

Generation of B Cells from Cord Blood-Derived CD34+ Cells Using StemSpan™ Medium and Supplements

B cells play a central role in adaptive immunity, and generating them in vitro is critical for studying human hematopoietic development, modeling immunodeficiencies, and exploring the origins of B cell-related diseases such as leukemia and lymphoma. Researchers also use B cell differentiation systems to investigate immune reconstitution, test immunotherapies, and examine the molecular pathways that guide lymphoid lineage commitment. Traditionally, generating mature B cells from hematopoietic stem and progenitor cells (HSPCs) has required co-culture with stromal cells or the use of serum-containing media, both of which introduce variability and limit reproducibility.

The [StemSpan™ B Cell Generation Kit](#) addresses these challenges by offering a defined, feeder-free and serum-free culture system that supports the efficient differentiation of cord blood-derived CD34+ HSPCs into immunoglobulin-secreting B cells. The differentiation progresses through defined developmental stages, including pro-B, pre-B, and immature B cells. The result is a controlled, standardized in vitro model that supports functional B cell output for downstream applications in immunology, developmental biology, and disease modeling. The following protocol uses the StemSpan™ B Cell Generation Kit to facilitate the differentiation of cord blood-derived CD34+ cells into B cells in serum-free culture conditions, without the use of a stromal cell line. It also describes an optional protocol extension to further differentiate the generated B cells into antibody-secreting cells (ASCs), including B cells expressing the plasma cell marker CD138.



Why Use StemSpan™ for B Cell Generation?

EFFICIENT. Achieve a high yield and frequency of CD19+ B cells and antibody-secreting cells from CD34+ HSPCs.

CONSISTENT. Eliminate variation introduced by serum and stromal/feeder cell lines by utilizing serum- and feeder-free conditions.

RELEVANT. Obtain functional B cells capable of secreting antibodies.

CONVENIENT. Avoid the extra passaging steps required for stromal or feeder cell-based cultures.

StemSpan™ B Cell Generation Kit (Catalog #100-1250)

Component Name*	Catalog # (Size)
StemSpan™ SFEM II Medium	09605 (2 x 100 mL) **
StemSpan™ B Cell Differentiation Supplement 1 (20X)	100-1251 (2.5 mL)
StemSpan™ B Cell Differentiation Supplement 2 (20X)	100-1252 (2.5 mL)
StemSpan™ B Cell Differentiation Supplement 3 (20X)	100-1253 (0.625 mL)
StemSpan™ B Cell Differentiation Supplement 4 (20X)	100-1254 (0.625 mL)

*Also available for individual sale.

**500 mL format also available (Catalog #09655).

Media and Supplements for the Expansion and B Lineage Differentiation of Human HSPCs

The [StemSpan™ B Cell Generation Kit](#) contains serum-free medium as well as expansion and differentiation supplements required for the generation of B cells and antibody-secreting cells in a three-step protocol. In the first step, CD34+ HSPCs are cultured for 14 days in medium containing the first supplement, to promote their proliferation and differentiation into B lymphoid progenitors. In the second step, B lymphoid progenitors generated during the first 14 days are cultured for another 14 days in medium containing the second supplement, to promote their differentiation into CD19+ B cells. In the final step, the CD19+ B cells are cultured sequentially with the third and fourth supplements for 3 and 4 days, respectively, to promote their further differentiation into immature B cells and antibody-secreting B cells.

On average, this system yields more than 140 CD19+ cells per input CD34+ cell. Both fresh and cryopreserved cord blood-derived CD34+ cells may be used with the StemSpan™ B Cell Generation Kit. If specific developmental subsets of B cells are desired, components can be purchased individually.

Protocol

This protocol describes how to proliferate and differentiate cord blood-derived CD34+ cells into B cells and antibody-secreting cells over 35 days of culture. Figure 1 outlines the steps of the protocol.

