StemSpan™ NK Cell Generation Kit

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

Catalog #09960	1 Kit
Catalog #09915	5 mL
Catalog #09925	250 µL
Catalog #09950	500 µL

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Product Description

StemSpan™ NK Cell Generation Kit has been developed to differentiate CD34+ cells isolated from cord blood (CB) and bone marrow (BM) to natural killer (NK) cells, without use of stromal cells. This kit typically promotes the expansion of thousands of CD56+ NK cells in cultures initiated with CD34+ human CB cells.

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 and BM samples to lymphoid progenitor cells when used in combination with StemSpan™ SFEM II medium, and on plates coated with StemSpan™ Lymphoid Differentiation Coating Material (100X). Subsequently, StemSpan™ NK Cell Differentiation Supplement (100X) enables differentiation of lymphoid progenitor cells to NK cells.

Product Information

The following products are components of the StemSpan™ NK Cell Generation Kit (Catalog #09960) 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	250 µL	Store at -20°C.	Stable for 12 months from date of manufacture (MFG) on label.
StemSpan™ NK Cell Differentiation Supplement (100X)	09950	500 µL	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.

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

Materials Required But Not Included

PRODUCT NAME	CATALOG #
D-PBS (Without Ca++ and Mg++)	37350
Trypan Blue	07050
UM729	72332

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™ NK Cell Differentiation Medium

Use sterile techniques to prepare StemSpan™ NK Cell Differentiation Medium (StemSpan™ SFEM II + StemSpan™ NK Cell Differentiation Supplement [100X] + UM729). 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™ NK Cell Differentiation Supplement 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 supplement. After thawing aliquots, use immediately. Do not re-freeze.

3. Add 100 µL of Differentiation Supplement to 9.9 mL of SFEM II. Mix thoroughly.
4. Add UM729 to a final concentration of 1 µM. Mix thoroughly.

   NOTE: If not used immediately, store StemSpan™ NK Cell Differentiation Medium at 2 - 8°C for up to 3 weeks. 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.

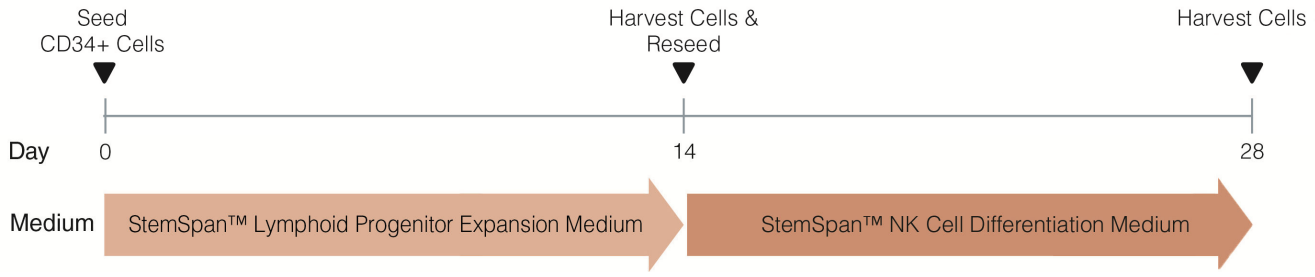
1. Thaw StemSpan™ Lymphoid Differentiation Coating Material (100X) at room temperature (15 - 25°C). Mix thoroughly.

   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.

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 DIFFERENTIATION MEDIUM	NUMBER OF CD34+ CELLS/WELL (cord blood)	NUMBER OF CD34+ CELLS/WELL (bone marrow)
96-well plate (e.g. Catalog #38044)	100 µL/well	100 µL/well	1 x 10^3	5 x 10^3
12-well plate (e.g. Catalog #38041)	1 mL/well	1 mL/well	1 x 10^4	5 x 10^4
6-well plate (e.g. Catalog #38040)	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 24-well plate. Rinse the well with D-PBS (Without Ca++ and Mg++). Aspirate D-PBS just prior to use.
4. If using frozen CD34+ cells from human CB or BM, thaw cells and proceed to step 5.

   OR

   If using fresh (less than 72 hours old) human CB or BM, isolate CD34+ cells 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)
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 (from step 4) to 500 µL of StemSpan™ Lymphoid Progenitor Expansion Medium (see Preparation of Reagents and Materials) as follows:

   • Human CB: 1 x 10^4 CD34+ cells/mL (5 x 10^3 CD34+ cells/well)

   • Human BM: 5 x 10^4 CD34+ cells/mL (2.5 x 10^4 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 lymphoid progenitors.

    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. Perform a viable cell count using Trypan Blue and a hemocytometer.
15. Add cells at 1 x 10^5 cells/mL to 500 µL of StemSpan™ NK Cell Differentiation 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.
16. Add 500 µL of cell suspension (prepared in step 15) to 1 well (5 x 10^4 cells/well) of a tissue-culture treated (non-coated) 24-well plate (e.g. Catalog #38017). Incubate at 37°C.

Day 17 or 18

17. Carefully add 500 µL of StemSpan™ NK Cell Differentiation Medium per well. Incubate at 37°C.

Day 21

Perform a half-medium change as follows:

18. Carefully remove 500 µL of medium from the well. Do not disturb cells.
19. Add 500 µL of StemSpan™ NK Cell Differentiation Medium per well. Incubate at 37°C.

Day 24 or 25

Perform a half-medium change as follows:

20. Carefully remove 500 µL of medium from the well. Do not disturb cells.
21. Add 500 µL of StemSpan™ NK Cell Differentiation Medium per well. Incubate at 37°C.

Day 28 - Harvest cells

22. Gently pipette cells up and down to ensure all cells are in suspension. Transfer cells to an appropriate tube. These NK cells are ready for assays or analysis as required.

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.
- UM729 is required in StemSpan™ NK Cell Differentiation Medium at a final concentration of 1 µM (as indicated in the Preparation section) to achieve optimal frequency and yield of CD56+ NK cells. UM171 (Catalog #72912) can be used as an alternative to UM729, at a final concentration of 100 nM.
- 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 NK cells by flow cytometry, use the following fluorochrome-conjugated antibodies:
  - Anti-Human CD16 Antibody, Clone 3G8 (Catalog #60041)
  - Anti-Human CD56 Antibody, Clone HCD56 (Catalog #60021)
  - Anti-human CD94 antibody, clone DX22
  - Anti-human CD158 (KIR) antibody, clone 180704 and/or HP-MA4
  - Anti-human CD335 (NKp46) antibody, clone 9E2
  - Anti-human CD336 (NKp44) antibody, clone P44-8
  - Anti-human CD337 (Nkp30) antibody, clone P30-15
  - Anti-human NKG2D antibody, clone 1D11