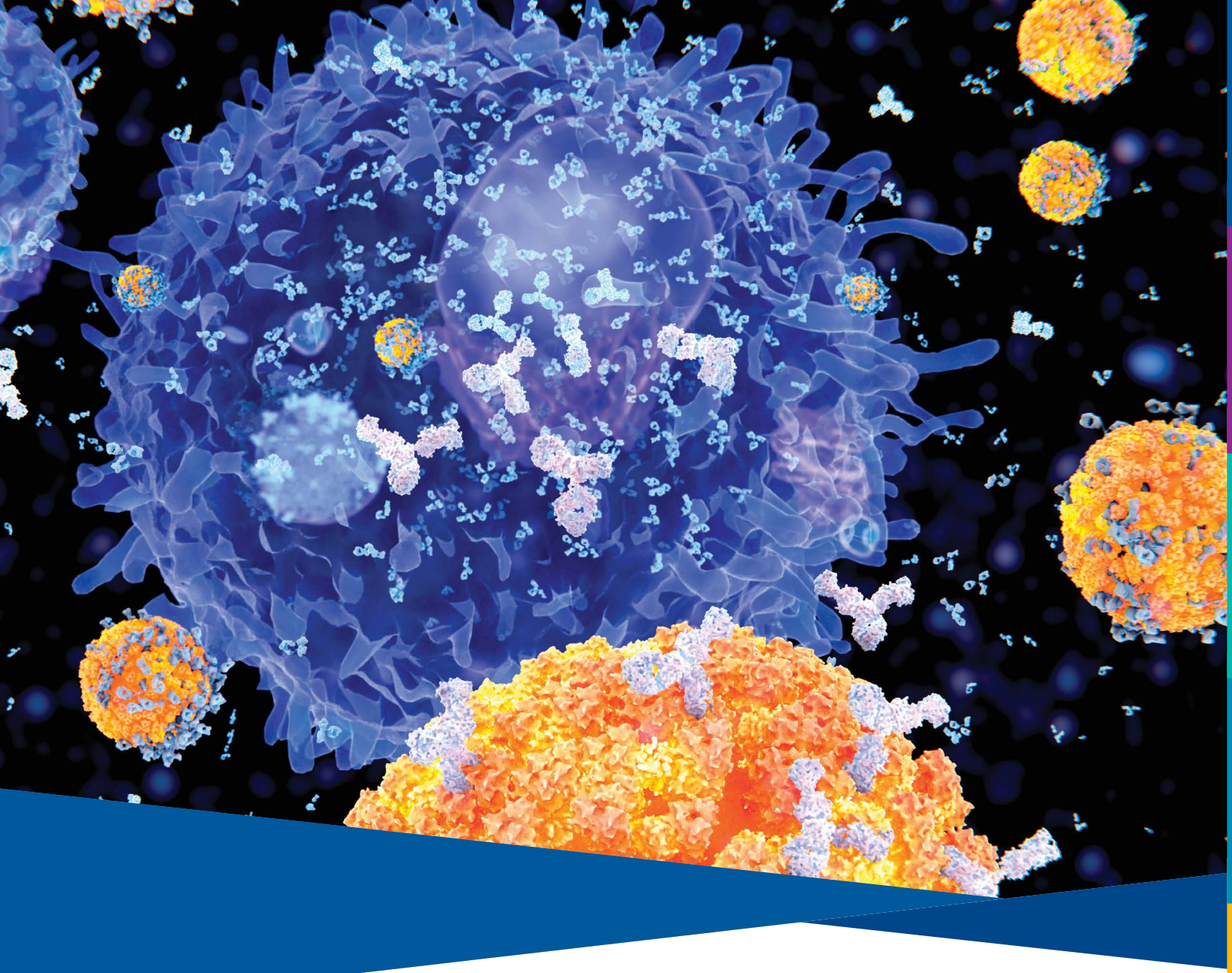


B cell research

Flow cytometry tools for the study of B cell biology

BD

Supporting B cell research

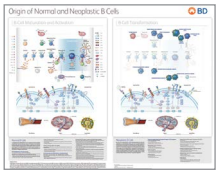
Providing innovative and flexible ways to study B cell phenotypes and functions

B cells are adaptive immune cells that play critical roles in humoral immune responses against infectious pathogens. While T cells govern cell-mediated immune responses, B cells secrete antibodies that may neutralize pathogen-derived antigens. Among other functions, B cells also act as professional antigen-presenting cells for T cells contributing to the generation of long-term immunological memory. B cell biology remains an active area of research since perturbations in B cell development and function may promote illnesses such as autoimmune diseases, immunodeficiency disorders and malignancies. Thus, a better understanding of B cell biology and responses to different diseases can accelerate the development of new therapies and aid in the design of effective vaccines.

Over the past several decades researchers have made significant progress in defining B cell phenotypes and unraveling B cell functions using flow cytometry. Ever a leader in flow cytometry applications, BD Life Sciences offers new solutions in the growing field of multiomics to further support B cell research. BD Life Sciences' main offerings include:

- Antibodies targeting a range of markers from cell surface to transcription factors for a comprehensive analysis of B cell developmental pathways and diverse immune functions.
- Antibodies and buffer solutions optimized for detection of critical intracellular components.
- Novel fluorochromes to simplify panel design and enable the detection of higher number of parameters in a single sample than previously possible.
- Innovative technologies to support the concurrent investigation of proteins and mRNA transcripts in thousands of single cells to accelerate discovery.

Origin of Normal and Neoplastic B Cells



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This brochure illustrates how BD Life Sciences supports the following areas in B cell research:

Cell Surface Marker Analysis to define cell populations in heterogenous samples

Bright Fluorochromes to detect low expression antigens or rare cell populations

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

Multiomic Analysis using BD® AbSeq Antibodies and targeted mRNA workflow to simultaneously interrogate proteins and mRNA transcripts

BD® Cytometric Bead Array (CBA) to analyze secreted cellular products including immunoglobulins and cytokines

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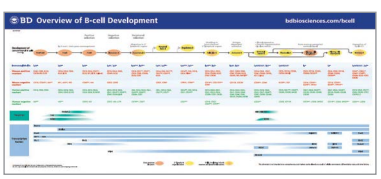
A dynamic area of research

B cell development overview

B cells undergo several 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.

Overview of B Cell Development



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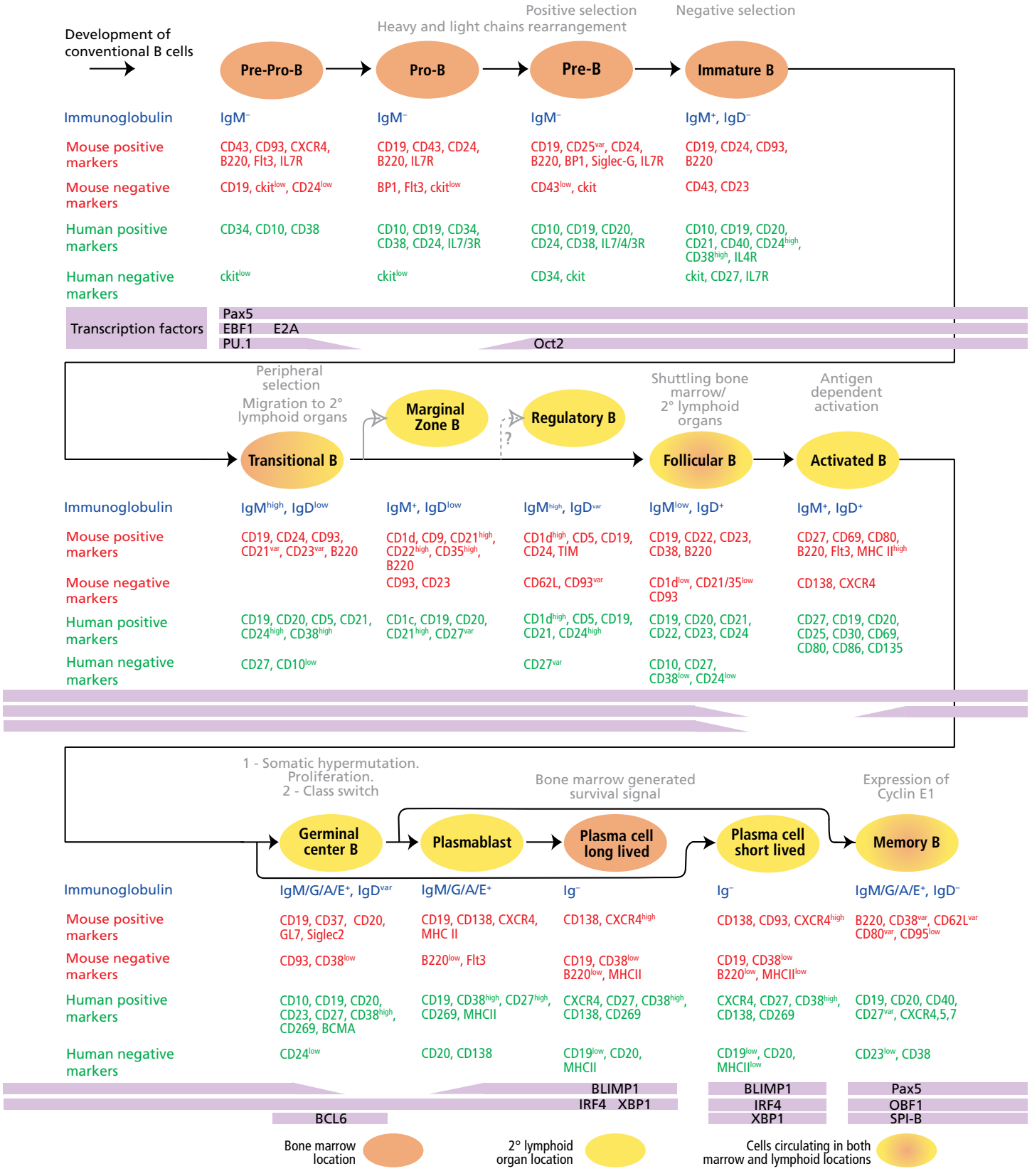


