse flow cytometer.
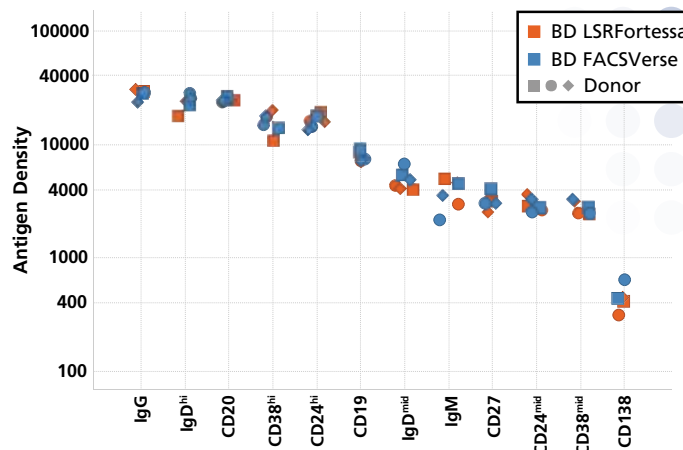
Fast multiplexed quantitation

Measurement of Secreted Immunoglobulins and Cytokines

Measurement of the types and amounts of immunoglobulins and cytokines that are secreted by B cells provides insight into the quality and quantity of their immune response—features that are also frequently altered in disease states. Methods that allow multiplexed measurements are increasingly being used to measure immunoglobulins (Igs) and cytokines. In addition to the benefits of multiplex analysis, they have several unique advantages compared to classically used techniques.

Table 1. Cytokine-producing B cells

Cell type	Cytokines Secreted
B effector 1 (Be-1)	IFN-γ, IL-12, TNF
B effector 2 (Be-2)	IL-2, IL-4, IL-6, TNF
Regulatory B	IL-10, TGF-β1

BD Cytometric Bead Array: Multiplexed Quantitation

BD Cytometric Bead Array (CBA) is a bead-based immunoassay that can simultaneously quantify multiple analytes from the same sample. The BD CBA system uses antibody-coated beads to efficiently capture analytes, and flow cytometry for read-out.

The broad dynamic range of fluorescence detection, and multiplexed measurement, allow for small sample volume, fewer sample dilutions, and substantially less time to establish the value of an unknown, versus a conventional ELISA approach.

The BD CBA portfolio includes assays for measurement of a variety of soluble and intracellular proteins, including immunoglobulins and cytokines.

Visit **bdbiosciences.com/cba** for more information.
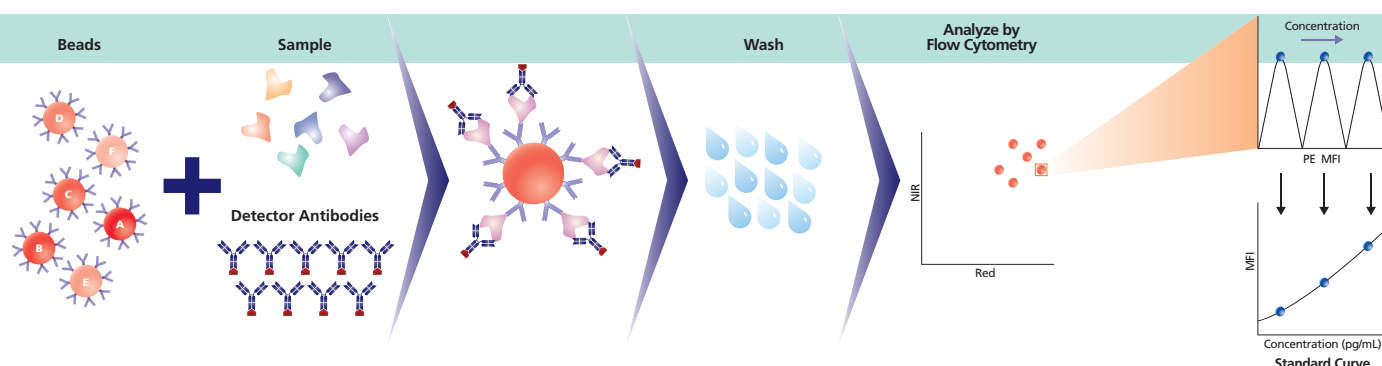
BD CBA Assay Principle

