

STEMPRO® MSC SFM

GIBCO® STEMPRO MSC SFM has been developed for the growth and expansion of human mesenchymal stem cells (MSCs). Using STEMPRO MSC SFM, human MSCs can be expanded for multiple passages while maintaining their multipotential phenotype (i.e. ability to differentiate into osteogenic, chondrogenic and adipogenic lineages).

Description	Cat. No.	Size
STEMPRO MSC SFM Contains:	A10332-01	1 Kit
STEMPRO MSC SFM Basal Medium	A10334-01	1 x 500mL
STEMPRO MSC SFM Supplement	A10333-01	1 x 75mL

Note: STEMPRO MSC SFM is sold as a complete kit, components are not sold separately.

Intended Use

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

Precautions

It is recommended to thaw STEMPRO MSC SFM Supplement overnight at 2 to 8°C prior to use. Use thawed material immediately or aliquot (i.e. 15mL) unused material and store at -5 to -20°C. Avoid additional freeze-thaw cycles.

STEMPRO MSC SFM complete medium (basal, supplement and L-glutamine) is stable for 4 weeks when stored in the dark at 2 to 8°C.

Storage

STEMPRO MSC SFM Basal Medium: Store at 2 to 8°C. Protect from light

STEMPRO MSC SFM Supplement: Store at -5 to -20°C. Protect from light

Shelf Life

STEMPRO MSC SFM Basal Medium: 12 months

STEMPRO MSC SFM Supplement: 12 months

Physical Conditions

Standard physical conditions for human mesenchymal stem cells grown in STEMPRO MSC SFM complete medium are 36 - 38°C in a humidified atmosphere of 4 to 6% CO₂ in air. Using standard aseptic conditions, cultures may be grown in complete STEMPRO MSC SFM complete medium on CELLstart™-coated tissue culture treated vessels (i.e. T25 flask). Ensure that proper gas exchange is achieved in culture vessels. Avoid overexposure of cultures to light.

Medium Preparation

STEMPRO MSC SFM Basal Medium requires supplementation with STEMPRO MSC SFM Supplement and L-glutamine.

- For 100mL complete medium, aseptically add 15mL of STEMPRO MSC SFM Supplement to 84mL of STEMPRO MSC SFM Basal Medium.
- Aseptically add 1.0 mL of L-Glutamine (200mM) (25030) or GLUTAMAX™ -I (200mM) (35050) to the complete medium before use.
- If so desired, add 50 µL of Gentamicin reagent solution (15710) to the complete medium.

Recovery of Cryopreserved Human MSCs:

- Rapidly thaw frozen vial of cells in a 37°C water bath.
- Pipet the entire contents of the cryovial into a 50 mL conical tube.
- Carefully add to conical tube 5 to 10mL of pre-warmed (37°C) STEMPRO MSC SFM complete medium at an approximate rate of 3 to 5 drops per 10 seconds and gently swirl after every addition.
- Transfer the entire contents of the conical tube into a CELLstart-coated tissue culture flask **See CELLstart Coating of Culture Flask section.**
- Incubate at 36 to 38°C in a humidified atmosphere of 4 to 6% CO₂ in air.
- Fluid change flasks 24 hrs post-thaw. **See Subculturing Cells in STEMPRO MSC SFM section.**

Subculturing Cells in STEMPRO MSC SFM

STEMPRO MSC SFM has been developed for the primary isolation and multi-passage expansion of human bone marrow-derived MSCs. STEMPRO MSC SFM has been developed for human MSC expansion at greater than clonal densities, **with optimal cell expansion observed at $\geq 1.0 \times 10^4$ cells/cm²** (see **Cell Propagation in STEMPRO MSC SFM section, step 11**). **Reduced seeding densities may result in suboptimal cell expansion.** Optimal growth conditions must be determined for each application.

It is recommended that when subculturing human MSCs in STEMPRO MSC SFM that input cell confluency be 60 to 80%, cell viability be at least 90% and the growth rate be in mid-logarithmic phase prior to subculture. Initiating cultures under suboptimal conditions may affect product performance.

For optimal performance and cell growth, cultures should be re-fed every 2 days with fresh complete STEMPRO MSC SFM.

NOTE: Procedures detailed below are for cultures in a T25 culture flask (25cm²). Volumes should be adjusted accordingly for desired vessel size.

A. CELLstart Coating of Culture Flask:

- Dilute CELLstart (A10142) 1:100 in DPBS (14190) (i.e. 50µL CELLstart into 5mL of DPBS). Pipet gently to mix. **DO NOT VORTEX.** Coat T25 culture flasks by adding 5mL of the CELLstart solution to each flask.

