Rev. 1

hPSC-derived Endothelial Cells (H9-EC)

Catalog #1680

Cell Specification

Human Endothelial Cells (H9-EC) from ScienCell Research Laboratories are differentiated from a human pluripotent stem cell line (H9), which is human blastocyst derived. The monolayer H9 efficiently generate mesoderm cells via small-molecule activation of WNT signaling in chemically defined media, and subsequently converted to endothelial cells through endothelial cell specification using vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF).

The derived endothelial cells are characterized by immunofluorescence with antibodies specific to CD31 and vWF. The cell population is highly pure: >80% of cells express CD31 (PECAM-1) & vWF positive. H9-EC are cryopreserved at passage one and delivered frozen. Each vial contains >1 x 10^6 cells in 1 ml volume. Cells are negative for mycoplasma, bacteria, yeast and fungi. After reviving, H9-EC can be maintained in Endothelial Cell Medium as an adherent culture. H9-EC are not recommended for expanding or long-term cultures.

Product Content

Cat. #	# of vials	Product	Quantity	Storage
1680	1	H9-EC	1 x 10^6 cells	Liquid Nitrogen
1001	1	Endothelial Cell Medium-basal (ECM)	500mL	4°C
1052	1	Endothelial Cell Growth Supplement (ECGS)	5mL	-20°C
0025	1	Fetal Bovine Serum (FBS)	25mL	-20°C
0503	1	Penicillin-Streptomycin (P/S)	5mL	-20°C

Recommended Medium

It is recommended to use the provided Endothelial Cell Medium (ECM, Cat. #1001) for plating H9-EC and culturing them.

Additional Materials Recommended (Not provided)

Cat. #	Product	Vendor
8248	Bovine Plasma Fibronectin	ScienCell Research Laboratories
0303	DPBS without Ca^{2+} and Mg^{2+}	ScienCell Research Laboratories

Product Use

H9-EC are for research use only. It is not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

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Storage

Upon receiving, directly and immediately transfer Cat. #1680 from dry ice to liquid nitrogen and keep the cells in liquid nitrogen until they are needed for experiments. Store Cat. #1001 at 4°C, Cat. #1052, 0025 and 0503 at -20°C.

Shipping

Cat. #1680, 1052, 0025 and 0503 are shipped on dry ice. Cat. #1001 is shipped at room temperature.

References

[1] Kennedy Crystal C., Brown Erin E., Abutaleb Nadia O., Truskey George A. (2021) "Development and Application of Endothelial Cells Derived from Pluripotent Stem Cells in Microphysiological Systems Models." *Frontiers in Cardiovascular Medicine*.

[2] Gu M (2018) "Efficient Differentiation of Human Pluripotent Stem Cells to Endothelial Cells." *Curr Protoc Hum Genet*.

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Instructions for culturing cells

Caution: Cryopreserved cells are very delicate. Thaw the vial in a 37°C water bath and return them to culture as quickly as possible with minimal handling!

***Note**: H9-EC are not recommended for expanding or long-term cultures. Experiments should be well organized before thawing the cells. It is recommended that H9-EC are used as early as possible for experiments after initial plating with minimal expansion. We do not recommend subculturing the cells.*

Initiating the culture as an adherent culture:

1. Dilute fibronectin (Cat #8248) in $Ca2^+$, $Mg2^+$-free phosphate buffered saline (Cat #0303). Coat the culture surface at 1-5 µg/cm^2 with a minimal volume. Incubate at 37°C incubator or at room temperature for at least 2 hours.
2. Prepare complete Endothelial Cell Medium (ECM): thaw the FBS, ECGS supplement and P/S at room temperature; decontaminate the external surfaces of medium bottle and supplement tube with 70% ethanol and transfer them to a sterile field. Aseptically open the supplement tube and add to the basal medium with a pipette. Rinse the tube with medium to recover the entire volume.

   Warm the medium to room temperature prior to thawing the cells.
3. Take one vial of endothelial cells out of the liquid nitrogen. Immediately transfer the vial to a 37°C water bath and gently swirl it until most of contents are thawed and only a small piece of ice remains.

   ***Note**: The viability of the cells will decrease if the vial contents are completely thawed.*
4. Immediately remove the vial from the water bath, wipe it dry, rinse the vial with 70% ethanol and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads. Using a 2 mL pipette, gently resuspend the contents of the vial. Transfer cell suspension to 15 mL tube containing 10 mL of endothelial cell medium. Wash the emptied vial with 1 mL medium and combine with the cell suspension in the tube.