Each capture bead in the array has a unique fluorescence intensity and is coated with a capture antibody specific for a single analyte. A combination of different beads is mixed with a sample (25 to 50 µL) or standard and a mixture of detection antibodies that are conjugated to a reporter molecule (PE). Following incubation and subsequent washing, the samples are acquired on a flow cytometer.

FCAP Array™ analysis software gates on each bead population and determines the median fluorescence intensity (MFI) for each analyte in the array. It generates a standard curve and performs interpolation of sample concentrations compared to the standard curve, and generates an analysis report.

8

BD CBA Assays for Igs

The BD™ CBA Human Immunoglobulin Flex Set system provides ready-to-use reagents that serve as building blocks for the multiplexed quantitation of multiple Ig subclasses.

Because the amount and type of antibody in a particular immune response can vary greatly, measurements of these parameters can provide insight into the response after immunization or vaccination, or serve as an indicator to assess immunoglobulin deficiency disorders. For example, BD CBA assays have been used to analyze the Igs and cytokines secreted by isolated B cells from HIV-infected individuals after CpG stimulation.[6]

BD CBA Assays for Cytokines

BD™ CBA Flex Sets for cytokines provide an open and configurable menu of bead-based reagents designed for easy and efficient multiplexing. The specificities include a wide range of human and mouse cytokines. Data comparing results using BD CBA Standards to the NIBSC/WHO International Standards is available as a guideline, to facilitate comparisons of cytokine concentration values determined by different laboratories or methods.

Measuring which cytokines are secreted can also provide information about the activity of different functional B-cell subsets in a sample, such as B effector-1 or -2, or regulatory B cells (Bregs) secreting anti-inflammatory cytokines such as IL-10.[3] BD CBA Flex Sets have been used to quantitate the secretion of several B-cell–related cytokines and chemokines after manipulation of exhausted tissue-like B-memory cells in HIV-infected individuals.[7]
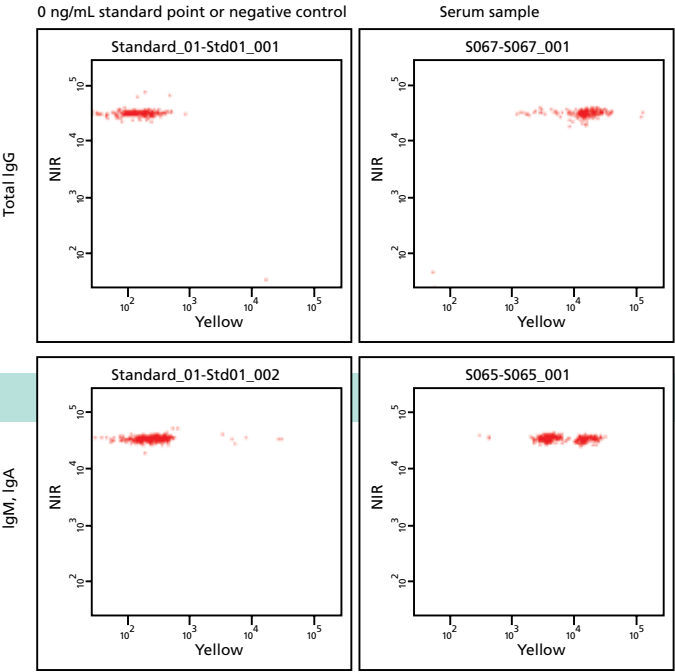
IgG Standard Curve
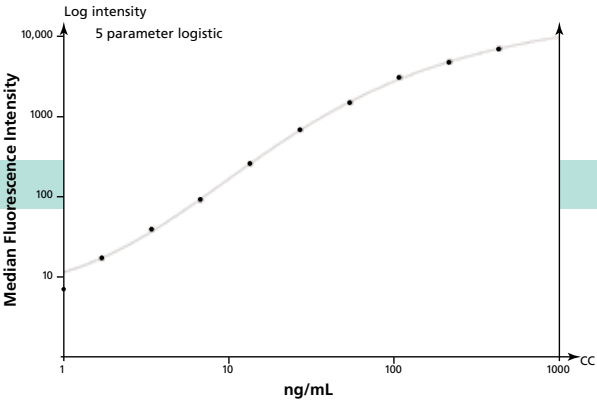
Quantitation of immunoglobulins in human serum samples