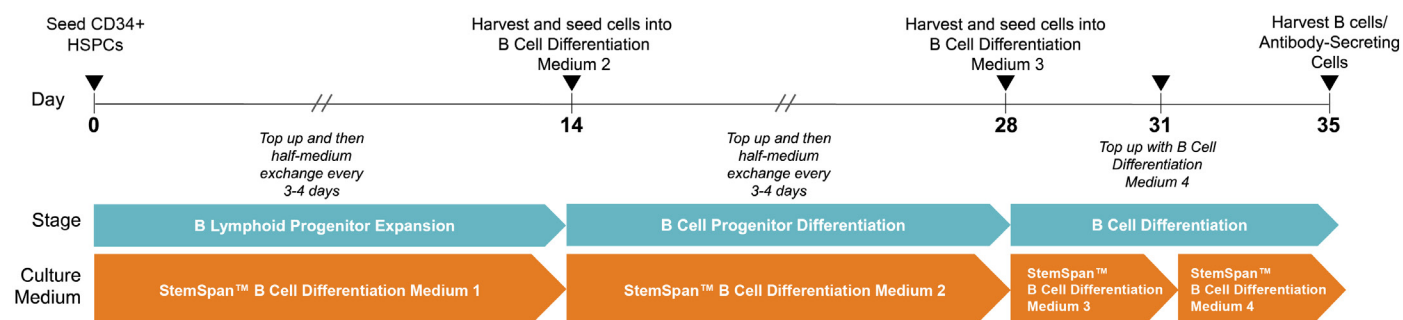


Figure 1. StemSpan™ B Cell Generation Protocol

Cord blood-derived CD34+ HSPCs are seeded on Day 0 in [StemSpan™ SFEM II](#) containing [StemSpan™ B Cell Differentiation Supplement 1](#) and cultured for 14 days followed by culture in [StemSpan™ SFEM II](#) containing [StemSpan™ B Cell Differentiation Supplement 2](#) for an additional 14 days. Medium should be topped up after 3 - 4 days of culture followed by two half-medium changes every 3 - 4 days. On Day 28, cells are harvested and reseeded in [StemSpan™ SFEM II](#) containing [StemSpan™ B Cell Differentiation Supplement 3](#). After 3 days, the medium should be topped up with [StemSpan™ SFEM II](#) containing [StemSpan™ B Cell Differentiation Supplement 4](#) and cultured for 4 more days to complete differentiation into B cells.

Optimal cell yields depend on maintenance of proper cell health, which largely depends on following the recommended schedule of feeding and medium changes.

1. Thaw [frozen cord blood CD34+ cells](#), or isolate CD34+ cells separately from fresh cord blood using the [EasySep™ Human Cord Blood CD34 Positive Selection Kit II](#).
2. Prepare StemSpan™ B Cell Differentiation Medium 1 ([StemSpan™ SFEM II](#) + [StemSpan™ B Cell Differentiation Supplement 1 \[20X\]](#)). Refer to the [product information sheet \(PIS\)](#) for details.
3. Dilute CD34+ cells to 1×10^4 cells/mL in StemSpan™ B Cell Differentiation Medium 1 and seed into a 24-well tissue culture-treated plate.
4. Incubate at 37°C and 5% CO₂ for 14 days, following instructions in the PIS for necessary half-medium changes.
5. On Day 14, harvest cells. They can be used for downstream applications if B lymphoid progenitors are desired (see Figure 2) or reseeded for further differentiation to B cell progenitors.
6. Before reseeding cells, prepare StemSpan™ B Cell Differentiation Medium 2 (StemSpan™ SFEM II + [StemSpan™ B Cell Differentiation Supplement 2 \[20X\]](#)).
7. Harvest and dilute B lymphoid progenitors to 2×10^5 cells/mL in StemSpan™ B Cell Differentiation Medium 2, and seed into a 24-well tissue culture-treated plate.
8. Incubate at 37°C and 5% CO₂ for 14 days, following instructions in the PIS for necessary half-medium changes.
9. On Day 28, harvest cells. They can be used for downstream applications if B cell progenitors are desired (see Figure 3) or reseeded for further differentiation to IgM-expressing B cells and antibody-secreting cells.
10. Before reseeding cells, prepare StemSpan™ B Cell Differentiation Medium 3 (StemSpan™ SFEM II + [StemSpan™ B Cell Differentiation Supplement 3 \[20X\]](#)).
11. Harvest and dilute cells to 5×10^5 cells/mL in StemSpan™ B Cell Differentiation Medium 3. Seed into a 24-well tissue culture-treated plate and incubate at 37°C and 5% CO₂ for 3 days.
12. Before topping up the medium, prepare StemSpan™ B Cell Differentiation Medium 4 (StemSpan™ SFEM II + [StemSpan™ B Cell Differentiation Supplement 4 \[20X\]](#)).
13. On Day 31, top up culture wells with StemSpan™ B Cell Differentiation Medium 4 and incubate at 37°C and 5% CO₂ for 4 days.
14. On Day 35, harvest cells containing CD19+IgM+ B cells and ASCs for analysis (see Figures 4 and 5) or downstream applications.

