StemSpan™ Leukemic Cell Culture Kit

For culture, expansion, and drug screening of chronic and acute myeloid leukemia cells

Catalog #09720 1 Kit

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Description

StemSpan™ Leukemic Cell Culture Kit has been developed for the in vitro culture and expansion of malignant cells and has been tested on chronic myeloid leukemia (CML) and acute myeloid leukemia (AML) samples. This optimized protocol allows users to expand, culture, and use malignant cells for drug screening. StemSpan™ Leukemic Cell Culture Kit includes the serum-free medium SFEM II (Catalog #09605), StemSpan™ CD34+ Expansion Supplement (10X; Catalog #02691), and small molecule UM729 (Catalog #72332).

Product Information

The following components are sold as part of the StemSpan™ Leukemic Cell Culture Kit (Catalog #09720) and are also available for individual sale. For storage and stability information, please refer to the Product Information Sheets for the individual products.

PRODUCT NAME	CATALOG #	SIZE
StemSpan™ SFEM II	09605	100 mL
StemSpan™ CD34+ Expansion Supplement (10X)	02691	10 mL
UM729	72332	250 µg

Materials Required

PRODUCT	CATALOG #
StemSpan™ SFEM II	09605
StemSpan™ CD34+ Expansion Supplement (10X)	02691
UM729	72332
Dimethyl sulfoxide (DMSO)	e.g. Fisher Scientific D128-500
Iscove's Modified Dulbecco's Medium (IMDM)	36150
DNase I Solution (1 mg/mL)	07900
EasySep™ Human Cord Blood CD34 Positive Selection Kit II	17896
Trypan Blue	07050
15 mL conical tubes	e.g. 38009
Tissue culture-treated plates	38052 (12-well) and/or 38022 (96-well)
245 mm x 245 mm Square Treated Tissue Culture Dishes	27140
35 mm Culture Dishes	27100

Preparation of StemSpan™ Leukemic Cell Culture Medium

Use sterile techniques to prepare StemSpan™ Leukemic Cell Culture Medium (StemSpan™ SFEM II + StemSpan™ CD34+ Expansion Supplement (10X) + UM729). The following example is for preparing 100 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. After thawing aliquots, use immediately. Do not re-freeze.
2. Thaw StemSpan™ CD34+ Expansion Supplement (10X) at room temperature (15 - 25°C) until just thawed. Mix thoroughly.

   NOTE: If not used immediately, store at 2 - 8°C for up to 1 month. Alternatively, aliquot and store at -20°C. After thawing aliquots, use immediately. Do not re-freeze.
3. Add 10 mL of StemSpan™ CD34+ Expansion Supplement (10X) to 90 mL of StemSpan™ SFEM II. Mix thoroughly.
4. Add 250 µg of UM729 to 68 µL of fresh DMSO (final concentration 10 mM).

   NOTE: If not used immediately, aliquot into tubes and store at -20°C. After thawing aliquots, use immediately. Do not re-freeze.
5. Prepare a 1 in 100 dilution of the 10 mM UM729 stock solution by performing serial 1 in 10 dilutions, as follows:
   a. Dilute UM729 stock solution 1 in 10 in 30% DMSO in IMDM.
   b. Dilute the solution prepared in step (a) 1 in 10 in IMDM. This will give a final concentration of 100 µM UM729.
6. Add 10 µL of diluted UM729 (prepared in step 5) per mL of medium to give a final concentration of 1.0 µM UM729.

   NOTE: Titration of UM729 may be required to determine the optimal concentration for CD34+ cell expansion. UM729 enhances expansion of CD34+cells, in addition to more primitive subsets such as CD34+CD45RA-CD90+cells.

Directions for Use

Day 0

A. SAMPLE PREPARATION AND CD34+ CELL ISOLATION

NOTE: Do not wash cells prior to using EasySep™ kits, as this may result in cell loss.

1. Isolate CD34+ cells from AML or CML peripheral blood mononuclear cells (PBMCs) using EasySep™ Human Cord Blood CD34 Positive Selection Kit II.
2. 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.
3. For expansion of CD34+ cells, proceed to section B. Alternatively, proceed to section C for analysis.

B. EXPANSION OF CD34+ AML OR CML CELLS

1. Add purified CD34+ AML or CML cells (prepared in section A) to cultureware containing StemSpan™ Leukemic Cell Culture Medium as follows:
   - 12-well plate: 1 x 10^3 - 1 x 10^4 CD34+ AML or CML cells in 1 mL of medium per well
   - 96-well plate: 1 x 10^3 CD34+ AML or CML cells in 100 µL of medium per well
2. Incubate at 37°C for 7 days.

Day 7

3. Gently pipette cells up and down to ensure all cells are in suspension.
4. Transfer cells to 15 mL conical tubes. Add 1 mL IMDM and centrifuge at 300 x *g* for 10 minutes. Remove and discard supernatant.
5. Add IMDM to resuspend cells, as follows:
   - 12-well plate culture: Add 1 mL IMDM
   - 96-well plate culture: Add 100 µL IMDM
6. For analysis of harvested cells by flow cytometry or CFU assay, proceed to section C. If desired, proceed to step 7 for further expansion of CD34+ AML/CML cells.

OPTIONAL: Further expansion of CD34+ AML/CML cells

7. Add cell suspension to cultureware containing StemSpan™ Leukemic Cell Culture Medium as follows:
   - 12-well plate: Add 100 µL of cell suspension to a new plate containing 1 mL of medium per well
   - 96-well plate: Add 10 µL of cell suspension to a new plate containing 100 µL of medium per well
8. Incubate at 37°C for 7 days.

