TECHNICAL BULLETIN

# Generation of Natural Killer Cells and T Cells from Human Pluripotent Stem Cells Using STEMdiff™ and StemSpan™ Media and Supplements

## Background

Natural killer (NK) cells and T cells are lymphocytes that provide defense against pathogens and tumors. NK cells play a critical role in innate immunity due to their secretion of proinflammatory cytokines and ability to kill cancerous or virus-infected cells. As part of the adaptive immune system, T cells recognize a wide range of targets through their antigen-specific T cell receptors (TCRs) and exert effector functions including cytokine secretion and cell killing. A crucial feature of T cells is the formation of memory cells, which helps establish a much faster response to reinfection than the primary response. While both NK and T cells can be isolated from peripheral blood, human pluripotent stem cells (PSCs) offer a potentially unlimited source of immune cells. The ability to differentiate PSCs into NK and T cells provides a useful tool for developing adoptive immunotherapy in cancer patients and for research into the basic biology of these cells.

STEMdiff™ NK Cell Kit and STEMdiff™ T Cell Kit facilitate the differentiation of PSCs into NK and T cells, respectively, without the use of stromal cells and in serum-free culture conditions.

### Why Use STEMdiff™ for Generating NK and T Cells?

**CONSISTENT.** Eliminate variation introduced by serum and stromal cell lines by using serum- and feeder-free conditions.

**UNIFORM.** Reduce variability by producing uniform aggregates for EB formation with AggreWell™.

**HIGH YIELD.** Produce approximately 210 NK cells or 60 DP T cells per input PSC-derived $CD34^+$ cell.

**CONVENIENT.** Avoid extra passaging steps required with stromal cell-based cultures.

## Differentiate PSCs into NK or T Cells

STEMdiff™ NK Cell and STEMdiff™ T Cell Kits are comprised of STEMdiff™ Hematopoietic - EB reagents paired with StemSpan™ NK Cell Generation Kit or StemSpan™ T Cell Generation Kit, respectively. These STEMdiff™ kits are used in a four-stage protocol to differentiate PSCs into either NK or T cells. In the first two stages, the STEMdiff™ Hematopoietic - EB reagents are used to differentiate PSCs into $CD34^+$ hematopoietic progenitor cells (Figure 1). First, PSCs are cultured for 3 days in EB Formation Medium and EB Medium A to induce mesoderm specification. This is done in AggreWell™, allowing for the formation of uniformly sized embryoid bodies (EBs). In the second stage, the cells are cultured in EB Medium B for 9 days, promoting their specification into hematopoietic progenitor cells. The generated EBs are then dissociated into single cells, and $CD34^+$ cells are enriched using EasySep™ immunomagnetic isolation.

In the third stage, $CD34^+$ cells are cultured for 14 days in StemSpan™ Lymphoid Progenitor Expansion Medium on plates coated with StemSpan™ Lymphoid Differentiation Coating Material to stimulate their proliferation and differentiation into lymphoid progenitor (LP) cells. Finally, LP cells are differentiated into either $CD56^+$ NK cells (Figure 3) or $CD4^+CD8^+$ double-positive (DP) T cells (Figure 8).

For NK cell differentiation, the LP cells are cultured for 14 days in non-coated plates in StemSpan™ NK Cell Differentiation Medium containing the small molecule UM729 (Catalog #72332; sold separately) to promote their further differentiation into $CD56^+$ NK cells. UM729 is necessary during this last stage of the culture to achieve high NK cell frequencies and yields. For DP T cell differentiation, LP cells are cultured for 14 days in StemSpan™ T Cell Progenitor Maturation Medium on plates coated with StemSpan™ Lymphoid Differentiation Coating Material. In these systems, approximately 210 NK cells or 60 DP T cells can be generated per $CD34^+$ cell (average of results from multiple embryonic stem (ES)/induced pluripotent stem (iPS) cell lines; see Figures 5 and 10).

# Product Information

## STEMdiff™ NK Cell Kit and STEMdiff™ T Cell Kit Components

| Product Name | Catalog # (Size) | Product Name | Catalog # (Size) |
|---|---|---|---|
| STEMdiff™ NK Cell Kit | 100-0170 (1 Kit) | STEMdiff™ T Cell Kit | 100-0194 (1 Kit) |
| **Kit Components** | **Component # (Size)** | **Kit Components** | **Component # (Size)** |
| STEMdiff™ Hematopoietic - EB Basal Medium | 100-0171 (120 mL) | STEMdiff™ Hematopoietic - EB Basal Medium | 100-0171 (120 mL) |
| STEMdiff™ Hematopoietic - EB Supplement A | 100-0172 (265 µL) | STEMdiff™ Hematopoietic - EB Supplement A | 100-0172 (265 µL) |
| STEMdiff™ Hematopoietic - EB Supplement B | 100-0173 (7 mL) | STEMdiff™ Hematopoietic - EB Supplement B | 100-0173 (7 mL) |
| StemSpan™ SFEM II | 09605 (100 mL)* | StemSpan™ SFEM II | 09605 (2 x 100 mL)* |
| StemSpan™ Lymphoid Progenitor Expansion Supplement (10X) | 09915 (5 mL) | StemSpan™ Lymphoid Progenitor Expansion Supplement (10X) | 09915 (5 mL) |
| StemSpan™ Lymphoid Differentiation Coating Material (100X) | 09925 (250 µL) | StemSpan™ Lymphoid Differentiation Coating Material (100X) | 09925 (2 x 250 µL) |
| StemSpan™ NK Cell Differentiation Supplement (100X) | 09950 (500 µL) | StemSpan™ T Cell Progenitor Maturation Supplement (10X) | 09930 (12.5 mL) |

**StemSpan™ SFEM II (Catalog #09655, 500 mL) can also be used.*

Note: The small molecule UM729 (Catalog #72332) is required for optimal differentiation of NK cells with the STEMdiff™ NK Cell Kit. This product is not included in the kit and must be purchased separately. See PIS for more details.

