

StemPro™ MSC SFM

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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](https://www.thermofisher.com/support).

Product description

StemPro™ MSC SFM is a serum-free medium specially formulated for the growth and expansion of human mesenchymal stem cells (MSC) or adipose-derived stem cells (ADSC). Using StemPro™ MSC SFM, human MSC or ADSC can be expanded for multiple passages while maintaining their tri-lineage mesoderm potential (i.e., ability to differentiate into osteogenic, chondrogenic and adipogenic lineages). Each container is sterile filtered.

Contents and storage

Contents	Amount	Storage	Shelf life ^[1]
StemPro™ MSC SFM Basal Medium	1 × 500 mL	2°C to 8°C. Protect from light.	24 months
StemPro™ MSC SFM Auxiliary Concentrated Medium	1 × 75 mL	–20°C to –5°C. Protect from light.	

^[1] Shelf-Life duration is determined from Date of Manufacture.

Culture conditions

Media: Complete StemPro™ MSC SFM

Cell line(s): Human mesenchymal stem cells (MSC) or adipose-derived stem cells (ADSC)

Culture type: Adherent

Culture vessels: CELLStart™ Substrate coated T-Flasks.

Temperature range: 36°C to 38°C

Incubator atmosphere: Humidified atmosphere of 4–6% CO₂ 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 cm²). Volumes should be adjusted accordingly for desired vessel size.

Procedural guidelines

- Thaw StemPro™ MSC SFM Auxiliary Concentrated Medium overnight at 2°C to 8°C. Use thawed material immediately or aliquot (i.e., 15 mL) unused material and store at –20°C to –5°C protected from light. Avoid additional freeze-thaw cycles.
- Complete StemPro™ MSC SFM medium (basal medium, auxiliary concentrated medium, and L-glutamine or CTS™ GlutaMAX™-I Supplement) is stable for 4 weeks when stored in the dark at 2°C to 8°C, within the expiration date of all three components.

Prepare complete medium

StemPro™ MSC SFM Basal Medium requires supplementation with StemPro™ MSC SFM Auxiliary Concentrated Medium and L-glutamine or CTS™ GlutaMAX™-I Supplement. For 100 mL complete medium:

- Aseptically add 15 mL of StemPro™ MSC SFM Auxiliary Concentrated Medium to 84 mL of StemPro™ MSC SFM Basal Medium.
- Aseptically add 2 mM L-glutamine or CTS™ GlutaMAX™-I Supplement to the complete medium before use.
- Antibiotics can be used if desired, we recommend Gentamicin Reagent Solution (5 µg/mL final concentration).

General recommendations subculture MSC

- StemPro™ MSC SFM has been developed for the primary isolation and multi-passage expansion of human bone marrow-derived MSC and ADSC at greater than clonal densities, with optimal cell expansion observed at $\geq 5 \times 10^3$ cells/cm². (see Subculture in StemPro™ MSC SFM, step 11).
- Reduced seeding densities may result in suboptimal cell expansion. Optimal growth conditions must be determined for each application.

- It is recommended that human MSC in StemPro™ MSC SFM be subcultured when cell confluency reaches 60–80%, cells are in mid-logarithmic phase of growth and cell viability is at least 90%. Initiating cultures under suboptimal conditions may affect product performance. Transitioning MSC or ADSC from serum-containing medium to complete StemPro™ MSC SFM does not require an adaptation protocol.
- For optimal performance and cell growth, cultures should be re-fed every 2 days with fresh complete StemPro™ MSC SFM.

Coat culture flasks with CELLStart™ Substrate

1. Dilute CELLStart™ Substrate 1:100 in CTS™ DPBS with calcium, magnesium (i.e., 100 µL CELLStart™ Substrate into 10 mL of CTS™ DPBS with calcium, magnesium). Mix by gentle pipetting, do not vortex. Add 10 mL of the CELLStart™ Substrate to each flask, ensure complete surface coverage.

Note: Do not store diluted CELLStart™ Substrate solution; prepare fresh before each use.

2. Incubate at 37°C in a humidified atmosphere of 5% CO₂ in air for 60 minutes.
3. After incubation, remove flasks from the incubator and temporarily place them in a laminar flow hood until use. Immediately before use, remove all CELLStart™ Substrate and replace with complete medium.

Subculture in StemPro™ MSC SFM

1. Observe stock culture flask (cells growing in current medium formulation or in StemPro™ MSC SFM) with an inverted microscope and confirm that the cells are ready to be subcultured (60–80% confluent).
2. Pre-warm CTS™ TrypLE™ Select Enzyme and Complete StemPro™ MSC SFM to 37°C before use.
3. Aspirate spent medium from the flask and discard.
4. Wash cell monolayer with 5 mL pre-warmed CTS™ DPBS without calcium chloride, without magnesium chloride, aspirate and discard.

5. Add 3–5 mL CTS™ TrypLE™ Select Enzyme to each flask, ensure complete coverage of cell monolayer.

6. Incubate for 5–10 minutes at 37°C.

Note: Cells coming out of serum-containing medium may require a longer incubation time.

7. Check flasks with an inverted microscope for cell detachment. Firmly tap the flask as necessary to facilitate complete cell detachment.
8. Upon cell detachment, add 5 mL pre-warmed CTS™ DPBS with calcium, magnesium to each flask to completely cover the surface area.
9. Transfer cell suspension to a sterile 15-mL conical tube. Tap flask firmly, re-wash with 5 mL pre-warmed CTS™ DPBS with calcium, magnesium and collect.
10. Centrifuge tubes at 100–200 × g for 5 minutes at room temperature. Aspirate and discard DPBS being careful not to disturb cell pellet.
11. Resuspend cell pellet in a minimal volume of pre-warmed complete StemPro™ MSC SFM for cell counting, using a preferred counting method (e.g., Countess™ II Automated Cell Counter).
12. Remove CELLStart™ Substrate coating solution from each coated flask, add 15 mL pre-warmed complete StemPro™ MSC SFM.
13. Add 5×10^3 viable cells/cm² to each flask (i.e., 3.75×10^5 viable cells/T-75 flask).
Gently swirl cell suspension to ensure even distribution.
14. Incubate at 37°C in a humidified atmosphere of 5% CO₂.
15. Replace spent culture medium every 2–3 days with fresh, pre-warmed complete StemPro™ MSC SFM for optimal performance and cell growth.

Related products

Item	Source
CELLStart™ Substrate	A10142
CTS™ DPBS without calcium chloride, without magnesium chloride	A12856
CTS™ DPBS with calcium, magnesium	A12858
Gentamicin Reagent Solution (10mg/mL) liquid	15710
L-Glutamine (200 mM)	A29168
CTS™ GlutaMAX™-I Supplement	A1286001
CTS™ TrypLE™ Select Enzyme (1X), no phenol red	A12859
CTS™ Synth-a-Freeze	A13713
StemPro™ MSC SFM XenoFree	A10675-01
StemPro™ Adipogenesis Differentiation Kit	A10070
StemPro™ Chondrogenesis Differentiation Kit	A10071
StemPro™ Osteogenesis Differentiation Kit	A10072
StemPro™ Human Adipose-Derived Stem Cells	R7788
StemPro™ BM Mesenchymal Stem Cells, 1 × 10 ⁶ cells	A15652
Fetal Bovine Serum, MSC-Qualified	12662
Trypan Blue Stain	15250
Countess™ II Automated Cell Counter	AMQAX1000

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