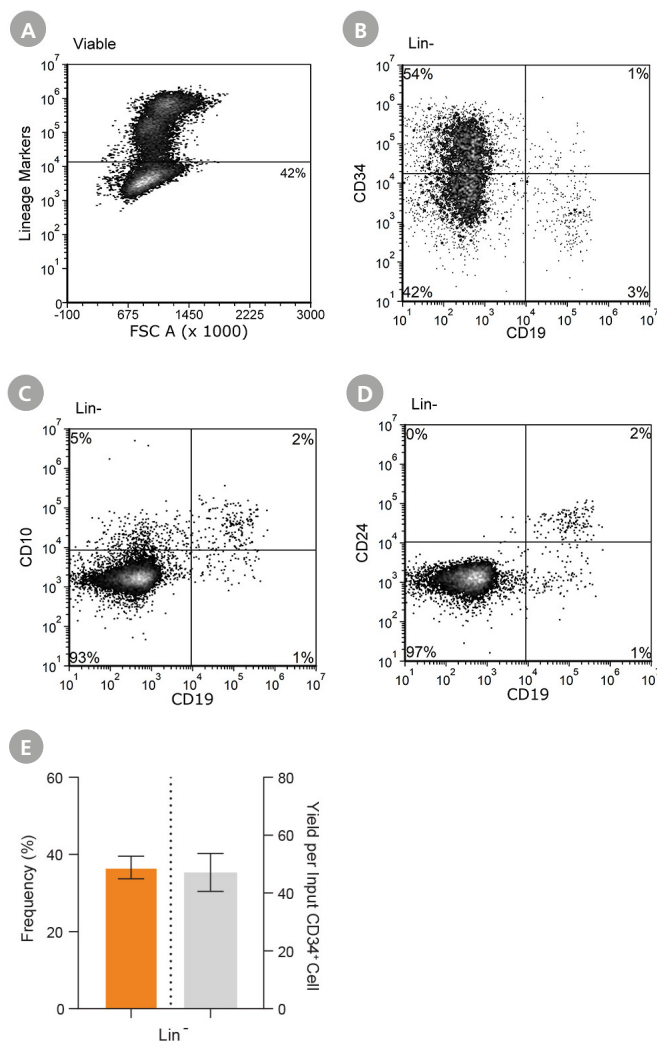


Figure 2. B Lymphoid Progenitors Are Contained Within the Lineage Negative Cell Population After 14 Days of Culture with StemSpan™ Reagents

Cord blood-derived CD34+ cells (freshly isolated or from cryopreserved stock) were cultured in [StemSpan™ SFEM II](#) containing [StemSpan™ B Cell Differentiation Supplement 1](#) for 14 days. (A-D) Cells were harvested and analyzed by flow cytometry for the presence of lineage negative cells (Lin-, to exclude non-B lineage cells) and expression of B lymphoid progenitor markers, including CD34, CD10, CD19, and CD24. A small population of early B cell progenitors (which may contain pro-B and pre-B cells) can be detected on gated Lin- cells. (E) The average frequency of Lin- cells on Day 14 was $36.6 \pm 1.4\%$ with a yield of 47.1 ± 3.2 Lin- cells per input CD34+ cell. The graph shows the mean \pm standard error (n = 36).

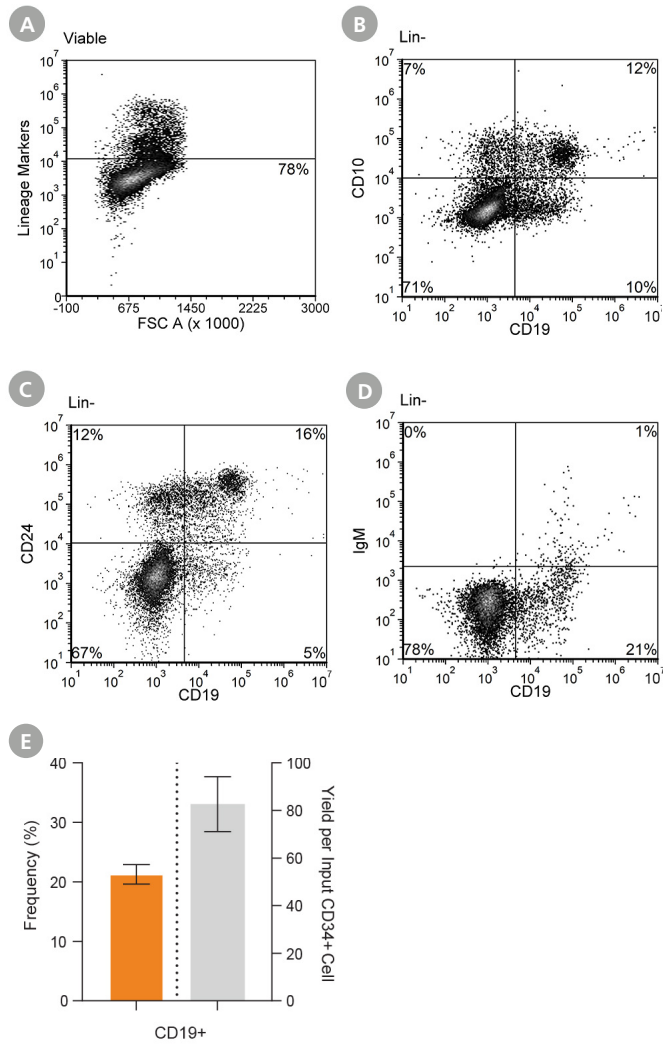


Figure 3. Pre-B Cells and Immature B Cells Are Generated After 28 Days of Culture with StemSpan™ Reagents

