Human iPSC-Derived Endothelial Cells

Catalog #200-0907 1 x 10^6 cells

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Product Description

Human iPSC-Derived Endothelial Cells (ECs) are highly pure cells derived and manufactured from the human induced pluripotent stem cell (iPSC) line, Healthy Control Human iPSC Line, Female, SCTi003-A (Catalog #200-0511), using STEMdiff™ Mesoderm Induction Medium (Catalog #05221) and STEMdiff™ Endothelial Differentiation Kit (Catalog #08005). ECs express high levels of endothelial markers CD31, CD144, and CD309, and are negative for hematopoietic and epithelial markers CD45 and CD326, respectively.

ECs can be expanded for at least 3 passages using serum- and xeno-free STEMdiff™ Endothelial Expansion Culture Kit (Catalog #100-1218), allowing for workflow flexibility. Expanded ECs may be used for co-culture applications, microfluidics, vascular disease modeling, drug discovery, and the development of regenerative medicine.

Cells were obtained using Institutional Review Board (IRB)-approved consent forms and protocols.

Stability and Storage

Cells are frozen in a cryopreservation medium containing dimethyl sulfoxide (DMSO). Product stable at -135°C or colder for 12 months from date of receipt. Thawed samples must be used immediately.

Precautions

Cell Screening: iPSC master cell banks are screened for AAV2, BK virus, Epstein-Barr Virus, Hepatitis A, Hepatitis B, Hepatitis C, Herpes Simplex 1 and 2, Herpes Virus Type 6, 7, and 8, HIV-1, HIV-2, HPV-16, HPV-18, Human Adenovirus, Human Cytomegalovirus, Human Foamy Virus, Human T-Lymphotropic Virus, John Cunningham Virus, LCMV, Parvovirus B19, Sarbecovirus (SARS Virus), Seoul Virus, Corynebacterium Bovis, and Mycoplasma (Human Comprehensive CLEAR Panel) by PCR. As testing cannot completely guarantee that the donor was virus-free, THIS PRODUCT SHOULD BE TREATED AS POTENTIALLY INFECTIOUS and only used following appropriate handling precautions such as those described in biological safety level 2.

Storage of frozen cell products in the vapor phase of a liquid nitrogen storage tank is recommended. Storage in the liquid phase can result in cross-contamination if the vial breaks or is not sealed properly. Storage in the liquid phase also increases the potential for liquid nitrogen to penetrate the vial and cause it to explode when removed from storage. Use of a face shield is required as a safety precaution when transferring cells from one container to another. When handling this product, do not use sharps such as needles and syringes.

STEMCELL cannot guarantee the biological function or any other properties associated with performance of cells in a researcher's individual assay or culture systems. STEMCELL assures the cells will meet the specifications only when assessed immediately after thawing by our test methods.

FOR IN VITRO RESEARCH USE ONLY. NOT APPROVED FOR DIAGNOSTIC, THERAPEUTIC, OR CLINICAL APPLICATIONS. NOT APPROVED FOR HUMAN OR VETERINARY USE IN VIVO.

Materials Required but Not Included

PRODUCT NAME	CATALOG #
Animal Component-Free Cell Dissociation Kit • ACF Enzymatic Dissociation Solution • ACF Enzyme Inhibition Solution	05426
Conical tubes, 15 mL and 50 mL	e.g. 38009 and 38010
Costar® 6-Well Flat-Bottom Plate, Tissue Culture-Treated	38015
D-PBS (Without Ca++ and Mg++)	37350
Hausser Scientific™ Bright-Line Hemocytometer	e.g. 100-1181
Heparin Solution	07980
Low protein binding polyethersulfone (PES) filter unit (0.2 - 0.22 µm)	e.g. Fisher 09-741-04 (0.2 µm, 250 mL) OR Fisher SCGP00525 (0.22 µm, 50 mL)
STEMdiff™ Endothelial Expansion Culture Kit	100-1218
T-75 cm^2 flasks, tissue culture-treated	e.g. 200-0501
Trypan Blue	07050

Preparation of Reagents and Materials

A. COATING CULTUREWARE WITH ANIMAL COMPONENT-FREE CELL ATTACHMENT SUBSTRATE

Use sterile technique when coating cultureware with Animal Component-Free Cell Attachment Substrate (included in STEMdiff™ Endothelial Expansion Culture Kit; Catalog #100-1218).

NOTE: Use only tissue culture-treated cultureware.