Figure 1. Summary of the key surface and intracellular markers expressed throughout B cell maturation in mice and humans.

B cell hematopoiesis initiates in the fetal liver and continues in the bone marrow during adult life. The immature B cells then migrate to the spleen, where they may undergo further development into mature B cells. Most mature B cells are recirculating cells that home mainly to B cell follicles of secondary lymphoid tissues. These follicular B cells when activated can easily interact with activated T cells and contribute to T cell-dependent immune responses. Such interactions may also induce B cell differentiation into antibody-secreting plasma cells. B cells may also follow alternative differentiation pathways from conventional follicular B cells, resulting in subsets that have distinct functions and marker expression patterns such as marginal zone B cells. Unlike follicular B cells, marginal zone B cells are non-recirculating cells and can rapidly mount antibody responses to both T cell-dependent and T cell-independent antigens. A variety of B cell subsets have been documented as regulatory B cells, which function via IL-10 dependent and independent mechanisms.

Characterization of B cell subsets

BD carries a comprehensive selection of antibodies against mouse and human targets for the routine cell surface and intracellular analyses of B cells by flow cytometry. A growing portfolio of BD®AbSeq Antibody-Oligonucleotides paired with the BD Rhapsody™ Single-Cell Analysis System allows further examination of B cell phenotypes and function through an integrated analysis of proteins and mRNA transcripts.

Earliest B cell precursors in mouse bone marrow

A combination of seven key cell surface markers that are differentially expressed during the earliest B cell developmental stages was used to discriminate cell progenitors and precursors from more mature B cell subsets derived from mouse bone marrow.

Pre-pro-B, Pro-B and Pre-B cells within the CD45R/B220^{low} population were further defined based on the expression of CD43, CD249 (BP-1) and CD24 (Figure 2A and Figure 2B). Immature and recirculating mature B cells could be distinguished based on the different expression profiles of surface IgM and IgD (Figure 2C and Figure 2D). This panel also enabled the detection of IL-7 receptor alpha chain (IL-7Rα or CD127) expression, which is known to be downregulated during B cell maturation and thus recirculating mature B cells expressed the lowest levels of this marker (Figure 2E).

Figure 2A

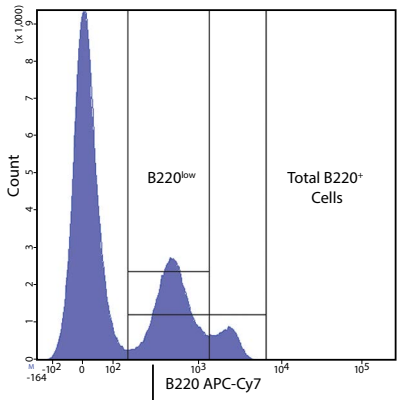


Figure 2B

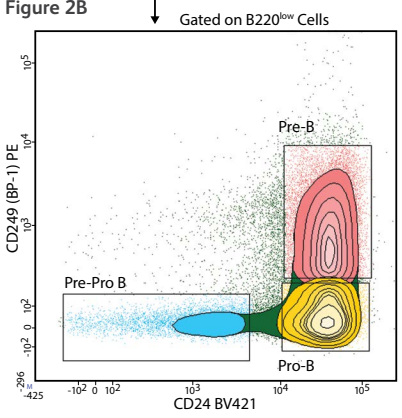


Figure 2C

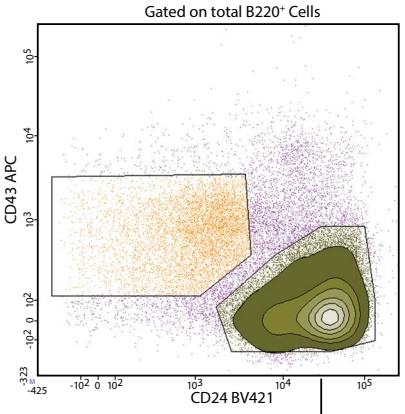


Figure 2D

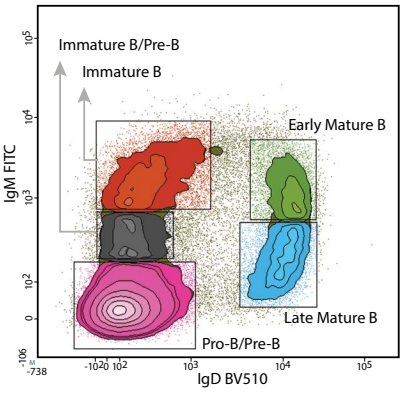


Figure 2E

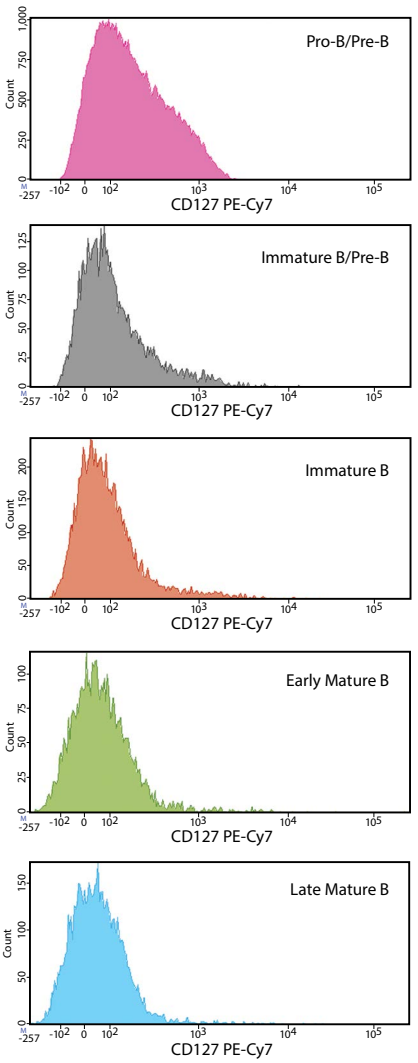


Figure 2. A 7-color panel enables discrimination of five B cell subsets in the bone marrow. C57BL/6 mouse bone marrow cells were stained with the following fluorescent antibodies, IgM FITC, CD43 APC, BP-1 PE, CD127 PE-Cy7, CD45R/B220 APC-Cy7, CD24 BD Horizon Brilliant Violet™ 421 (BV421), and IgD BD Horizon Brilliant Violet™ 510 (BV510) Reagents, and analyzed using a BD FACSCanto™ II Flow Cytometer.