- Place culture flasks with CELLstart in the incubator at 36 to 38°C in a humidified atmosphere of 4 to 6% CO₂ in air for 60 minutes.
- After incubation, remove flasks from the incubator until use. Immediately before use, remove all CELLstart solution and replace with complete STEMPRO MSC SFM.

B. Cell Propagation in STEMPRO MSC SFM:

- Observe stock culture flask (cells growing in current medium formulation or in STEMPRO MSC SFM) under the microscope and confirm that the cells are ready to be sub-passaged (\leq 80% confluent).
- Pre-warm TrypLE™ and complete STEMPRO MSC SFM to 37°C before use.
- Remove spent medium from the flask using a pipet and discard.
- Wash cell surface with DPBS, remove and discard.
- Add 1.0 mL TrypLE to the T25 flask, tilt flask in all directions to evenly distribute. Incubate cells in TrypLE for 5 to 10 minutes in the incubator at 36 to 38°C.
- After incubation, check the flask under the microscope to see if the cells have detached. Firmly tap the flask as necessary to facilitate cell detachment.
- Upon cell detachment, add enough DPBS to each flask to completely cover the surface area. Collect the cell suspension in a sterile 15 mL conical tube. Tap flask firmly, re-wash with DPBS and collect.
- Centrifuge tubes at 1500 rpm (466 x g) for 5 minutes.
- Resuspend cells in a minimal volume of warmed complete STEMPRO MSC SFM. Take a sample from the cell suspension for cell counting using preferred counting method (i.e. Hemacytometer).
- Remove CELLstart from each coated flask (*see **CELLstart Coating of Culture Flask section***) and add 5 mL of complete STEMPRO MSC SFM.
- Add enough cell suspension to each flask to provide 1×10^4 cells/cm² (i.e. 2.5×10^5 cells/T25 flask). Mix or swirl cell suspension to ensure even distribution.
- Place culture flask in the incubator at 36 to 38°C in a humidified atmosphere of 4 to 6% CO₂ in air.
- Replace spent culture medium every 2 days with fresh, pre-warmed complete STEMPRO MSC SFM.

Cryopreservation of Cells in STEMPRO MSC SFM

- Prepare cryopreservation solution by supplementing STEMPRO MSC SFM Basal Medium with 25% STEMPRO MSC SFM Supplement and 10% Dimethyl Sulfoxide (DMSO).
- To harvested cell pellet, add cold cryopreservation solution, gently resuspend pellet and immediately add suspension to cryovials (recommended 1.0×10^6 cells/mL; 0.5mL/vial).
- Place cryovials at -70°C in a freezing container overnight.
- Transfer cryovials to liquid nitrogen for long-term storage.
- See Recovery of Cryopreserved Human MSCs section** for thawing of frozen cells.

NOTE: For recovery of cells grown in STEMPRO MSC SFM, it is recommended to **seed cells at $\geq 2 \times 10^4$ cells/cm²** for the initial recovery passage.

Quality Control

STEMPRO MSC SFM Basal Medium and STEMPRO MSC SFM Supplement are performance tested using human MSCs in a cell expansion assay. Additional standard evaluations for STEMPRO MSC SFM Basal Medium and STEMPRO MSC SFM Supplement are endotoxin, pH, osmolality and tests for the absence of bacterial and fungal contaminants.

Related Products

CELLstart (A10142)

Dulbecco's Phosphate Buffered Saline (DPBS) without calcium, magnesium, or phenol red (1X), liquid (14190)

Gentamicin Reagent Solution (10mg/mL) liquid (15710)

L-Glutamine, 200mM (100X), liquid (25030)

GLUTAMAX-I, 200mM (100X), liquid (35050)

TrypLE Express (1X), liquid without phenol red (12604)

Trypan Blue Stain (15250)

Fetal Bovine Serum, MSC-Qualified (12662)

STEMPRO Adipogenesis Differentiation Kit (A10070-01)

STEMPRO Osteogenesis Differentiation Kit (A10072-01)

STEMPRO Human Adipose-Derived Stem Cell Kit (R7788)

MesenPRO™ RS Medium (12746)

Contacts

For further information on this or other GIBCO products, contact Technical Services at the following:

United States TECH-LINESM : 1-800-955-6288

Canada TECH-LINE: 1-800-757-8257

Europe: eurotech@invitrogen.com

Outside the U.S. and Canada, refer to the GIBCO products catalog for the TECH-LINE in your region.

You may also contact your Invitrogen Sales Representative or our World Wide Web site at www.invitrogen.com.

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