

# StemSpan™ T Cell Generation Kit

**For expansion and differentiation of human CD34+ hematopoietic progenitor cells to T cells**

Catalog #09940	1 Kit
Catalog #09915	5 mL
Catalog #09925	250 µL
Catalog #09930	12.5 mL



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TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713

[INFO@STEMCELL.COM](mailto:INFO@STEMCELL.COM) • [TECHSUPPORT@STEMCELL.COM](mailto:TECHSUPPORT@STEMCELL.COM)

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## Product Description

StemSpan™ T Cell Generation Kit has been developed to differentiate CD34+ cells isolated from cord blood (CB) to T cells, without use of stromal cells. This kit typically promotes the expansion of thousands of CD3+TCRαβ+ T cells in cultures initiated with CD34+ human CB cells. At 6 weeks, the majority of these cells are CD4+CD8+ double-positive (DP) cells, and can mature to CD8+ single-positive (CD8 SP) T cells after an additional week of stimulation.

StemSpan™ Lymphoid Progenitor Expansion Supplement (10X) contains a combination of recombinant human cytokines and other additives formulated to selectively promote the expansion and differentiation of CD34+ cells isolated from human CB samples to CD7+CD5+ progenitor T (pro-T) cells. Additionally, StemSpan™ T Cell Progenitor Maturation Supplement (10X) enables further maturation of pro-T cells to DP cells. It also supports maturation of DP cells to CD8 SP T cells with additional stimuli. StemSpan™ Lymphoid Progenitor Expansion Supplement (10X) and StemSpan™ T Cell Progenitor Maturation Supplement (10X) are intended for use in combination with StemSpan™ SFEM II medium, and on plates coated with StemSpan™ Lymphoid Differentiation Coating Material (100X).

## Product Information

The following products are components of StemSpan™ T Cell Generation Kit (Catalog #09940) and are also available for individual sale.

PRODUCT NAME	CATALOG #	SIZE	STORAGE	SHELF LIFE
StemSpan™ Lymphoid Progenitor Expansion Supplement (10X) <sup>†</sup>	09915	5 mL	Store at -20°C.	Stable for 18 months from date of manufacture (MFG) on label.
StemSpan™ Lymphoid Differentiation Coating Material (100X) <sup>†</sup>	09925	2 x 250 µL	Store at -20°C.	Stable for 18 months from date of manufacture (MFG) on label.
StemSpan™ T Cell Progenitor Maturation Supplement (10X)	09930	12.5 mL	Store at -20°C.	Stable for 12 months from date of manufacture (MFG) on label.
StemSpan™ SFEM II <sup>††</sup>	09605	2 x 100 mL	Store at -20°C.	Stable for 18 months from date of manufacture (MFG) on label.

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

<sup>†</sup>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.

## Materials Required But Not Included

PRODUCT NAME	CATALOG #
D-PBS (Without Ca++ and Mg++)	37350
EasySep™ Human Cord Blood CD34 Positive Selection Kit II	17896
Trypan Blue	07050

## Preparation of Reagents and Materials

### StemSpan™ Lymphoid Progenitor Expansion Medium

Use sterile techniques to prepare StemSpan™ Lymphoid Progenitor Expansion Medium (StemSpan™ SFEM II + StemSpan™ Lymphoid Progenitor Expansion Supplement [10X]). The following example is for preparing 10 mL of medium. If preparing other volumes, adjust accordingly.

1. Thaw StemSpan™ SFEM II at room temperature (15 - 25°C) or overnight at 2 - 8°C. Mix thoroughly.  
NOTE: If not used immediately, aliquot into tubes and store at -20°C. Do not exceed the shelf life of the medium. After thawing aliquots, use immediately. Do not re-freeze.
2. Thaw StemSpan™ Lymphoid Progenitor Expansion Supplement (10X) at room temperature (15 - 25°C). Mix thoroughly.  
NOTE: If not used immediately, 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 supplement. After thawing aliquots, use immediately. Do not re-freeze.
3. Add 1 mL of Expansion Supplement to 9 mL of SFEM II. Mix thoroughly.  
NOTE: If not used immediately, store StemSpan™ Lymphoid Progenitor Expansion Medium at 2 - 8°C for up to 1 month. Do not freeze.

