TECHNICAL BULLETIN

StemSpan™ Medium and Supplements for the Generation of T Cells from Cord Blood-Derived CD34+ Cells

Background

Investigators studying hematopoiesis require standardized culture conditions to promote the proliferation and/or lineage-specific differentiation of hematopoietic stem and progenitor cells (HSPCs). Methods to differentiate and expand HSPCs into T cells offer a source of cells for research into this lineage. The in vitro differentiation of HSPCs to T cells has previously only been possible by co-culture of HSPCs with a stromal or "feeder" cell line that has been engineered to express Notch ligands. In such cultures, $CD34^+CD38^{-/lo}$ HSPCs develop into $CD7^+CD5^+$ progenitor T (pro-T) cells that further differentiate to CD4 immature single-positive (CD4 ISP) cells. These cells give rise to $CD4^+CD8^+$ double-positive (DP) cells which finally mature into $CD3^+TCR\alpha\beta^+$ single-positive (SP) T cells that express either CD4 or CD8.

The StemSpan™ T Cell Generation Kit (Catalog #09940) facilitates the differentiation of cord blood (CB)-derived $CD34^+$ cells into T cells without the use of a stromal cell line, and in serum-free culture conditions.

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

The StemSpan™ T Cell Generation Kit contains serum-free medium, coating material, expansion and maturation supplements (see table to the right) required for the generation of pro-T cells and DP cells in a two-step protocol. In the first step, $CD34^+$ HSPCs are cultured for 14 days in medium containing expansion supplement to promote their proliferation and differentiation into $CD7^+CD5^+$ pro-T cells. In the second step, pro-T cells generated during the first 14 days are cultured for another 4 weeks in medium containing the maturation supplement to promote their maturation into DP cells. In this system, more than 20,000 DP cells can be generated per input $CD34^+$ cell (See Figures 3 and 4). Both fresh and cryopreserved CB-derived $CD34^+$ cells may be used with the StemSpan™ T Cell Generation Kit. If pro-T cells are the desired cell type, only materials for the first step of the protocol are necessary and can be purchased individually.

Why Use StemSpan™ for T Cell Generation?

DEFINED. Serum- and feeder-free conditions eliminate variation introduced by serum and stromal cell lines.

SUPERIOR PERFORMANCE. Produce more than 20,000 DP cells per input CB-derived $CD34^+$ cell.

CONVENIENT. Eliminate extra passaging steps required for stromal cell-based cultures.

StemSpan™ T Cell Generation Kit

Product Name	Catalog #
StemSpan™ T Cell Generation Kit	09940 (1 kit)
Components*	
StemSpan™ SFEM II Medium	09605 (2 x 100 mL)**
StemSpan™ Lymphoid Progenitor Expansion Supplement (10X)	09915 (5 mL)
StemSpan™ Lymphoid Differentiation Coating Material (100X)	09925 (2 x 0.25 mL)
StemSpan™ T Cell Progenitor Maturation Supplement (10X)	09930 (12.5 mL)

**Available for sale individually.*

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

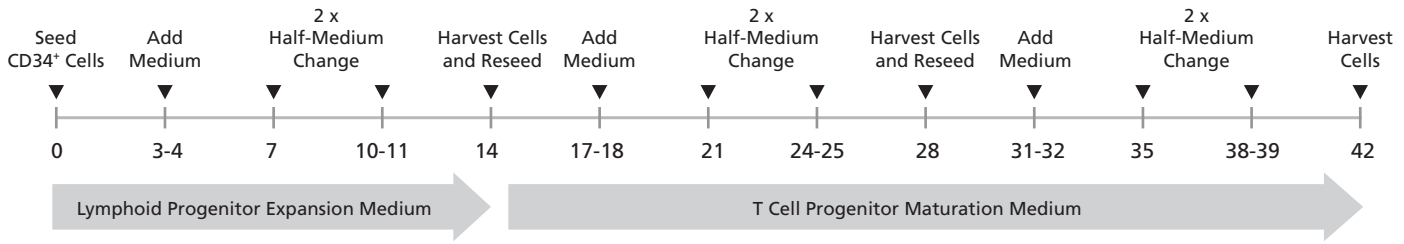

Figure 1. General StemSpan™ T Cell Generation Protocol

CB-derived CD34+ cells are seeded on day 0. Medium should be topped up after 3 - 4 days of culture followed by two half-medium changes every 3 - 4 days. On day 14, the CD7+CD5+ pro-T cells are harvested and can be reseeded if further maturation into DP cells is desired. Top-up and half-medium changes should be performed every 3 - 4 days after each harvest and reseed, as illustrated in the figure. For more information see protocol below.

