StemSpan™ B Cell Generation Kit

Serum-free culture kit for expansion and differentiation of human cord blood-derived CD34+ hematopoietic stem and progenitor cells to B cells

Catalog #100-1250 1 Kit

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Product Description

StemSpan™ B Cell Generation Kit has been developed to differentiate CD34+ hematopoietic stem and progenitor cells (HSPCs) isolated from fresh and frozen cord blood (CB) to B cells.

Product Information

The following components are sold as a complete kit (Catalog #100-1250) and are also available for individual sale.

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE
StemSpan™ B Cell Differentiation Supplement 1 (20X)	100-1251	2.5 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
StemSpan™ B Cell Differentiation Supplement 2 (20X)	100-1252	2.5 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
StemSpan™ B Cell Differentiation Supplement 3 (20X)	100-1253	0.625 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
StemSpan™ B Cell Differentiation Supplement 4 (20X)	100-1254	0.625 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
StemSpan™ SFEM II†*	09605	2 x 100 mL	Store at -20°C.	Stable until expiry date (EXP) on label.

† This product contains material derived from human plasma. Donors have been tested and found negative for hepatitis B surface antigen (HBsAg) and HIV-1 antibodies and/or HIV-1 antigen. However, this product should be considered potentially infectious and treated in accordance with universal handling precautions.

* StemSpan™ SFEM II is also available for individual sale in 500 mL format (Catalog #09655).

Preparation of Media

A. StemSpan™ B Cell Differentiation Medium 1

Use sterile technique to prepare StemSpan™ B Cell Differentiation Medium 1 (StemSpan™ SFEM II + StemSpan™ B Cell Differentiation Supplement 1 [20X]). The following example is for preparing 10 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw StemSpan™ SFEM II at room temperature (15 - 25°C) or at 2 - 8°C overnight. Mix thoroughly.

   NOTE: Once thawed, use immediately or store at 2 - 8°C for up to 1 month. Alternatively, aliquot and store at -20°C. Do not exceed the shelf life of the medium. After thawing the aliquots, use immediately. Do not re-freeze.
2. Thaw StemSpan™ B Cell Differentiation Supplement 1 (20X) at room temperature. Mix thoroughly.

   NOTE: Once thawed, use immediately or store at 2 - 8°C for up to 2 weeks. Alternatively, aliquot and store at -20°C. Do not exceed the shelf life of the medium. After thawing the aliquots, use immediately. Do not re-freeze.
3. Add 0.5 mL of StemSpan™ B Cell Differentiation Supplement 1 (20X) to 9.5 mL of StemSpan™ SFEM II. Mix thoroughly.

   NOTE: If not used immediately, store complete StemSpan™ B Cell Differentiation Medium 1 at 2 - 8°C for up to 2 weeks. Do not freeze.

B. StemSpan™ B Cell Differentiation Medium 2

Use sterile technique to prepare StemSpan™ B Cell Differentiation Medium 2 (StemSpan™ SFEM II + StemSpan™ B Cell Differentiation Supplement 2 [20X]). The following example is for preparing 10 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw StemSpan™ SFEM II at room temperature or at 2 - 8°C overnight. Mix thoroughly.

   NOTE: Once thawed, use immediately or store at 2 - 8°C for up to 1 month. Alternatively, aliquot into tubes and store at -20°C. Do not exceed the shelf life of the medium. After thawing the aliquots, use immediately. Do not re-freeze.
2. Thaw StemSpan™ B Cell Differentiation Supplement 2 (20X) at room temperature. Mix thoroughly.

   NOTE: If not used immediately, store at 2 - 8°C for up to 2 weeks. Alternatively, aliquot into tubes and store at -20°C. Do not exceed the shelf life of the medium. After thawing the aliquots, use immediately. Do not re-freeze.