# Protocol for Differentiation of PSCs to CD34⁺ Cells

This protocol is designed to promote the differentiation of PSCs into CD34⁺ hematopoietic progenitor cells over 12 days of culture. The two stages—mesoderm formation and hematopoietic specification—are shown in Figure 1.

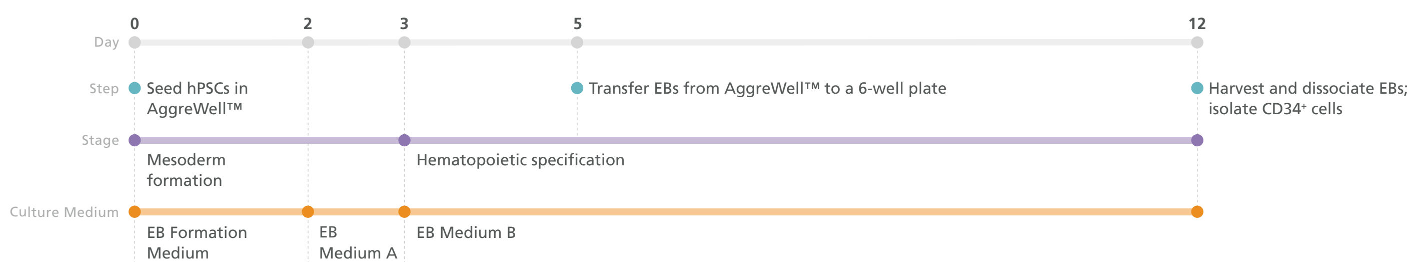


**Figure 1.** STEMdiff™ Hematopoietic Progenitor Differentiation Protocol

PSCs are harvested and dissociated into a single-cell suspension prior to seeding into AggreWell™ plates in EB Formation Medium (EB Medium A + 10 µM Y-27632) to form 500 cell aggregates. After 3 days of mesoderm formation, the medium is changed to EB Medium B to induce hematopoietic lineage differentiation. On day 5, EBs are transferred onto non-tissue culture-treated plates. After a total of 12 days, the EBs are harvested and dissociated, then CD34⁺ cells are enriched by EasySep™ positive selection.

## I. Protocol for Differentiation of PSCs to CD34⁺ Cells

This procedure has been optimized for use with ES and iPS cells; refer to the Technical Manual (Document #10000007537 or #10000007541) for complete instructions.

1. Prepare EB Medium A (STEMdiff™ Hematopoietic - EB Basal Medium + STEMdiff™ Hematopoietic - EB Supplement A). Prepare EB Formation Medium by adding Y-27632 (Catalog #72302) at 10 µM to EB Medium A.
2. Prepare an AggreWell™400 (Catalog #34421) plate by rinsing with Anti-Adherence Rinsing Solution (Catalog #07010), washing with DMEM/F-12 with 15 mM HEPES (Catalog #36254), and adding a half-volume of EB Formation Medium.
3. Harvest PSCs and generate a single-cell suspension using ACCUTASE™ (Catalog #07922).
4. Dilute PSCs to 1.4 x $10^6$ cells/mL in 2.5 mL of EB Formation Medium, then seed into the AggreWell™ plate that was prepared in step 2.
5. Perform a half-medium change with EB Medium A on day 2.
6. Prepare EB Medium B (STEMdiff™ Hematopoietic - EB Basal Medium + STEMdiff™ Hematopoietic - EB Supplement B).
7. Perform a half-medium change with EB Medium B on day 3.
8. Harvest EBs on day 5, then filter and elute these with EB Medium B, using a 37 µm reversible strainer (Catalog #27250).
9. Transfer eluted EBs to a non-tissue culture-treated plate.
10. Add EB Medium B on day 7.
11. Perform a half-medium change with EB Medium B on day 10.
12. Harvest EBs and dissociate into a single-cell suspension using Collagenase Type II (Catalog #07418) and TrypLE™ Express (Thermo Fisher #12605010). Isolate CD34⁺ cells using EasySep™ Human CD34 Positive Selection Kit II (Catalog #17856).
13. Proceed to the protocol for NK cell generation or T cell generation as desired: See II. Protocol for NK Cell Generation or IIIa. Protocol for T Cell Generation.

# Cell Analysis

PSCs are differentiated into CD34⁺ hematopoietic progenitors over 12 days. The frequency and yield of CD34⁺ cells are shown in Figure 2.

