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by life technologies

StemPro® MSC SFM XenoFree

Description

StemPro® MSC SFM XenoFree is specially formulated for the growth and expansion of human mesenchymal stem cells (MSCs) and adipose-derived stem cells (ADSCs) under completely serum-free and xeno-free conditions. Using StemPro® MSC SFM XenoFree, human MSCs or ADSCs can be expanded for multiple passages while maintaining their multipotent phenotype (i.e., ability to differentiate into osteogenic, chondrogenic and adipogenic lineages).

Product	Cat. no./Part no.	Amount	Storage	Shelf Life*
StemPro® MSC SFM XenoFree Kit Contains:	**A10675-01****	1 kit	—	—
StemPro® MSC SFM Basal Medium	A13829-01	1 × 500 mL	2°C to 8°C; Protect from Light	24 months
StemPro® MSC SFM XenoFree Supplement	A11577-01	1 × 5 mL	–20°C to –5°C; in the dark	12 months

* Shelf Life has been determined from Date of Manufacture.

** StemPro® MSC SFM XenoFree is sold as a complete kit, components are not sold separately.

Product use

For Research Use Only. Not for use in diagnostic procedures.

Important information

- Thaw StemPro® MSC SFM XenoFree Supplement overnight at 2°C to 8°C prior to use (thawed supplement will have a slightly cloudy appearance). Use thawed material immediately or aliquot (i.e., 1 mL) unused material and store at –20°C to –5°C. Avoid additional freeze-thaw cycles.
- StemPro® MSC SFM XenoFree complete medium (StemPro® MSC SFM Basal Medium, StemPro® MSC SFM XenoFree Supplement, and GlutaMAX™-I CTS™ supplement) is stable for 2 weeks when stored in the dark at 2°C to 8°C.

Safety information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Caution: Human origin materials are non-reactive (donor level) for anti-HIV 1 & 2, anti-HCV, and HBsAg. Handle in accordance with established bio-safety practices.

Prepare complete medium

StemPro® MSC SFM Basal Medium requires supplementation with StemPro® MSC SFM XenoFree Supplement and GlutaMAX™-I CTS™ supplement or L-Glutamine.

1. For 500 mL complete medium, aseptically add 5 mL of StemPro® MSC SFM XenoFree Supplement to StemPro® MSC SFM Basal Medium (500 mL).
2. Aseptically add 5 mL GlutaMAX™-I CTS™ supplement or 200 mM L-Glutamine to the complete medium before use.
3. If so desired, add 50 µL of 50 mg/mL Gentamicin Reagent Solution to the complete medium.

Culture conditions

Media: StemPro® MSC SFM XenoFree complete medium

Cell Line(s): Human mesenchymal stem cells (MSCs) or adipose-derived stem cells (ADSCs)

Culture Type: Adherent

Culture Vessels: CELLstart™ CTS™ Substrate-coated T-Flasks.

Temperature Range: 36°C to 38°C

Incubator Atmosphere: Humidified atmosphere of 4–6% CO_2 in air. Ensure proper gas exchange and minimize exposure of cultures to light.

Note: Procedures detailed in the following sections are for cultures in T-75 culture flasks (75 cm^2). Volumes should be adjusted accordingly for desired vessel size.

Recovering cryopreserved human MSCs

1. Rapidly thaw a frozen vial of human MSCs in a 37°C water bath until a small amount of ice remains.
2. Pipet the entire contents of the cryovial into a 50-mL conical tube.
3. Carefully add 5–10 mL of pre-warmed (37°C) StemPro® MSC SFM XenoFree complete medium to the conical tube at an approximate rate of 3 to 5 drops per 5 seconds and gently swirl after every addition.
4. Centrifuge the tubes at 100–200 × *g* for 5 minutes at room temperature.
5. Resuspend the cell pellet in pre-warmed (37°C) StemPro® MSC SFM XenoFree complete medium and add the cell suspension to an appropriate CELLstart™ CTS™ substrate -coated flask at a density of ≥ 5×10^3 cells/cm^2 (see **Coating culture flasks with CELLstart™ CTS™ Substrate**, next page).

   Note: For the initial isolation of MSCs, supplementation of the complete medium with 2.5% human AB serum facilitates cell attachment and growth. For subsequent passages, human AB serum is not required.
6. Incubate at 36°C to 38°C in a humidified atmosphere of 4–6% CO_2 in air.
7. Replace the medium in the flasks every 2 days.

Guidelines for subculturing cells in StemPro® MSC SFM XenoFree complete medium

- StemPro® MSC SFM XenoFree has been developed for the multi-passage expansion of human bone marrow-derived MSCs and Adipose-derived Stem Cells (ADSCs) at greater than clonal densities (**≥ 5×10^3 cells/cm^2**). Reduced seeding densities may result in suboptimal cell expansion, although optimal growth conditions must be determined for each application.
- When subculturing human MSCs in StemPro® MSC SFM XenoFree, input cell confluence should be 60–90%, cell viability should be at least 90% and the growth rate should be in mid-logarithmic phase prior to subculture. Initiating cultures under suboptimal conditions may affect product performance. Transitioning MSCs or ADSCs from serum-containing medium to StemPro® MSC SFM XenoFree does not require an adaptation protocol.
- For optimal performance, re-feed the cultures **every 2 days** with StemPro® MSC SFM XenoFree complete medium.

  Note: Procedures detailed on the next page are for cultures maintained in a T-75 culture flask (75 cm^2). Adjust the volumes accordingly for desired vessel size.

