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by life technologies

StemPro® hESC SFM

Description

StemPro® hESC SFM is a defined serum-free medium (SFM) developed for the culture of human Embryonic Stem Cells (hESCs) without the need for mouse or human fibroblast feeder layers or feeder-conditioned medium. Using StemPro® hESC SFM, human ESCs and induced pluripotent stem cells (iPSC) can be expanded for up to 80 passages while maintaining their multipotential phenotype and normal karyotype.

Product	Catalog no.	Amount	Storage	Shelf life*
StemPro® hESC SFM Kit Contains:	A10007-01	1 kit	—	—
DMEM/F12 + GlutaMAX™-I	10565-018	1 × 500 mL	2°C to 8°C; Protect from light	12 months
StemPro® hESC Supplement	A10006-01	1 × 10 mL	–20°C to –5°C; Protect from light	12 months
Bovine Serum Albumin 25% (BSA)	A10008-01	1 × 40 mL	2°C to 8°C; Protect from light	12 months

*Shelf life duration is determined from Date of Manufacture.

Product use

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

Important information

- Thaw StemPro® hESC Supplement at 37°C (minimize dwell time). Thawed material can be stored at 2°C to 8°C for up to 7 days before use or aliquot (i.e., 2 mL) unused material and store at –20°C to –5°C protected from light.
  Avoid additional freeze-thaw cycles.
- Complete StemPro® hESC SFM medium is stable for up to 7 days when stored in the dark at 2°C to 8°C. Add 2-Mercaptoethanol daily during storage (see Prepare Media).
- Additional reagents required but not supplied with the kit.

Product*	Amount
FGF-basic, Human recombinant	50 µg
2-Mercaptoethanol	50 mL
Collagenase, Type IV	1 g
Geltrex® LDEV-Free hESC-qualifed Reduced Growth Factor Basement Membrane Matrix	5 mL
Dulbecco's Phosphate Buffered Saline (DPBS)	500 mL

* See Related Products for ordering information.

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 HB_sAg. Handle in accordance with established bio-safety practices.

Important guidelines for hESC culture

- To prevent differentiation and slow growth of hESCs grown in StemPro® hESC SFM, follow these guidelines:
- **Starter culture:** This must be a high-quality culture, with a high density of cells, and primarily undifferentiated. The starter culture should be hESCs maintained on Matrigel™ or Geltrex® in Mouse Embryonal Fibroblast-Conditioned Medium (MEF-CM), or on CELLstart™ without MEF-CM. The cells should not be maintained on MEF feeder cells.
  Note: Cells can be transferred from MEF-CM into StemPro® hESC SFM complete medium without prior adaptation.
- **Passaging:** It is critical to achieve high plating/survival of colony pieces. The pieces must be a bit smaller than typical collagenase passaging on Matrigel™ or Geltrex®/MEF-CM.
- Some cell death at passaging is normal, but not wide-scale cell death (i.e., <20% survival); this indicates poor passaging.
- **Timing of passaging:** It is critical the cultures grow to near-confluence (i.e., 1–2 days after the colonies are just touching), before cultures are harvested. This usually results in a cell density of $2.5–4 \times 10^5$ cells/cm^2 at time of harvest. **Important:** Do not over-expose cells to collagenase; we recommend ≤3 minutes, even with lower amounts of collagenase.
- **Density:** The cultures must be maintained at a high density (200+ colonies in a 60-mm dish). hESCs grown in culture are always under selection pressure of proliferation vs. differentiation. The cultures should be fed every day; do not exhaust medium by not feeding. Scrape clearly differentiated areas out with a 21½-gauge needle.

Prepare supplements

FGF-basic: Dissolve the entire contents FGF-basic (50 µg) in 5 mL Wash Medium (DMEM/F-12 with 0.1% BSA, see Prepare Media) for a final concentration of 10 µg/mL. Dispense 80-µL aliquots per tube and store frozen at –20°C to –5°C until use.

Collagenase: Dissolve the entire contents Collagenase Type IV (1 g) in 100 mL DMEM/F-12 for a final concentration of 10 mg/mL. Filter to sterilize and dispense 1-mL aliquots per tube and store frozen at –20°C to –5°C until use.

Geltrex® LDEV-Free: Thaw Geltrex® LDEV-Free hESC-qualifed Matrix overnight at 2°C to 8°C. Dispense 1-mL aliquots into 50-mL conical tubes and store frozen at –80°C to –20°C until use.

Prepare media

Wash medium (DMEM/F-12, 0.1% BSA)**:** Add 0.4 mL BSA 25% to 99.6 mL DMEM/F-12.

Complete medium: StemPro® hESC SFM complete medium requires supplementation of DMEM/F12 + GlutaMAX™-I, with StemPro® hESC Supplement, BSA, basic Fibroblast Growth Factor (FGF-basic), and 2-Mercaptoethanol according to the following table.

