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StemPro®-34 Medium and CD34+ Cell Kit

Catalog Numbers A14059 **Publication Part Number** MAN0005808 **Revision Date** 9 November 2011

Description

StemPro®-34 SFM is a serum-free medium developed to support the growth of human hematopoietic progenitor cells (e.g., CD34+). The formulation was optimized using freshly isolated CD34+ bone marrow cells from normal donors. A 40X nutrient supplement is supplied as a frozen liquid (–5 to –20°C). These two components are combined at the time of use. L-Glutamine (Cat. no. 25030) must be added at the time of use to a final concentration of 2 mM. StemPro®-34 SFM is manufactured without cytokines and hematopoietic growth factors, giving the investigator the freedom to use any factor or combination of factors required in ones' studies.

StemPro® CD34+ cells are human hemopoietic progenitor cells (HPCs) derived from the human umbilical cord blood of mixed donors. After the cord blood is pooled, an enriched CD34+ cell population is isolated using immunomagnetic CD34 MicroBeads. StemPro® CD34+ cells have ≥90% purity as determined by Flow Cytometry and validated for use in reprogramming to generate iPSCs with the Cytotune™-iPS Reprogramming Kit (Cat. no. A13780-01), in addition to their use in hematopoietic stem cell studies.

Kit Name/Components*	Catalog no./Part no.	Amount	Storage
StemPro®-34 Medium and CD34+ Cell Kit includes:	**A14059**	**1 kit**	
StemPro®-34 Nutrient Supplement (40X)	10641-025	13 mL	Store at –5°C to –20°C. Protect from light.
StemPro®-34 SFM (1X)	10640-019	500 mL	Store at 2–8°C. Protect from light.
StemPro® CD34+ cells (0.5 × 10^6 cells/vial)**	A14058	1 vial	Liquid nitrogen

* The nutrient supplement may be thawed, aliquoted, and refrozen once. Do not subject the nutrient supplement to repeated freeze/thaw cycles. When aliquoting, care must be taken to reduce the potential for dehydration. We recommend storing the nutrient supplement in a well sealed container with minimal head space. Most frost-free freezers have heat/cool cycles that will impact the product. We recommend storing the nutrient supplement in a **non-frost-free** freezer at –20°C.

** StemPro® CD34+ cells are available only as part of the StemPro®-34 Medium and CD34+ Cell Kit and are not sold separately. For additional custom formats, contact Technical Support at **techsupport@lifetech.com**.

Product Use

For research use only. CAUTION: Not intended for human or animal diagnostic or therapeutic uses.

Features

The StemPro®-34 Medium and Cells Kit has the following features and benefits:

- StemPro®-34 SFM is used to study hematopoietic cells from bone marrow, peripheral blood, or cord blood
- Offers superior expansion of HSCs
- Formulated with human, human recombinant, or synthetic materials

Preparing Complete StemPro®-34 Medium

1. Thaw the frozen StemPro®-34 Nutrient Supplement at 4°C overnight.
2. After thawing, mix the supplement well by gently inverting the vial a couple of times, and then aseptically transfer the entire contents of the vial to the bottle of StemPro®-34 SFM. Swirl the bottle to mix and to obtain a homogenous complete medium.
3. Aseptically add L-Glutamine (Cat. no. 25030) to a final concentration of 2 mM (5 mL of 200 mM L-Glutamine to 500 mL of medium).

   Note: For hemopoietic stem cell (HSC) culture, we recommend adding the cytokines SCF, IL-3, and GM-CSF into the medium. The recommended concentrations are 100 ng/mL for SCF, 50 ng/mL for IL-3, and 25 ng/mL for GM-CSF.

Shelf Life

The complete medium has a shelf life of 30 days when stored at 2–8°C, in the dark. The thawed nutrient supplement has a shelf life of 14 days when stored at 2–8°C, in the dark.

Thawing Cryopreserved StemPro® CD34+ cells

We recommend seeding the cells at **≥1 × 10^4 cells/mL** for the initial recovery passage. Thawing medium for StemPro® CD34+ cells is D-PBS (without Ca^{2+} or Mg^{2+}) supplemented with 0.1% BSA. To avoid cell clumping, DNAse I may be added to the thawed cells (see Step 4, below).