Day 14

9. Gently pipette cells up and down to ensure all cells are in suspension.
10. Transfer cells to 15 mL conical tubes. Add 1 mL IMDM and centrifuge at 300 x *g* for 10 minutes. Remove and discard supernatant.
11. Add an appropriate volume of IMDM to resuspend cells.
12. For analysis of harvested cells, proceed to section C.

C. ANALYSIS

Flow Cytometry

The percentage and total cell numbers of hematopoietic stem and progenitor cell (HSPC) populations (CD45+, CD34+, CD34+CD90+CD45RA-) as well as aldehyde dehydrogenase (ALDH) activity (optional) can be analyzed simultaneously using flow cytometry.

NOTE: If labeling multiple surface markers (e.g. HSPC markers) in parallel with the ALDEFLUOR™ assay, at least 1 x 10^4 CD34+ AML/CML cells are required.

1. Obtain non-expanded or expanded CD34+ AML or CML cells as described in section A or B, respectively.
2. OPTIONAL: Using the ALDEFLUOR™ Kit (Catalog #01700), perform an ALDEFLUOR™ assay as described in section C of Directions For Use in the corresponding Product Information Sheet (PIS).
3. Incubate cells (resuspended in IMDM or ALDEFLUOR™ Assay Buffer) with surface marker antibodies at 4°C for 15 minutes.
4. Proceed with flow cytometry.

Colony-Forming Unit (CFU) Assay

For complete instructions on setup of human CFU assays and counting and classification of colonies, refer to the Technical Manual: Human Colony-Forming Unit (CFU) Assays Using MethoCult™, available at www.stemcell.com or contact us to request a copy.

1. Obtain non-expanded or expanded CD34+ AML or CML cells as described in section A or B, respectively.
2. Perform a viable cell count using Trypan Blue and a hemocytometer.
3. Prepare a CD34+ AML or CML cell suspension in MethoCult™ H4435 Enriched (Catalog #04435) as follows:
   - For non-expanded purified CD34+ AML or CML cells: 500 - 1500 cells/mL
   - For 7-day-expanded CD34+ AML or CML cells: 750 - 1500 cells/mL
   - For 14-day-expanded CD34+ AML or CML cells: 1500 - 2000 cells/mL
4. Add 1.1 mL of CD34+ AML or CML cell suspension (prepared in step 3) per 35 mm Culture Dish or per well of a SmartDish™ plate (Catalog #27370).

   NOTE: Yield will be approximately 40 - 100 colonies per well or dish.
5. Incubate at 37°C for 14 days.
6. Count colonies.

   NOTE: Single-colony quantitative reverse transcription PCR (qRT-PCR) may be performed for genotyping (e.g. quantifying BCR-ABL+ colonies).

Drug Screening Assay

Both non-expanded and expanded (7 days and 14 days) CD34+ AML or CML cells can be used in drug screening to assess test compounds against leukemic cells. Analysis methods in drug screening assay may include automated cell counting, plate reader-based, or absolute cell counting of labeled cells (e.g. 7-ADD-, CD45+, or CD34+ cells) using a flow cytometer.

1. Prepare StemSpan™ Leukemic Cell Culture Medium.
2. Dissolve or dilute the test compound in an appropriate solvent, to at least 1000X the concentration at which it will be tested in culture. This is the test compound stock solution. Different dilutions may need to be prepared depending on the solubility of the test compound and the required concentration range.

   NOTE: A stock solution ≥ 1000X the test concentration will ensure that the final solvent concentration in the culture will be ≤ 0.1%. If DMSO is the solvent, a DMSO concentration of ≤ 0.1% will not affect cell growth.
3. Prepare a 2X test compound solution by diluting the test compound stock solution (prepared in step 2) in StemSpan™ Leukemic Cell Culture Medium.

   NOTE: Prepare sufficient volume of 2X test compound solution for replicate wells at 100 µL/well. Three replicate wells are recommended for each test compound. The IC_{50} of a test compound can be determined by using a 7-point 3-fold compound dilution series.

4. Prepare a solvent control by diluting solvent in StemSpan™ Leukemic Cell Culture Medium to the same concentration as the solvent in the 2X test compound solution.
5. Obtain non-expanded or expanded CD34+ AML or CML cells as described in section A or B, respectively.
6. Prepare a cell suspension in StemSpan™ Leukemic Cell Culture Medium at a concentration of 1000 - 8000 viable CD34+ AML or CML cells/100 µL (10,000 - 80,000 cells/mL).

   NOTE: Prepare at least 10 mL of cell suspension for each 96-well plate to ensure that there is a sufficient volume to seed the required number of wells for the experiment (100 µL/well).
7. Mix the cell suspension (prepared in step 6) immediately before use. Add 100 µL of the cell suspension to each well of the 96-well flat-bottom plate.
8. Add 100 µL of the appropriate 2X test compound solution or solvent control to each well.

   NOTE: Three replicate wells are recommended for each test compound.
9. Mix the cell suspension with test compound solution by pipetting up and down.
10. Place each 96-well plate in a 245 mm x 245 mm Square Treated Tissue Culture Dish. Within this outer tissue culture dish, surround the 96-well plate with 4 x 35 mm Culture Dishes containing ~3 mL of sterile water.
11. Incubate at 37°C in 5% CO_2 and > 95% humidity for 7 days.
12. Label cells with multiple surface markers (e.g. HSPC markers) in parallel with the ALDEFLUOR™ assay and proceed with flow cytometry or an alternative analysis method for the drug screening readout.

Related Products

For related products, including specialized cell culture and storage media, matrices, antibodies, cytokines, and small molecules, visit www.stemcell.com/hPSCworkflow or contact us at techsupport@stemcell.com.