3. Add 0.5 mL of StemSpan™ B Cell Differentiation Supplement 2 (20X) to 9.5 mL of StemSpan™ SFEM II. Mix thoroughly.

   NOTE: If not used immediately, store complete StemSpan™ B Cell Differentiation Medium 2 at 2 - 8°C for up to 2 weeks. Do not freeze.

C. StemSpan™ B Cell Differentiation Medium 3

Use sterile technique to prepare StemSpan™ B Cell Differentiation Medium 3 (StemSpan™ SFEM II + StemSpan™ B Cell Differentiation Supplement 3 [20X]). The following example is for preparing 2.5 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw StemSpan™ SFEM II at room temperature or overnight at 2 - 8°C. Mix thoroughly.

   NOTE: Once thawed, use immediately or store at 2 - 8°C for up to 1 month. Alternatively, aliquot and store at -20°C. Do not exceed the shelf life of the medium. After thawing the aliquots, use immediately. Do not re-freeze.

2. Thaw StemSpan™ B Cell Differentiation Supplement 3 (20X) at room temperature. Mix thoroughly.

   NOTE: Once thawed, use immediately or store at 2 - 8°C for up to 2 weeks. Alternatively, aliquot and store at -20°C. Do not exceed the shelf life of the medium. After thawing the aliquots, use immediately. Do not re-freeze.

3. Add 0.125 mL of StemSpan™ B Cell Differentiation Supplement 3 (20X) to 2.375 mL of StemSpan™ SFEM II. Mix thoroughly.

   NOTE: If not used immediately, store complete StemSpan™ B Cell Differentiation Medium 3 at 2 - 8°C for up to 2 weeks. Do not freeze.

D. StemSpan™ B Cell Differentiation Medium 4

Use sterile technique to prepare StemSpan™ B Cell Differentiation Medium 4 (StemSpan™ SFEM II + StemSpan™ B Cell Differentiation Supplement 4 [20X]). The following example is for preparing 2.5 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw StemSpan™ SFEM II at room temperature or overnight at 2 - 8°C. Mix thoroughly.

   NOTE: Once thawed, use immediately or store at 2 - 8°C for up to 1 month. Alternatively, aliquot and store at -20°C. Do not exceed the shelf life of the medium. After thawing the aliquots, use immediately. Do not re-freeze.

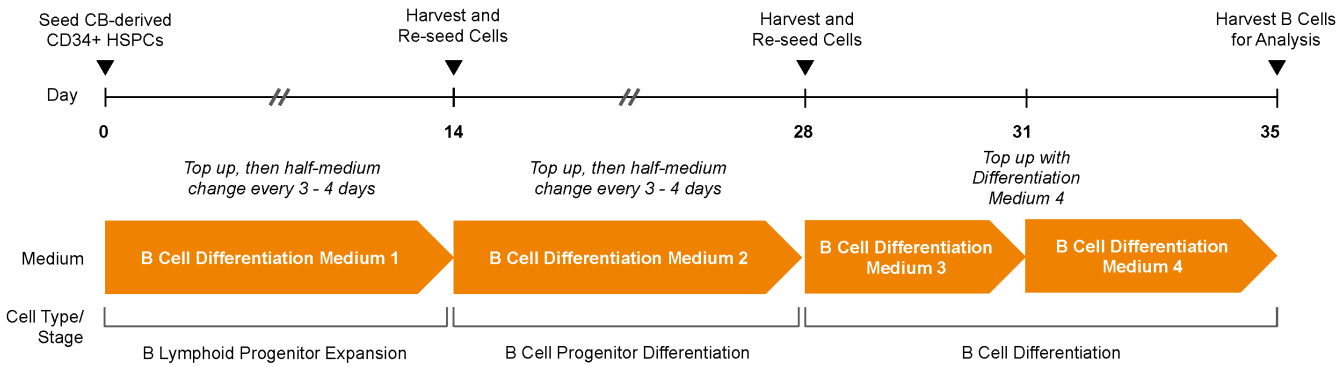
2. Thaw StemSpan™ B Cell Differentiation Supplement 4 (20X) at room temperature. Mix thoroughly.