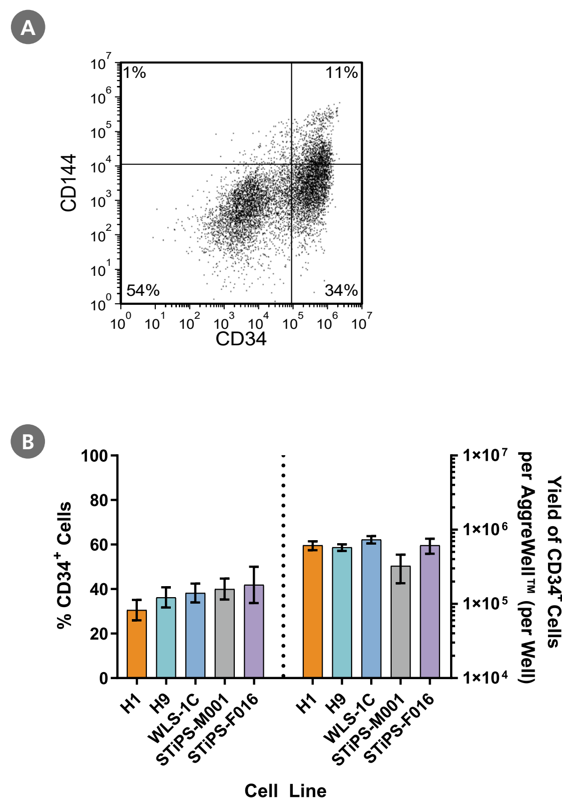


**Figure 2.** PSCs Differentiate to CD34⁺ Hematopoietic Progenitor Cells After 12 Days of Culture

Human ES and iPS cells were induced to differentiate into CD34⁺ cells using a 12-day protocol shown in Figure 1. At the end of the culture period, cells were harvested, dissociated into a single-cell suspension, and analyzed by flow cytometry for CD34 and CD144 expression. Dead cells were excluded by light scatter profile and DRAQ7™ staining. (**A**) Representative flow cytometry plot for ES (H1)-derived cells analyzed on day 12. (**B**) The average frequency of viable CD34⁺ cells on day 12 for two ES cell lines (H1 and H9) and three iPS cell lines (WLS-1C, STiPS-M001, and STiPS-F016) ranged between 31% and 42%. The average yield of CD34⁺ cells produced per well of a 6-well AggreWell™400 plate ranged between 3.3 x $10^5$ and 7.3 x $10^5$. Data are shown as mean ± SEM (n = 7 - 22).

## Protocol for Generating NK Cells

This protocol is designed to promote the proliferation and differentiation of PSC-derived CD34⁺ cells into CD56⁺ NK cells over 28 days of culture. Figure 3 shows a general overview of this protocol. Please refer to the Technical Manual for complete instructions.

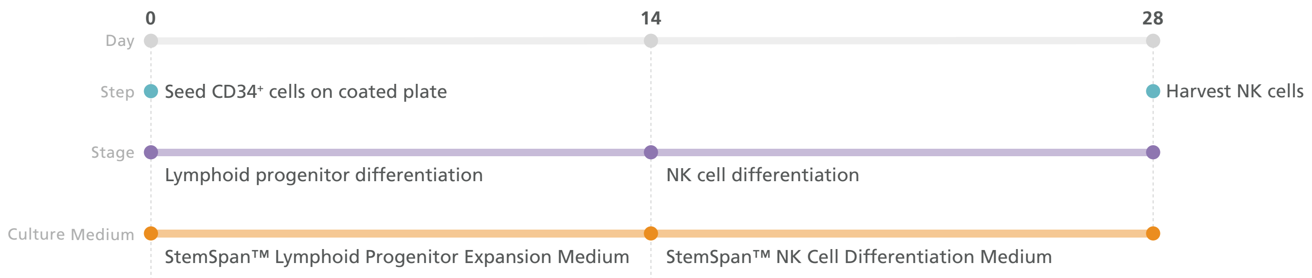


**Figure 3.** NK Cell Generation Protocol

PSC-derived CD34⁺ cells are seeded in StemSpan™ Lymphoid Progenitor Expansion Medium on plates coated with StemSpan™ Lymphoid Differentiation Coating Material. On day 14, cells at the lymphoid progenitor stage are harvested and reseeded in StemSpan™ NK Cell Differentiation Medium for further differentiation into NK cells. Note: UM729 should only be added to the StemSpan™ NK Cell Differentiation Medium (see step 5), but not the StemSpan™ Lymphoid Progenitor Expansion Medium. NK cells are harvested after 28 days. For further details see the step-by-step protocol below.

### II. Protocol for NK Cell Generation

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

1. Coat non-tissue culture-treated plates with StemSpan™ Lymphoid Differentiation Coating Material.

2. Prepare StemSpan™ Lymphoid Progenitor Expansion Medium (StemSpan™ SFEM II + StemSpan™ Lymphoid Progenitor Expansion Supplement).

3. Dilute CD34⁺ cells to 5 x $10^4$ cells/mL in StemSpan™ Lymphoid Progenitor Expansion Medium and seed onto the coated plate.

4. Incubate at 37°C for 7 days, following instructions in the Technical Manual (Document# 10000007537) for required half-medium changes and plate transfer on day 7. On day 14, harvest lymphoid progenitor cells (containing CD5⁺CD7⁺ cells; see Figure 4) for further differentiation to NK cells.

5. Prepare StemSpan™ NK Cell Differentiation Medium (StemSpan™ SFEM II + StemSpan™ NK Cell Differentiation Supplement + UM729*).