On Day 14, B lymphoid progenitors were further differentiated into CD19+ B cells by culturing for an additional 14 days in [StemSpan™ SFEM II](#) containing [StemSpan™ B Cell Differentiation Supplement 2](#). (A-D) Cells were harvested and analyzed by flow cytometry for expression of CD10, CD19, CD20, CD24, and IgM within the Lin- cell population to detect later stage B cell progenitors, containing Pre-B cells and a small population of IgM+ immature B cells. (E) The average frequency of CD19+ cells on Day 28 was 21.3 ± 1.6% with a yield of 82.7 ± 11.5 CD19+ cells per input CD34+ cell. The graph shows the mean +/- standard error (n = 36).

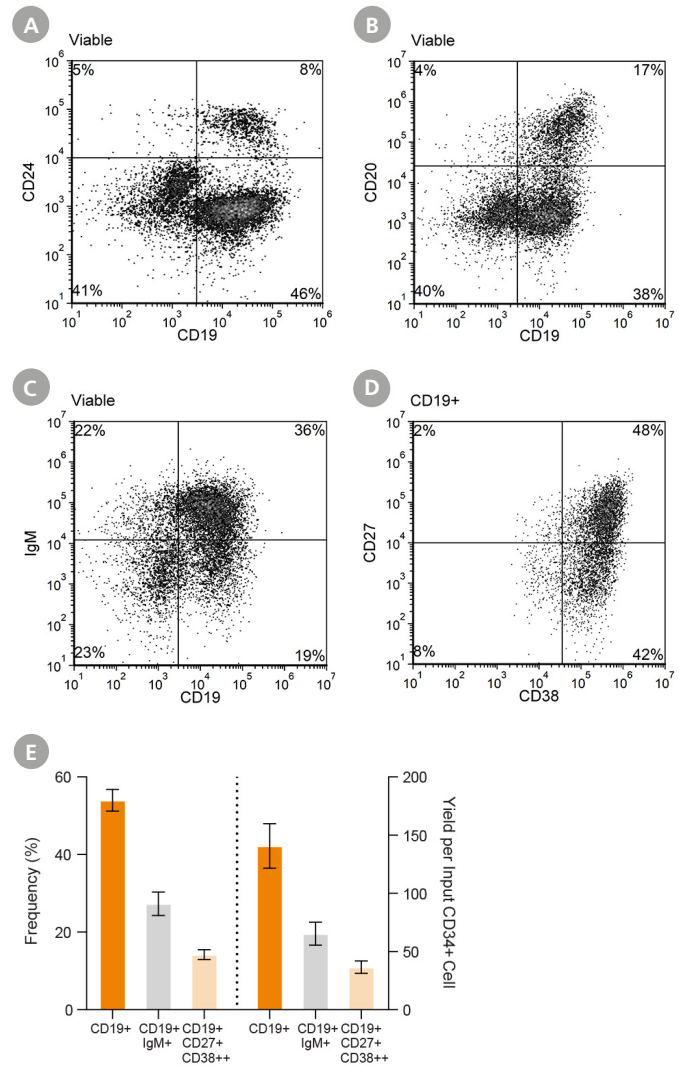


Figure 4. Culturing B Cell Progenitors in StemSpan™ Reagents Results in High Yield and Frequency of CD19+IgM+ and Antibody-Secreting B Cells After 35 Days

On Day 28, CD19+ B cells were further differentiated into CD19+IgM+ and antibody-secreting cells (ASCs) by culturing for an additional 7 days in [StemSpan™ SFEM II](#) containing [StemSpan™ B Cell Differentiation Supplement 3](#) followed by [StemSpan™ SFEM II](#) containing [StemSpan™ B Cell Differentiation Supplement 4](#). (A-D) On Day 35, cells were analyzed by flow cytometry for the expression of CD19, CD20, CD24, CD27, CD38, and IgM. (E) The frequency and yield of CD19+ (Pan B cells), CD19+IgM+ (IgM+ B cells), and CD19+CD27+CD38++ B cells (memory-like B cells and ASCs) are shown. On average, the frequencies were 54.0 ± 2.7%, 27.3% ± 1.3%, and 14.2% ± 1.3%, respectively. The average yield per input CD34+ cell was 140.7 ± 19.1, 65.3 ± 9.9, and 36.5 ± 5.3, respectively. The graph shows the mean +/- standard error (n = 36).