B cell subsets across different mouse tissues

Using a 28-color broad immunophenotyping panel (Table 1) for characterization of various mouse immune cell populations, including B cell subsets, we can monitor the status of B cell maturation across different tissues. As B cells maturate, the IgM levels on their cell surface decrease while IgD expression increases, as shown in Figure 3A. In the spleen, three transitional stages (T1, T2 and T3) are detected before the cells turn into $IgM^{low}IgD^{high}$ follicular mature B cells (Figure 3B).

Table 1. 28-Color mouse broad immunophenotyping panel

Laser	Fluorochrome	Marker
Ultraviolet 355 nm 40 mW	BUV805	CD45R/B220
	BUV737	Ly-6C
	BUV661	CD21
	BUV615	CD314/NKG2D
	BUV563	F4/80
	BUV496	IgD
	BUV395	CD8a
Violet 405 nm 100 mW	BV786	IgM
	BV750	CD25
	BV711	CD27
	BV650	CD23
	BV605	CD192
	BV570	CD4
	BV480	I-A/I-E
	BV421	CD44
Blue 488 nm 100 mW	BB790	NK1.1
	BB755	Ly-6G
	7-AAD	live/dead
	BB660	CD127
	BB630	CD11c
	BB515	CD62L
	SSC	
Yellow-Green 561 nm 150 mW	PE-Cy7	CD43
	PE-Cy5	NKG2A/C/E
	PE-CF594	CD93
	PE	CD5
Red 628 nm 200 mW	APC-H7	CD19
	APC-R700	CD11b
	APC	CD3ε

BB, BD Horizon Brilliant™ Blue; BUV, BD Horizon Brilliant™ UV; BV, BD Horizon Brilliant Violet™

Figure 3A

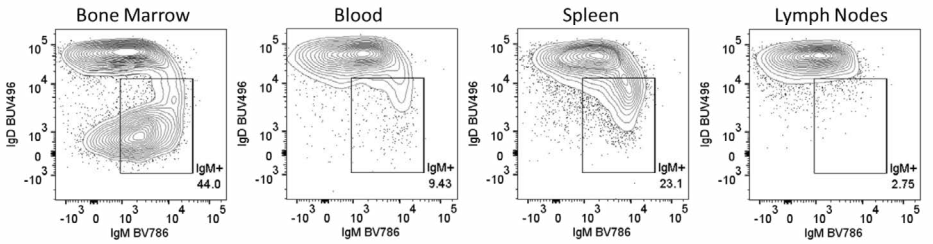


Figure 3B

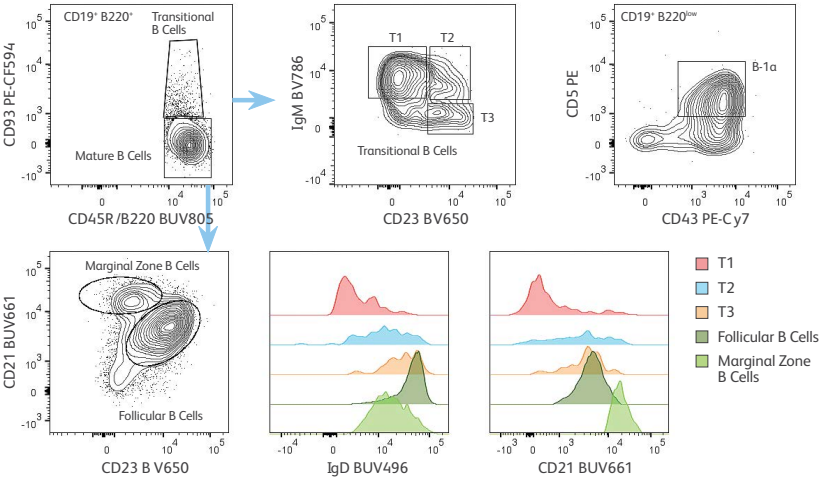


Figure 3. 28-color broad immunophenotyping panel for high-dimensional B cell analysis. C57BL/6 mouse bone marrow, blood, spleen and lymph nodes cells were stained with a cocktail of 27 antibodies (Table 1) in the presence BD Horizon™ Brilliant Stain Buffer Plus and Mouse BD Fc Block™ Reagent. **A)** Analysis of the correlated expression of IgM versus IgD in gated 7-AAD$^-$B220$^+$CD19$^+$ B cells shows B cell populations that express higher levels of IgM (depicted in the gate) that are found in bone marrow, blood and spleen and mature recirculating follicular B cells $IgM^{low}IgD^{high}$ present in all tissues. **B)** Analysis of B cell subsets in spleen. Data were acquired in a FACSymphony™ A5 Cell Analyzer and analyzed using FlowJo™ Software v10.7.1.

Peripheral Mature B Cells In Mouse Spleen

Multiomic applications constitute an in-depth approach for interrogating B cell heterogeneity and complexity at the single-cell level. In this example, six immune cell populations, including follicular and marginal zone B cells, were simultaneously purified from mouse spleen using the BD FACSymphony™ S6 Cell Sorter. Individually tagging the sorted populations with BD® Mouse Immune Single-Cell Multiplexing Antibodies enabled the loading of all cells onto the same cartridge of the BD Rhapsody™ Single-Cell Analysis System. Subsequent analysis of a total of 33 proteins (detected with AbSeq antibodies) and 397 mRNA transcripts revealed a series of components that were differentially expressed in marginal zone B cells compared to follicular B cells (Figure 4).

Figure 4A

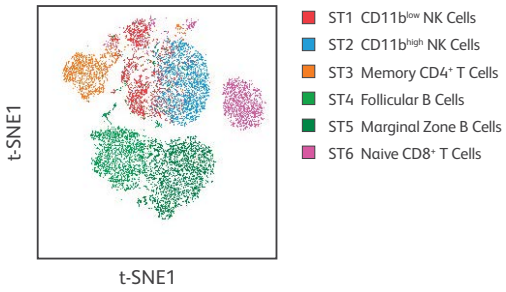


Figure 4B

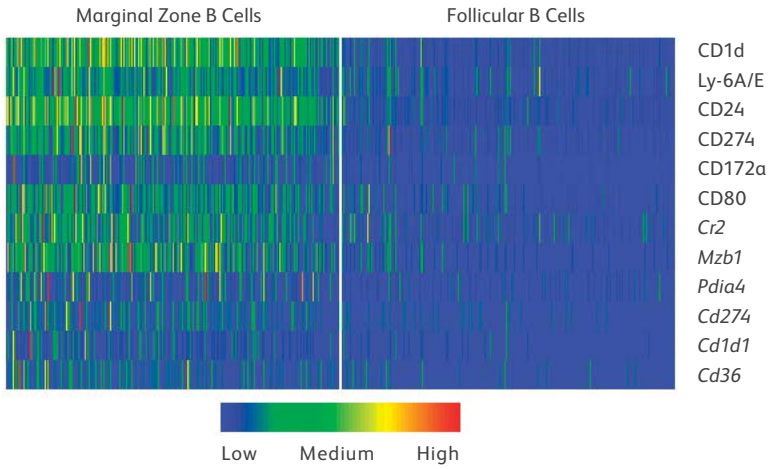


Figure 4. Single-cell multiomics for comparison between distinct B cell subsets

C57BL/6 mouse splenocytes were stained with a 20-color panel of fluorescent antibodies and 33 BD® AbSeq Antibodies (not shown). After simultaneous sorting of six immune cell populations using a BD FACSymphony™ S6 Cell Sorter, each sorted population was individually labeled with multiplexing antibodies (sample tags – ST1 through 6), pooled and loaded onto the BD Rhapsody™ Single-Cell Analysis System for single-cell capture. BD® AbSeq Antibodies, mRNA (BD Rhapsody™ Mouse Immune Response Panel) and sample tag libraries were prepared for sequencing. The sequencing results were analyzed using the BD Rhapsody™ Analysis Pipeline and SeqGeq™ Software v1.6. **A)** Application of t-distributed stochastic neighbor embedding (t-SNE) for identification of the sorted subpopulations using sample tags. **B)** Single-cell heatmap showing representative proteins and mRNA transcripts that are highly expressed in marginal zone B cells compared to follicular B cells.