### StemSpan™ T Cell Progenitor Maturation Medium

Use sterile techniques to prepare StemSpan™ T Cell Progenitor Maturation Medium (StemSpan™ SFEM II + StemSpan™ T Cell Progenitor Maturation Supplement [10X]). The following example is for preparing 10 mL of medium. If preparing other volumes, adjust accordingly.

1. Thaw StemSpan™ SFEM II at room temperature (15 - 25°C) or overnight at 2 - 8°C. Mix thoroughly.  
NOTE: If not used immediately, aliquot into tubes and store at -20°C. Do not exceed the shelf life of the medium. After thawing aliquots, use immediately. Do not re-freeze.
2. Thaw StemSpan™ T Cell Progenitor Maturation Supplement at room temperature (15 - 25°C). Mix thoroughly.  
NOTE: If not used immediately, 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 supplement. After thawing aliquots, use immediately. Do not re-freeze.
3. Add 1 mL of Maturation Supplement to 9 mL of SFEM II. Mix thoroughly.  
NOTE: If not used immediately, store StemSpan™ T Cell Progenitor Maturation Medium at 2 - 8°C for up to 1 month. Do not freeze.

### StemSpan™ Lymphoid Differentiation Coating Material

Use sterile techniques to prepare StemSpan™ Lymphoid Differentiation Coating Material (Coating Material [100X] + D-PBS [Without Ca++ and Mg++]). The following example is for preparing 1 mL of Coating Material. If preparing other volumes, adjust accordingly.

1. Thaw StemSpan™ Lymphoid Differentiation Coating Material (100X) at room temperature (15 - 25°C). Mix thoroughly.  
NOTE: If necessary, centrifuge vial in a microfuge for 30 seconds to collect liquid from cap.  
NOTE: If not used immediately, 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 product. After thawing aliquots, use immediately or store at 2 - 8°C for up to 1 month. Do not re-freeze.
2. Add 10 µL of Coating Material to 990 µL of D-PBS (Without Ca++ and Mg++). Mix thoroughly. Use immediately.

## Protocol Diagram



## Directions for Use

Please read the entire protocol before proceeding. Use non-tissue culture-treated cultureware.

The following instructions are for 1 well of a 24-well plate. If using alternative cultureware, refer to Table 1 and adjust cell numbers and volumes accordingly.

For optimal performance, 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. Add 500 µL of StemSpan™ Lymphoid Differentiation Coating Material (see Preparation of Reagents and Materials) per well of a non-tissue culture-treated 24-well plate (e.g. Catalog #38042). Refer to Table 1 for volumes required for other types of cultureware.

**Table 1. Recommended Volumes of Coating Material and Medium and Recommended Cell Numbers for Various Cultureware**

NON-TISSUE CULTURE-TREATED CULTUREWARE	VOLUME OF COATING MATERIAL	VOLUME OF EXPANSION MEDIUM OR MATURATION MEDIUM	NUMBER OF CD34+ CELLS/Well
96-well plate (e.g. Catalog #38044)	100 µL/well	100 µL/well	$1 \times 10^3$
12-well plate (e.g. Catalog #38041)	1 mL/well	1 mL/well	$1 \times 10^4$
6-well plate (e.g. Catalog #38040)	2.5 mL/well	2.5 mL/well	$2.5 \times 10^4$

