STEMdiff™ Endothelial Differentiation and Expansion Kits

Catalog #08005 1 Kit
#08007 1 Kit
#100-1218 1 Kit

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Product Description

STEMdiff™ Endothelial Differentiation Kit is a serum- and xeno-free system for the robust and efficient generation of highly pure human pluripotent stem cell (hPSC)-derived endothelial cells (ECs) without the need for cell enrichment. This kit includes animal component-free STEMdiff™ Endothelial Induction Medium, serum- and xeno-free STEMdiff™ Endothelial Expansion Basal and 5X Supplement, and Animal Component-Free Cell Attachment Substrate.

This kit is compatible with hPSCs maintained in mTeSR™1 (Catalog #85850), mTeSR™ Plus (Catalog #100-0276), or TeSR™-E8™ (Catalog #05990) on Corning® Matrigel®. hPSCs are cultured for 2 days in STEMdiff™ Mesoderm Induction Medium (Catalog #05220) on Corning® Matrigel® to generate early mesoderm cells, then cultured for 4 days in STEMdiff™ Endothelial Induction Medium for differentiation to hemogenic/vascular progenitor cells. By Day 7, 50 - 80% of cells are positive for CD31, CD34, and CD144 and are ready for use in downstream applications.

These cells can then be expanded for 5 - 6 passages in STEMdiff™ Endothelial Expansion Medium and Animal Component-Free Attachment Substrate to generate large numbers of highly pure, mature ECs that typically reach ≥ 99% CD144+CD31+ by passage 2 and maintain this phenotype until passage 5. STEMdiff™ Endothelial Expansion Medium is available in a kit with (Catalog #100-1218) or without (Catalog #08007) Animal Component-Free Cell Attachment Substrate. For passaging, Animal Component-Free Cell Dissociation Kit (Catalog #05426) is required.

NOTE: Heparin Solution (Catalog #07980) is required for preparation of complete STEMdiff™ Endothelial Expansion Medium. See Preparation of Reagents and Materials.

Ordering Information

PRODUCT NAME	CATALOG #	COMPONENTS
STEMdiff™ Endothelial Differentiation Kit	08005	• STEMdiff™ Endothelial Induction Medium • STEMdiff™ Endothelial Expansion Basal Medium • STEMdiff™ Endothelial Expansion 5X Supplement • Animal Component-Free Cell Attachment Substrate
STEMdiff™ Endothelial Expansion Medium Kit	08007	• STEMdiff™ Endothelial Expansion Basal Medium • STEMdiff™ Endothelial Expansion 5X Supplement
STEMdiff™ Endothelial Expansion Culture Kit	100-1218	• STEMdiff™ Endothelial Expansion Basal Medium • STEMdiff™ Endothelial Expansion 5X Supplement • Animal Component-Free Cell Attachment Substrate

Component Storage and Stability

The following components are sold as part of complete kits (Catalog #08005 and 08007) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
STEMdiff™ Endothelial Induction Medium	08006	100 mL	Store at -20°C.	Stable for 3 years from date of manufacture (MFG) on label.
STEMdiff™ Endothelial Expansion Basal Medium	08008	120 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Endothelial Expansion 5X Supplement*	08009	30 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
Animal Component-Free Cell Attachment Substrate	07130	1 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.

*This component contains material derived from human plasma. Donors have been tested and found negative for HIV-1 and -2, hepatitis B, and hepatitis C prior to donation. However, this product should be considered potentially infectious and treated in accordance with universal handling precautions.

Materials Required but Not Included

PRODUCT NAME	CATALOG #
ACCUTASE™	07920
Animal Component-Free Cell Dissociation Kit • ACF Enzymatic Dissociation Solution • ACF Enzyme Inhibition Solution	05426
Conical tubes, 15 or 50 mL	e.g. 38009 or 38010
Corning® Matrigel® hESC-Qualified Matrix	Corning 354277
D-PBS (Without Ca++ and Mg++)	37350
Hausser Scientific™ Bright-Line Hemocytometer	100-1181
Heparin Solution	07980
mTeSR™1 OR mTeSR™ Plus OR TeSR™-E8™	85850 OR 100-0276 OR 05990
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™ Mesoderm Induction Medium	05220
Tissue culture-treated 6-well plates	e.g. 38016
Trypan Blue	07050
Y-27632 (Dihydrochloride)	72302

Preparation of Reagents and Materials

A. STEMdiff™ ENDOTHELIAL INDUCTION MEDIUM

Thaw STEMdiff™ Endothelial Induction Medium at 2 - 8°C overnight or at room temperature (15 - 25°C). If not used immediately, store at 2 - 8°C for up to 2 weeks.