Human B cell maturation

Human B cell maturation also occurs in a stepwise process that involves upregulation and downregulation of certain cell surface markers. A 12-color panel revealed three major B cell populations in adult bone marrow: $CD38^{high}CD10^{-}$, $CD38^{high}CD10^{high}$ and $CD38^{low}CD10^{-}$. As B cells maturate from $CD10^{high}$ to $CD10^{-}$ mature B cells, they also upregulate the expression of CD20. Analysis of CD10 versus CD20 expression in bivariate plots showed the various stages of B cell maturation from precursors or hematogones to more mature B cells: early-($CD10^{high}$ $CD20^{-}$), intermediate- ($CD10^{+}CD20^{-/low}$) and late-hematogones ($CD10^{+}CD20^{+}$) and mature B cells ($CD10^{-}CD20^{+}$). Notably, t-SNE clustering analysis provided a more robust separation of the cell populations than the bivariate plot analysis, demonstrating the significance of high-dimensional analysis for visualization of rare cell populations (Figure 5A).

In humans, transitional B cells in peripheral tissues are hardly distinguishable from mature B cells based on the expression of cell surface markers. However, mature B cells uniquely express the ATP-binding cassette B1 (ABCB1) transporter, which extrudes drugs, metabolites and other compounds from cells. By staining the cells with MitoTracker™ Green FM (MTG, Thermo Fisher Scientific), a green fluorescent mitochondrial stain, it was possible to distinguish MTG-negative mature B cells from transitional subsets such as T3 cells, which similarly to mature B cells express surface IgD (Figure 5B).

Table 2. B cell immunophenotyping panel

Laser (nm)	Fluorochrome	B Cell Panel
355 Ultraviolet	BUV737	CD138
	BUV395	IgM
405 Violet	BV786	CD20
	BV711	CD10
	BV650	IgG
	BV605	CD27
	BV480	IgD
	BV421	CD38
488 Blue	PE-Cy7	CD19
	PerCP-Cy5.5	CD3 and 7-AAD
	PE	CD24
	MTG*	MTG

*MitoTracker™ Green FM

Figure 5A

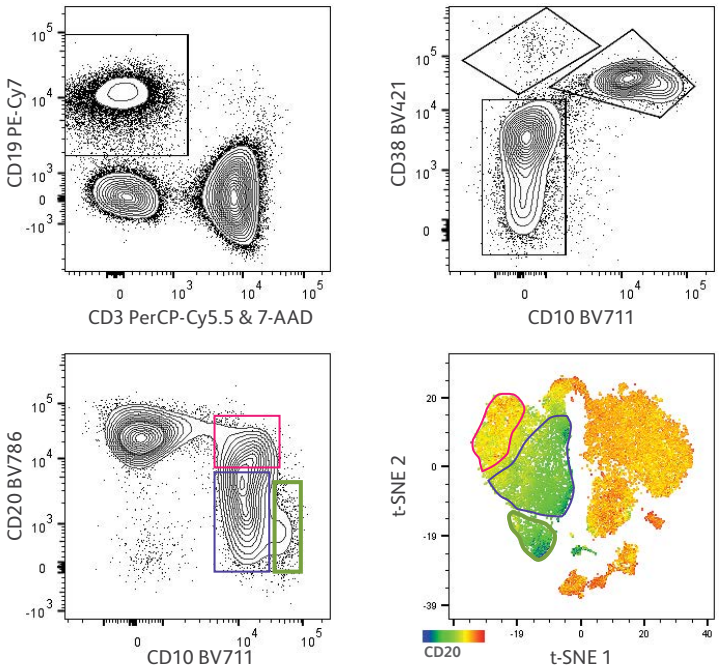


Figure 5B

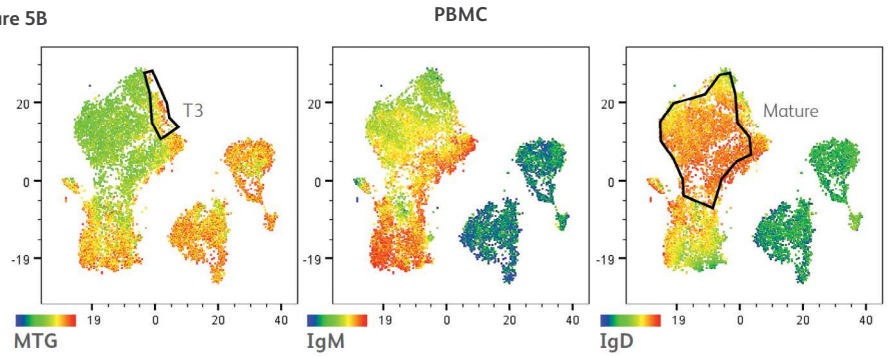


Figure 5. 12-color panel for assessment of B cell subsets in human bone marrow and blood. Frozen bone marrow and peripheral blood mononuclear cells (BMMC and PBMC) were prelabeled with MitoTracker™ Green FM (MTG) and stained with a cocktail of antibodies (Table 2) in the presence of Brilliant Stain Buffer Plus. The cells were acquired in a BD FACSCelesta™ Flow Cytometer and analyzed using FlowJo™ Software v10.7.1. **A)** Analysis of B cell progenitors in bone marrow based on the correlated expression of CD38 and CD10 or CD20 and CD10. Application of t-SNE clustering also distinguishes the three main subsets of progenitors B cells (circled with the indicated colors) from mature re-circulating B cells ($CD20^{high}$). **B)** The t-SNE plots depicts the location of transitional T3 and mature B cells based on the analysis of MTG, IgM and IgD.

Flexibility in panel design

Deeper B cell phenotyping

CD19 is expressed very early in B cell ontogeny and used to identify cells that are committed to the B cell lineage. A majority of human B cells also express CD20 while IgD, CD27, CD38 and CD24 are differentially expressed markers and can be used for analysis of the major B cell subsets of transitional, naïve and memory B cells and plasmablasts (Figure 6).

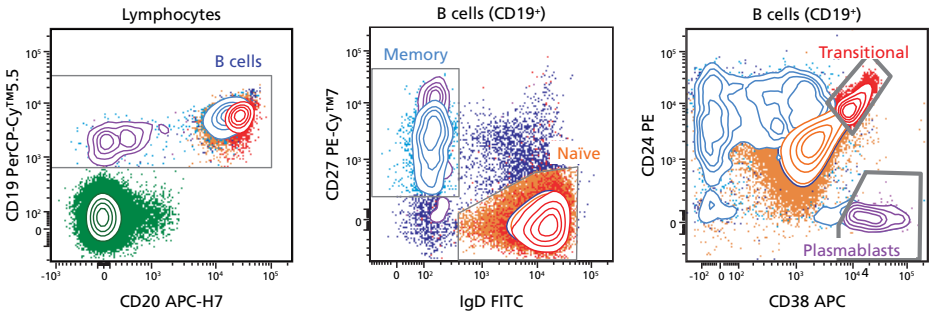


Figure 6. Six-color analysis of human peripheral blood B cells.
PBMCs were stained with the following fluorescent antibodies: CD19 PerCP-Cy5.5, CD20 APC-H7, IgD FITC, CD27 PE-Cy7, CD38 APC and CD24 PE.

As the complexity of flow cytometry panels increases, it can be challenging to define combinations of conjugated antibodies that efficiently allow the best resolution of B cell subpopulations. CD19, CD20, IgD, CD27, CD38 and CD24 are sufficient to identify B-lineage cells in the blood and may constitute a low parameter backbone panel. For deeper B cell phenotyping, this panel was expanded to include IgG, IgM and CD138. The antigen density of each marker was calculated using BD Quantibrite™ Beads. The antigen density information from the BD Quantibrite™ Bead results along with known co-expression profiles aided in the design of the new deeper 10-color panel (Figure 7).

