Human Cardiac Micrvascular Endothelial cells (HCMEC) provided by Innoprot are isolated by ScienCell Research Laboratories from human heart tissue. HCMEC are cryopreserved immediately after purification and delivered frozen. HCMEC are guaranteed to further expand for 15 population doublings at the conditions provided by ScienCell Research Laboratories.

The endothelial cells modulate vascular tone by release of several endothelium-derived contracting and relaxing factors, by regulation and degradation of vasoactive peptides, and by enzymes located on the endothelial surface. These functions vary within different spaces, within blood vessels of different organs, and between the macro- and microvasculations. They release cardioactive substances include nitric oxide, endothelin-1, prostanoids, adenylpurines, natriuretic peptides, and other agents, that modify and regulate cardiac myocyte function. Such effects of endothelial cells have been demonstrated both in vivo and in vitro.

Recommended Medium

Endothelial Cell Medium
(Reference: P60104)

Product Characterization

Immunofluorescent method
- vWF/Factor VIII and
- CD31 (P-CAM)
- Uptake of Dil-Ac-LDL

The cells test negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi

Product Use

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in vitro diagnostic or clinical procedures.
**INSTRUCTIONS FOR CULTURING CELLS**

**IMPORTANT:** Cryopreserved cells are very delicate. Thaw the vial in a 37 ºC waterbath and return them to culture as quickly as possible with minimal handling!

### Set up culture after receiving the order:

1. Prepare a fibronectin coated flask (2 μg/cm², T-75 flask is recommended). Add 10 ml of sterile Dulbecco’s phosphate buffered saline (DPBS) to a T-75 flask and then add 150 μl of fibronectin stock solution (1 mg/ml, Sigma cat. no. F1141). Leave the flask in incubator overnight.

2. Prepare complete medium: decontaminate the external surfaces of medium and medium supplements with 70% ethanol and transfer them to sterile field. Aseptically open each supplement tube and add them to the basal medium with a pipette. Rinse each tube with medium to recover the entire volume.

3. Aspirate fibronectin solution and add 20 ml of complete medium to the flask. Leave the flask in the hood and go to thaw the cells. The fibronectin solution can be used twice.

4. Place the vial in a 37°C waterbath, hold and rotate the vial gently until the contents are completely thawed. Remove the vial from the waterbath immediately, 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 with fingers. Using a 1 ml eppendorf pipette gently resuspend the contents of the vial.

5. Dispense the contents of the vial into the equilibrated, fibronectin-coated flask. A seeding density of 5,000 cells/cm² is recommended. Note: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of DMSO residue in the culture. It is also important that endothelial cells are plated in fibronectin coated flask that promotes cell attachment.

6. Replace the cap or cover of flask, and gently rock the flask to distribute the cells evenly. Loosen cap if necessary to permit gas exchange.

7. Return the culture vessels to the incubator.

8. For best results, do not disturb the culture for at least 16 hours after the culture has been initiated. Change the growth medium the next day to remove the residual DMSO and unattached cells, then every other day thereafter. A healthy culture will display cobblestone or spindle shaped morphology, nongranular cytoplasm and the cell number will be double after two to three days in culture.

### Maintenance of Culture:

1. Change the medium to fresh supplemented medium the next morning after establishing a culture from cryopreserved cells. For subsequent subcultures, change medium 48 hours after establishing the subculture.

2. Change the medium every other day thereafter, until the culture is approximately 50% confluent.

3. Once the culture reaches 50% confluence, change medium every day until the culture is approximately 80% confluent.
Subculture:

1. Subculture the cells when they are 80% confluent.

2. Prepare poly-L-lysine coated cell culture flasks.

3. Warm medium, trypsin/EDTA solution, trypsin neutralization solution, and DPBS to room temperature. We do not recommend warming the reagents and medium at 37°C waterbath prior to use.

4. Rinse the cells with DPBS.

5. Incubate cells with 3 ml of trypsin/EDTA solution (in the case of T-25 flask) until 80% of cells are rounded up (monitored with microscope). Add 3 ml of trypsin neutralization solution to the digestion immediately and gently rock the culture vessel.

6. Harvest and transfer released cells into a 15 ml centrifuge tube. Rinse the flask with another 3 ml of growth medium to collect the residue cells. Examine the flask under microscope to make sure the harvesting is successful by looking at the number of cells left behind. There should be less than 5%.

7. Centrifuge the harvested cell suspension at 1000 rpm for 5 min and resuspend cells in growth medium.

8. Count cells and plate them in a new, fibronectin-coated flask with cell density as recommended.

Caution: Handling human derived products is potentially biohazardous. Although each cell strain tests negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions must be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].