   NOTE: Once thawed, use immediately or store at 2 - 8°C for up to 2 weeks. Alternatively, aliquot and store at -20°C. Do not exceed the shelf life of the medium. After thawing the aliquots, use immediately. Do not re-freeze.

3. Add 0.125 mL of StemSpan™ B Cell Differentiation Supplement 4 (20X) to 2.375 mL of StemSpan™ SFEM II. Mix thoroughly.

   NOTE: If not used immediately, store complete StemSpan™ B Cell Differentiation Medium 4 at 2 - 8°C for up to 2 weeks. Do not freeze.

Protocol Diagram



Directions for Use

The following protocol is for differentiating CB-derived CD34+ cells to B cells in one well of a 24-well plate. For other cultureware, refer to Table 1 and adjust volumes accordingly.

For optimal performance, warm media to room temperature (15 - 25°C) prior to use and follow the recommended schedule of feeding and passaging. However, the schedule may be adjusted as needed, as long as a feeding interval of 3 - 4 days is maintained.

Day 0

1. Prepare CD34+ cells as follows:
   a. If using frozen CD34+ cells from human CB (Catalog #70008), thaw cells according to manufacturer's protocol and proceed to step 2.
   b. If using fresh (< 72 hour-old) human CB, isolate CD34+ cells using EasySep™ Human Cord Blood CD34 Positive Selection Kit II (Catalog #17896) or flourescence-activated cell sorting. Proceed to step 2.

2. Perform a viable cell count using a hemocytometer (e.g. Catalog #100-1181) and Trypan Blue (Catalog #07050) or an automated cell counter. Determine the % CD34+ cells by flow cytometry, using one of the following fluorochrome-conjugated antibodies:
   - Anti-Human CD34 Antibody, Clone 581 (Catalog #60013), or
   - Anti-Human CD34 Antibody, Clone 8G12 (Catalog #60121)

   Determine the concentration of CD34+ cells by multiplying the % CD34+ cells by the viable cell count.
3. Prepare StemSpan™ B Cell Differentiation Medium 1 (Preparation of Media, section A).
4. Dilute CD34+ cells in StemSpan™ B Cell Differentiation Medium 1 to prepare a cell suspension of 1 x 10^4 cells/mL.

   NOTE: This cell suspension is for one well of a 24-well plate. If using other cultureware, refer to Table 1 for recommended volumes.
5. Add 0.5 mL of cell suspension (prepared in step 4) or 5 x 10^3 CD34+ cells/well to one well of the tissue culture-treated 24-well plate.
6. Incubate at 37°C and 5% CO_2 for 3 or 4 days.

Table 1. Recommended Volumes of Media for Various Cultureware

TISSUE CULTURE-TREATED CULTUREWARE	SEED & FEED VOLUMES OF MEDIUM	FINAL CULTURE VOLUME OF MEDIUM
96-well plate (e.g. Catalog #38022)	0.1 mL/well	0.2 mL/well
24-well plate (e.g. Catalog #38021)	0.5 mL/well	1 mL/well
12-well plate (e.g. Catalog #38052)	1 mL/well	2 mL/well
6-well plate (e.g. Catalog #38016)	2.5 mL/well	5 mL/well

Day 3 or 4

7. Carefully add 0.5 mL of pre-warmed StemSpan™ B Cell Differentiation Medium 1 per well. Incubate at 37°C and 5% CO_2 for 3 - 4 days.

Day 7

8. Perform a half-medium change as follows:
   a. Carefully remove 0.5 mL of medium from the top of the well. Do not disturb cells.
   b. Add 0.5 mL of pre-warmed StemSpan™ B Cell Differentiation Medium 1 per well.
9. Incubate at 37°C and 5% CO_2 for 3 - 4 days. Repeat half-medium changes every 3 - 4 days until day 14.