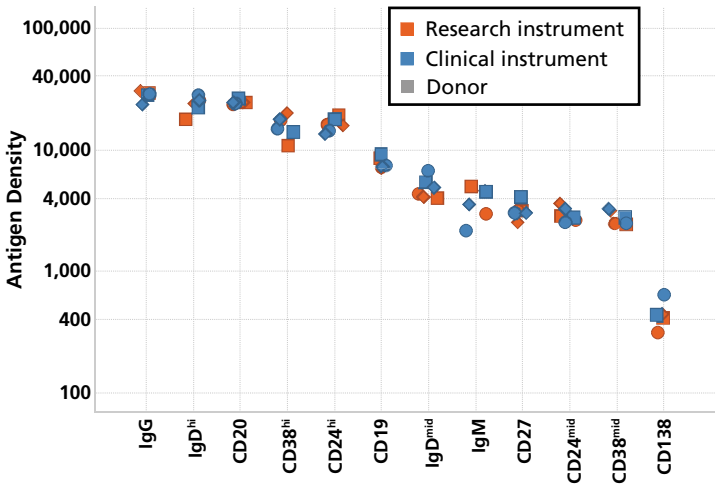


Figure 7. Antigen density of B cell markers on human PBMCs.
The antigen density of B cell markers on human PBMCs from three healthy donors was calculated using BD Quantibrite™ Beads and analyzed in two different flow cytometer instruments.

Following basic panel design principals, PE, a bright fluorochrome, was chosen for the low density antigen CD138. PE-CF594 and APC provided resolution of populations expressing different levels of IgD and CD38, respectively, while the BD Horizon Brilliant Violet™ Reagents were used to obtain optimal detection of CD27 and IgM. This panel also allowed the 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^- $CD20^-$ $CD38^{++}CD138^+$) (Figure 8).

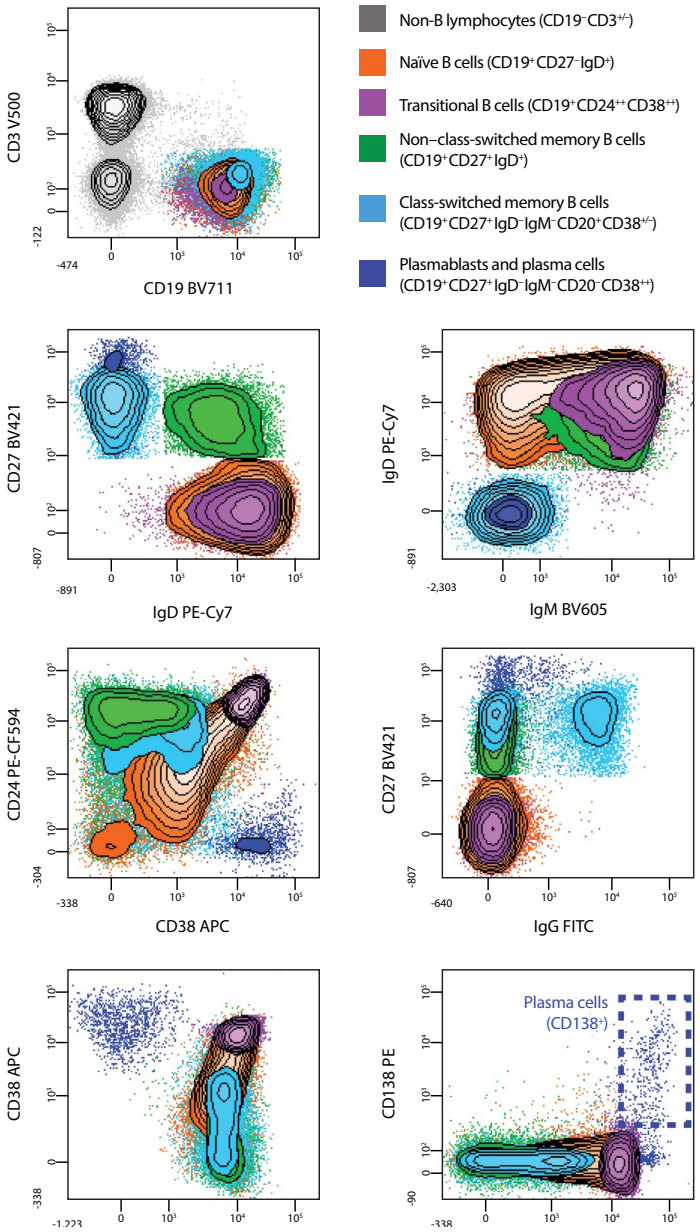


Figure 8. Ten-color analysis of human peripheral blood cells.
Human PBMCs were stained with the following fluorescent antibodies, CD19 BD Horizon Brilliant Violet™ 711 (BV711), CD20 Alexa Fluor™ 700, IgD PE-Cy7, CD27 BV421, CD38 APC, CD24 BD Horizon™ PE-CF594, CD3 BD Horizon™ V500, CD138 PE, IgM BV605, and IgG FITC, and analyzed using a BD LSRFortessa™ Flow Cytometer.

Enabling intracellular staining

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. Advances in buffer systems and methodologies now make it easier to simultaneously measure both surface and intracellular markers, enabling a better understanding of pathways regulating B cell function.

Specialized buffers and antibodies

To facilitate the detection of intracellular markers by flow cytometry, BD has developed specialized buffers and kits that are optimized for detection of different types of intracellular targets, such as phosphorylated proteins or transcription factors.

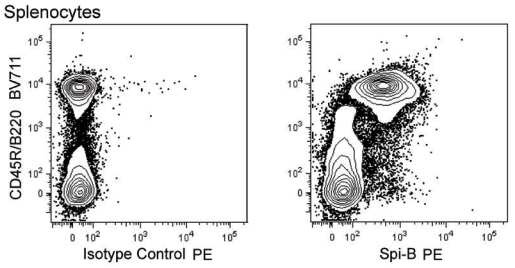
- The BD Pharmingen™ Transcription Factor Buffer permeabilizes cells sufficiently to allow exposure of intranuclear epitopes, while still being gentle enough to allow the detection of most cell surface proteins, and it can also be applied for detection of intracellular cytokines.
- Flow cytometry–validated antibodies enable the analysis of transcription factor networks that coordinate B cell development and differentiation.
- A selection of antibody reagents can be utilized for immunohistochemistry or immunofluorescence to provide visual details about distribution and localization of B cells in tissues.

Detection of key B cell intracellular markers

Bcl-6 and Blimp-1 transcription repressors are crucial for B cell fate decisions. Bcl-6 regulates germinal center formation while Blimp-1 drives B cell differentiation into antibody-secreting plasma cells. Blimp-1 also inhibits the expression of genes required for antibody class switch recombination (CSR) and affinity maturation, including activation-induced cytidine deaminase (AID). On the other hand, Blimp-1 expression can be regulated by Bcl-6, Spi-B and other factors to maintain a balance between CSR and plasma cell differentiation.

Transcription factors involved in class switching

The transcription factor Spi-B was detected in the majority of B cells in mouse spleen and bone marrow (Figure 9). In contrast, Bcl-6 was detected in a restricted IgD^{low}CD95^{+} B cell population, which corresponds to proliferating B cells that have entered germinal centers (Figure 10).



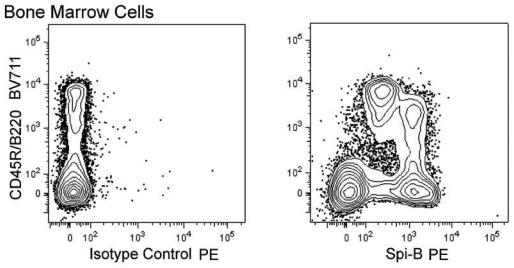


Figure 9. Spi-B expression in mouse leucocytes.
C57BL/6 mouse splenocytes (left panel) and bone marrow cells (right panel) were preincubated with Mouse BD Fc Block™ Reagent and stained with anti-CD45R/B220 BD Horizon Brilliant Violet™ 711 Dye. Then, the cells were fixed and permeabilized using the BD Pharmingen™ Transcription Factor Buffer Set and stained with either PE Rat IgG2a, κ Isotype Control or anti-Spi-B PE or matching isotype control. Flow cytometric analysis was performed using a BD LSRFortessa™ X-20 Flow Cytometer System.

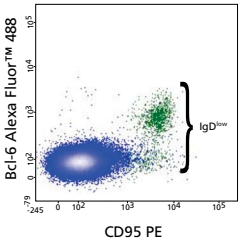


Figure 10. Bcl-6 expression in mouse lymph node cells.
The cells were stained with anti CD45R/B220, CD4, IgD and CD95, fixed and permeabilized using the BD Pharmingen™ Transcription Factor Buffer Set, and stained with anti-Bcl-6 Alexa Fluor™ 488. Flow cytometry was performed using a BD® LSR II System. Bcl-6 expression was observed in germinal center B cells, identified using the CD4^{-} B220^{+}IgD^{low}CD95^{high} phenotype.