2. Incubate at room temperature (15 - 25°C) for 2 hours.  
NOTE: See Notes and Tips for an overnight coating method.
3. Aspirate Coating Material from the 24-well plate. Rinse well with D-PBS (Without Ca++ and Mg++). Aspirate D-PBS just prior to use.
4. Isolate CD34+ cells from fresh (less than 72 hours old) human CB using EasySep™ Human Cord Blood CD34 Positive Selection Kit II.  
NOTE: Frozen CD34+ cells from human CB may also be used.
5. Perform a viable cell count using Trypan Blue and a hemocytometer. Determine the % CD34+ cells by flow cytometry. To determine the concentration of CD34+ cells, multiply the % CD34+ cells by the viable cell count.
6. Add human CD34+ cells (isolated in step 4) to 500 µL of StemSpan™ Lymphoid Progenitor Expansion Medium (see Preparation of Reagents and Materials) at  $1 \times 10^4$  CD34+ cells/mL ( $5 \times 10^3$  CD34+ cells/well).  
NOTE: This cell suspension is for 1 well of a 24-well plate. If using other cultureware, refer to Table 1 for volumes and cell numbers required.
7. Add 500 µL of cell suspension (prepared in step 6) to 1 coated well of the 24-well plate prepared in steps 1 - 3. Incubate at 37°C.

### Day 3 or 4

8. Carefully add 500 µL of StemSpan™ Lymphoid Progenitor Expansion Medium per well of the 24-well plate. Incubate at 37°C.

### Day 7

Perform a half-medium change as follows:

9. Carefully remove 500 µL of medium from the well. Do not disturb cells.
10. Add 500 µL of StemSpan™ Lymphoid Progenitor Expansion Medium per well. Incubate at 37°C.

### Day 10 or 11

Perform a half-medium change as follows:

11. Carefully remove 500 µL of medium from the well. Do not disturb cells.
12. Add 500 µL of StemSpan™ Lymphoid Progenitor Expansion Medium per well. Incubate at 37°C.

### Day 14 - Harvest cells and reseed

13. 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 pro-T cells.

NOTE: Cells can be cryopreserved at this stage using CryoStor® CS10 (Catalog #07930). Refer to the CryoStor® CS10 Product Information Sheet (Document #29941) for freezing and thawing instructions. Once cells are thawed, continue to step 14.

14. Coat a non-tissue culture-treated 24-well plate with StemSpan™ Lymphoid Differentiation Coating Material (see steps 1 - 3).
15. Perform a viable cell count using Trypan Blue and a hemocytometer.
16. Add cells at  $1 \times 10^5$  cells/mL to 500 µL of StemSpan™ T Cell Progenitor Maturation Medium (see Preparation of Reagents and Materials).

NOTE: This cell suspension is for 1 well of a 24-well plate. If using other cultureware, refer to Table 1 for volumes required.

17. Add 500 µL of cell suspension (prepared in step 16) to 1 coated well of the 24-well plate prepared in step 14 ( $5 \times 10^4$  cells/well). Incubate at 37°C.

**Day 17 or 18**

18. Carefully add 500 µL of StemSpan™ T Cell Progenitor Maturation Medium per well. Incubate at 37°C.

**Day 21**

Perform a half-medium change as follows:

19. Carefully remove 500 µL of medium from the well. Do not disturb cells.  
20. Add 500 µL of StemSpan™ T Cell Progenitor Maturation Medium per well. Incubate at 37°C.

**Day 24 or 25**

Perform a half-medium change as follows:

21. Carefully remove 500 µL of medium from the well. Do not disturb cells.  
22. Add 500 µL of StemSpan™ T Cell Progenitor Maturation Medium per well. Incubate at 37°C.

**Day 28 - Harvest cells and reseed**

23. Coat a non-tissue culture-treated 24-well plate with StemSpan™ Lymphoid Differentiation Coating Material (see steps 1 - 3).  
24. Gently pipette cells up and down to ensure all cells are in suspension. Transfer cells to an appropriate tube.  
25. Perform a viable cell count using Trypan Blue and a hemocytometer.  
26. Add cells at  $5 \times 10^5$  cells/mL to 500 µL of StemSpan™ T Cell Progenitor Maturation Medium.  
NOTE: This cell suspension is for 1 well of a 24-well plate. If using other cultureware, refer to Table 1 for volumes required.  
27. Add 500 µL of cell suspension (prepared in step 26) to 1 coated well of the 24-well plate prepared in step 23 ( $2.5 \times 10^5$  cells/well). Incubate at 37°C.

