StemPro™-34 medium and CD34+ cell kit

Catalog Number A14059

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Description

StemPro™-34 SFM™ is a serum-free medium developed to support the growth of human hematopoietic progenitor cells (for example, 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 (-20℃ to -5℃). These two components are combined at the time of use. L-Glutamine 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, in addition to their use in hematopoietic stem cell studies.

Table 1 StemPro™-34 Medium and CD34+ Cell Kit (A14059)

Kit Components	Catalog no./Part no.	Amount	Storage	Shelf life[1]
StemPro™-34 Nutrient Supplement (40X)	10641-025	13 mL	–20℃ to –5℃; Protect from light	30 months
StemPro™-34 SFM™ (1X)	10640-019	500 mL	Store at 2℃ to 8℃; Protect from light	18 months
StemPro™ CD34+ cells (0.5 × 10^6 cells/vial)[2]	A14058	1 vial	Liquid nitrogen, vapor-phase	—

[1] Shelf life duration is determined from Date of Manufacture.

[2] 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.

Important guidelines for thawing and storing cells

- Upon receipt, immediately thaw cells or place into vapor-phase liquid nitrogen storage until ready to use. **Do not store the cells at –80°C**.

Important information

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

Prepare complete StemPro™-34 medium

1. Thaw the frozen StemPro™-34 Nutrient Supplement at 4℃ 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 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.

Use

Thaw cryopreserved StemPro™ CD34+ cells

We recommend seeding the cells at **≥1 × 10^4 cells/mL** for the initial recovery passage. The 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 on page 2).

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.

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.

Reprogram 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 medium 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

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

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

1. 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.

2. Harvest the cells and seed the wells of a 24-well plate with 2.5 × 10^5 cells/well for transduction.

3. 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

1. 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.

2. 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

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

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

1. 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 products listed in the Related products Table on page 3.

KnockOut™ D-MEM™/F-12	78 mL
KnockOut™ Serum Replacement	20 mL
MEM™ Non-Essential™ Amino Acids Solution	1 mL
GlutaMAX™-I Supplement	1 mL
ß-Mercaptoethanol	182 µL
Penicillin-Streptomycin (*optional*)	1 mL
Basic FGF[1]	40 µL

[1] Prepare the iPSC medium without bFGF, and then supplement with fresh bFGF when the medium is used.

2. 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

1. Completely remove the media from the cells and replace with 10 mL of human iPSC medium.

2. Continue culturing the cells and replace the spent medium every day. Transformed colonies should become apparent by day 15 post-transduction.

Related products

Product	Catalog no.
L-glutamine, 200 mM	25030
Recombinant human SCF	PHC211
Recombinant human IL-3	PHC00
Recombinant human GM-CSF	PHC201
StemPro™-34 SFM™ (1X), liquid	10639
KnockOut™ D-MEM™/F-12	12660
KnockOut™ Serum Replacement	10828
MEM™ Non-Essential™ Amino Acids Solution (10 mM)	11140
GlutaMAX™-I Supplement	35050
ß-Mercaptoethanol (1000X), liquid	21985
Penicillin-Streptomycin, liquid	15140
FGF-basic, AA 1-155 Recombinant Human	PHG0264
CytoTune™-iPS Reprogramming Kit	A13780

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Limited product warranty

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