Transcription factors involved in plasma cell differentiation

In vitro induction of B cell terminal differentiation with lipopolysaccharide (LPS) resulted in Blimp-1 expression in B220^{low} mouse B cells (Figure 11). Similarly, human PBMCs cultured with cytosine-phosphorothioate-guanine oligodeoxynucleotides (CpG-ODN) differentiated into plasma cells as demonstrated by analysis of spliced XBP-1 expression on CD20^{low} B cells (Figure 12) or Blimp-1 on CD38^{+}CD138^{+} B cells (Figure 13). Detection of CpG-induced signaling events and other signaling pathways can be assessed with BD Phosflow™ Antibodies and buffer systems that are formulated for optimal measurement of phosphorylated epitopes (Figure 14).

Figure 11

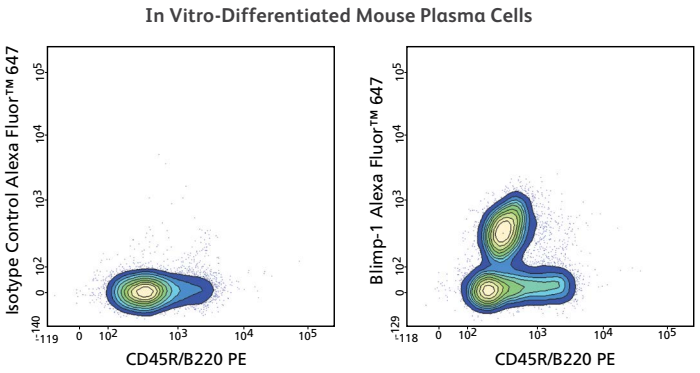


Figure 11. Blimp-1 staining in activated mouse splenocytes.
B6 mouse splenocytes activated with LPS for 3 days were analyzed using the BD Pharmingen™ Transcription Factor Buffer Set, anti-CD45R/B220 PE, and either anti-Blimp-1 Alexa Fluor™ 647 or a matching isotype control. Flow cytometry was performed using a BD FACSCanto™ II System.

Figure 12

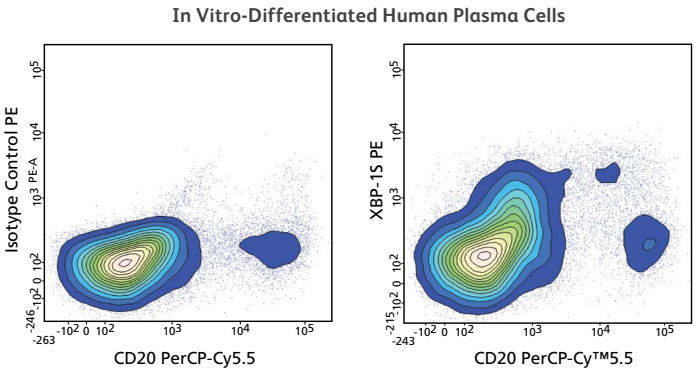


Figure 12. XBP-1s expression in CpG-stimulated human PBMCs.
CpG-stimulated human PBMCs were incubated with BD Horizon™ Fixable Viability Stain 450, fixed and permeabilized using the BD Pharmingen™ Transcription Factor Buffer Set, and stained with anti-CD20 PerCP-Cy5.5 and either anti-XBP-1S PE or a matching isotype control. Flow cytometry was performed using a BD FACSCanto™ II System.

Figure 13

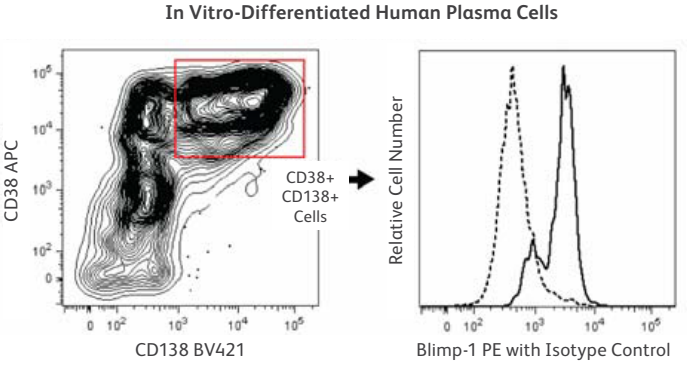


Figure 13. In vitro-differentiated human plasma cells.
CD19^{+}CD27^{+} cells were sorted from PBMCs using the BD IMag™ Human B Lymphocyte Enrichment Set followed by FACS sorting with a BD FACSAria™ III Cell Sorter. Sorted cells were cultured (10 days) per Jourdan M, et al. (2009) for differentiation of plasma cells. Cells were initially stained with BD Horizon™ Fixable Viability Stain 510 (FVS510) for dead cell exclusion. Cells were surface stained with anti-CD38 APC and anti-CD138 BD Horizon™ BV421. Cells were then fixed and permeabilized with the transcription factor buffer set and stained with Blimp-1 PE or matching isotype control.

Figure 14

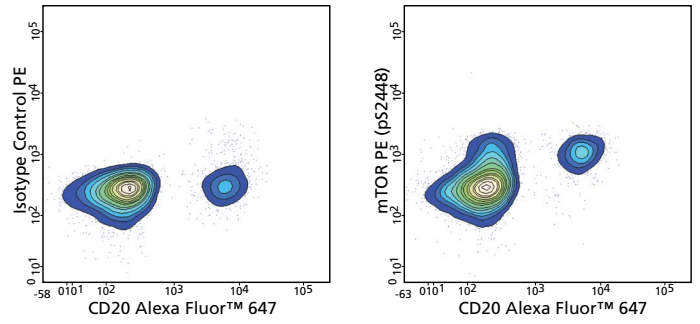
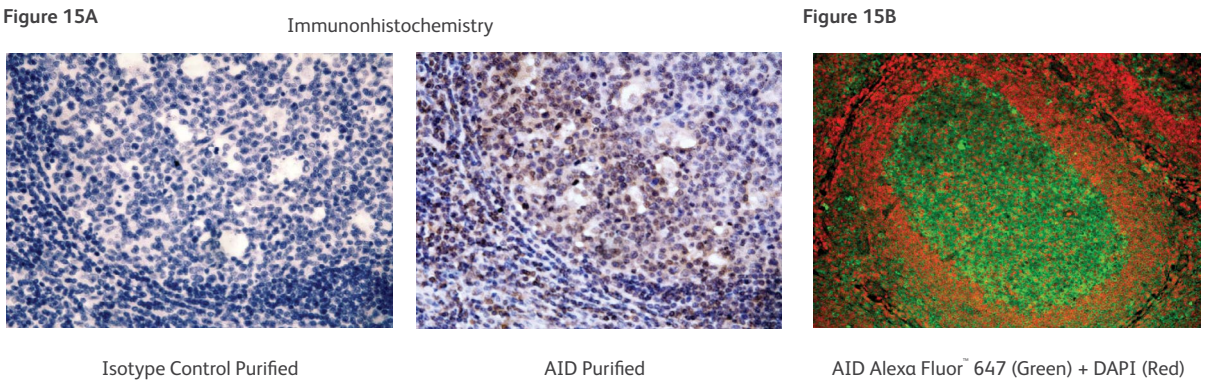


Figure 14. Phospho-mTOR staining of human cells.
Human peripheral B lymphocytes were stimulated with CpG ODN2395, then fixed with BD Cytofix™ Fixation Buffer, permeabilized using BD Phosflow™ Perm Buffer III and stained with anti-mTOR (pS2448) PE or a matching isotype control and anti-CD20 Alexa Fluor™ 647. Cells were analyzed using a BD FACSCanto™ II System.

AID expression in germinal centers

AID expression, CSR and somatic hypermutation are confined largely to germinal center B cells present in the follicles of secondary lymphoid tissues. In this example, AID was detected in the follicles of human tonsil using immunohistochemistry or immunofluorescence and also in a human Burkitt lymphoma cell line by western blot or flow cytometry (Figure 15).



