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StemPro® MSC SFM CTS™

Description

StemPro® MSC SFM CTS™ 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 CTS™, 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). StemPro® MSC SFM CTS™ is intended for human *ex vivo* tissue and cell culture processing applications. Each container is sterile filtered.

Product	Catalog No.	Amount	Storage	Shelf Life*
StemPro® MSC SFM CTS™	A10332-01**	1 kit		
Kit Contains (components shipped separately):				
StemPro® MSC SFM Basal Medium CTS™	A10334-01	1 × 500 mL	2°C to 8°C; Protect from light	24 months
StemPro® MSC SFM Supplement CTS™	A10333-01	1 × 75 mL	–20°C to –5°C; Protect from light	24 months

* Shelf Life has been determined from Date of Manufacture.

** Cell Therapy Systems™ StemPro® MSC SFM is sold as a complete kit, components are not sold separately.

Intended Use

For human *ex-vivo* tissue and cell culture processing applications. CAUTION: When used as a medical device, Federal Law restricts this device to sale by or on the order of a physician.

Important Information

- We recommend thawing StemPro® MSC SFM Supplement CTS™ 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 CTS™ medium (basal medium, supplement and L-glutamine or GlutaMAX™-I CTS™) is stable for 4 weeks when stored in the dark at 2°C to 8°C, within the expiration date of all three components.

Safety Information

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

Prepare Complete Medium

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

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

Culture Conditions

Media: Complete StemPro® MSC SFM CTS™

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

Culture Type: Adherent

Culture Vessels: CELLstart™ CTS™-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.

Recovery

1. Rapidly thaw (<1 minute) frozen cells in a 37°C water bath.
2. Pipet the entire contents of the cryovial into a sterile 50-mL conical tube.
3. Carefully, by dropwise addition (one drop every two seconds), add 5–10 mL of pre-warmed (37°C) Complete StemPro® MSC SFM CTS™. Ensure homogeneity of the cell suspension by regular gentle swirling of the tube.
4. Centrifuge cell suspension at 100–200 × *g* for 5 minutes at room temperature. Aspirate and discard supernatant being careful not to disturb the cell pellet.
5. Resuspend the cell pellet in a minimal volume of pre-warmed Complete StemPro® MSC SFM CTS™ for cell counting. Determine total viable cell density with a Countess® Automated Cell Counter (alternative automated or manual procedures may be used). Calculate the volume of cell suspension required to seed cells at a density of ≥5 × 10^3 cells/cm^2.
6. Add cell suspension to an appropriate CELLstart™ CTS™-coated T-75 flask containing 10–15 mL pre-warmed Complete StemPro® MSC SFM CTS™ at a density of ≥5 × 10^3 cells/cm^2 (see **Coating Culture Flasks with CELLstart™ CTS™**).
7. Incubate at 37°C in a humidified atmosphere of 5% CO_2 in air.
8. Replace the medium in the flasks every 2–3 days with fresh pre-warmed Complete StemPro® MSC SFM CTS™.

Subculture MSC

General Recommendations

- StemPro® MSC SFM CTS™ 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 ≥5 × 10^3 cells/cm^2. (see **Subculture in StemPro® MSC SFM CTS™**, 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 CTS™ 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 CTS™ 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 CTS™.

Coating Culture Flasks with CELLstart™ CTS™

1. Dilute CELLstart™ CTS™ 1:100 in Dulbecco's Phosphate Buffered Saline (DPBS) CTS™ with calcium and magnesium (i.e., 100 µL CELLstart™ CTS™ into 10 mL of DPBS CTS™). Mix by gentle pipetting, **do not vortex**. Add 10 mL of the CELLstart™ CTS™ solution to each flask, ensure complete surface coverage. **Note:** Do not store diluted CELLstart™ CTS™ solution; prepare fresh before each use.
2. Incubate at 37°C in a humidified atmosphere of 5% CO_2 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™ CTS™ solution and replace with complete medium.

Subculture in StemPro® MSC SFM CTS™

1. Observe stock culture flask (cells growing in current medium formulation or in StemPro® MSC SFM CTS™) with an inverted microscope and confirm that the cells are ready to be subcultured (60–80% confluent).
2. Pre-warm TrypLE™ Select CTS™ and Complete StemPro® MSC SFM CTS™ to 37°C before use.
3. Aspirate spent medium from the flask and discard.
4. Wash cell monolayer with 5 mL pre-warmed DPBS CTS™ without calcium and magnesium, aspirate and discard.
5. Add 3–5 mL TrypLE™ Select CTS™ to each flask, ensure complete coverage of cell monolayer. Incubate for 5–10 minutes at 37°C. **Note:** Cells coming out of serum-containing medium may require a longer incubation time.
6. Check flasks with an inverted microscope for cell detachment. Firmly tap the flask as necessary to facilitate complete cell detachment.
7. Upon cell detachment, add 5 mL pre-warmed DPBS CTS™ with calcium and magnesium to each flask to completely cover the surface area. Transfer cell suspension to a sterile 15-mL conical tube. Tap flask firmly, re-wash with 5 mL pre-warmed DPBS CTS™ with calcium and magnesium and collect.
8. Centrifuge tubes at 100–200 × *g* for 5 minutes at room temperature. Aspirate and discard DPBS being careful not to disturb cell pellet.
9. Resuspend cell pellet in a minimal volume of pre-warmed complete StemPro® MSC SFM CTS™ for cell counting, using a preferred counting method (e.g., Countess® Automated Cell Counter).
10. Remove CELLstart™ CTS™ coating solution from each coated flask, add 15 mL pre-warmed complete StemPro® MSC SFM CTS™.
11. Add 5×10^3 viable cells/cm^2 to each flask (i.e., 3.75×10^5 viable cells/T-75 flask). Gently swirl cell suspension to ensure even distribution.
12. Incubate at 37°C in a humidified atmosphere of 5% CO_2.
13. Replace spent culture medium every 2–3 days with fresh, pre-warmed complete StemPro® MSC SFM CTS™ for optimal performance and cell growth.

Cryopreserve MSC

1. Prepare cryopreservation solution on day of use by supplementing StemPro® MSC SFM Basal Medium CTS™ with 25% StemPro® MSC SFM Supplement CTS™ and 10% Dimethyl Sulfoxide (DMSO). Keep on ice until use.
2. Harvest cells for cryopreservation (see **Subculture in StemPro® MSC SFM CTS™** steps 1–8).
3. Reconstitute the harvested cells to 1×10^6 viable cells/mL with cold cryopreservation solution. Immediately dispense the desired volume of cell suspension (i.e., 1 mL) into cryovials.
4. Cryopreserve cells in an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
5. After 24 hours transfer 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™, 200mM (100X), liquid	A12860
TrypLE™ Select CTS™ (1X), liquid, without Phenol Red	A12859
Synth-a-Freeze CTS™	A13713
StemPro® MSC SFM XenoFree	A10675-01
MesenPRO RS™ Medium	12746
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
StemPro® BM Mesenchymal Stem Cells, 5×10^6 cells	A15653
Fetal Bovine Serum, MSC-Qualified	12662
Trypan Blue Stain	15250
Countess® Automated Cell Counter	C10227

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