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Human Endothelial-SFM

Description

Human Endothelial Serum Free Medium (SFM) has been developed to support the long-term propagation of human umbilical vein endothelial cells (HUVEC). Complete Human Endothelial-SFM has been demonstrated to successfully support primary isolation and subsequent secondary growth of HUVEC for ≤15 passages. HUVEC cultured in complete Human Endothelial-SFM exhibit the histotypic "cobblestone" morphology and retain endothelial specific markers including: expression of Factor VIII-related antigen, UEA-1 lectin binding, uptake of DiI-acetylated LDL, vimentin, and IL-1a-induced ICAM-1 expression. Additionally, HUVEC cultured in Human Endothelial-SFM have been shown to maintain cAMP and prostacyclin signal transduction systems. Human Endothelial-SFM also supports the growth and retention of physiological markers for human umbilical arterial and dermal microvascular endothelial cells.

Product	Catalog No.	Amount	Storage	Shelf Life*
Human Endothelial-SFM	11111-044	500 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

- We have found primary HUVEC cultures established in SFM to be very sensitive to antibiotics. We recommend using penicillin at a concentration of 10 U/mL and streptomycin at a concentration of 10 µg/mL (equivalent to 0.1X penicillin-streptomycin 100X solution).
- Antibiotics should be removed from the culture medium 48–72 hours after the establishment of primary cultures. The use of antibiotics for secondary cultures should be avoided. However, if antibiotics must be used for secondary cultures we recommend using 0.1X penicillin-streptomycin 100X solution.
- Endothelial cells cultured in SFM are very sensitive to proteolysis. It is critically important that all residual trypsin be removed prior to cell detachment and that the cells are washed in SFM at least two times prior to replating.
- Care should be exercised in the handling of endothelial cells. Avoid centrifugation forces in excess of 100 × *g* as well as vigorous pipeting to resuspend cell pellets following centrifugation.

Safety Information

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

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 Medium

Human Endothelial-SFM requires supplementation with human plasma fibronectin (20 µg/mL for primary culture, 10 µg/mL for secondary cultures) as an attachment factor, human recombinant basic Fibroblast Growth Factor (rbFGF) at 20 ng/mL, and human recombinant Epidermal Growth Factor (rEGF) at 10 ng/mL. Do not add supplements to the bottle of Human Endothelial–SFM but rather supplement media in culture vessles immediately prior to adding cells.

Prepare Growth and Attachment Factors

1. Reconstitute human plasma fibronectin in sterile water for injection (WFI) grade water to yield a stock solution of 1 mg/mL. Mix with gentle swirling. **Do not** vortex, heat, or filter–sterilize.
2. Prepare stock solutions of human rbFGF and rEGF in DPBS with 0.1% human serum albumin at 2 µg/mL. Do not filter sterilize.
3. Dispense appropriate volume aliquots into polypropylene tubes and store at –70°C until use. Avoid repeated freeze/thaw cycles and use immediately once thawed.

Isolation and Establishment of Primary Cultures

1. Before beginning, prepare culture vessels by precoating with fibronectin. Add 3 mL Human Endothelial-SFM and 100 µg (100 µl of 1 mg/mL stock) human plasma fibronectin per 25 cm^2 flask. Incubate at 37°C for 1–1.5 hours before use (see step 6 of this procedure).
2. Flush canulated, untraumatized umbilical cord segments with 50 mL of Medium 199 supplemented with penicillin (10 U/mL) and streptomycin (10 µg/mL).
3. Incubate umbilical veins with 0.1% collagenase in Medium 199 for 25 minutes at 22°C. Flush, and collect in a sterile conical tube, umbilical vein segments with 50 mL of Medium 199. The resulting cell suspension contains the HUVEC.
4. Centrifuge the cell suspension at 100 × *g* for 5 minutes at room temperature to collect cells. Discard the supernatant and resuspend the cell pellet in 50 mL Medium 199.
5. Repeat step 4.
6. Resuspend the cell pellet in 2 mL Human Endothelial-SFM. Add cell suspension directly to the pre-coated 25 cm^2 culture flasks (step 1) containing 3 mL Human Endothelial-SFM and 100 µg fibronectin (results in 20 µg/mL fibronectin).
7. Supplement each flask with human rbFGF (20 ng/mL, 100 ng/flask), human rEGF (10 ng/mL, 50 ng/flask), penicillin (10 U/mL), and streptomycin (10 µg/mL). Incubate at 37°C in a humidified atmosphere of 5% CO_2 in air.
8. Aspirate and discard spent media 24 hours after plating. Wash attached monolayer of attached HUVEC with DPBS to remove contaminating adherent blood cells. Add 5 mL Human Endothelial-SFM and supplement with human plasma fibronectin (20 µg/mL, 100 µg/flask), human rbFGF (20 ng/mL, 100 ng/flask), human rEGF (10 ng/mL, 50 ng/flask), penicillin (10 U/mL), and streptomycin (10 µg/mL). Incubate at 37°C in a humidified atmosphere of 5% CO_2 in air.
9. Re-feed with medium and supplements or subculture cells after 48–72 hours in culture. Remove antibiotics from the culture at this time.