Cell Analysis

After 14 days of culture, B lymphoid progenitors are produced from the cord blood-derived CD34+ cells (Figure 2). These cells further differentiate into B cell progenitors during a second 14-day culture step (Figure 3). In the third step, the B cell progenitors can be further differentiated into CD19+IgM+ B cells and ASCs after 7 more days of culture (Figure 4 and 5). Cells may be stained with antibodies directed against cell surface markers [CD3](#), [CD14](#), CD15, [CD16](#), [CD56](#), [CD66b](#) (a lineage cocktail for Day 14 & 28), CD10, [CD19](#), IgM and CD34 for analysis by flow cytometry. In the representative flow cytometry plots shown, dead cells were excluded by light scatter profile and viability staining. Further analysis of diversity of surface receptors and gene expression profiles can be performed on the cultured cells using V(D)J sequencing (Figure 6) and single cell RNA sequencing (Figure 7).

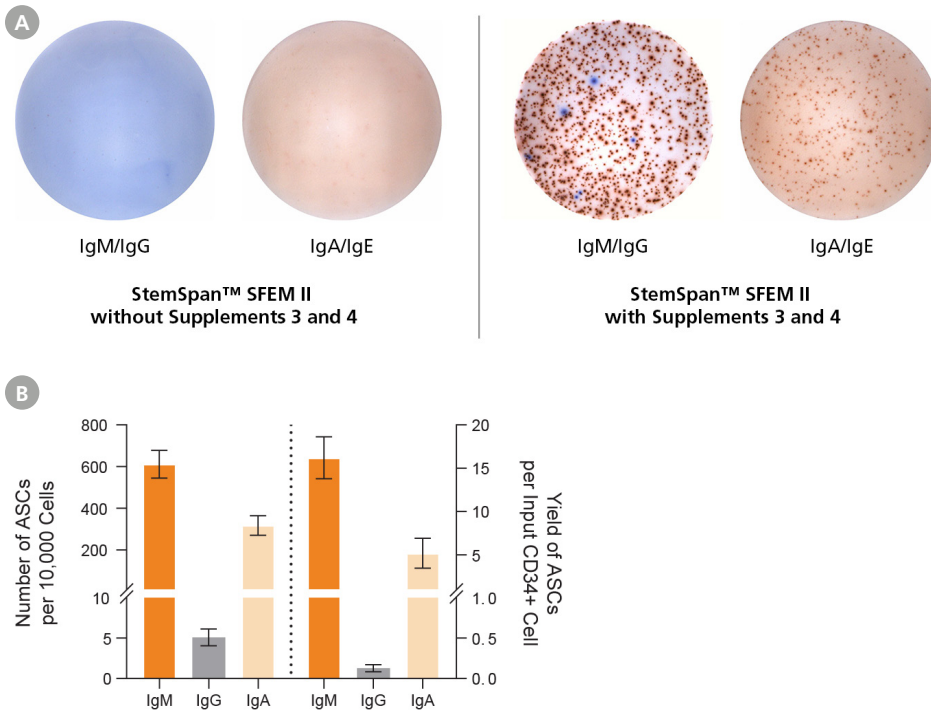


Figure 5. Functional Antibody-Secreting B Cells Are Generated After 35 Days of Culture with StemSpan™ Reagents

On Day 35, the frequencies of immunoglobulin-secreting cells generated from cultured B cells were determined by ELISpot assay. (A) Images of dual ELISpot assays (CTL ImmunoSpot®, IgM/IgG, and IgA/IgE) for detection of IgM (red) and IgG (blue) or IgA (red) and IgE (blue) antibody-secreting B cells. Images on the right depict cells cultured in [StemSpan™ SFEM II](#) with [StemSpan™ B Cell Differentiation Supplement 3](#) and [StemSpan™ B Cell Differentiation Supplement 4](#) added, as described in the culture protocol (Figure 1). Those on the left depict a basal medium control where cells were cultured in StemSpan™ SFEM II without adding StemSpan™ B Cell Differentiation Supplements 3 and 4; 10,000 cells per well were used. IgE ASCs were not detected. (B) The frequency and yield of ASCs are shown. On average, 610 ± 67 , 5 ± 1 , and 317 ± 47 cells per 10,000 cells secrete IgM, IgG, and IgA antibodies with a yield of 16.2 ± 2.4 , 0.1 ± 0.04 , and 5.2 ± 1.7 per input CD34+ cell, respectively. The graph shows the mean \pm the standard error ($n = 8 - 33$).