Serum from five donors was tested using BD CBA Human Immunoglobulin Flex Sets to analyze total IgG, IgM, and IgA. Data was acquired on a BD FACSArray™ flow cytometer and analyzed using FCAP Array software. Dot plots for a negative control (detector alone) and one of the samples are shown. The results calculated (in g/L) on the basis of standard curves are shown, as well as the expected range.

	Total IgG	IgM	IgA
Donor 1	16.9	1.3	1.6
Donor 2	10.9	2.4	0.9
Donor 3	8.9	0.7	1.5
Donor 4	14.6	2.8	1.5
Donor 5	11.6	0.3	1.1
Expected Range	7–14	0.5–3.3	0.8–3.9

Analysis of B-Cell Activation and Signaling

The development, activation, and differentiation of B cells are accompanied by a number of changes in intracellular molecules, including transcription factors, phospho-signaling proteins, and cytokines. BD offers solutions that uniquely enable analysis of these intracellular molecules, to support researchers in deciphering the interconnected pathways regulating B-cell biology.

Intracellular Detection

Multicolor flow cytometry is a powerful technique for the analysis of intracellular molecules. Simultaneous analysis with markers for specific cell subsets can reveal information about the differentiation and activation state of distinct populations of B cells. While techniques for cell surface staining are relatively standard, optimal staining for intracellular markers often depends on the biology of the target protein.

Specialized Buffers and Antibodies

To facilitate the detection of these intracellular molecules by flow cytometry, BD has developed monoclonal antibodies, specialized buffer systems, and kits that are optimized for detection of specific types of intracellular targets. Flow cytometry has several advantages over commonly used methods such as Western blot: multiple parameters can be measured simultaneously from heterogeneous samples such as blood. This conserves precious samples and provides detailed results at the cellular level.

Detection of Transcription Factors

The BD Pharmingen™ transcription factor buffer permeabilizes the cells sufficiently to allow the exposure of intranuclear epitopes, while still being gentle enough to allow the detection of most cell surface proteins. It can be used alone or in combination with cell surface markers and cytokines for an improved detection of a number of different transcription factors.

BD offers several B-cell–relevant, flow-validated transcription factor antibodies, including unique specificities. Some, such as Oct-2 and Pax-5, are expressed through the follicular or germinal center (GC) stages. Bcl-6 characterizes proliferating GC B cells, while others, such as Blimp-1 and XPB-1s, are typical for plasma cells.

Detection of Phosphoproteins

BD Phosflow™ antibodies are monoclonal phospho-epitope–specific antibodies validated for flow cytometric detection. The recommended permeabilization buffer for most BD Phosflow antibodies is BD Phosflow perm buffer III, but alternative permeabilization buffers also are available to meet particular experimental needs.

A number of BD Phosflow antibody specificities are available for analysis of signaling pathways involved in B-cell development and activation, including phospho-Akt, phospho-Btk, phospho-Syk, phospho-BLNK, phospho-CD20/BL-CAM, phospho-IKKγ, phospho-NFκB, and phospho-mTOR.

Basic principles of intracellular staining

Cells are fixed and permeabilized (symbolized by dashed line membrane), stained, and then analyzed by flow cytometry. For studies of secreted proteins, cells are first treated with a protein transport inhibitor to allow accumulation of the target protein inside the cell.