B. 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 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.

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

C. COATING CULTUREWARE WITH CORNING® MATRIGEL®

For complete instructions for coating cultureware with Corning® Matrigel®, refer to the Technical Manual: Maintenance of Human Pluripotent Stem Cells in mTeSR™1, available at www.stemcell.com or contact us to request a copy.

D. COATING CULTUREWARE WITH ANIMAL COMPONENT-FREE (ACF) CELL ATTACHMENT SUBSTRATE

For passaging and expansion of ECs, coat cultureware with ACF Cell Attachment Substrate as described below.

NOTE: Use sterile technique; only use tissue culture-treated cultureware.

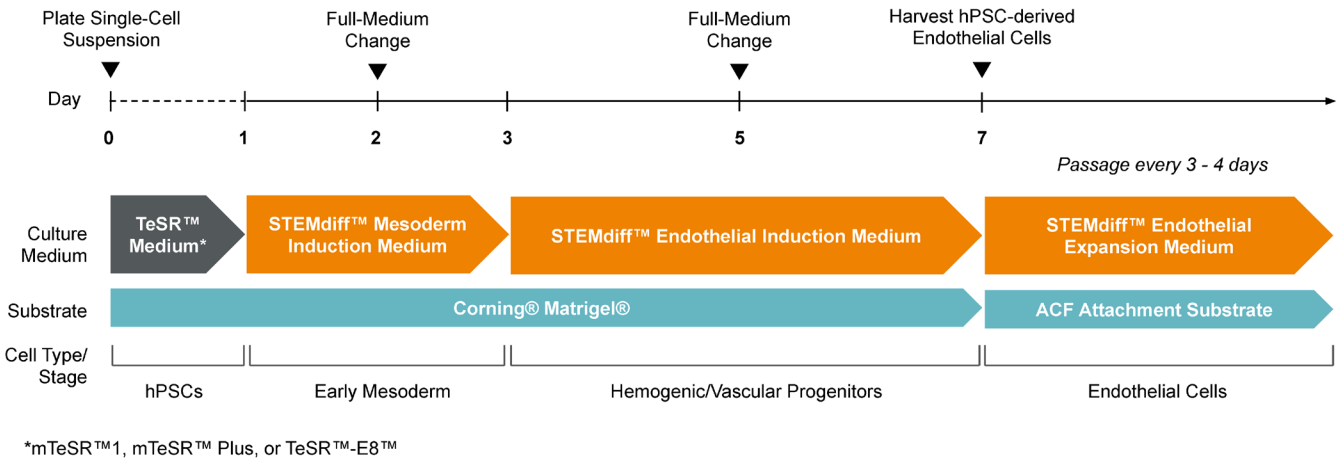
1. Dilute ACF Cell Attachment Substrate 1 in 100 in D-PBS (Without Ca++ and Mg++).
   For example, add 100 µL of ACF Cell Attachment Substrate to 9.9 mL of D-PBS.
2. Gently mix diluted ACF Cell Attachment Substrate. Do not vortex.
3. Immediately use diluted ACF Cell Attachment Substrate to coat cultureware. Refer to Table 1 for recommended coating volumes.

Table 1. Recommended Volumes for Coating Cultureware with Diluted ACF Cell Attachment Substrate

CULTUREWARE	VOLUME OF DILUTED ACF CELL ATTACHMENT SUBSTRATE
24-well plate	0.4 mL/well
6-well plate	1 mL/well
T-25 cm^2 flask	2 - 3 mL/flask
T-75 cm^2 flask	5 - 6 mL/flask

4. Gently rock cultureware back and forth to spread ACF Cell Attachment Substrate evenly across the surface. Seal with Parafilm®.
5. Incubate sealed cultureware at room temperature (15 - 25°C) for at least 2 hours before use. Do not let ACF Cell Attachment Substrate evaporate.
   NOTE: Sealed coated cultureware can be stored at 2 - 8°C for up to 3 days after coating. Allow stored coated cultureware to warm to room temperature for 30 minutes before proceeding to step 6.
6. Gently tilt cultureware onto one side and allow excess ACF Cell Attachment Substrate to collect at the edge. Remove excess solution using a serological pipette or by aspiration. Ensure that the coated surface is not scratched.
7. Wash cultureware twice with D-PBS (e.g. use 2 x 2 mL/well if using a 6-well plate).
8. Aspirate D-PBS. The coated cultureware is now ready for use.

Protocol Diagram



*mTeSR™1, mTeSR™ Plus, or TeSR™-E8™

Directions for Use

For differentiation of hPSCs to ECs, proceed to section A; for passaging and expansion of ECs, proceed to section B.

A. DIFFERENTIATION OF hPSCs TO ECs (MONOLAYER PROTOCOL)

The following protocol is for differentiation of hPSCs that have been maintained in mTeSR™1 on Corning® Matrigel®. mTeSR™ Plus or TeSR™-E8™ may also be used for hPSC maintenance; in the protocol below, use the same TeSR™ medium that was used for hPSC maintenance. When hPSC colonies are confluent and ready for passaging, proceed to Day 0: hPSC seeding.