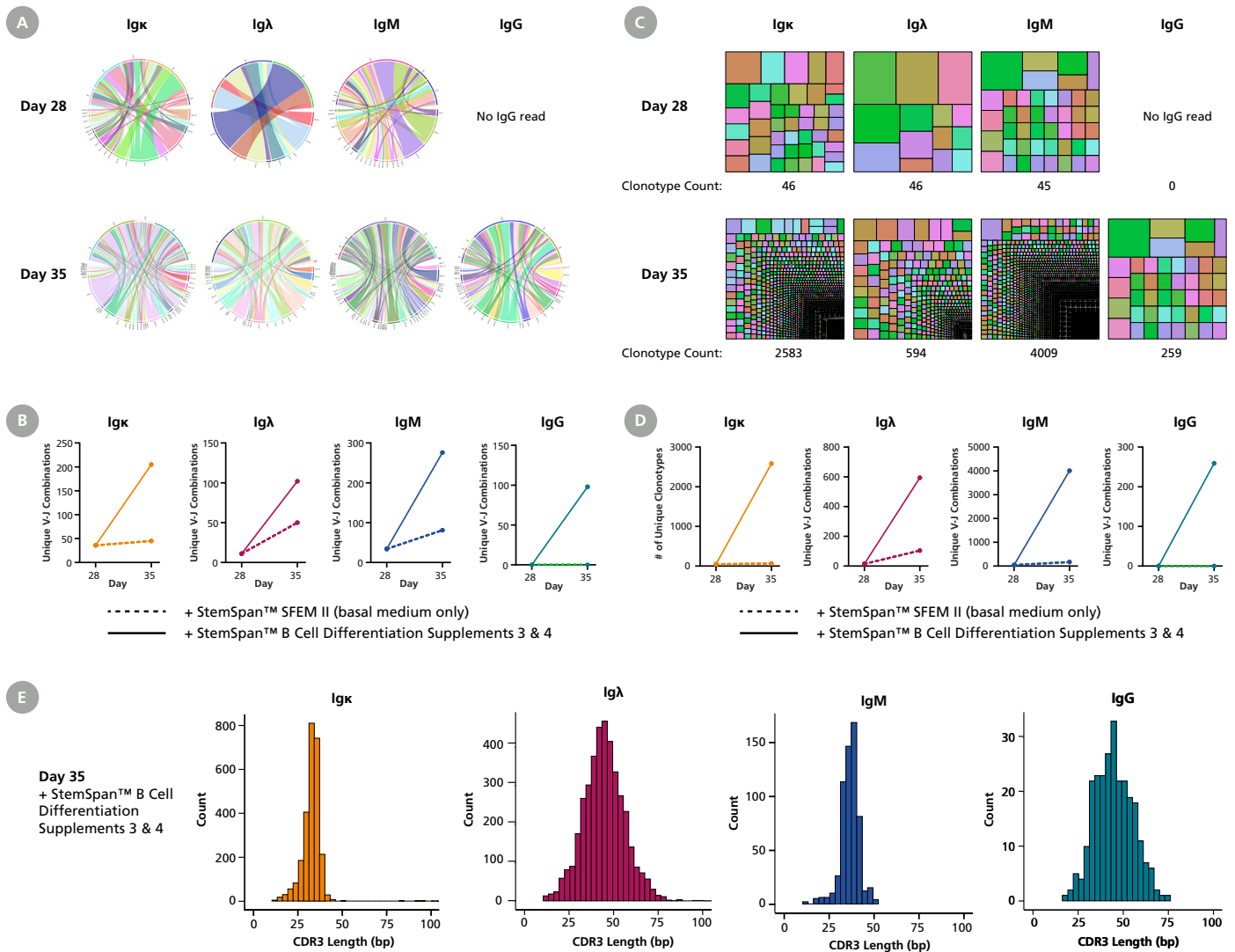


Figure 6. B Cell Receptor Profiling of Cord Blood CD34+ HSPC-Derived B Cells Shows Generation of Diverse V-J Rearrangement and CDR3 Clonotypes

On Day 28 and 35, the B cell receptor profile of the cultured cells was determined via V(D)J sequencing. Analysis of the kappa and lambda immunoglobulin light chains (Igκ and Igλ) and heavy chains (IgM and IgG) was performed before and after culture in [StemSpan™ B Cell Differentiation Supplement 3](#) and [StemSpan™ B Cell Differentiation Supplement 4](#). (A) Representative images of V-J junctional diversity. Each colored band within a circle connecting a V segment (bottom half of circle) to a J segment (top half of circle) represents a unique V-J combination with the width of the band representing the relative frequency. (B) Representation of V-J combination demonstrated that V-J diversity increased in cells cultured in StemSpan™ B Cell Differentiation Supplements 3 and 4 compared to [StemSpan™ SFEM II](#), basal medium control. (C) Representative images of complementary-determining region 3 (CDR3) sequencing show generation of diverse clonotypes before and after culture in StemSpan™ B Cell Differentiation Supplements 3 and 4. Each rectangle represents a unique CDR3 sequence, and the area of the rectangle represents its relative frequency within the culture. (D) Quantification of CDR3 sequence diversity demonstrated that clonotype diversity increased in cells cultured in StemSpan™ B Cell Differentiation Supplements 3 and 4 compared to no supplements. (E) CDR3 length analysis confirmed that cord blood-derived B cells cultured in StemSpan™ B Cell Differentiation Supplements 3 and 4 had normal CDR3 length distribution when harvested at Day 35.