6. Dilute lymphoid progenitor cells to 1 x $10^5$ cells/mL in StemSpan™ NK Cell Differentiation Medium. Seed onto a non-coated tissue culture plate, incubate at 37°C, and follow instructions in the Technical Manual for required half-medium changes.

7. On day 28, harvest cells containing CD56⁺ NK cells (see Figure 5–7) for use in downstream assays.

**UM729 Purchased Separately*

### Applications for STEMdiff™ NK Cell Kit and STEMdiff™ T Cell Kit

- Research the differentiation of PSCs into NK cells or T lymphoid lineage cells
- Assess the efficacy and toxicity of candidate therapeutics on NK or T cell differentiation during drug development
- Research the use of NK or T cells for potential development of cellular therapeutics
- Develop in vitro models to study diseases that involve NK or T cells
- Perform gene editing of PSCs prior to differentiation into NK or T cells

## Cell Analysis

CD7⁺CD5⁺ lymphoid progenitor cells generated after 14 days of culture of PSC-derived CD34⁺ cells (Figure 4A) and CD56⁺ NK cells generated during an additional 14 days of culture (Figures 5 and 6) are identified by flow cytometry. Expression of other NK specific cell surface markers, such as NKp46, NKp44, NKp30, NKG2D, and CD16 can also be examined (Figures 5A, 5B, and 6). In the examples shown in Figure 6, staining for KIR molecules was performed using two different antibodies; 180704 and HP-MA4, which recognize distinct KIR molecules. The average frequency and yield of CD7⁺CD5⁺ LP cells and CD56⁺ NK cells generated in culture, after 14 and 28 days, respectively, are shown in Figures 4B and 5C.

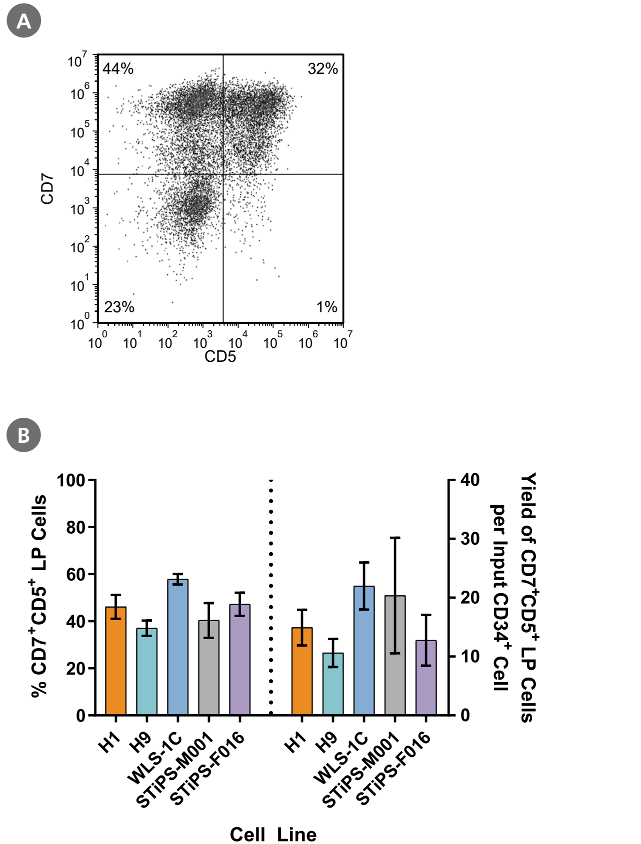


**Figure 4.** PSC-Derived CD34⁺ Cells Differentiate into CD5⁺CD7⁺ Lymphoid Progenitor Cells Over 14 Days of Culture

PSC-derived CD34⁺ cells were cultured for 14 days in StemSpan™ SFEM II + StemSpan™ Lymphoid Progenitor Expansion Supplement on plates coated with StemSpan™ Lymphoid Differentiation Coating Material (Figures 3 and 8). Cells were harvested and analyzed for CD7 and CD5 expression by flow cytometry. (**A**) Representative flow cytometry plot for ES (H1)-derived cells. (**B**) The average frequency of viable CD7⁺CD5⁺ lymphoid progenitor cells on day 14 ranged between 40% and 58% and the average yield of lymphoid progenitor cells produced per input PSC-derived CD34⁺ cell was between 11 and 22. Data are shown as mean ± SEM (n = 5 - 21).