Treat with protein transport inhibitor (for secreted proteins)

Fix and permeabilize cells
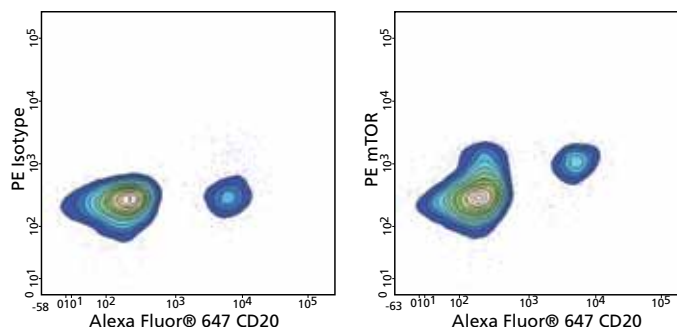
Stain cells

Flow cytometry analysis
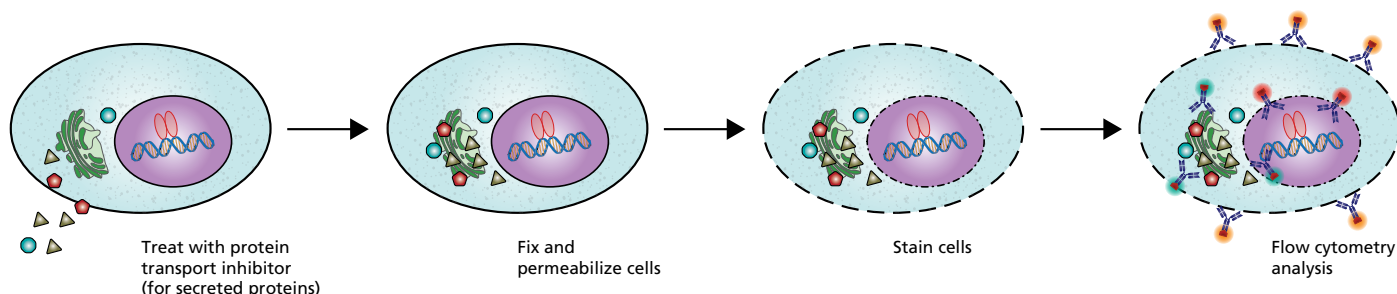
Detection of Cytokines

Using a protein transport inhibitor to block secretion, researchers can detect cytokines in the cell in which they are being produced. This makes it possible to easily determine if the cytokine production by an activated cell population is the result of a few cells producing large amounts of cytokine or a large population of cells producing small quantities of cytokine per cell.

BD's well established kits, buffer systems, and protocols for intracellular cytokine staining include BD Cytofix/Cytoperm™ fixation/permeabilization solution, which is suitable for staining most cytokines and cell surface markers. A wide selection of direct conjugates is available, covering cytokines often used to distinguish Bregs, and those secreted by Be-1 and Be-2 cells.

Additional information regarding the compatibilities of markers with the different buffer systems can be found at our online buffer compatibility resource.

Phospho-mTOR staining of human cells

Human peripheral B lymphocytes were stimulated with CpG ODN2395, then fixed with BD Cytofix™ fixation butter, permeabilized using BD Phosflow perm buffer III, and stained with anti-mTOR (pS2448) PE or a matching isotype control, and anti-CD20 Alexa Fluor® 647. Cells were analyzed using a BD FACSCanto II system.
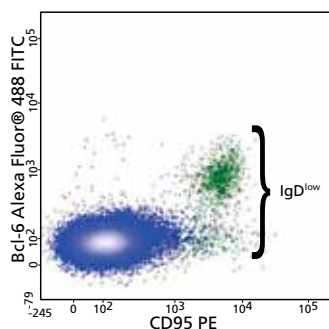
Bcl-6 expression in mouse lymph node cells