Day 14: Harvest and re-seed cells

10. Gently pipette cells up and down in the well to ensure all cells are in suspension. Transfer all cells to an appropriate tube; these cells include B lymphoid progenitors containing proB and preB cell progenitors (Figure 1).
11. Centrifuge at 300 x g for 5 - 10 minutes. Carefully aspirate the supernatant.
12. Resuspend in 0.25 mL or appropriate volume of StemSpan™ SFEM II.
13. Perform a viable cell count using a hemocytometer and Trypan Blue or an automated cell counter. Flow cytometry can be performed to assess surface expression, refer to the Phenotype Assessment section for recommended surface markers.
14. Prepare StemSpan™ B Cell Differentiation Medium 2 (Preparation of Media, section B).
15. Dilute cells in StemSpan™ B Cell Differentiation Medium 2 to prepare a cell suspension at 2 x 10^5 cells/mL.

    NOTE: This cell suspension is for one well of a 24-well plate. If using other cultureware, refer to Table 1 for volumes and cell numbers required.
16. Add 0.5 mL of cell suspension (prepared in step 15) or 1 x 10^5 cells/well to one well of a 24-well tissue culture-treated plate.
17. Incubate at 37°C and 5% CO_2 for 3 or 4 days.

Day 17 or 18

18. Carefully add 0.5 mL/well of pre-warmed StemSpan™ B Cell Differentiation Medium 2. Incubate at 37°C and 5% CO_2 for 3 - 4 days.

Day 21

19. Perform a half-medium change as follows:
    a. Carefully remove 0.5 mL of medium from the top of the well. Do not disturb cells.
    b. Add 0.5 mL of pre-warmed StemSpan™ B Cell Differentiation Medium 2 per well.
20. Incubate at 37°C and 5% CO_2 for 3 or 4 days. Repeat half-medium changes every 3 - 4 days until day 28.

Day 28: Harvest and re-seed cells

21. Gently pipette cells up and down to ensure all cells are in suspension. Transfer cells to an appropriate tube; these differentiated cells contain preB cells and a small population of immature B cells (Figure 2).
22. Centrifuge at 300 x g for 5 - 10 minutes. Carefully aspirate the supernatant.
23. Resuspend in 0.25 mL or appropriate volume of StemSpan™ SFEM II.
24. Perform a viable cell count using a hemocytometer and Trypan Blue or an automated cell counter. Flow cytometry can be performed to assess surface expression, refer to the Phenotype Assessment section for recommended surface markers.
25. Prepare StemSpan™ B Cell Differentiation Medium 3 (Preparation of Media, section C).
26. Dilute cells in StemSpan™ B Cell Differentiation Medium 3 to prepare a suspension at 4 - 6 x 10^5 cells/mL.

    NOTE: This cell suspension is for one well of a 24-well plate. If using other cultureware, refer to Table 1 for volumes and cell numbers required.
27. Add 0.5 mL of cell suspension (prepared in step 26) or 2 - 3 x 10^5 cells/well to one well of a 24-well tissue culture-treated plate. Incubate at 37°C and 5% CO_2 for 3 days.

Day 31

28. Prepare StemSpan™ B Cell Differentiation Medium 4 (Preparation of Media, section D).
29. Carefully add 0.5 mL/well of pre-warmed StemSpan™ B Cell Differentiation Medium 4 . Incubate at 37°C and 5% CO_2 for 4 days.

Day 35: Harvest cells

30. Gently pipette cells up and down in the well to ensure all cells are in suspension. Transfer cells to an appropriate tube; these cells include immature B cells and antibody-secreting cells (ASCs; Figures 3 and 4).
31. Centrifuge at 300 x g for 5 - 10 minutes. Carefully aspirate the supernatant.
32. Resuspend in 0.25 mL or appropriate volume of StemSpan™ SFEM II.
33. Perform a viable cell count using a hemocytometer and Trypan Blue or an automated cell counter. Cells are now ready for assays or analysis by flow cytometry to assess surface expression. Refer to the Phenotype Assessment section for recommended surface markers.