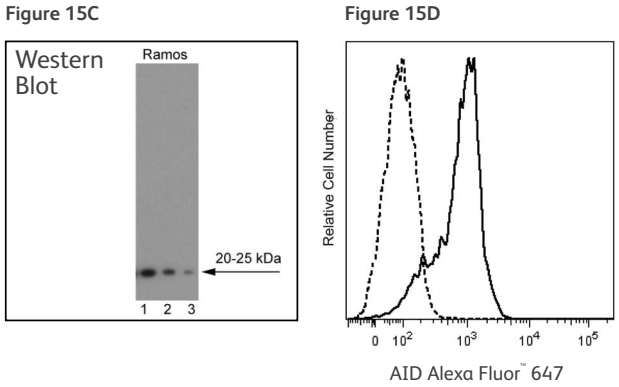


Figure 15. Analysis of AID expression.
A) Following antigen retrieval with BD Pharmingen™ Retrievagen A Buffer, the formalin-fixed paraffin-embedded human tonsil sections were stained with either purified anti-AID or matching isotype control. A three-step staining procedure that employs a biotin goat anti-rat immunoglobulin, streptavidin-horseradish peroxidase (HRP) and a DAB substrate kit was used to develop the primary staining reagents. Original magnification: 40×. **B)** The tonsil sections were blocked with an avidin/biotin blocking kit (Vector Laboratories; as recommended by the manufacturer), stained with anti-AID Alexa Fluor™ 647 (pseudo-colored green) and counterstained with BD Pharmingen™ DAPI Solution (pseudo-colored red). The images were captured on a standard epifluorescence microscope. Original magnification, 20×. **C)** Lysate prepared from Ramos (Burkitt lymphoma, ATCC CRL-1596) cell line was analyzed with 2, 1 and 0.5 µg/mL of purified anti-AID (lanes 1, 2 and 3, respectively). The protein was detected after subsequent labeling with HRP goat anti-rat Ig and detection with ECL western blotting detection reagents. AID was identified in protein bands of ~20–25 kDa. **D)** Ramos cells were preincubated with BD Pharmingen™ Human BD Fc Block™ Reagent, fixed with BD Cytofix™ Fixation Buffer and permeabilized with BD Phosflow™ Perm Buffer III. Cells were stained with either anti-AID Alexa Fluor™ 647 (solid line histogram) or matching isotype control (dashed line histogram). Flow cytometric analysis was performed using a BD LSRFortessa™ X-20 Cell Analyzer System.

From simplified design to information-rich analysis

Enabling multicolor flow

Simplifying Multicolor Setup

With so many fluorochrome options, managing spillover between reagents can be one of the more difficult elements of multicolor panel design. BD Horizon Brilliant™ Ultraviolet and BD Horizon Brilliant Violet™ Reagents aid in distributing markers over multiple lasers, which can decrease spillover and spread. For example, by assigning one marker to each laser line, a 5-color panel with minimal spillover and resulting spread can be run on an instrument equipped with UV (355 nm), violet (405 nm), blue (488 nm), yellow-green (561 nm) and red (640 nm) lasers. This is illustrated by an example of a 5-color panel discriminating naive and memory B cells. For this minimal compensation panel, little panel design was needed, only the fluorochrome assignment to marker expression (Figure 16).

Figure 16

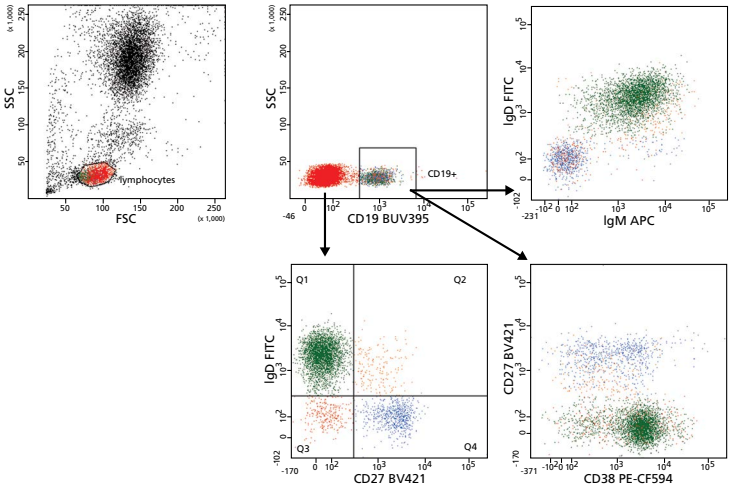


Figure 16. Five-color B cell panel: minimal compensation.
Human PBMCs were stained with the following fluorescent antibodies: CD19 BUV395, CD27 BV421, IgD FITC, CD38 PE-CF594 and IgM APC. Samples were acquired using a 5-laser BD LSRFortessa™ Flow Cytometry System.

The following multicolor panel illustrates the resolution of cell populations when markers are assigned to the first fluorochrome excited by each laser (BUV395, BV421, FITC, PE, APC). Then, additional markers are distributed across the laser lines. This particular example includes the expression of the transcription factor Pax-5 in different B cell subsets (Figure 17).

Figure 17

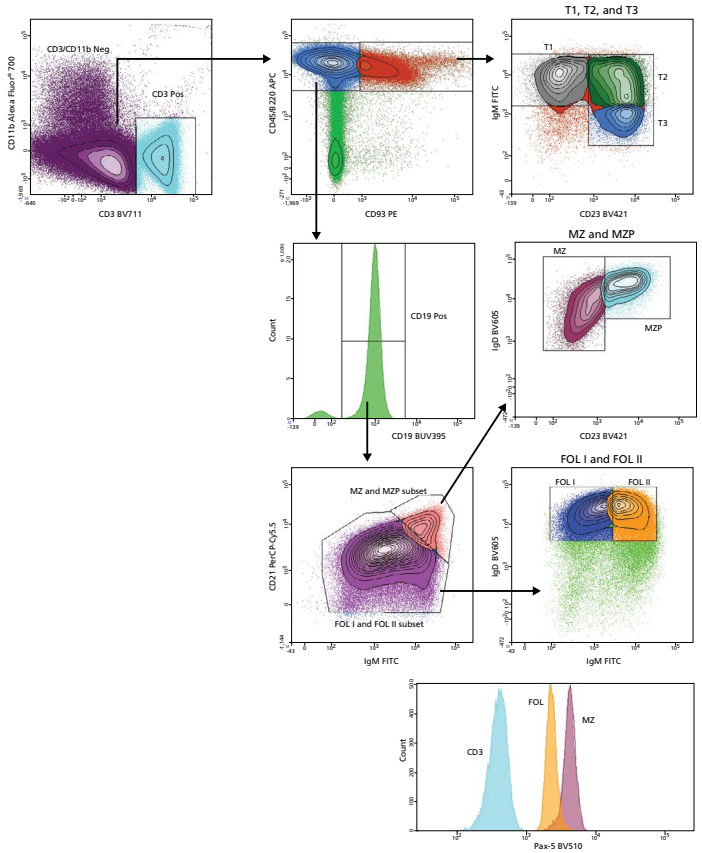


Figure 17. Multicolor panel enabling the analysis of Pax-5 expression in mouse splenic B cell subpopulations.
C57BL/6 mouse splenocytes were stained with the following fluorescent antibodies: CD11b Alexa Fluor™ 700, IgM FITC, CD19 BUV395, CD45R/B220 APC, CD93 PE, IgD BV605, CD21 PerCP-Cy5.5, CD23 BV421, 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 Brilliant Violet™ 510 (BV510) Anti Pax-5 Reagent, and analyzed using a special order BD LSRFortessa™ X-20 System.

Multiple parameters at the single-cell level

Cytokine detection

Flow cytometry is routinely used for cytokine detection as an alternative for ELISA (enzyme-linked immunosorbent assay) and ELISPOT assays. Since it is possible to stain both surface markers and intracellular cytokines on the same cells, this is a powerful technique for analyzing cytokine expression in defined cell populations. Appropriate cell stimulation is usually required for analysis of cytokines as well as the addition of reagents that inhibit protein secretion during the stimulation. BD GolgiPlug™ Protein Transport Inhibitor (containing Brefeldin A) and BD GolgiStop™ Protein Transport Inhibitor (containing Monensin) have different modes of action and can be used alone or in combination for retention of a broad variety of cytokines in the cytosol.