Day 0: hPSC seeding

1. Prepare a single-cell suspension as described in section 7.2 of the Technical Manual: Maintenance of Human Pluripotent Stem Cells in mTeSR™1, available at www.stemcell.com or contact us to request a copy.
2. Seed hPSCs at a density of 5000 to 10,000 cells/cm^2 in mTeSR™1 + 10 µM Y-27632 on a Matrigel®-coated 6-well plate.

   NOTE: The seeding density may need to be adjusted depending on the cell line and hPSC maintenance medium used. Refer to Table 2 for recommended plating densities.

Table 2. Recommended Cell Plating Densities for Setting up Endothelial Induction

hPSC MAINTENANCE MEDIUM	PLATING DENSITY (cells/cm^2)	EXAMPLE OF NUMBER OF CELLS PER WELL OF A 6-WELL PLATE
mTeSR™1	5000 to 10,000 cells/cm^2	5.0 x 10^4 7.5 x 10^4 10.0 x 10^4
mTeSR™ Plus	3500 to 7,500 cells/cm^2	3.5 x 10^4 5.0 x 10^4 7.5 x 10^4
TeSR™-E8™	5000 to 10,000 cells/cm^2	5.0 x 10^4 7.5 x 10^4 10.0 x 10^4

Day 1: Mesoderm induction

For complete instructions on storage, handling, and thawing of STEMdiff™ Mesoderm Induction Medium, refer to the corresponding Product Information Sheet. The mesoderm induction stage generates early mesoderm cells from hPSCs.

The following instructions are for one well of a 6-well plate. For other cultureware, adjust volumes accordingly.

1. Warm STEMdiff™ Mesoderm Induction Medium to room temperature (15 - 25°C).
2. Aspirate medium from the well. Slowly add 3 mL of STEMdiff™ Mesoderm Induction Medium down the side of the well.
3. Incubate at 37°C for 24 hours.

   NOTE: Some degree of cell loss is expected at this stage.

Day 2

4. Repeat steps 1 - 3 above.

Day 3: Endothelial induction

5. Thaw STEMdiff™ Endothelial Induction Medium (see Preparation section).
6. Warm STEMdiff™ Endothelial Induction Medium to room temperature.
7. Aspirate medium from the well. Slowly add 4 mL of STEMdiff™ Endothelial Induction Medium down the side of the well.
8. Incubate at 37°C for 48 hours. Observe the cultures daily for changes in morphology.

Day 5

9. Repeat steps 6 - 8.

Day 7: Harvest & subculture of hPSC-derived ECs

10. Prepare complete STEMdiff™ Endothelial Expansion Medium (see Preparation section).
11. Coat the desired culture vessel with ACF Cell Attachment Substrate (see Preparation section).
12. Harvest cells using ACCUTASE™ as follows:
    a. Warm ACCUTASE™ to room temperature. Do not incubate at 37°C.
    b. Gently wash the well twice with D-PBS (Without Ca++ and Mg++).
    c. Add 1 mL ACCUTASE™ per well.
    d. Incubate at 37°C for 4 - 5 minutes.

e. Gently tap the cultureware to detach cells. If less than 90% of the cells have detached, incubate at 37°C for an additional 1 - 2 minutes and tap again. Do not exceed 7 minutes of incubation.
f. Add 1 mL D-PBS to each well.
g. Gently pipette up and down to create a single-cell suspension and to lift any remaining cells. Add the cell suspension to a conical tube. Duplicate wells may be combined at this time.
h. Wash the well or flask with D-PBS and add the wash to the corresponding tube from step g.
i. Centrifuge the cell suspension at 300 x *g* for 5 minutes with the brake on.
j. Remove and discard the supernatant.
k. Resuspend each cell pellet in STEMdiff™ Endothelial Expansion Medium.

13. Perform a viable cell count using Trypan Blue and a Hausser Scientific™ Bright-Line Hemocytometer.

    OPTIONAL: Perform flow cytometry on the harvested cells to assess surface marker expression.

14. Add cells to coated cultureware (prepared in step 11) at the desired density in STEMdiff™ Endothelial Expansion Medium. Refer to Table 3 for recommended volumes. The optimal density is 10,000 cells/cm^2 for cells to reach confluency in 3 - 5 days.

    NOTE: Appropriate seeding density range is 5000 - 15,000 cells/cm^2; this may require optimization depending on the proliferative potential of the hPSC-derived ECs.

