StemSpan™ T Cell Progenitor Differentiation Kit

For expansion and differentiation of human CD34+ hematopoietic progenitor cells to the T cell lineage

Catalog #09900	1 Kit
Catalog #09910	10 mL
Catalog #09920	0.5 mL

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Product Description

StemSpan™ T Cell Progenitor Differentiation Kit has been developed for the differentiation of CD34+ cord blood (CB) and bone marrow (BM) hematopoietic progenitor cells to T cell progenitor (pro-T) cells. StemSpan™ T Cell Progenitor Differentiation Kit typically promotes greater than 2000-fold expansion of CD5+CD7+ pro-T cells in cultures seeded with CD34+ human CB cells. After 3 weeks, on average 84% of the cultured cells express CD5 and CD7, indicative of pro-T cells, with a further subset expressing CD1a, a marker used to characterize CD5+CD7+CD1a+ pre-T cells.

StemSpan™ T Cell 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 and BM samples to T lineage cells. StemSpan™ T Cell Progenitor Expansion Supplement is intended for use in combination with StemSpan™ SFEM II medium, and on plates coated with StemSpan™ T Cell Differentiation Coating Material (100X).

Product Information

The following products are components of StemSpan™ T Cell Progenitor Differentiation Kit (Catalog #09900) and are also available for individual sale.

PRODUCT NAME	CATALOG #	SIZE	STORAGE	SHELF LIFE
StemSpan™ T Cell Progenitor Expansion Supplement (10X)	09910	10 mL	Store at -20°C.	Stable for 12 months from date of manufacture (MFG) on label.
StemSpan™ T Cell Differentiation Coating Material (100X)	09920	0.5 mL	Store at -20°C.	Stable for 12 months from date of manufacture (MFG) on label.
StemSpan™ SFEM II*	09605	100 mL	Store at -20°C.	Stable for 18 months from date of manufacture (MFG) on label.

*StemSpan™ SFEM II is also available in a 500 mL size (Catalog #09655).

Preparation of Reagents and Materials

StemSpan™ T Cell Progenitor Expansion Medium

Use sterile techniques to prepare StemSpan™ T Cell Progenitor Expansion Medium (StemSpan™ SFEM II + StemSpan™ T Cell 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. Once aliquots are thawed, do not re-freeze.
2. Thaw StemSpan™ T Cell Progenitor Expansion Supplement (10X) at room temperature (15 - 25°C). Mix thoroughly.

   NOTE: If not used immediately, aliquot and store at 2 - 8°C for up to 1 month. Alternatively, store aliquots at -20°C. Do not exceed the shelf life of the supplement. After thawing aliquots, use immediately or store at 2 - 8°C for up to 1 month. 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™ T Cell Progenitor Expansion Medium at 2 - 8°C for up to 1 month. Do not freeze.

StemSpan™ T Cell Differentiation Coating Material

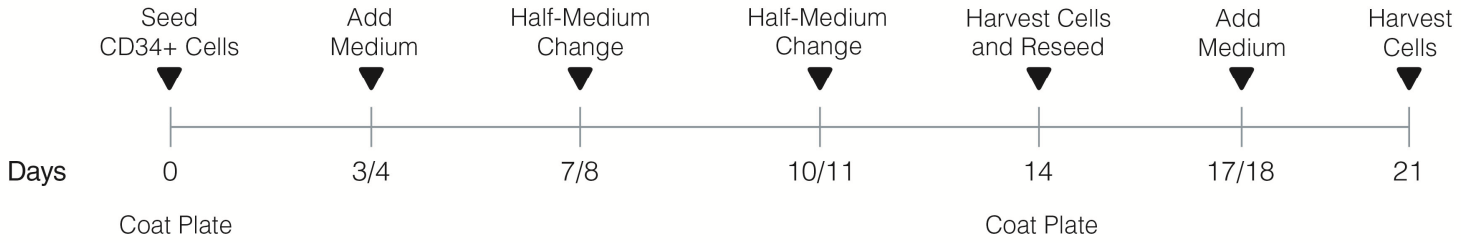
Use sterile techniques to prepare StemSpan™ T Cell Differentiation Coating Material (Coating Material [100X] + phosphate-buffered saline [PBS; Catalog #37350]). The following example is for preparing 1 mL of StemSpan™ T Cell Differentiation Coating Material. If preparing other volumes, adjust accordingly.

1. Thaw StemSpan™ T Cell 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, aliquot and store at 2 - 8°C for up to 1 month. Alternatively, store aliquots at -20°C. Do not exceed the shelf life of the Coating Material (100X). 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 (100X) to 990 µL of PBS. 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 one well of a 96-well plate. If using alternative cultureware, refer to Table 1 and adjust cell numbers and volumes accordingly.