Protocol

This protocol is designed to promote the proliferation and differentiation of CB-derived CD34+ cells into CD7+CD5+ pro-T cells, followed by their subsequent differentiation into CD4+CD8+ DP cells after 42 days of culture. See Figure 1 for a general representation of this protocol. An optional protocol extension for further maturation of DP cells into CD8 single-positive (SP) T cells is also illustrated in Figure 2.

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

1. Non-tissue culture treated plates must be coated with StemSpan™ Lymphoid Differentiation Coating Material prior to culture. Prepare coating material following instructions in the Product Information Sheet (PIS; Document #10000003538).
2. Thaw frozen CB CD34+ cells (Catalog #70008), or isolate CD34+ cells separately from fresh CB using the EasySep™ Human Cord Blood CD34 Positive Selection Kit II (Catalog #17896).
3. Prepare complete lymphoid progenitor expansion medium (StemSpan™ SFEM II + Lymphoid Progenitor Expansion Supplement). Refer to PIS for details.
4. Dilute CD34+ cells to 1 x 10^4 cells/mL in complete lymphoid progenitor expansion medium and seed into the pre-coated plate.
5. Incubate at 37 °C for 14 days, following instructions in the PIS for necessary half-medium changes. Cells are harvested on day 14 and can be used for downstream applications if pro-T cells are desired (See Figure 3), or reseeded to further mature into DP cells.
6. Before reseeding cells, prepare a freshly coated plate (See step 1).
7. Prepare complete T cell progenitor maturation medium (StemSpan™ SFEM II + T Cell Progenitor Maturation Supplement).
8. Dilute pro-T cells to 1 x 10^5 cells/mL in complete T cell progenitor maturation medium, seed into the coated plate and incubate at 37°C following instructions in the PIS for necessary half-medium changes and replating.
9. On day 28, harvest and dilute cells to 5 x 10^5 cells/mL in complete T cell progenitor maturation medium.
10. Reseed on a freshly coated plate and incubate at 37 °C.
11. Harvest cells containing CD4+CD8+ DP cells on day 42 (See Figure 4) for use in downstream assays, or follow the optional protocol extension for further maturation to CD8 SP T cells.

Optional Protocol Extension

An optional protocol is available to mature DP cells to CD8 SP T cells. This extended protocol uses complete T cell progenitor maturation medium made with reagents included in the StemSpan™ T Cell Generation Kit and must be combined with additional components including ImmunoCult™ CD3/CD28/CD2 T Cell Activator (Catalog #10970) and IL-15 (Catalog #78031).

1. Prepare a freshly coated plate (See step 1 of Protocol).
2. Prepare complete CD8 SP T cell maturation medium (StemSpan™ SFEM II + T Cell Progenitor Maturation Supplement + ImmunoCult™ T Cell Activator + IL-15). Refer to PIS for details.
3. Dilute cells to 1 x 10^6 cells/mL in complete CD8 SP T cell maturation medium, seed into the coated plate and incubate at 37°C.
4. After 3 - 4 days of culture, top up with CD8 SP T cell maturation medium, without ImmunoCult™ T Cell Activator.
5. Incubate at 37°C and harvest cells on day 49 (See Figure 5).

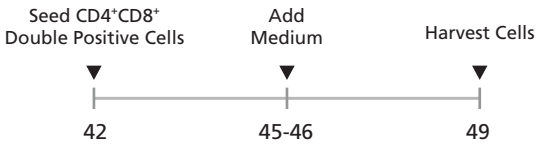

Figure 2. Optional CD8 SP T Cell Maturation Protocol

DP cells are seeded and medium is added after 3 - 4 days. CD8 SP T cells can be harvested on day 49.