Phenotype Assessment

NOTE: Use of a lineage marker cocktail consisting of several antibodies conjugated with the same fluorochrome (e.g. FITC) to gate out non-B lineage cells is recommended for day 14 and 28 flow cytometry assessments.

Use the following antibodies for the lineage marker cocktail:

- Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011)
- Anti-Human CD14 Antibody, Clone M5E2 (Catalog #60004)
- Anti-Human CD15 Antibody (Clone HI98)
- Anti-Human CD16 Antibody, Clone 3G8 (Catalog #60041)
- Anti-Human CD56 (NCAM) Antibody, Clone HCD56 (Catalog #60021)
- Anti-Human CD66b Antibody, Clone G10F5 (Catalog #60086)

Use the following antibodies specific to the B cell lineage:

- Anti-Human CD10 Antibody, Clone HI10a
- Anti-Human CD19 Antibody, Clone HIB19 (Catalog #60005)
- Anti-Human CD20 Antibody, Clone 2H7 (Catalog #60008)
- Anti-Human CD24 Antibody, Clone ML5
- Anti-Human CD27 Antibody, Clone O323
- Anti-Human CD38 Antibody, Clone HIT2 (Catalog #60014)
- Anti-Human IgM Antibody, Clone MHM-88
- DRAQ7™ dye live/dead staining

Data

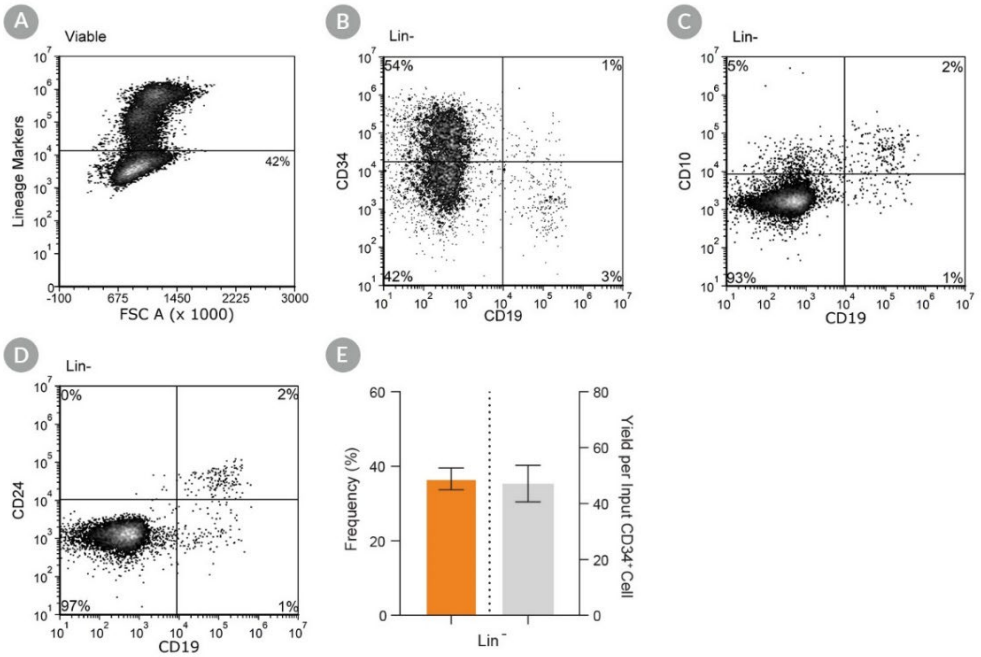


Figure 1. B Lymphoid Progenitors are Contained Within Lineage-Negative Cell Population After 14 Days of Culture

CB-derived CD34+ cells (freshly isolated or from cryopreserved stock) were cultured with StemSpan™ B Cell Differentiation Medium 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 proB and preB cells) can be detected on gated Lin- cells. **(E)** The average frequency of Lin- cells on day 14 was 36.6 ± 1.4% with a yield of 47.1 ± 3.2 Lin- cells per input CD34+ cell. The graph shows mean with standard error of mean (n = 36).