1. Warm up thawing medium in 37°C water bath.
2. Remove cryotube from liquid nitrogen, place the tube on ice and immediately transfer into a 37°C water bath. Hold the tube in the surface of the water bath while gently swirling.

   Note: Do not leave the cryotube unattended during the thawing process and do not thaw more than two tubes at the same time.
3. Take out the cryotube from water bath when only a tiny ice crystal is left and transfer it into a biosafety hood. Disinfect the outside of the cryotube with 70% isopropyl alcohol.
4. *Optional*: To avoid cell clumping, add 300 µg of DNAse I to a 15-mL conical tube into which you will transfer the cells.

   Note: DNAse I is not needed if cells are used for purification of genomic DNA or RNA.

Thawing Cryopreserved StemPro® CD34+ cells, continued

5. Aseptically transfer the cells in the cryotube to the 15-mL conical tube. Rinse the cryotube with 1 mL of warm thawing medium, and slowly add the rinse to the cells drop-wise (5 seconds per drop), while gently flicking the tube to mix.
6. Slowly add warm thawing medium to the cells drop-by-drop until the total volume is 15 mL. Drop-wise addition of media prevents osmotic damage to the cells by gradually diluting the DMSO in the freezing medium and allows sufficient time for cells to rehydrate.

   Note: Do not use cold thawing medium because it will cause cell damage. Warm medium will prevent loss in cell viability.
7. Centrifuge the cells at 200 × *g* at room temperature for 10 minutes.
8. Discard the supernatant and gently tap the tube to dislodge the pellet.
9. Wash the cell pellet with 10–15 mL of warm thawing medium.
10. Centrifuge the tube at 200 × *g* at ambient temperature for 10 minutes.
11. Carefully remove all but 2–3 mL of the supernatant. Gently resuspend the cells in the remaining supernatant and proceed to cell count.
12. Aseptically remove 10 µL of the cell suspension from the tube and mix with 10 µL of trypan blue. Count the number of cells using a hemacytometer or the Countess® Automated Cell Counter to determine the viability and total number of cells recovered from the vial.
13. Seed the cells at a density of 1×10^4 cells/mL in complete StemPro®-34 medium for the initial recovery passage and incubate at 37°C. For subsequent passages, seed the cells at 0.5×10^6 cells/mL.

Reprogramming StemPro® CD34+ cells using the CytoTune™-iPS Reprogramming Kit

Day –3: Seed cells

1. 3 days before transduction, thaw 1 vial of StemPro® CD34+ cells (0.5×10^6 cells) and gently transfer into one well of 24-well culture plate. Drop-wise add 1 mL of StemPro®-34 SFM to the cells while gently agitating to mix.

   Note: We recommend using the wells in the middle section of the 24-well plate to prevent excessive evaporation of the mediun during incubation.
2. Centrifuge the cell suspension at 200 × *g* for 10 minutes, discard the supernatant, and resuspend the cells in 1 mL of complete StemPro®-34 medium containing cytokines (i.e., SCF, IL-3, and GM-CSF).

   Note: The recommended final concentrations for the cytokines are 100 ng/mL for SCF, 50 ng/mL for IL-3, and 25 ng/mL for GM-CSF.

Day –2: Observe cells and add fresh medium

3. 2 days before transduction, count the cells to ensure that they are expanding and add 0.5 mL of fresh complete StemPro®-34 medium containing cytokines without disturbing the cells.

Day –1: Observe cells and add fresh medium

4. A day before transduction, count the cells to ensure that they are continuing to expand, gently remove 0.5 mL of media, and add 1.0 mL of fresh complete StemPro®-34 medium containing cytokines without disturbing the cells.

Day 0: Count cells and perform transduction

5. Count the number of cells using a hemacytometer or the Countess® Automated Cell Counter to determine the viability and total number of cells; the cells should have more than doubled in number.
6. Harvest the cells and seed the wells of a 24-well plate with 2.5×10^5 cells/well for transduction.
7. Transduce the cells overnight by adding each of the four CytoTune™ Sendai viruses at an MOI of 3–5 with 4 µg/mL of Polybrene in a volume of <0.3 mL.