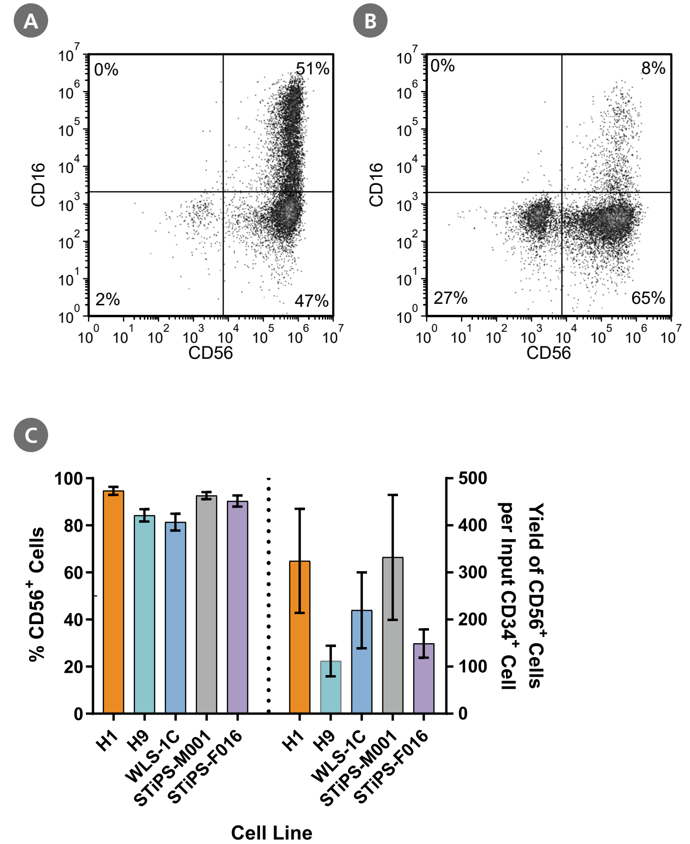


**Figure 5.** PSC-Derived Lymphoid Progenitor Cells Differentiate into CD56⁺ NK Cells After 14 Days of Culture

PSC-derived lymphoid progenitor cells were cultured in StemSpan™ NK Cell Differentiation Medium on non-coated plates for 14 days. Cells were harvested and analyzed for expression of CD56 and CD16 by flow cytometry. Representative flow cytometry plots are shown for both (**A**) ES (H1)-derived and (**B**) iPS (WLS-1C)-derived cells. (**C**) After 28 days of culture, the average frequency of viable CD56⁺ NK cells from PSC-derived CD34⁺ cells ranged between 81% and 95%. The average yield of CD56⁺ cells produced per PSC-derived CD34⁺ cell was between 112 and 332. Data are shown as mean ± SEM (n = 4 - 13).

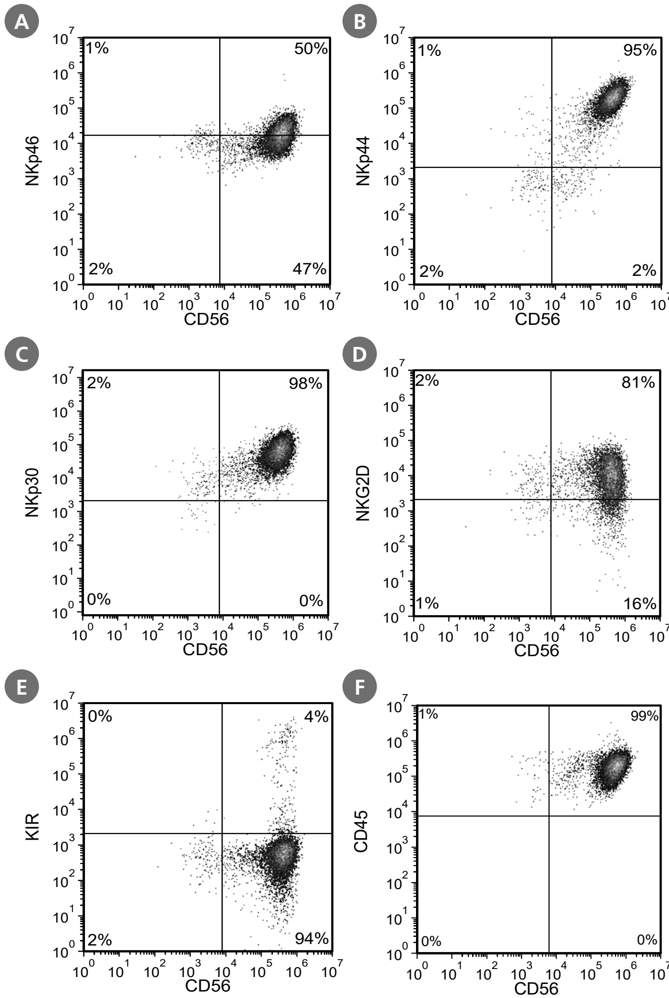


**Figure 6.** Cell Surface Marker Expression on PSC-Derived CD56+ NK Cells After 28 Days of Culture

PSC-derived CD34+ cells were cultured in StemSpan™ Lymphoid Progenitor Expansion Medium on plates coated with StemSpan™ Lymphoid Differentiation Coating Material for 14 days, followed by 14 days of culture in StemSpan™ NK Cell Differentiation Medium on non-coated plates to generate CD56+ NK cells. Cells were harvested and analyzed for CD56, NKp46, NKp44, NKp30, NKG2D, KIR, and CD45 expression by flow cytometry. Dead cells were excluded by light scatter profile and DRAQ7™ staining. Data shown are from a representative culture intiated with ES (H1) cells.