Mouse lymph node cells were stained with anti CD45R/B220, CD4, IgD, and CD95, fixed and permeabilized using the BD Pharmingen transcription factor buffer set, and stained with anti-Bcl-6 Alexa Fluor® 488. Flow cytometry was performed using a BD™ LSR II system. Bcl-6 expression was observed in germinal center B cells, identified using the $CD4^-B200^+IgD^{lo}CD95^{hi}$ phenotype.
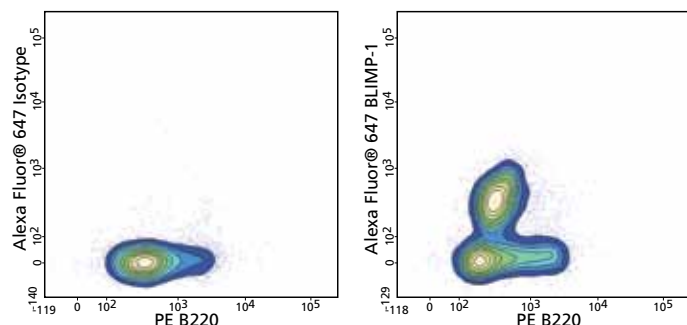
Blimp-1 staining in activated mouse splenocytes

B6 mouse splenocytes activated with LPS for 3 days were analyzed using the BD Pharmingen transcription factor buffer set, anti-CD45R/B220 PE, and either anti-Blimp-1 Alexa Fluor® 647 or a matching isotype control. Flow cytometry was performed using a BD FACSCanto II system.
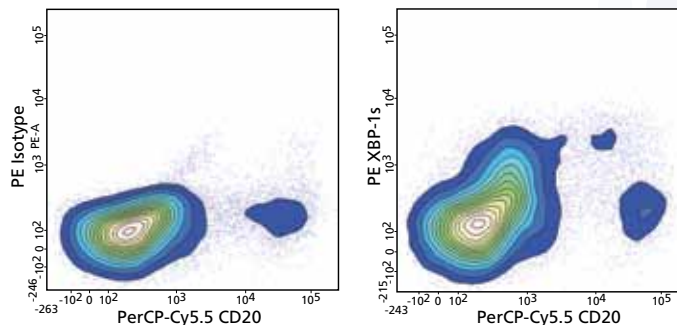
XBP-1s expression in CpG-stimulated human PBMCs

CpG-stimulated human PBMCs were incubated with BD Horizon™ fixable viability stain 450, fixed and permeabilized using the BD Pharmingen transcription factor buffer set, and stained with anti-CD20 PerCP-Cy5.5, and either anti-XBP-1S PE or a matching isotype control. Flow cytometry was performed using a BD FACSCanto II system.

Enabling Multicolor Flow

Simplifying Multicolor Setup

Instrument setup for multicolor experiments using antibody panels of more than four colors can be time consuming. When a panel of five markers is measured on two lasers, the fluorochromes that are typically used exhibit spillover into other fluorescence channels, thus requiring compensation. Researchers who have access to an instrument with four or five lasers can use this capability to their advantage, to simplify setup and minimize the need for compensation.

Five-laser instruments are often equipped with a 355-nm UV laser, which is typically used for side population analysis, calcium measurement using indo-1, or viability staining. To allow users to take full advantage of this equipment for phenotyping experiments, BD has developed a new UV-excited polymer-based dye, BD Horizon Brilliant™ Ultraviolet 395 (BUV395). Incorporating antibodies conjugated to BUV395 into a panel in which each fluorochrome is excited by a different laser results in minimal or no spillover between them.

This is illustrated by an example of a 5-color panel discriminating naive and memory B cells. Using this panel, only minimal setup and virtually no compensation were needed.

Combining Measurements to Maximize Results

Advances in buffer systems and methodologies now make it easier to simultaneously measure both surface and intracellular markers, and help researchers move toward information-rich, combined measurements. For example, several cell types in a heterogeneous sample of differentiating B cells can be monitored using a combination of cell surface and intracellular markers, to efficiently get more relevant data out of each sample.