   ***Note**: Minimize the time for step 5-6.*
5. Centrifuge the tube at 1000 rpm for 5 minutes at room temperature.
6. Aspirate supernatant carefully. Be careful not to disturb the cell pellet
7. Tighten the cap of the tube and loosen the cell pellet by tapping the bottom of the tube. Add 1 mL of the endothelial cell medium and mix well. If a large visible cell pellet is present, try to break them into small pieces by gently pipetting 2 – 3 times with a 2 mL pipette.
8. The recommended cell seeding density is 10000 – 20000 cells/cm^2. We recommend following Table 1 for seeding H9-EC onto 24 well plates or T-25 flasks, or a T-75 flask.

Table 1.

Recommended seeding volume per vial using 24 well plate and T-25 flask.

Well format	Surface area/well (approx. values)	Volume of media/well	Volume of cell suspension from vial/well #	# of wells/vial
24 well	1.9 cm^2	1.0 mL	30 ul	30 wells
T-25	25 cm^2	7.0 mL	330 ul	3 flasks
T-75	75 cm^2	20 mL	1 mL	1 flask

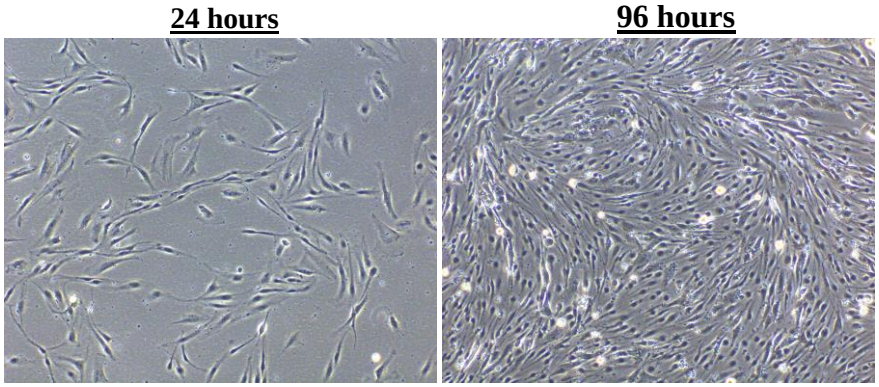
9. Bring the fibronectin coated plate to the hood and aspirate the fibronectin from the well or flask. Gently pipet the correct volume of cell suspension into each well or flask containing complete medium. Replace the cover and gently rock the vessel to distribute the cells evenly.
10. Return the culture vessel to the incubator.
11. For best results, do not disturb the culture for 24 hours after the culture has been initiated. Change the medium the next day to remove unattached cells, then every other day thereafter.

    Note: To maintain the purity of endothelial cells, we do not recommend passaging cells.

Caution: Handling human-derived products is potentially biohazardous. Always wear gloves and safety glasses when working with these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].

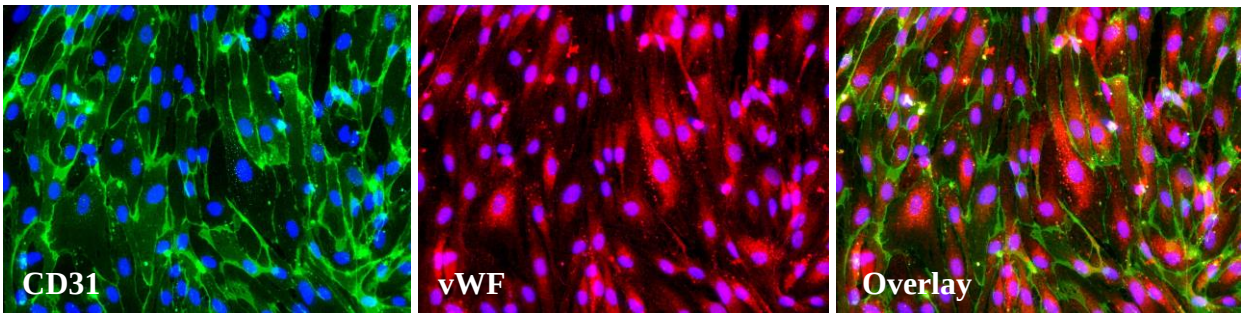
[1] Grizzle, W. E., and Polt, S. S. (1988) "Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues." *J Tissue Culture Methods*. 11(4).

Figure 1. Revived H9-EC at 24 hours and 96 hours of post-plating.



Pictures were taken 24 hours and 96 hours of post-plating. 100x

Figure 2. Revived H9-EC express endothelial cell markers: CD31 and vWF.



The revived H9-EC were characterized by immunostaining with antibodies against CD31 (PECAM-1) (green) and vWF (Red). Nuclei were stained with DAPI (blue). 200x