Coating culture flasks with CELLstart™ CTS™ Substrate

1. Dilute CELLstart™ CTS™ Substrate 1:100 in Dulbecco's Phosphate Buffered Saline (DPBS) CTS™ with Ca^{2+} and Mg^{2+} (i.e., 100 µL CELLstart™ CTS™ substrate into 10 mL of DPBS CTS™). Pipet gently to mix. **Do not vortex.** Coat T-75 culture flasks by adding 10 mL of the CELLstart™ CTS™ substrate solution to each flask.
2. Place culture flasks with the CELLstart™ CTS™ substrate solution in the incubator at 36°C to 38°C in a humidified atmosphere of 4–6% CO_2 for 60 minutes.
3. After incubation, remove the flasks from the incubator and temporarily place them in a laminar flow hood until use. Immediately before use, remove all CELLstart™ CTS™ substrate solution and replace it with complete medium.

   Note: Do not store diluted CELLstart™ CTS™ substrate solution; prepare it freshly before each use.

Propagating cells in StemPro® MSC SFM XenoFree

1. Observe the stock culture flask (cells growing in current medium formulation or in StemPro® MSC SFM XenoFree) under the microscope and confirm that the cells are ready to be sub-passaged (~60–90% confluent).
2. Pre-warm TrypLE™ Select CTS™ reagent and StemPro® MSC SFM XenoFree complete medium to 37°C before use.
3. Add 10 mL of pre-warmed StemPro® MSC SFM XenoFree complete medium to a 50-mL conical tube for each flask being harvested.
4. Remove the spent medium from the T-75 flask and discard.
5. Wash the cell surface with 10 mL of DPBS CTS™ without Ca^{2+} and Mg^{2+}, remove and discard.
6. Add 3–5 mL of TrypLE™ CTS™ reagent to the T-75 flask, tilt the flask in all directions to evenly distribute. Incubate the cells in TrypLE™ CTS™ reagent for 2–10 minutes in the incubator.

   Note: Cells coming out of serum-containing medium may require a longer incubation time (5–10 minutes), while cells growing under serum-free conditions should detach more readily (2–3 minutes).
7. After incubation, check the flask under the microscope for cell detachment. Firmly tap the flask as necessary to facilitate complete cell detachment.
8. Add 7 mL of pre-warmed StemPro® MSC SFM XenoFree to each flask and collect the cell suspension in the 50-mL conical tube containing complete medium. Firmly tap the flask, re-wash with 10 mL StemPro® MSC SFM XenoFree and collect.

   Note: The addition of StemPro® MSC SFM XenoFree complete medium to harvested cells is critical for preventing the cells from adhering to the wall of the conical tube during centrifugation.
9. Centrifuge the tubes at 100–200 × *g* for 5 minutes at room temperature.
10. Resuspend the cells in a minimal volume of pre-warmed StemPro® MSC SFM XenoFree complete medium for cell counting, using a preferred counting method (e.g., Countess® Automated Cell Counter).
11. Remove the CELLstart™ CTS™ substrate solution from each coated flask and add 15 mL of StemPro® MSC SFM XenoFree complete medium.
12. Add enough cell suspension to each flask to provide **≥ 5 × 10^3 cells/cm^2** (i.e. 3.75 × 10^5 cells per T-75 flask). Mix or swirl the cell suspension to ensure even distribution.
13. Place the culture flask(s) in the incubator at 36°C to 38°C with a humidified atmosphere of 4–6% CO_2.
14. Replace the spent culture medium **every 2 days** with pre-warmed StemPro® MSC SFM XenoFree complete medium.

Cryopreservation

1. Prepare cryopreservation solution (2X) by supplementing StemPro® MSC SFM XenoFree complete medium with 20% Dimethyl Sulfoxide (DMSO). Store at 4°C until use; make cryopreservation medium on day of intended use.
2. Reconstitute the harvested cell pellet to twice the desired final cell concentration (i.e., 2 × 10^6 cells/mL) in pre-warmed StemPro® MSC SFM XenoFree complete medium.
3. Slowly add cryopreservation solution to the cell suspension, and gently mix to ensure even cell distribution.
4. Immediately add the desired volume of cell suspension (i.e., 1 mL) to pre-chilled (2°C to 8°C) cryovials.
5. Place the cryovials at –70°C in a cryogenic freezing container (e.g., "Mr. Frosty" (1°C) Freezing Container).
6. After 24 hours, transfer the frozen cells to liquid nitrogen (vapor phase); storage at –200°C to –125°C is recommended.

Related products

Product	Catalog no.
CELLstart™ CTS™	A10142
Dulbecco's Phosphate Buffered Saline (DPBS) CTS™ without calcium, magnesium	A12856
Dulbecco's Phosphate Buffered Saline (DPBS) CTS™ with calcium, magnesium	A12858
Gentamicin Reagent Solution (10mg/mL) liquid	15710
L-Glutamine, 200 mM (100X), liquid	25030
GlutaMAX-I CTS™ Supplement, 200mM (100X), liquid	A12860
TrypLE™ Select CTS™ (1X), liquid, without Phenol Red	A12859
Synth-a-Freeze CTS™ Cryopreservation Medium	A13713
StemPro® MSC SFM CTS™ Medium	A10332-01
MesenPRO RS™ Medium	12746
StemPro® BM Mesenchymal Stem Cells	A15652
StemPro® Adipogenesis Differentiation Kit	A10070
StemPro® Chondrogenesis Differentiation Kit	A10071
StemPro® Osteogenesis Differentiation Kit	A10072
StemPro® Human Adipose-Derived Stem Cells	R7788
Fetal Bovine Serum, MSC-Qualified	12662
Trypan Blue Stain	15250
Countess® Automated Cell Counter	C10227

Explanation of symbols and warnings

The symbols present on the product label are explained below:

					
Use By:	Keep away from light	Sterilized using aseptic processing techniques	Temperature Limitation	Consult instructions for use	Caution, consult accompanying documents

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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For further assistance, email **techsupport@lifetech.com**