The availability of antibodies to both B-cell surface markers and specific transcription factors in many different conjugates, including new BD Horizon™ dyes, adds flexibility when designing multicolor experiments.

### Monitoring Pax-5 in Splenic B-cell Subpopulations

In the example shown, nine cell surface markers were used to analyze B-cell subsets in mouse spleen. They allowed discrimination between the different developmental phases present in this tissue. Follicular B I and II and marginal cell subsets (Marginal Zone and Marginal Zone Progenitor) were identified based on expression of IgM, IgD, CD21, and CD23. The different levels of IgM and CD23 expression helped distinguish the stages of transitional subsets (T1, T2, and T3).

Measurement of the expression of Pax-5 in the different subsets revealed differential Pax-5 expression in follicular and marginal zone B cells.

**Five-color B-cell panel: minimal compensation**
Human PBMCs were stained with the following fluorescent antibodies: CD19 BUV395, CD27 BV421, IgD FITC, CD38 PE-CF594, and IgM APC. Samples were acquired using a 5-laser BD LSRFortessa flow cytometry system.
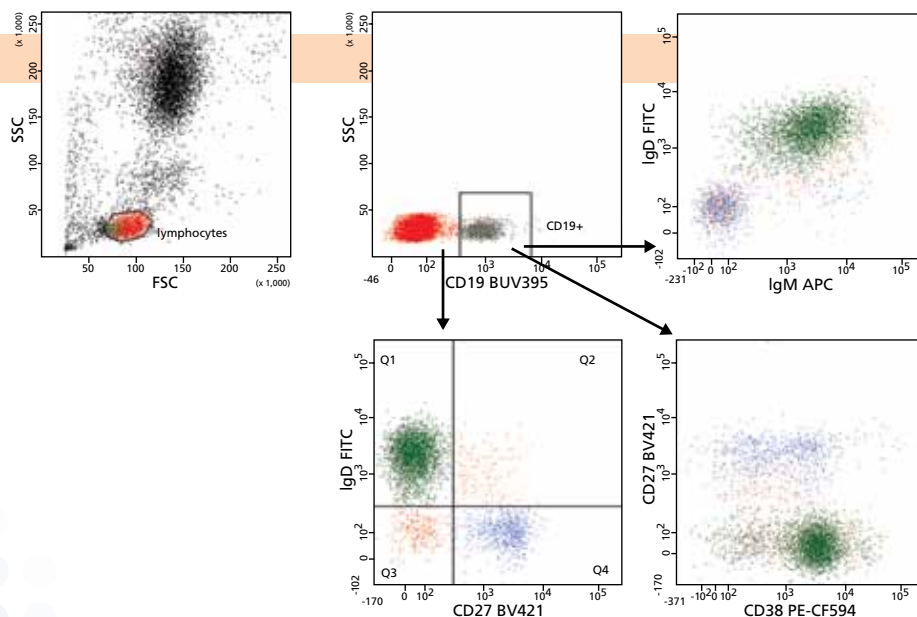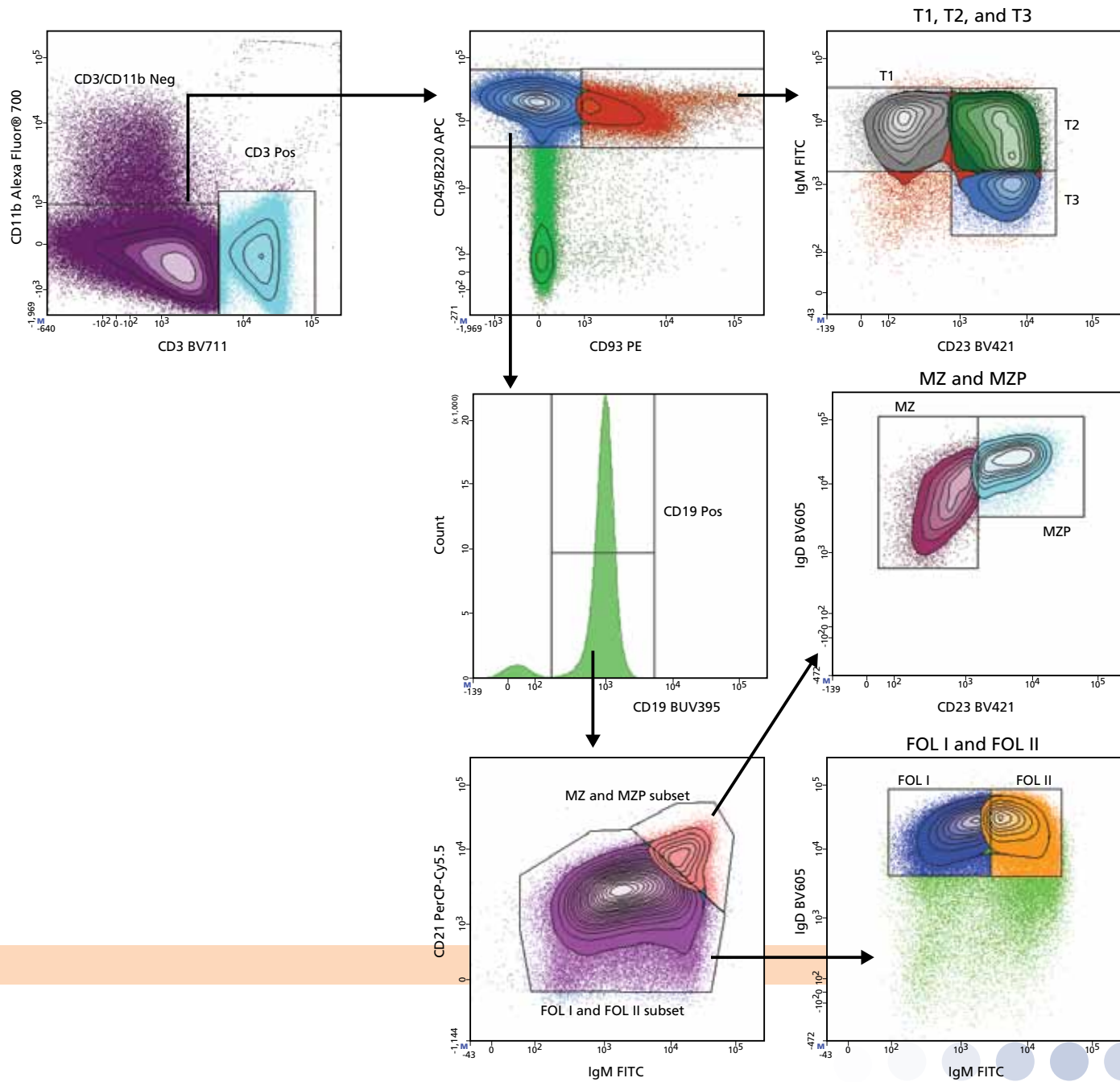
Analysis of Pax-5 expression in mouse splenic B-cell subpopulations