1. Dilute Animal Component-Free Cell Attachment Substrate 1 in 100 in D-PBS (Without Ca++ and Mg++).
   For example, add 100 µL of ACF Cell Attachment Substrate to 9.9 mL of PBS.
2. Gently mix diluted substrate solution. Do not vortex.
3. Immediately use the diluted substrate solution to coat cultureware. Refer to Table 1 for recommended coating volumes.
4. Gently tilt the cultureware to spread the diluted substrate solution evenly across the surface.
5. Incubate at room temperature (15 - 25°C) for at least 2 hours before use. Do not let the diluted substrate solution evaporate.
   NOTE: If not used immediately, the cultureware must be sealed to prevent evaporation of the diluted substrate solution (e.g. with Parafilm®). Sealed cultureware can be stored at 2 - 8°C for up to 3 days after coating. Warm to room temperature before use.
6. Gently tilt the cultureware onto one side and allow excess substrate solution to collect at the edge. Remove the excess solution using a serological pipette or by aspiration. Ensure that the coated surface is not scratched.
7. Rinse the cultureware twice using D-PBS (e.g. use 2 mL/well if using a 6-well plate).
8. Aspirate wash solution immediately prior to seeding cells. Do not let the surface dry.

Table 1. Recommended Volumes for Coating Cultureware with Diluted Animal Component-Free Cell Attachment Substrate

CULTUREWARE	VOLUME OF DILUTED ANIMAL COMPONENT-FREE CELL ATTACHMENT SUBSTRATE SOLUTION
6-well plate	1 mL/well
T-25 cm^2 flask	2 - 3 mL/flask
T-75 cm^2 flask	5 - 6 mL/flask

B. PREPARATION OF STEMDIFF™ ENDOTHELIAL EXPANSION MEDIUM

Use sterile technique to prepare STEMdiff™ Endothelial Expansion Medium (STEMdiff™ Endothelial Expansion Basal Medium + STEMdiff™ Endothelial Expansion 5X Supplement + Heparin Solution). The following example is for preparing 100 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw 5X Supplement either at 2 - 8°C overnight or at 37°C until fully thawed. Mix thoroughly but do not vortex.

   NOTE: Some precipitate may form. This will not affect product performance and will be removed when the complete medium is filtered (step 4).

   NOTE: Once thawed, use immediately or aliquot and store at -20°C. Do not exceed the shelf life of the supplement. After thawing the aliquots, use immediately. Do not re-freeze.
2. Warm STEMdiff™ Endothelial Expansion Basal Medium and Heparin Solution to room temperature (15 - 25°C).
3. Add 20 mL of 5X Supplement to 79.4 mL of Basal Medium. Add 625 µL of Heparin Solution (final concentration of 12.5 µg/mL). Mix thoroughly.

   NOTE: Since Heparin Solution contains non-human animal-derived components, the complete medium will not be xeno-free.
4. Filter the complete medium through a 0.2 - 0.22 µm PES filter unit. Warm complete medium to room temperature before use.

   NOTE: If not used immediately, store STEMdiff™ Endothelial Expansion Medium at 2 - 8°C for up to 2 weeks. Do not freeze complete medium. If precipitate is observed, filter again as described above. This will not affect the performance of the medium.

Directions for Use

A. THAWING AND PLATING iPSC-DERIVED ENDOTHELIAL CELLS

Generally, 1 x 10^6 ECs are sufficient to seed one T-75 cm^2 flask. The following instructions are for thawing one vial of ECs and seeding into a T-75 cm^2 flask. If using other cultureware, adjust volumes accordingly. Refer to Table 2 for recommended volumes and seeding densities for various cultureware.

NOTE: Do not centifuge ECs directly after thawing. This may result in low post-thaw viability and reduced cell attachment. The cryopreservation medium containing DMSO will be removed from the culture by performing a full-medium change 24 hours after seeding.

1. Coat a tissue culture-treated T-75 cm^2 flask with ACF Cell Attachment Substrate (Preparation section A) and prepare complete STEMdiff™ Endothelial Expansion Medium (Preparation section B).
2. Warm STEMdiff™ Endothelial Expansion Medium and coated cultureware to room temperature (15 - 25°C) before starting the protocol to ensure that the thawing procedure is done as quickly as possible.
3. Add 2 mL of warm STEMdiff™ Endothelial Expansion Medium to a 15 mL conical tube.
4. Wipe the outside of the vial of cells with 70% ethanol or isopropanol.
5. In a biosafety hood, twist the cap a quarter-turn to relieve internal pressure and then retighten.
6. Quickly thaw cells in a 37°C water bath by gently shaking the cryovial continuously until only a small frozen cell pellet remains. Do not vortex cells.
7. Remove the cryovial from the water bath and wipe it with 70% ethanol or isopropanol.

   NOTE: It is important to work quickly in the following steps to ensure high cell viability and recovery.
8. Transfer cells from the cryovial to the 15 mL conical tube (prepared in step 3). Mix gently.
9. Rinse the cryovial with 1 mL of warm complete STEMdiff™ Endothelial Expansion Medium and add the rinse to the 15 mL tube.
10. Count viable cells using Trypan Blue and a hemocytometer.
11. Plate cells at a density of 1 - 1.5 x 10^4 cells/cm^2 into the pre-coated T-75 cm^2 flask (prepared in step 1) containing ~15 mL of warm complete STEMdiff™ Endothelial Expansion Medium. Refer to Table 2 for recommended volumes. Move the flask in several quick, short, back-and-forth and side-to-side motions to evenly distribute the ECs across the surface.
12. Incubate cells at 37°C and 5% CO_2 for 24 hours.
13. Perform a full-medium change with STEMdiff™ Endothelial Expansion Medium to remove the cryopreservation medium from the culture.
14. Incubate cells at 37°C and 5% CO_2 for approximately 3 - 5 days. When the culture is 90 - 100% confluent, proceed to section B to passage the cells.