Cell Analysis

$CD7^+CD5^+$ pro-T cells are produced after 14 days of culture of CB-derived $CD34^+$ cells (Figure 3). These cells differentiate further into CD4 immature single-positive (CD4 ISP) cells and $CD4^+CD8^+$ DP cells during a second 28-day culture step (Figure 4). By following the optional protocol extension, DP cells can finally mature into CD8 SP T cells (Figure 5). Cells may be stained with antibodies directed against cell surface markers CD3, CD4, CD5, CD7, CD8 and TCRαβ for analysis by flow cytometry. In the representative flow cytometry plots shown, dead cells were excluded by light scatter profile and viability staining.

Applications

- Research into the differentiation of T lineage cells from HSPCs
- Development of procedures to expand T cells from $CD34^+$ cells in culture
- Assessment of efficacy and toxicity of candidate therapeutics on T cell differentiation during drug development
- Development of in vitro models to study diseases which involve T cell development

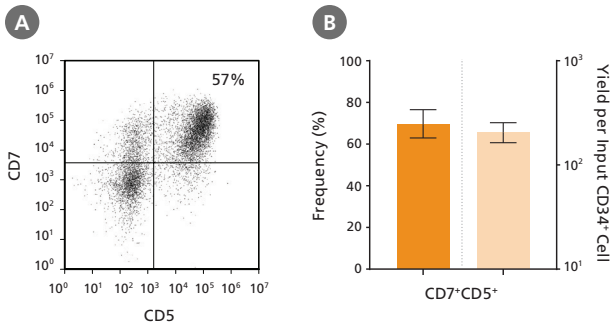

Figure 3. Frequency and Yield of $CD7^+CD5^+$ Pro-T Cells After 14 Days of Culture

CB-derived $CD34^+$ cells (freshly isolated or frozen) were cultured for 14 days in StemSpan™ SFEM II containing Lymphoid Progenitor Expansion Supplement (Catalog #09915) on plates coated with Lymphoid Differentiation Coating Material (Catalog #09925). Cells were harvested and analyzed for CD7 and CD5 expression by (A) flow cytometry. The (B) average frequency of viable $CD7^+CD5^+$ pro-T cells on day 14 was 70%, with ~200 $CD7^+CD5^+$ cells produced per input $CD34^+$ cell. Shown are means with 95% confidence intervals (n = 33).

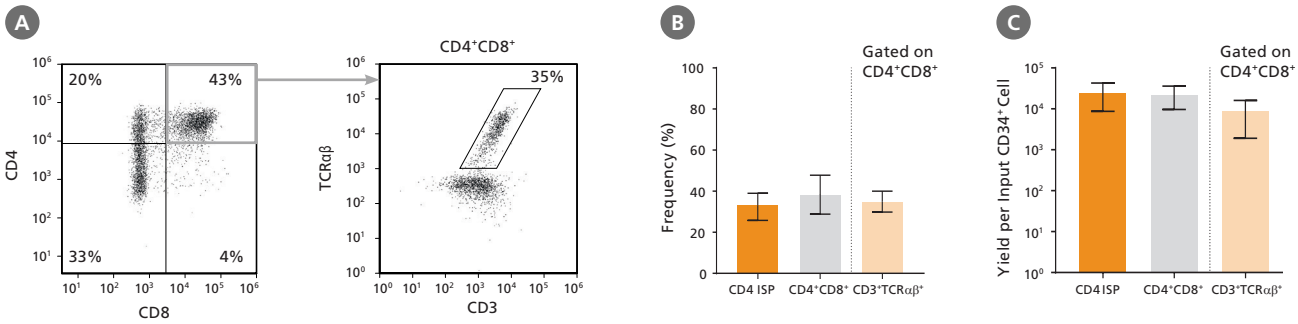

Figure 4. Frequency and Yield of CD4 ISP and $CD4^+CD8^+$ DP Cells After 42 Days of Culture