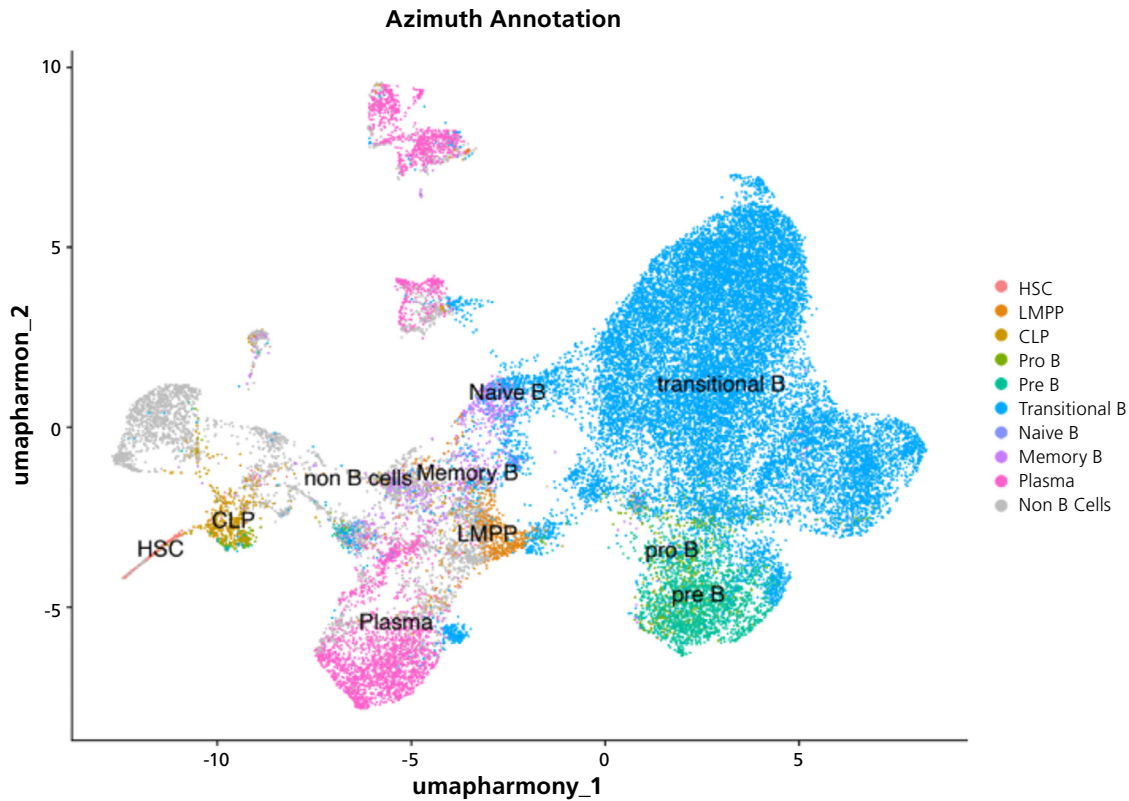


Figure 7. Single Cell RNA Sequencing Analysis of Cord Blood CD34+ HSPC-Derived Cells Shows Generation of Different B Cell Populations

[Cord blood-derived CD34+ cells](#) were cultured with [StemSpan™ B Cell Generation Kit](#) for 35 days. (A) Single cells were harvested and their transcriptome was analyzed using 10x Genomics Cell Ranger v7.0.0. Cell identities were annotated by reference-based mapping to a bone marrow dataset generated from 39 healthy donors, available through the Human BioMolecular Atlas Program Data Portal (HuBMAP) and processed using the Azimuth reference mapping tool. Data represents a compilation of three separate timepoints (Days 28, 31, and 35) and was provided courtesy of the Renée Beekman lab at the Centre for Genomic Regulation (CRG). HSC = hematopoietic stem cell; LMPP = lymphoid-primed multipotent progenitor; CLP = common lymphoid progenitor; Plasma = plasma cell.

Applications of B Cells Derived from Cord Blood CD34+ Cells

Research into the differentiation of B lineage cells from HSPCs

Development of procedures to expand B cells from CD34+ cells in culture

Assessment of efficacy and toxicity of candidate therapeutics on B cell differentiation during drug development research

Development of in vitro models to study diseases that involve B cell development

Optional Protocol Extension for Further Differentiation into CD19+CD138+ Cells

An optional protocol is available to further differentiate the CD19+IgM+ B cells and ASCs into CD19+CD138+ cells. This extended protocol requires [StemSpan™ SFEM II](#) supplemented with [ImmunoCult™-ACF Human B Cell Expansion Supplement](#).

1. Prepare complete Human B Cell Expansion Medium ([StemSpan™ SFEM II](#) + [ImmunoCult™-ACF Human B Cell Expansion Supplement](#)). Refer to [PIS](#) for details.
2. Dilute cells to 1×10^6 cells/mL in Human B Cell Expansion Medium, seed into a 24-well tissue culture-treated plate, and incubate at 37°C and 5% CO₂.
3. After 3 - 4 days of culture, top up with Human B Cell Expansion Medium.
4. Incubate at 37°C and 5% CO₂, and harvest cells on Day 42 (see Figure 8).

