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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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)†	09915	5 mL	Store at -20°C.	Stable for 12 months from date of manufacture (MFG) on label.
StemSpan™ Lymphoid Differentiation Coating Material (100X)†	09925	2 x 250 µL	Store at -20°C.	Stable for 12 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*†	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).
†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 x 10^3
12-well plate (e.g. Catalog #38041)	1 mL/well	1 mL/well	1 x 10^4
6-well plate (e.g. Catalog #38040)	2.5 mL/well	2.5 mL/well	2.5 x 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 x 10^4 CD34+ cells/mL (5 x 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 x 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 x 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 x 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 x 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. Carefuly 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. Carefuly 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 x 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 x 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