**Day 31 or 32**

28. Carefully add 500 µL of StemSpan™ T Cell Progenitor Maturation Medium per well. Incubate at 37°C.

**Day 35**

Perform a half-medium change as follows:

29. At this stage there may be an accumulation of cellular debris floating at the center of the well. Carefully aspirate this debris, removing ~500 µL of medium. Be careful not to disturb cells.  
30. Add 500 µL of StemSpan™ T Cell Progenitor Maturation Medium per well. Incubate at 37°C.

**Day 38 or 39**

Perform a half-medium change as follows:

31. At this stage there may be an accumulation of cellular debris floating at the center of the well. Carefully aspirate this debris, removing ~500 µL of medium. Be careful not to disturb cells.  
32. Add 500 µL of StemSpan™ T Cell Progenitor Maturation Medium per well. Incubate at 37°C.

**Day 42**

33. Gently pipette cells up and down to ensure all cells are in suspension. Transfer cells to an appropriate tube. These DP cells are ready for assays or analysis as required. A protocol for CD8 SP T cell maturation is provided below.

**Further Maturation to CD8 SP T Cells (Optional):**

34. Coat a non-tissue culture-treated 24-well plate with StemSpan™ Lymphoid Differentiation Coating Material (see steps 1 - 3).  
35. Perform a viable cell count on cells harvested in step 33 using Trypan Blue and a hemocytometer.  
36. To prepare CD8 SP T Cell Maturation Medium for 1 well of a 24-well plate, combine the following:

- 500 µL StemSpan™ T Cell Progenitor Maturation Medium
  - 6.25 µL ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator (Catalog #10970)
- OR
- 6.25 µL ImmunoCult™ Human CD3/CD28 T Cell Activator (Catalog #10971)

NOTE: This is half the concentration recommended in the Product Information Sheets (Document #DX20348 and DX20349).

- 10 ng/mL Human Recombinant IL-15 (Catalog #78031)

37. Add cells at  $1 \times 10^6$  cells/mL to 500 µL of CD8 SP T Cell Maturation Medium (prepared in step 36).

NOTE: This cell suspension is for 1 well of a 24-well plate. If using other cultureware, refer to Table 1 for volumes required.

38. Add 500 µL of cell suspension (prepared in step 37) to 1 coated well of the 24-well plate prepared in step 34 ( $5 \times 10^5$  cells/well). Incubate at 37°C.

**Day 45 or 46**

39. Add 500 µL of StemSpan™ T Cell Progenitor Maturation Medium containing 10 ng/mL Human Recombinant IL-15 to each well. Incubate at 37°C.

NOTE: Do not add T Cell Activator at this step.

**Day 49**

40. Gently pipette cells up and down to ensure all cells are in suspension. Transfer cells to an appropriate tube. These cells should include CD8 SP T cells.

## Notes and Tips

- When coating cultureware with StemSpan™ Lymphoid Differentiation Coating Material, it may be incubated at 2 - 8°C overnight or at room temperature (15 - 25°C) for 2 hours, if desired.
- If cells reach confluency prior to the recommended harvest timepoint, reduce cell density by pipetting up and down, removing half of the medium including cells, and replacing with fresh medium.
- For determining % CD34+ cells prior to plating, use one of the following fluochrome-conjugated antibodies:
  - Anti-Human CD34 Antibody, Clone 581 (Catalog #60013)
  - Anti-Human CD34 Antibody, Clone 8G12 (Catalog #60121)
- For phenotype assessment of pro-T cells by flow cytometry, use the following fluorochrome-conjugated antibodies:
  - Anti-Human CD5 Antibody, Clone UCHT2 (Catalog #60082)
  - Anti-human CD7 antibody, clone CD7-6B7
- For phenotype assessment of more mature T cells by flow cytometry, use the following fluorochrome-conjugated antibodies:
  - Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011)
  - Anti-human CD4 antibody, clone RPA-T4
  - Anti-Human CD8a Antibody, Clone RPA-T8 (Catalog #60022)
  - Anti-human TCRαβ antibody, clone IP26

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