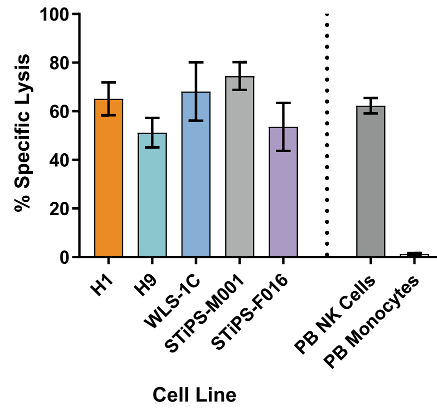


**Figure 7.** Cultured NK Cells Exhibit Cytotoxicity Toward K562 Cell Line

CD56+ NK cells were generated from PSC-derived CD34+ cells after 28 days of culture and then co-cultured with calcein AM (CAM)-labeled K562 cells at a ratio of 2.5:1 for 4 hours. Isolated peripheral blood (PB) NK cells and monocytes were also co-cultured with labeled K562 cells as positive and negative controls, respectively. Before the co-culture, frozen PB NK cells were thawed and cultured overnight with the NK Cell Differentiation Supplement and StemSpan™ SFEM II, while PB monocytes were cultured overnight in SFEM II only. To measure maximum release, the labeled K562 cells were treated with 1% Triton™ X-100. Culture supernatants were assessed for fluorescence released by dead cells after 4 hours using a SpectraMax® microplate reader (excitation 485 nm / emission 520 nm). The % specific lysis was calculated as follows: [(test release - spontaneous release) / (maximum release - spontaneous release)] X 100%. The average specific lysis by PSC-derived NK cells ranged between 51% and 75% as compared to 62% specific lysis by PB NK cells. Cultures of ES (H1 and H9) and iPS (WLS-1C, STiPS-M001, and STiPS-F016) cells are shown. Data are shown as mean ± SEM (n = 3 - 7).

## Protocol for Generating T Cells

This protocol is designed to promote the proliferation and differentiation of PSC-derived CD34⁺ cells into CD4CD8 DP T cells over 28 days of culture. Figure 8 shows a general overview of this protocol. An optional protocol extension for further maturation of DP T cells into CD8⁺ single-positive (SP) T cells is shown in Figure 9. Please refer to the Technical Manual for complete instructions.

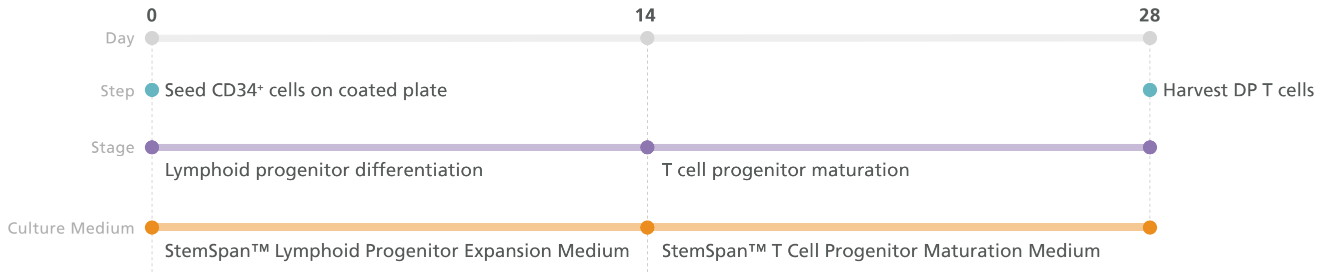


**Figure 8.** T Cell Generation Protocol

PSC-derived CD34⁺ cells are seeded in StemSpan™ Lymphoid Progenitor Expansion Medium on plates coated with StemSpan™ Lymphoid Differentiation Coating Material. On day 14, cells at the lymphoid progenitor stage are harvested and reseeded in StemSpan™ T Cell Progenitor Maturation Medium for further differentiation into CD4CD8 DP T cells. The DP T cells are harvested after 28 days. For further details see the step-by-step protocol below.

### IIIa. Protocol for T Cell Generation

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

1. Coat non-tissue culture-treated plates with StemSpan™ Lymphoid Differentiation Coating Material; refer to the Technical Manual (Document #10000007541) for complete instructions.

2. Prepare StemSpan™ Lymphoid Progenitor Expansion Medium (StemSpan™ SFEM II + StemSpan™ Lymphoid Progenitor Expansion Supplement).

3. Dilute CD34⁺ cells to 5 x $10^4$ cells/mL in StemSpan™ Lymphoid Progenitor Expansion Medium and seed onto the coated plate.

4. Incubate at 37°C for 7 days, following instructions in the Technical Manual (Document# 10000007541) for required half-medium changes and plate transfer on day 7. On day 14, harvest lymphoid progenitor cells (see Figure 4) for further differentiation to DP T cells.

5. Prepare StemSpan™ T Cell Progenitor Maturation Medium (StemSpan™ SFEM II + StemSpan™ T Cell Progenitor Maturation Supplement).

6. Dilute lymphoid progenitor cells to 0.5 - 1 x $10^6$ cells/mL in StemSpan™ T Cell Progenitor Maturation Medium. Seed onto a freshly coated plate (see step 1), incubate at 37°C, and follow instructions in the manual for required half-medium changes (removing dead cells using fluorescence-activated cell sorting [FACS] at this stage may improve frequency and yield of DP T cells).

7. On day 28, harvest cells containing DP T cells (see Figures 8 and 10) for use in downstream assays, or follow the optional protocol extension for further maturation to CD8 SP T cells (see Figures 9 and 11).