Subculture Cells

1. Subculture confluent monolayers of HUVEC using TrypLE™ Select or 0.05% Trypsin-EDTA.
2. Aspirate and discard spent media. Add 1–2 mL TrypLE™ Select (or 0.05% Trypsin-EDTA); ensure complete coverage of the flask surface.
3. Incubate at room temperature for 10–20 seconds. Stand the flask upright and aspirate cell dissociation reagent prior to cell detachment.
   Note: HUVEC cultured in SFM detach very readily from plastic surfaces. Following removal of trypsin, the flask should be observed using an inverted phase contrast microscope to confirm complete cell detachment.
4. Collect detached cells in 5 mL Human Endothelial-SFM (supplementd with Soybean Trypsin Inhibitor if using Trypsin) and transfer to a sterile conical tube. Rinse the flask with an additional 5 mL Human Endothelial-SFM and combine into the conical tube.
5. Centrifuge the cell suspension at 100 × *g* for 5 minutes at room temperature to collect cells. Discard the supernatant and resuspend the cell pellet in 5–10 mL Human Endothelial-SFM.
6. Repeat step 5.
7. Resuspend the cell pellet in 5 mL Human Endothelial-SFM.
8. Add 2.5 mL Human Endothelial-SFM to a new flask and supplement with fibronectin (10 µg/mL), human rbFGF (20 ng/mL), and human rEGF (10 ng/mL).
9. Add 2.5 mL HUVEC suspension to the new flask containing supplemented Human Endothelial-SFM. Incubate at 37°C in a humidified atmosphere of 5% CO_2 in air.
   Note: We recommend subculturing cells using a 1:2 ratio every 4–5 days.
10. Refeed HUVEC cultures 48–72 hours after subculturing by exchanging half of the spent media for fresh Human Endothelial-SFM supplemented with human rbFGF (20 ng/mL) and human rEGF (10 ng/mL), fibronectin can be omitted when refeeding cells.

Cryopreservation

1. Prepare the desired quantity of cells harvesting as above in mid-log phase of growth with viability >90%. Reserve the conditioned medium to prepare cryopreservation medium.
2. Wash cells two times in fresh Human Endothelial-SFM. Determine the viable cell density and calculate the required volume of cryopreservation medium to give a final cell density of 2–3 × 10^6 cells/mL.
3. Prepare the required volume of cryopreservation medium: 92.5% Human Endothelial-SFM (50:50 fresh to conditioned medium) + 7.5% DMSO. Alternatively, cells may be frozen in serum-supplemented medium consisting of 92.5% serum supplemented Medium 199 (4:1 Medium 199 to FBS) + 7.5% DMSO. Store freezing media at 4°C until use.
   Important: Prepare cryopreservation medium on the day of intended use.
4. Harvest cells by centrifugation at 100 × *g* for 5–10 minutes. Resuspend cell pellet in the pre-determined volume of 4°C cryopreservation medium.
5. Dispense 1 mL aliquots of this suspension into each cryovial.
6. Achieve cryopreservation in an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
7. Transfer frozen cells to liquid nitrogen, (vapor phase) storage at –200°C to –125°C is recommended.

Recovery

1. Rapidly thaw (<1 minute) frozen cells in a 37°C water bath.
2. Transfer the entire contents of the cryovial into a T-25 culture flask containing 5 mL prewarmed Human Endothelial-SFM supplemented with human plasma fibronectin (20 µg/mL), human rbFGF (20 ng/mL), human rEGF (10 ng/mL).
3. Incubate at 37°C in a humidified atmosphere of 5% CO_2 in air. Loosen flask caps (or use vented caps) to allow for gas exchange.
4. Subculture cells 3–5 days post thaw.

Related Products

Product	Catalog No.
FGF-basic, Recombinant Human	13256
EGF Recombinant Human	PHG0314
Fibronectin Human, Plasma	33016
Penicillin-Streptomycin, Liquid	15140
Water for Injection (WFI) for Cell Culture	A12873
Dulbecco's Phosphate Buffered Saline, without calcium and magnesium	14190
Collagenase Type II	17101
Medium 199	11150
TrypLE™ Select (1X), no Phenol Red	12563
Trypsin-EDTA (1X)	25300
Trypsin Inhibitor, Soybean	17075
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:	Manufacturer	Batch code	Protect from light	Temperature Limitation
				
Catalog number	Consult instructions for use	Caution, consult accompanying documents	Sterilized using aseptic processing techniques	

Limited Product Warranty

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