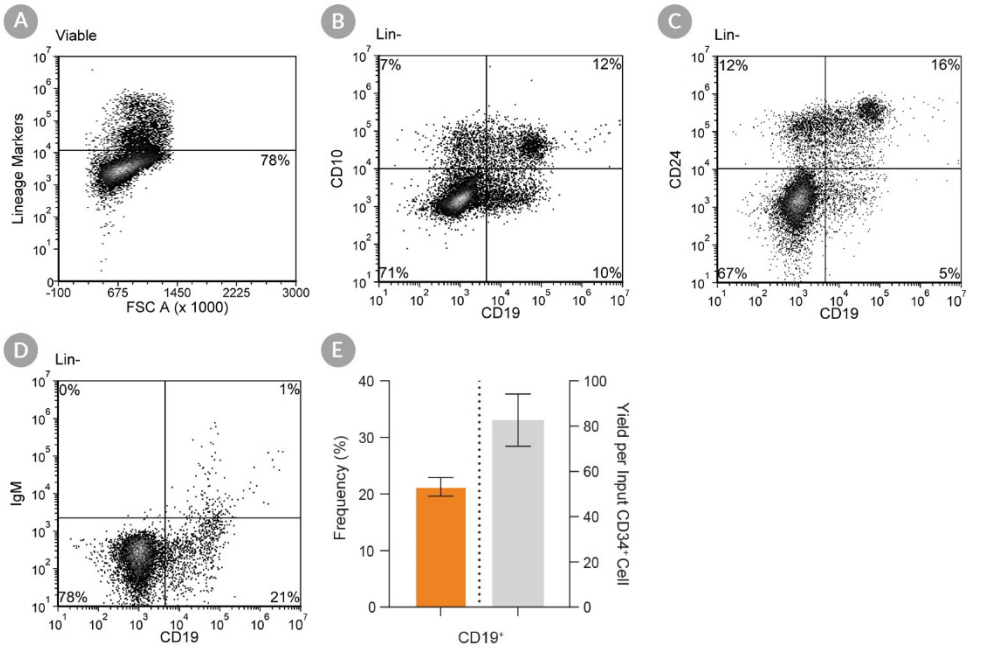


Figure 2. Frequency and Yield of CD19+ B Cells After 28 Days of Culture

Day 14 B lymphoid progenitors were further differentiated into CD19+ B cells by culturing for an additional 14 days in StemSpan™ B Cell Differentiation Medium 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 preB 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 mean with standard error of mean (n = 36).