StemPro® hESC SFM complete medium	Final Conc.	For 500 mL	For 100 mL
DMEM/F12 + GlutaMAX™-I	1X	454 mL	90.8 mL
StemPro® hESC Supplement	1X	10 mL	2 mL
BSA 25%	1.8%	36 mL	7.2 mL
FGF-basic (10 µg/mL)	8 ng/mL	400 µL	80 µL
2-Mercaptoethanol (55 mM)	0.1 mM	909 µL	182 µL

Culture conditions

Media: StemPro® hESC SFM complete medium

Cells: Human ESCs, iPSCs

Culture type: Adherent

Culture vessels: Geltrex® LDEV-Free hESC-qualified or CELLstart™ CTS™ substrate-coated 60-mm dishes.

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 below are for cultures in a 60-mm culture dish (28 cm^2). Adjust volumes accordingly for desired vessel size.

Coat culture vessels with Geltrex® LDEV-Free hESC-qualified Matrix

Geltrex® Matrix solutions should be maintained on ice to prevent premature gelling. Detailed protocols using Geltrex® LDEV-Free hESC-qualified Matrix with StemPro® hESC SFM can be found at **www.lifetechnologies.com/3D-cellculture**.

1. Thaw on ice, one 1-mL aliquot (in 50-mL conical tube) of Geltrex® LDEV-Free hESC-qualified Matrix solution.
2. Add 29 mL pre-chilled (4°C) DMEM/F-12 medium. Mix by slowly pipetting up and down; be careful not to introduce air bubbles.
3. Dispense 1.5 mL diluted Geltrex® LDEV-Free hESC-qualified Matrix solution into each 60-mm culture dish (1 mL per 35-mm dish). Tilt gently to cover the entire growth surface area.
4. Seal each dish with Parafilm® laboratory film to prevent drying and incubate at 37°C for 1 hour. The sealed, coated dish is stable for up to 7 days when stored at 2°C to 8°C.
5. At time of use transfer each dish to a laminar flow hood and equilibrate to room temperature (about 1 hour).
6. Before plating cells, tip the plate slightly and aspirate off the supernatant above the Geltrex® coating. Immediately plate cells in pre-equilibrated complete medium.

Coat culture vessels with CELLstart™ CTS™ Substrate

Detailed protocols using CELLstart™ substrate with StemPro® hESC SFM can be found at **www.lifetechnologies.com/cellstart**.

Passaging using collagenase

1. Warm appropriate amount of 10 mg/mL Collagenase IV solution, complete StemPro® hESC SFM Medium and Wash Medium to 37°C in a water bath. Minimize dwell time.
2. Set up hESC plate on a dissecting microscope in a biosafety cabinet or laminar flow hood to observe colonies.
3. Cut out and remove any overtly differentiated colonies with a 21½-gauge needle.
4. Aspirate the medium and gently add 1–2 mL of Collagenase IV solution. Incubate for ≤3 minutes at room temperature.
5. Aspirate the Collagenase IV solution, rinse with DPBS, and then add 3 mL Wash Medium.
6. Gently scrape dish using a sterile 1000-µL pipette tip.
7. Gently transfer clumps to a 15-mL tube using a 5-mL pipette. Wash plate with 3 mL of Wash Medium and combine.
8. Spin cells at 200 × *g* for 2 minutes at room temperature. Carefully aspirate supernatant and flick tube to loosen cell pellet.
9. Gently resuspend the cells in 5 mL pre-equilibrated complete StemPro® hESC SFM Medium using a 5-mL serological pipette.
10. Aspirate the Geltrex® solution from a Geltrex®-coated plate and immediately plate the cells. Do not allow the surface to dry out before plating.
11. Gently tilt plates to evenly spread cell clumps and incubate at 37°C, 5% CO_2 in air.
12. Gently exchange spent media every 24 hours.
13. Observe cells every day and passage (steps 1–12) whenever required (approximately every 5–7 days).

Related products

Product	Catalog no.
FGF-basic Recombinant Human	PHG0026
2-Mercaptoethanol, 55 mM	21985
Collagenase, Type IV, powder	17104
Geltrex® LDEV-Free hESC-qualifed Reduced Growth Factor Basement Membrane Matrix	A1413302
CELLstart™ CTS™	A10142
DPBS no calcium, no magnesium (1X), liquid	14190
DPBS calcium, magnesium (1X), liquid	14040

Explanation of symbols and warnings

The symbols present on the product label are explained below:

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

Important licensing information

These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

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**.

For additional technical information such as Safety Data Sheets (SDS), Certificates of Analysis, visit www.lifetechnologies.com/support
For further assistance, email **techsupport@lifetech.com**