Day 0

1. Add 100 µL of StemSpan™ T Cell Differentiation Coating Material (see Preparation of Reagents and Materials) per well of a non-tissue culture-treated 96-well plate. Refer to Table 1 for volumes required for other types of cultureware.

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

NON-TISSUE CULTURE-TREATED CULTUREWARE	VOLUME OF STEMSPAN™ T CELL DIFFERENTIATION COATING MATERIAL	VOLUME OF STEMSPAN™ T CELL PROGENITOR EXPANSION MEDIUM	NUMBER OF CD34+ CELLS/WELL (cord blood)	NUMBER OF CD34+ CELLS/WELL (bone marrow)
24-well plate	500 µL/well	500 µL/well	5 x 10^3	2.5 x 10^4
12-well plate	1 mL/well	1 mL/well	1 x 10^4	5 x 10^4
6-well plate	2.5 mL/well	2.5 mL/well	2.5 x 10^4	1.25 x 10^5

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 96-well plate. Add PBS to the coated wells. Aspirate PBS just prior to use.
4. Isolate CD34+ cells from fresh (less than 72 hours old) human CB or BM using one of the following EasySep™ positive selection cell separation kits:
   - Human CB : EasySep™ Human Cord Blood CD34 Positive Selection Kit II (Catalog #17896)
   - Human BM: EasySep™ Human CD34 Positive Selection Kit (Catalog #18056)

   NOTE: Frozen CD34+ cells from human CB or BM may also be used.
5. Perform a viable cell count using Trypan Blue (Catalog #07050) and a hemocytometer. Determine the % CD34+ cells by flow cytometry (see Notes and Tips). To determine the concentration of CD34+ cells, multiply the % CD34+ cells by the viable cell count.
6. Add purified human CD34+ cells to 100 µL of StemSpan™ T Cell Progenitor Expansion Medium (see Preparation of Reagents and Materials) as follows:
   - Human CB: 1 x 10^4 CD34+ cells/mL (1 x 10^3 CD34+ cells/well)
   - Human BM: 5 x 10^4 CD34+ cells/mL (5 x 10^3 CD34+ cells/well)

   NOTE: This cell suspension is for one well of a 96-well plate. If using other cultureware, refer to Table 1 for volumes and cell numbers required.

7. Add 100 µL of cell suspension (prepared in step 6) to one coated well of the 96-well plate (prepared in steps 1 - 3). Incubate at 37°C.

Day 3 or 4

8. Carefully add 100 µL of StemSpan™ T Cell Progenitor Expansion Medium per well of the 96-well plate. Incubate at 37°C.

Day 7 or 8

Perform a half-medium change as follows:

9. Carefully remove 100 µL of medium from well. Do not disturb cells.
10. Add 100 µL of StemSpan™ T Cell Progenitor Expansion Medium per well. Incubate at 37°C.

Day 10 or 11

Perform a half-medium change as follows:

11. Carefully remove 100 µL of medium from well. Do not disturb cells.
12. Add 100 µL of StemSpan™ T Cell Progenitor Expansion Medium per well. Incubate at 37°C.

Day 14

13. Gently pipette cells up and down to ensure all cells are in suspension. Transfer cells to an appropriate tube.

    NOTE: Harvested cells may now be used for downstream applications instead of reseeding and continuing culture for 1 week as described below. The optimal culture time is dependent on the application.
14. Coat a 96-well plate with StemSpan™ T Cell 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 100 µL of StemSpan™ T Cell Progenitor Expansion Medium.

    NOTE: This cell suspension is for one well of a 96-well plate. If using other cultureware, refer to Table 1 for volumes required.
17. Add 100 µL of cell suspension (prepared in step 16) to one coated well of the 96-well plate prepared in step 14 (1 x 10^4 cells/well). Incubate at 37°C.

Day 17 or 18

18. Carefully add 100 µL of StemSpan™ T Cell Progenitor Expansion Medium per well. Incubate at 37°C.

Day 21

19. Gently pipette cells up and down to ensure all cells are in suspension. Transfer cells to an appropriate tube. These pro/pre-T cells are ready for assays or analysis as required.

Notes and Tips

- When coating cultureware with StemSpan™ T Cell Differentiation Coating Material, it may be incubated at 2 - 8°C overnight instead of at room temperature for 2 hours, if desired.
- For determining % CD34+ cells prior to plating, use one of the following fluorochrome-conjugated antibodies:
  - Anti-Human CD34 Antibody, Clone 581 (Catalog #60013)
  - Anti-Human CD34 Antibody, Clone 8G12 (Catalog #60121)
- For phenotype assessment of pro/pre-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
  - Anti-human CD1a antibody, clone HI149