C57BL/6 mouse splenocytes were stained with the following fluorescent antibodies: CD11b Alexa Fluor® 700, IgM FITC, CD19 BUV395, CD45R/B220 APC, CD93 PE, IgD BV605, CD21 PerCP Cy5.5, CD23 BV421, and CD3 BD Horizon Brilliant™ Violet 711 (BV711). Following surface staining, cells were fixed and permeabilized using the BD Pharmingen transcription factor buffer set, intracellularly stained with BD Horizon™ BV510 anti Pax-5, and analyzed using a special order BD LSRFortessa™ X-20 system.
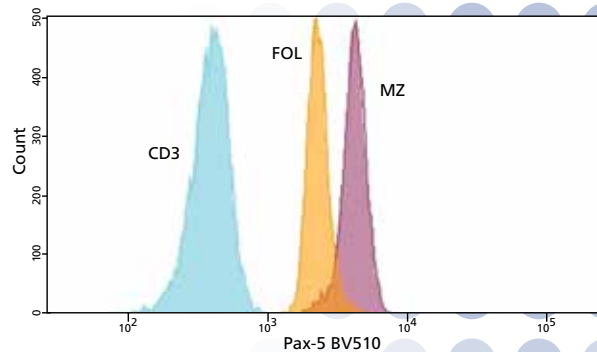
Service and Support