Day 1: Remove CytoTune™ Sendai virus and culture cells

8. Remove the CytoTune™ Sendai viruses by centrifugating the cells at 400 × *g* for 10 minutes and resuspending the cells in 0.5 mL of complete StemPro®-34 medium containing cytokines in the 24-well plate.
9. Culture the cells at 37°C for 2 days.

   Note: While the cells are incubating (1–2 days before passaging the transduced cells), prepare two 100-mm MEF culture dishes for each well containing transduced cells.

Day 3: Plate cells on MEF culture dishes

10. Count the cells using the desired method (e.g., Countess® Automated Cell Counter), and seed the MEF culture dishes with 5×10^4 and 2×10^5 cells per 100-mm dish in 10 mL of StemPro®-34 medium without cytokines. Incubate the cells at 37°C.

Day 3–6: Replace spent medium

11. Every other day, gently remove 5 mL of medium from the cells and replace with 5 mL of fresh StemPro®-34 medium without cytokines.

Day 7: Start transition to human iPSC medium

12. Prepare 100 mL of complete human iPSC medium by aseptically mixing the components listed below. Complete human iPSC medium can be stored at 2–8°C for up to 1 week.

    Note: The volumes given are accurate only for Life Technologies products listed in Related Products, below.

KnockOut™ D-MEM/F-12	89 mL
KnockOut™ Serum Replacement	10 mL
MEM Non-Essential Amino Acids Solution	1 mL
GlutaMAX™-I Supplement	1 mL
β-Mercaptoethanol	182 µL
Penicillin-Streptomycin (*optional*)	1 mL
Basic FGF*	40 µL

    * Prepare the iPSC medium without bFGF, and then supplement with fresh bFGF when the medium is used.
13. Remove 5 mL of medium from the cells and add 5 mL of human iPSC medium to transition the cells to the new culture medium.

Day 8: Complete transition to human iPSC medium and expand cells

14. Completely remove the media from the cells and replace with 10 mL of human iPSC medium.
15. Continue culturing the cells and replace the spent medium everyday. Transformed colonies should become apparent by day 15 post-transduction.

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Related Products

Product	Catalog number	Amount
L-glutamine, 200 mM	25030081 25030164	100 mL 20 × 100 mL
Recombinant human SCF	PHC2111 PHC2116 PHC2115	100 µg 25 µg 10 µg
Recombinant human IL-3	PHC0033 PHC0031 PHC0035 PHC0034	1 mg 100 µg 50 µg 10 µg
Recombinant human GM-CSF	PHC2013 PHC2011 PHC2015	1 mg 100 µg 10 µg
StemPro®-34 SFM (1X), liquid	10639-011	500 mL
KnockOut™ D-MEM/F-12	10660-012	500 mL
KnockOut™ Serum Replacement	10828-028	500 mL
MEM Non-Essential Amino Acids Solution (10 mM)	11140-050	100 mL
GlutaMAX™-I Supplement	35050-061	100 mL
β-Mercaptoethanol (1000X), liquid	21985-023	50 mL
Penicillin-Streptomycin, liquid	15140-122	100 mL
FGF-basic, AA 1-155 Recombinant Human	PHG0264	10 µg
Cytotune™-iPS Reprogramming Kit	A13780-01	1 kit

Performance Testing

StemPro®-34 SFM and StemPro®-34 Nutrient Supplement are tested at the time of manufacture for pH, osmolality, endotoxin and sterility. In addition, each lot of medium and 40X frozen nutrient supplement is performance tested in an application specific assay of cell growth under defined conditions, utilizing $CD34^+$ cells isolated from freshly drawn human bone marrow from normal donors.

For additional information and protocols, refer to **www.lifetechnologies.com**.

Technical Support

For additional product and technical information, such as Safety Data Sheets (SDS), Certificates of Analysis, etc., visit our website at **www.lifetechnologies.com**. For further assistance, email our Technical Support team at **techsupport@lifetech.com**.

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