Species	Cytokines	Transport Inhibitor
Human	IL-1α, IL-6, IL-8, TN-α	BD GolgiStop™
Human	IFN-γ, IL-2, IL-10, IL-12, MCP-1, MCP-3, MIG, MIP-1α, RANTES	BD GolgiStop™ or BD GolgiPlug™
Mouse	IL-6, IL-12, TNF-α	BD GolgiPlug™
Mouse	GM-CSF, IL-3, IL-4, IL-5, IL-10	BD GolgiStop™
Mouse	IFN-γ, IL-2	BD GolgiStop™ or BD GolgiPlug™

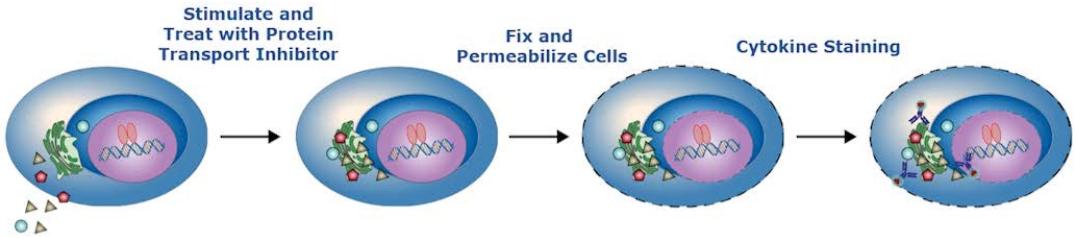


Figure 18. Intracellular staining for analysis of cytokine-producing cells.
In order to accumulate the cytokines within the cells, protein secretion is blocked by treating the cells with protein transport inhibitors during cell stimulation. Then, the cells are fixed and permeabilized (represented by dashed lines) and stained with fluorochrome-labeled antibodies. This figure depicts the staining of surface and intracellular makers after cell fixation and permeabilization. Alternatively, cell surface markers can be stained prior to cell fixation.

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 the immune response—features that are frequently altered in the course of immunization and disease states. Methods that allow multiplexed measurements are increasingly being used to simultaneously measure different types of immunoglobulins or cytokines.

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 compared to a conventional ELISA approach.

The BD® CBA portfolio includes BD® Cytometric Bead Array (CBA) Kits and BD CBA Flex™ Sets for measurement of a variety of soluble and intracellular proteins, including immunoglobulins (Igs), cytokines, chemokines and phosphorylated proteins.

Visit bdbiosciences.com/cba for more information.

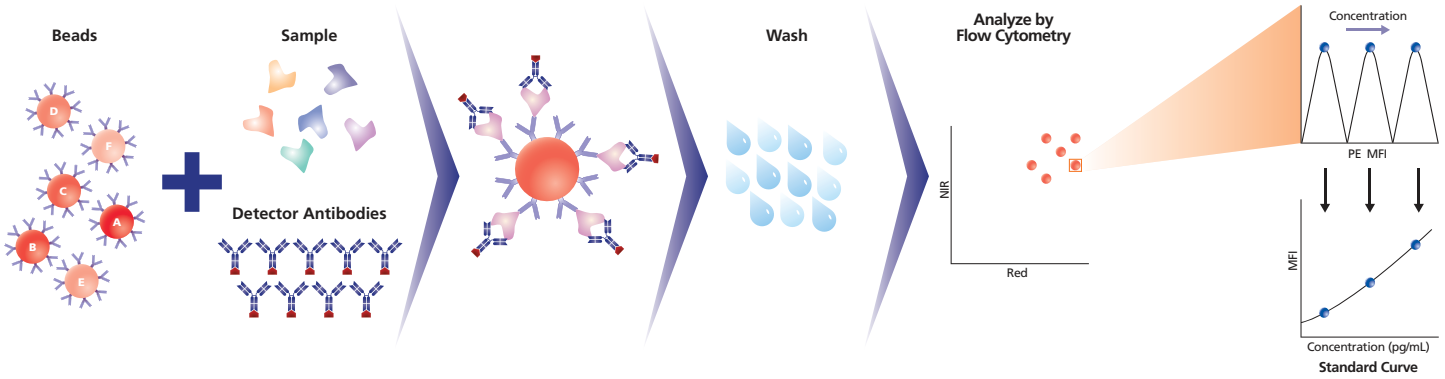
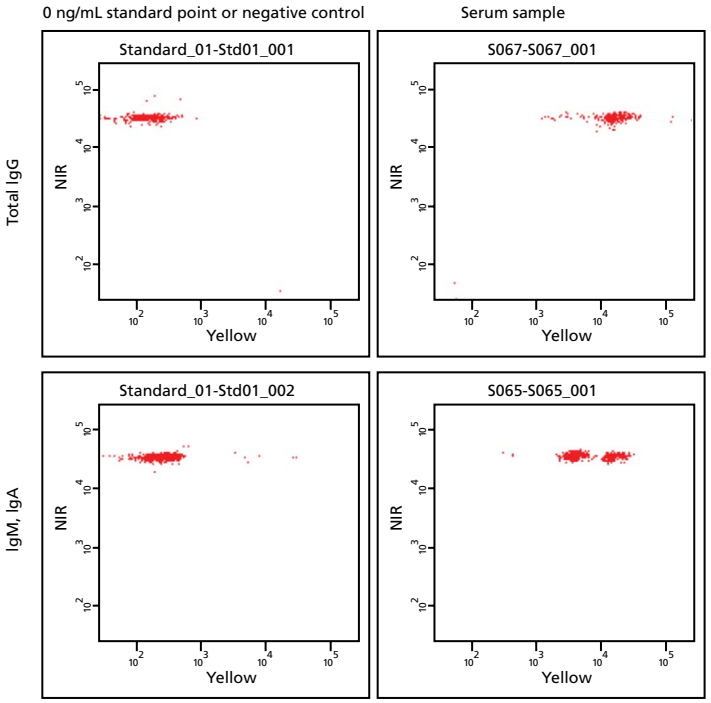


Figure 19. BD® Cytometric Bead Array (CBA) Assay Principle.
Each capture bead in the array has a unique fluorescence intensity and is coated with a capture antibody specific for a single analyte. A combination of different beads is mixed with a sample (25 to 50 µL) or standard and a mixture of detection antibodies that are conjugated to a reporter molecule (PE). Following incubation and subsequent washing, the samples are acquired on a flow cytometer. BD® Cytometric Bead Array (CBA) 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, performs interpolation of sample concentrations compared to the standard curve, and generates an analysis report.

BD® Cytometric Bead Array (CBA) assays for Igs

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

Since the amount and type of Igs 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. The figure below shows quantification of IgM, IgG and IgA in serum of healthy donors (Figure 20).



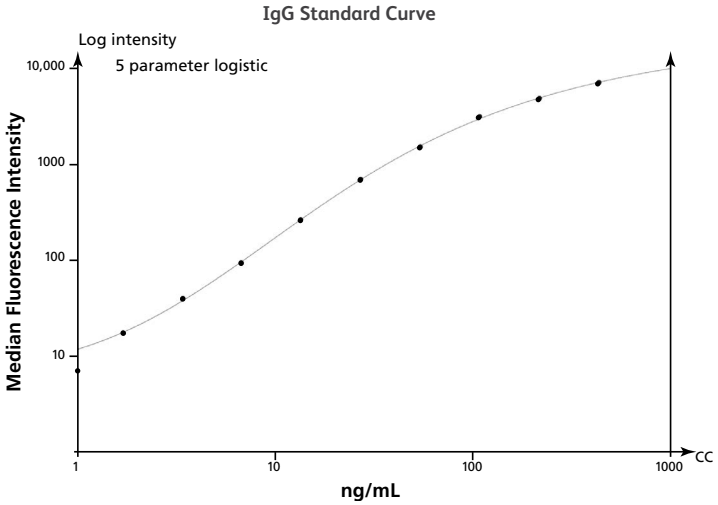


Figure 20. Quantitation of immunoglobulins in human serum samples. Serum from five donors was tested using BD CBA Flex™ Human Immunoglobulin Sets to analyze total IgG, IgM and IgA. Data were acquired on a BD FACSArray™ Flow Cytometer and analyzed using BD® Cytometric Bead Array (CBA) FCAP Array™ Software 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

BD® Cytometric Bead Array (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 are available as a guideline, to facilitate comparisons of cytokine concentration values determined by different laboratories or methods.

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

Table 3. Cytokine-producing B cells.

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.

Technical application support

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

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