## Optional Protocol Extension

An optional protocol to mature DP T cells to CD8 SP T cells is presented in Figure 9. This extended protocol uses StemSpan™ T Cell Progenitor Maturation Medium prepared with reagents included in the STEMdiff™ T Cell Kit and must be combined with additional components, including either ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator (Catalog #10970) or ImmunoCult™ Human CD3/CD28 T Cell Activator (Catalog #10971), and Human Recombinant IL-15 (Catalog #78031).

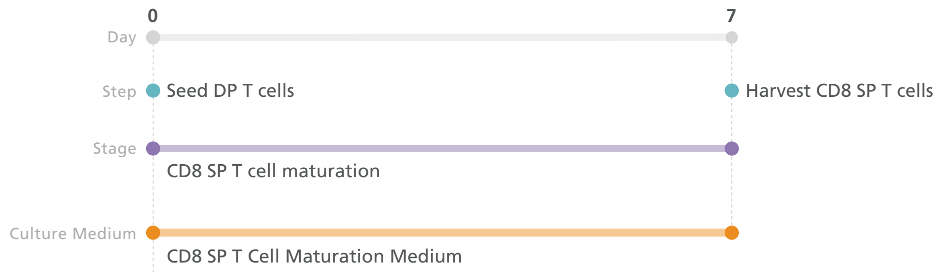


**Figure 9.** Optional CD8 SP T Cell Maturation Protocol

DP T cells are seeded in CD8 SP T Cell Maturation Medium with added ImmunoCult™ T Cell Activator on plates coated with StemSpan™ Lymphoid Differentiation Coating Material. CD8 SP T cells can be harvested on day 7.

### IIIb. Protocol for Optional CD8 SP T Cell Maturation

1. Prepare a freshly coated plate (See step 1 of IIIa. Protocol for T Cell Generation).

2. Prepare complete CD8 SP T Cell Maturation Medium (StemSpan™ SFEM II + StemSpan™ T Cell Progenitor Maturation Supplement + IL-15). See Technical Manual for details.

3. Add ImmunoCult™ T Cell Activator at half of the recommended concentration.

4. Dilute cells to 1 x $10^6$ cells/mL in complete CD8 SP T Cell Maturation Medium containing ImmunoCult™ T Cell Activator, seed onto the coated plate, and incubate at 37°C.

5. After 3 - 4 days of culture, add CD8 SP T Cell Maturation Medium, without ImmunoCult™ T Cell Activator.

6. Incubate at 37°C and harvest cells after 7 days.

## Cell Analysis

$CD7^+CD5^+$ lymphoid progenitor cells generated after 14 days of culture of PSC-derived $CD34^+$ cells (Figures 4 and 8), DP T cells generated during a second 14-day culture step (Figure 8), and CD8 SP T cells generated by following the optional protocol extension (Figure 9) are identified by flow cytometry. Cells may also be stained with antibodies directed against cell surface markers CD3, CD4, CD8α, CD8β, TCRαβ, CD45RA, and CD27 for analysis of T cell subsets (Figures 10 and 11). In the representative flow cytometry plots shown, dead cells were excluded by light scatter profile and DRAQ7™ staining.