CB-derived $CD34^+$ cells (freshly isolated or frozen) were cultured with the StemSpan™ T Cell Generation Kit (Catalog #09940) for 42 days and (A) analysed by flow cytometry for the expression of CD4, CD8, CD3 and TCRαβ. The (B) frequency and (C) yield of CD4 ISP, double-positive ($CD4^+CD8^+$) and $CD3^+TCR\alpha\beta^+$-expressing double-positive cells ($CD4^+CD8^+CD3^+TCR\alpha\beta^+$) are shown. On average, 38% of the total viable population were DP ($CD4^+CD8^+$), of which 35% co-expressed CD3 and TCRαβ. The yields of total DP cells and $CD3^+TCR\alpha\beta^+$ DP cells per input $CD34^+$ cell were ~23,000 and ~9,000, respectively. Shown are means with 95% confidence intervals (n = 31).

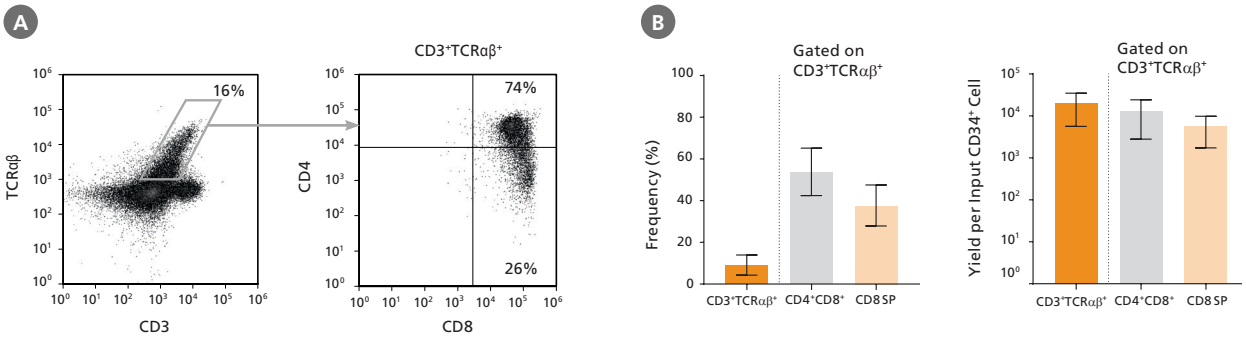

Figure 5. Frequency and Yield of CD8 SP T Cells After 49 Days of Culture

DP cells were further matured into CD8 SP T cells by culturing for an additional 7 days in StemSpan™ SFEM II with T Cell Progenitor Maturation Supplement (Catalog #09930), IL-15 (Catalog #78031) and ImmunoCult™ CD3/CD28/CD2 T Cell Activator (Catalog #10970) on coated plates. On day 49, cells were (A) analyzed by flow cytometry for the expression of CD3, TCRαβ, CD4 and CD8. The (B) frequency and yield of $CD3^+TCR\alpha\beta^+$-expressing cells and their subsets are shown. On average, 54% of the $CD3^+TCR\alpha\beta^+$ cells were DP ($CD4^+CD8^+$) and 38% were CD8 SP ($CD4^-CD8^+$). The average yield of CD8 SP T cells per input $CD34^+$ cell was ~6,000. $CD3^+TCR\alpha\beta^+$ CD4 SP ($CD4^+CD8^-$) T cells were detected at very low frequencies (data not shown). Shown are means with 95% confidence intervals (n = 12).

Suggested Antibodies for Analysis*

Product Name	Catalog #
Anti-Human CD34 Antibody Clone 581	60013
Anti-Human CD3 Antibody, Clone UCHT1	60011
Anti-Human CD4 Antibody, Clone RPA-T4	Coming soon
Anti-Human CD5 Antibody, Clone UCHT2	60082
Anti-Human CD7 Antibody, Clone CD7-6B7	Coming soon
Anti-Human CD8a Antibody, Clone RPA-T8	60022
Anti-Human TCRαβ Antibody, Clone IP26	Coming soon

*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

**Not included in the kit*

Products Required for Optional Protocol Extension*

Product Name	Catalog #
ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator	10970
Human Recombinant IL-15	78031

**Not included in the kit*
***Catalog #10971 may also be used for activation*