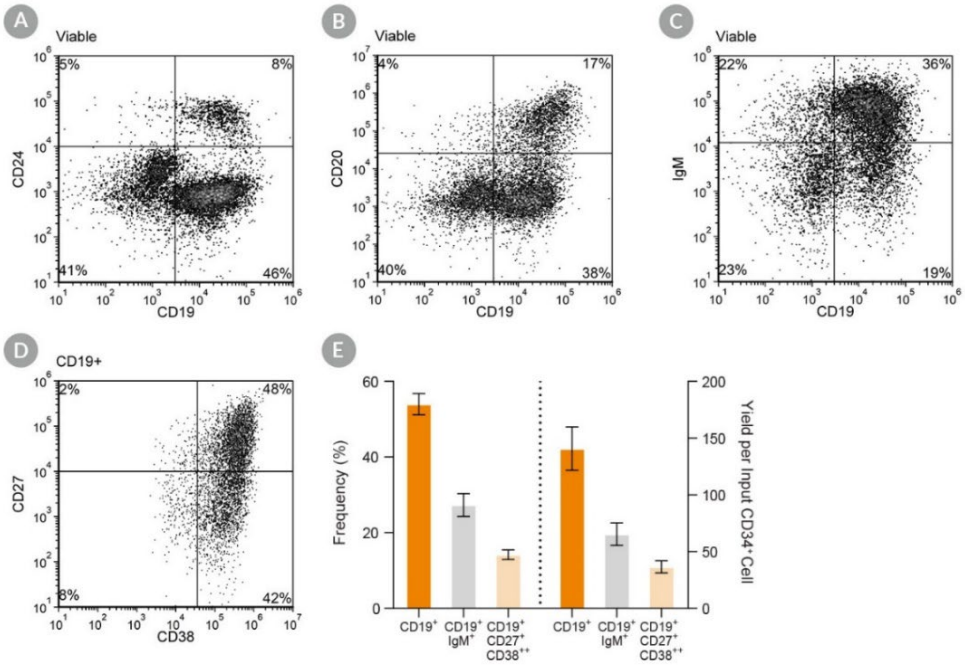


Figure 3. Frequency and Yield of CD19+IgM+ and Antibody-secreting B Cells After 35 Days of Culture

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™ B Cell Differentiation Medium 3 followed by StemSpan™ B Cell Differentiation Medium 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 and yield per input CD34+ cells were 54.0 ± 2.7% and 140.7 ± 19.1, 27.3% ± 1.3% and 65.3 ± 9.9, and 14.2% ± 1.3% and 36.5 ± 5.3, respectively. The graph shows means with standard error of mean (n = 36).

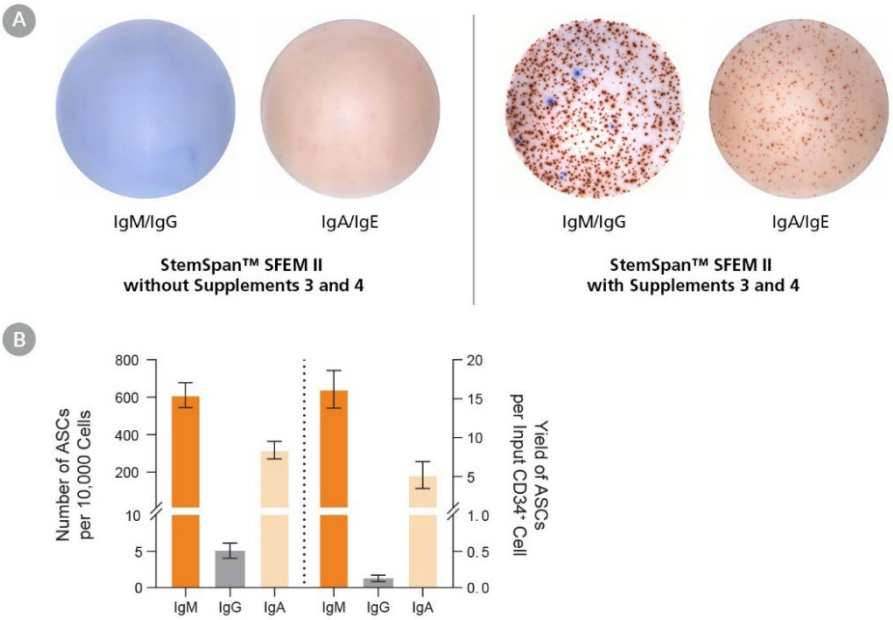


Figure 4. Antibody-secreting B Cells After 35 Days of Culture

On day 35, immunoglobulin-secreting cell frequencies of 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 (StemSpan™ SFEM II with StemSpan™ B Cell Differentiation Supplements 3 and 4; right) compared to negative control (StemSpan™ SFEM II without StemSpan™ B Cell Differentiation Supplements 3 and 4; left); 10,000 culture day 35 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 day 35 cells secreted 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 means with standard error of mean (n = 8 - 33).

Related Products

For related products, including specialized cell culture and storage media, supplements, antibodies, cytokines, and small molecules, visit www.stemcell.com/hPSCNCworkflow, or contact us at techsupport@stemcell.com.