Table 3. Recommended Volume of Medium for Various Cultureware

CULTUREWARE	VOLUME OF STEMdiff™ ENDOTHELIAL EXPANSION MEDIUM
6-well plate	2 mL/well
T-25 cm^2 flask	4 mL/flask
T-75 cm^2 flask	10 mL/flask

15. Incubate at 37°C and 5% CO_2. If the cells have not grown to confluence after 3 days, perform a full-medium change with STEMdiff™ Endothelial Expansion Medium and continue incubation. When cells have reached 90 - 100% confluency (see Figure 1), proceed to section B for passaging/expansion.

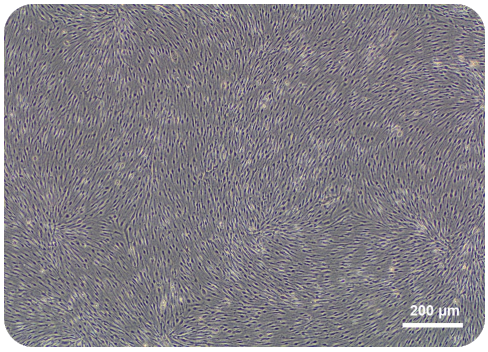


Figure 1. 90 - 100% Confluent Monolayer of H9-derived ECs at Passage 2 (4X Magnification)

B. PASSAGING AND EXPANSION OF ECs

1. Coat the desired culture vessel(s) for expansion (e.g. T-25 cm^2 or T-75 cm^2 flask) with ACF Cell Attachment Substrate (see Preparation section).
2. Detach ECs using Animal Component-Free Cell Dissociation Kit as follows:
   a. Warm ACF Enzymatic Dissociation Solution and ACF Enzyme Inhibition Solution to room temperature (15 - 25°C). Do not incubate at 37°C.
   b. Wash the well or flask twice with D-PBS (Without Ca++ and Mg++).
   c. Add ACF Enzymatic Dissociation Solution to the well or flask according to Table 4.

Table 4. Recommended Volume of ACF Enzymatic Dissociation Solution or ACF Enzyme Inhibition Solution for Various Cultureware

CULTUREWARE	VOLUME OF ACF ENZYMATIC DISSOCIATION SOLUTION OR ACF ENZYME INHIBITION SOLUTION
6-well plate	0.5 mL/well
T-25 cm^2 flask	1 mL/flask
T-75 cm^2 flask	2 mL/flask

d. Incubate at 37°C for 4 - 5 minutes.
e. Gently tap the cultureware to detach cells. If < 90% of cells have detached, incubate at 37°C for an additional 1 - 2 minutes and tap again.
f. Add ACF Enzyme Inhibition Solution to the well or flask as indicated in Table 4 and collect cells in a polypropylene tube (e.g. 15 mL conical tube [Catalog #38009]).
g. Wash the well or flask with D-PBS and pipette up and down to lift any remaining cells. Add the wash to the corresponding tube from step f.
h. Centrifuge the cell suspension at 300 x *g* for 5 minutes with the brake on.
i. Remove and discard the supernatants. Resuspend each cell pellet in STEMdiff™ Endothelial Expansion Medium as follows:
   - For one well of a 6-well plate, resuspend in 0.3 - 0.5 mL medium
   - For a T-25 cm^2 flask, resuspend in 1 mL medium
   - For a T-75 cm^2 flask, resuspend in 2 mL medium

3. Perform a viable cell count using Trypan Blue and a Hausser Scientific™ Bright-Line Hemocytometer.

   OPTIONAL: Perform flow cytometry on the harvested cells to assess surface marker expression.
4. Add cells to coated plates or flasks at the desired density in STEMdiff™ Endothelial Expansion Medium. The optimal density is 10,000 cells/cm^2 to reach confluency in 3 - 5 days.

   NOTE: Appropriate seeding density range is 5000 - 15,000 cells/cm^2; this may require optimization, depending on the proliferative potential of the hPSC-derived ECs.
5. Incubate at 37°C and 5% CO_2. If the cells have not grown to confluency after 3 days, perform a full-medium change.

Assessment of hPSC-Derived ECs

Assessment of hPSC-derived ECs can be verified on day 7 by flow cytometry after labeling with fluorochrome-conjugated antibodies (see list below for examples). On day 7, > 50% of cells express CD34, CD31, and CD144 and do not express lymphocyte marker CD45. The absence of undifferentiated cells can be confirmed by flow cytometry after labeling with fluorochrome-conjugated anti-OCT4 and anti-TRA-1-60. After two passages in STEMdiff™ Endothelial Expansion Medium, ≥ 99% of hPSC-derived ECs are $CD31^+CD144^+$. Results may vary depending on the cell line used.

- Anti-Human CD34 Antibody, Clone 581 (Catalog #60013)
- Anti-Human CD31 antibody, clone WM59
- Anti-Human CD144 antibody, clone 55-7H1
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018)
- Anti-Human CD309 antibody, clone 7D4-6
- Anti-Human CD105 Antibody, Clone 43A3 (Catalog #43A3)

Related Products

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