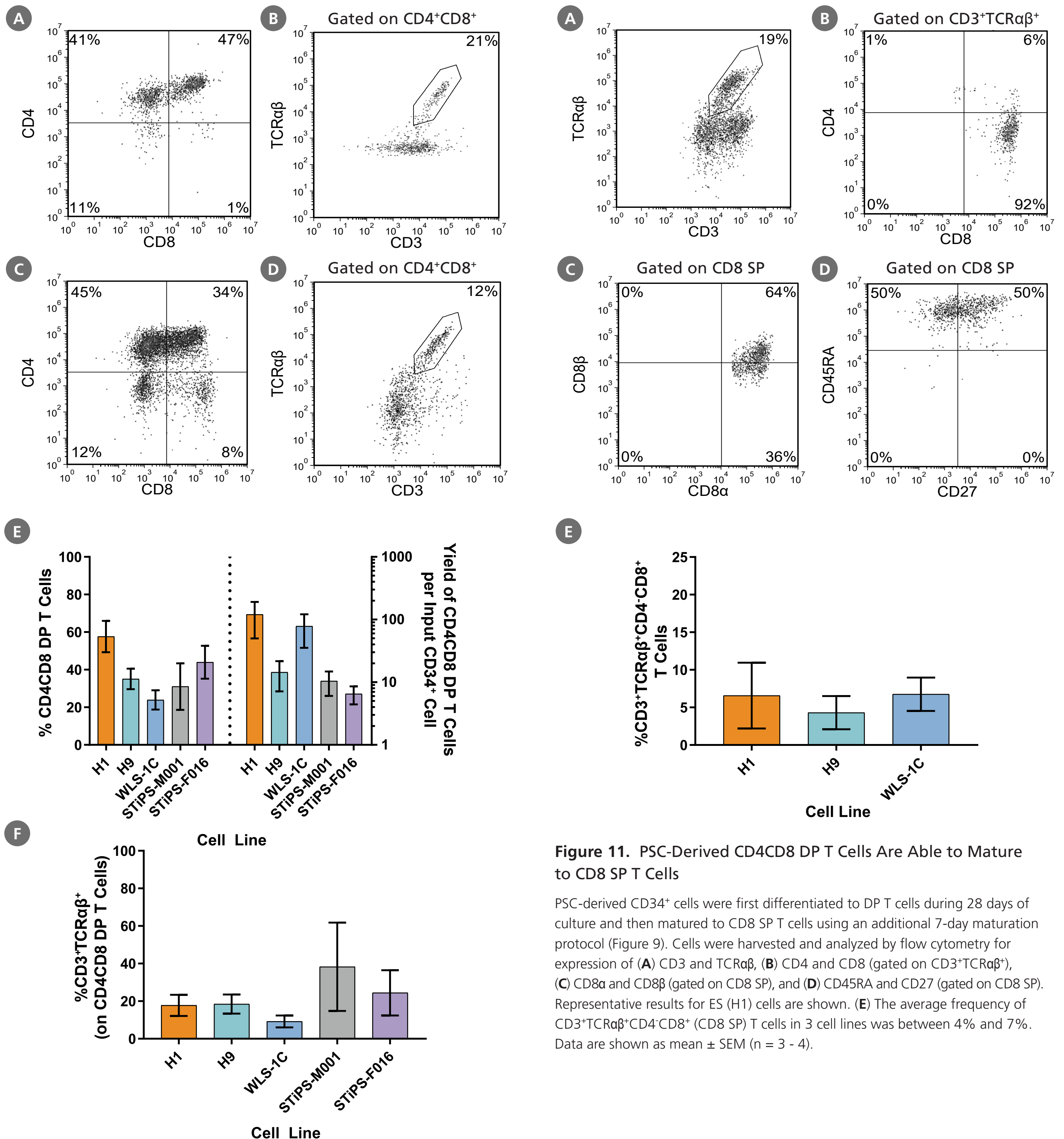


**Figure 10.** Generation of CD4CD8 DP T Cells From Human PSC-Derived CD34⁺ Cells After 28 Days of Culture

DP T cells were differentiated from PSC-derived CD34⁺ cells as described (Figure 8). Cells were harvested and analyzed for expression of CD3, CD4, CD8, and TCRαβ by flow cytometry. Representative flow cytometry plots are shown for (**A**, **B**) ES (H1)-derived and (**C**, **D**) iPS (WLS-1C)-derived cells. (**E**) The average frequency of viable CD4CD8 DP T cells on day 28 ranged between 24% and 58%, and the average yield of DP T cells produced per input PSC-derived CD34⁺ cell was between 7 and 120. (**F**) The average frequency of CD3⁺TCRαβ⁺ expressed on DP T cells ranged between 9% and 38%. Data are shown as mean ± SEM (n = 3 - 13).

**Figure 11.** PSC-Derived CD4CD8 DP T Cells Are Able to Mature to CD8 SP T Cells

PSC-derived CD34⁺ cells were first differentiated to DP T cells during 28 days of culture and then matured to CD8 SP T cells using an additional 7-day maturation protocol (Figure 9). Cells were harvested and analyzed by flow cytometry for expression of (**A**) CD3 and TCRαβ, (**B**) CD4 and CD8 (gated on CD3⁺TCRαβ⁺), (**C**) CD8α and CD8β (gated on CD8 SP), and (**D**) CD45RA and CD27 (gated on CD8 SP). Representative results for ES (H1) cells are shown. (**E**) The average frequency of CD3⁺TCRαβ⁺CD4⁻CD8⁺ (CD8 SP) T cells in 3 cell lines was between 4% and 7%. Data are shown as mean ± SEM (n = 3 - 4).

# Product Information

## Recommended Antibodies for Analysis*

| Product Name | Catalog # |
|---|---|
| Anti-Human CD34 Antibody, Clone 581 | 60013 |
| Anti-Human CD5 Antibody, Clone UCHT2 | 60082 |
| Anti-Human CD7 Antibody, Clone CD7-6B7 | N/A |
| Anti-Human CD16 Antibody, Clone 3G8 | 60041 |
| Anti-Human CD56 Antibody, Clone HCD56 | 60021 |
| Anti-Human CD158 (KIR) Antibody, Clone 180704 and/or HP-MA4 | N/A |
| Anti-Human CD335 (NKp46) Antibody, Clone 9E2 | N/A |
| Anti-Human CD336 (NKp44) Antibody, Clone P44-8 | N/A |
| Anti-Human CD337 (NKp30) Antibody, Clone P30-15 | N/A |
| Anti-Human NKG2D Antibody, Clone 1D11 | N/A |
| Anti-Human CD3 Antibody, Clone UCHT1 | 60011 |
| Anti-Human CD4 Antibody, Clone RPA-T4 | N/A |
| Anti-Human CD8a Antibody, Clone RPA-T8 | 60022 |
| Anti-Human CD8b Antibody, Clone SIDI8BEE | N/A |
| Anti-Human TCRαβ Antibody, Clone IP26 | N/A |

**Not included in the kit*

## Accessory Products*

| Product Name | Catalog # |
|---|---|
| Y-27632 | 72302 |
| AggreWell™400 6-Well (or 24-Well) Plate | 34421 |
| DMEM/F-12 with 15 mM HEPES | 36254 |
| Anti-Adherence Rinsing Solution | 07010 |
| ACCUTASE™ | 07920 |
| TrypLE™ Express | Thermo Fisher 12604013 |
| Collagenase Type II | 07418 |
| EasySep™ Human CD34 Positive Selection Kit II | 17856 |
| 37 µm Reversible Strainer, Large | 27250 |

**Not included in the kit*

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