    NOTE: If the culture is not 90 - 100% confluent after 3 days, perform a full-medium change with warm STEMdiff™ Endothelial Expansion Medium and continue incubation.

B. PASSAGING ENDOTHELIAL CELLS FOR EXPANSION

The following instructions are for passaging a T-75 cm^2 flask of ECs. If using other cultureware, adjust volumes accordingly. Refer to Table 2 for recommended volumes and seeding densities for various cultureware.

1. Coat a tissue culture-treated T-75 cm^2 flask with ACF Cell Attachment Substrate (Preparation section A).
2. Warm sufficient volumes of ACF Enzymatic Dissociation and ACF Enzyme Inhibition Solution, complete STEMdiff™ Endothelial Expansion Medium, and coated cultureware to room temperature (15 - 25°C). Do not incubate at 37°C.
3. Aspirate medium and wash the flask once with 6 mL of D-PBS (Without Ca++ and Mg++). Discard the wash.
4. Add 6 mL of ACF Enzymatic Dissociation Solution and incubate at 37°C for 4 - 5 minutes.
5. Tap the flask to detach the remaining cells. If less than 90% of cells have detached, incubate at 37°C for an additional 1 - 2 minutes and tap the flask again. Do not exceed 7 minutes of incubation.

   NOTE: Regardless of whether the cells detach, proceed to the next step.
6. Add 6 mL of ACF Enzyme Inhibition Solution and collect cells in a 50 mL conical tube.
7. Wash the flask with 6 mL of D-PBS and transfer the wash to the same tube.
8. Centrifuge the tube at 300 x g for 5 minutes.
9. Discard the supernatant. Resuspend the cell pellet in 2 mL complete STEMdiff™ Endothelial Expansion Medium.
10. Count viable cells using Trypan Blue and a hemocytometer.
11. Plate cells at a density of 1 - 1.5 x 10^4 cells/cm^2 onto pre-coated cultureware (prepared in step 1). Move the cultureware in several quick, short, back-and-forth, and side-to-side motions to evenly distribute the ECs across the surface.
12. Incubate cells at 37°C and 5% CO_2. Passage ECs every 3 - 5 days when the culture reaches 90 - 100% confluence. If the cells have not grown to confluency after 3 days, perform a full-medium change.

Table 2. Recommended Volumes and Cell Plating Densities for Passaging ECs

VOLUME OF SOLUTIONS AND CELL NUMBERS	6-WELL PLATE	T-25 cm^2 FLASK	T-75 cm^2 FLASK
D-PBS Wash	1 mL/well	2 mL/flask	6 mL/flask
ACF Enzymatic Dissociation Solution	1 mL/well	2 mL/flask	6 mL/flask
ACF Enzymatic Inhibition Solution	1 mL/well	2 mL/flask	6 mL/flask
Complete STEMdiff™ Endothelial Expansion Medium	2 mL/well	5 mL/flask	15 mL/flask
Recommended Cell Numbers	0.96 - 1.44 x 10^5	2.5 - 3.75 x 10^5	7.5 - 11.25 x 10^5

Assessment of Endothelial Cells

Human iPSC-Derived ECs can be assessed by flow cytometry after at least one passage in STEMdiff™ Endothelial Expansion Medium. The following fluorochrome-conjugated antibodies have been validated for the assessment of Human iPSC-Derived ECs:

- Anti-human CD31 (PECAM-1) antibody, clone WM59 (BioLegend #303115)
- Anti-human CD144 antibody, clone 55-7H1 (BD Biosciences #560874)
- Anti-human CD309 antibody, clone 7D4-6 (BioLegend #359903)
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018)
- Anti-human CD326 (EpCAM) antibody, clone 9C4 (BioLegend #324212)
- Anti-Human OCT4 (OCT3) Antibody, Clone 40 or Clone 3A2A20 (Catalog #60059 or #60093)
- Anti-Human TRA-1-60 Antibody, Clone TRA-1-60R (Catalog #60064)

≥ 70% of Human iPSC-Derived ECs express endothelial markers CD31, CD144, and CD309 and are negative for hematopoietic and epithelial markers CD45 and CD326, respectively. Expression of CD31, CD144, and CD309 will increase to ≥ 90% with subsequent passaging. The absence of undifferentiated cells can be confirmed by flow cytometry after labeling with fluorochrome-conjugated anti-OCT4 and anti-TRA-1-60.

Related Products

For related products, including specialized cell culture and storage media, supplements, antibodies, cytokines, and small molecules, visit www.stemcell.com, or contact us at techsupport@stemcell.com.