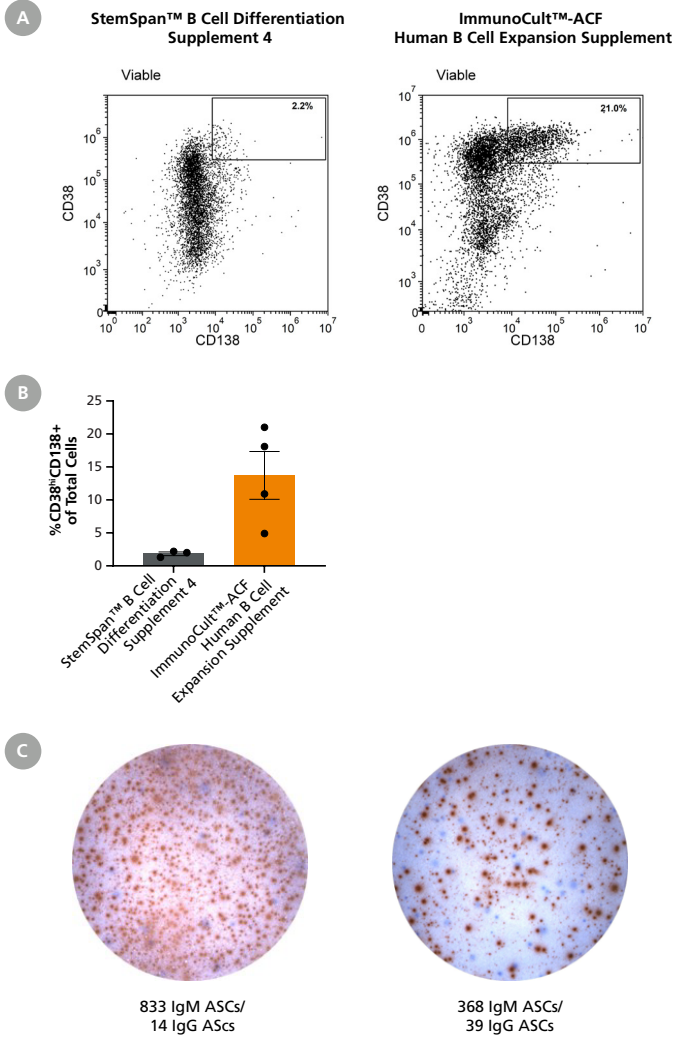


Figure 8. StemSpan™ B Cell Generation Kit-Derived B Cells Cultured for an Additional Week with ImmunoCult™-ACF Human B Cell Expansion Supplement Generated CD19+CD138+ Cells

On Day 35, B cells generated with [StemSpan™ B Cell Generation Kit](#) were cultured for an additional week in [StemSpan™ SFEM II](#) supplemented with [StemSpan™ B Cell Differentiation Supplement 4](#) or [ImmunoCult™-ACF Human B Cell Expansion Supplement](#). (A) Representative flow cytometry plots show the generation of CD38^{high}CD138⁺ cells, suggesting the presence of plasma cells when cultured in ImmunoCult™-ACF Human B Cell Expansion Supplement. (B) CD38^{high}CD138⁺ cells were detected at a frequency of 12.7 ± 3.1% (mean ± standard error, n = 3 - 4). (C) Images of dual ELISpot assays (CTL ImmunoSpot®, IgM/IgG) for detection of IgM (red) and IgG (blue) antibody-secreting B cells. The image on the left depicts cells supplemented with StemSpan™ B Cell Differentiation Supplement 4 during the additional week of culture, while the image on the right depicts cells supplemented with ImmunoCult™-ACF Human B Cell Expansion Supplement. 10,000 cells per well were used.

From sourcing your starting sample to downstream B cell analysis, explore our complete portfolio of products to support your [human B cell research workflow](#).

Suggested Antibodies*

PRODUCT NAME	Catalog #
Anti-Human CD3 Antibody, Clone UCHT1	60011
Anti-Human CD14 Antibody, Clone M5E2	60004
Anti-Human CD16 Antibody, Clone 3G8	60041
Anti-Human CD56 Antibody, Clone HCD56	60021
Anti-Human CD66b Antibody, Clone G10F5	60086
Anti-Human CD19 Antibody, Clone HIB19	60005

*Not included in the kit.

Accessory Products*

PRODUCT NAME	Catalog #
Human Cord Blood CD34+ Cells, Frozen**	70008
EasySep™ Human Cord Blood CD34 Positive Selection Kit II	17896
ImmunoCult™-ACF Human B Cell Expansion Supplement	10974

*Not included in the kit.

**Available in select regions; contact your sales representative for further information.