BD Biosciences instruments and reagents are backed by a world-class service and support organization with unmatched flow cytometry experience. Our integrated approach combines flow cytometry instrumentation with trusted, certified reagents, and advanced applications. BD Biosciences tools enable our customers to discover more and obtain the most complete picture of cell function, and at the same time experience improved workflow, ease of use, and optimal performance.

Researchers come to BD Biosciences not only for quality products, but for support as a trusted lab partner. Our repository of in-depth, up-to-date knowledge and experience is available to customers through comprehensive training, application and technical support, and expert field service.

For example, our website, bdbiosciences.com, offers a number of resources to support researchers in doing flow cytometry experiments. The BD FACSelect™ multicolor panel designer and other multicolor flow cytometry tools help when designing multicolor assays, and the BD FACSelect™ buffer compatibility resource helps navigate buffer choices to select the right combination for intracellular and surface marker experiments.

Technical Application Support
BD Biosciences technical application support specialists are available to provide field- or phone-based assistance and advice. Expert in a diverse array of topics, BD technical application specialists are well equipped to address customer needs in both instrument and application support.

References
1. Allman D, Pillai S. Peripheral B cell subsets. *Curr Opin Immunol.* 2008;20:149-157.
2. Shapiro-Shelef M, Calame K. Regulation of plasma-cell development. *Nature Rev Immunol.* 2005;5:230-242.
3. Lund FE. Cytokine-producing B lymphocytes–key regulators of immunity. *Curr Opin Immunol.* 2008;20:332-338.
4. Mauri C, Bosma A. Immune regulatory function of B cells. *Annu Rev Immunol.* 2012;30:221-241.
5. Maecker HT, McCoy JP, Nussenblatt R. Standardizing immunophenotyping for the Human Immunology Project. *Nature Rev Immunol.* 2012;12:191-200.
6. Malaspina A, Moir S, DiPoto AC, et al. CpG oligonucleotides enhance proliferative and effector responses of B cells in HIV-infected individuals. *J Immunol.* 2008;181:1199-1206.
7. Kardava L, Moir S, Wang W, et al. Attenuation of HIV-associated human B cell exhaustion by siRNA downregulation of inhibitory receptors. *J Clin Invest.* 2011;121:2614-2624.

BD Biosciences Regional Offices

Australia
Toll Free 1800 656 100
Tel   61.2.8875.7000
Fax 61.2.8875.7200
bdbiosciences.com/anz

Canada
Tel   866.979.9408
Fax 888.229.9918
bdbiosciences.com/ca

China
Tel   86.21.3210.4610
Fax 86.21.5292.5191
bdbiosciences.com/cn

Europe
Tel   32.2.400.98.95
Fax 32.2.401.70.94
bdbiosciences.com/eu

India
Tel   91.124.2383566
Fax 91.124.2383224/25/26
bdbiosciences.com/in

Japan
Nippon Becton Dickinson
Toll Free 0120.8555.90
Fax 81.24.593.3281
bd.com/jp

Latin America/Caribbean
Tel   55.11.5185.9995
Fax 55.11.5185.9895
bdbiosciences.com/br

New Zealand
Toll Free 0800 572.468
Tel   64.9.574.2468
Fax 64.9.574.2469
bdbiosciences.com/anz

Singapore
Tel   65.6861.0633
Fax 65.6860.1593
bdbiosciences.com/sg

United States
US Orders 855.236.2772
Technical Service 877.232.8995
Fax 800.325.9637
bdbiosciences.com

Office locations are available on our websites.