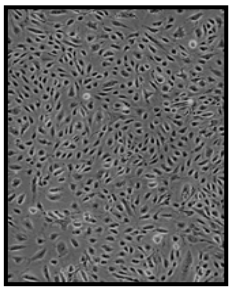
REF: P10995

FEMALE REPRODUCTIVE CELL SYSTEM INNOPROFILE™

HUMAN CERVICAL MICROVASCULAR ENDOTHELIAL CELLS



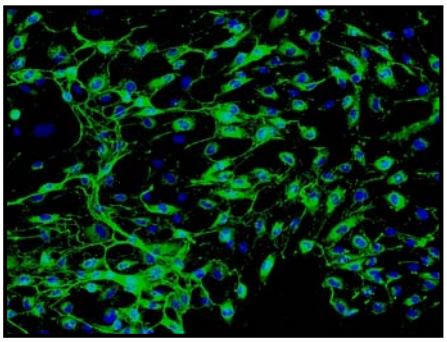
Product Type:	Cryo-preserved Microvascular Endothelial Cells
Catalog Number:	P10995
Source:	Human Cervix
Number of Cells:	5 x 10^5 Cells / vial (1ml)
Storage:	Liquid Nitrogen

Human Cervical Microvascular Endothelial Cells (HCerMEC) provided by Innoprot are isolated from human cervix. HCerMEC are cryopreserved at passage one and delivered frozen. HCerMEC are guaranteed to further expand for 5 population doublings under the conditions provided in this technical sheet.

The cervix is the lower portion of the uterus and functions to promote fertility and protect the female reproductive tract and fetus from potential pathogens. In addition, the cervix plays a critical role in childbirth by effacing and dilating to allow the baby to pass out of the uterus. Cervical Microvascular Endothelial Cells (CerMEC) limit the passage of cellular and soluble substances between the blood vessels and the surrounding tissues. CerMEC also play critical roles in angiogenesis, vasculogenesis, and the inflammatory response. CerMEC angiogenesis has been shown to contribute to the progression of cervical cancer. Recent studies suggest that CerMEC may even initiate signaling events which directly promote tumor growth.

Recommended Medium

- Endothelial Cell Medium (Reference: P60104)



Product Characterization

Immunofluorescent method

- vWF/Factor VIII
- CD31 (P-CAM)

Uptake of DiI-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

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INSTRUCTIONS FOR CULTURING CELLS

IMPORTANT: Cryopreserved cells are very delicate. Thaw the vial in a 37 °C 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 culture vessel (2 µg/cm², T-75 flask is recommended). Add 5 ml of sterile Dulbecco's phosphate buffered saline, Ca^{++}- and Mg^{++}free to a T-75 flask and then add 150 µl of fibronectin stock solution (Cat. #P8248). Leave the vessel in a 37°C incubator overnight.

2. Prepare complete medium. Decontaminate the external surfaces of medium bottle and medium supplement tubes with 70% ethanol and transfer them to a sterile field. Aseptically transfer supplement to the basal medium with a pipette. Rinse the supplement tube with medium to recover the entire volume.

3. Aspirate fibronectin solution and add 15 ml of complete medium to the culture vessel. The fibronectin solution can be used twice. Leave the vessel in the sterile field and proceed to thaw the cryopreserved cells.

4. Place the frozen vial in a 37°C water bath. Hold and rotate the vial gently until the contents completely thaw. Promptly remove the vial from the water bath, wipe it down with 70% ethanol, and transfer it to the sterile field.

5. Carefully remove the cap without touching the interior threads. Gently resuspend and dispense the contents of the vial into the equilibrated, fibronectin-coated culture vessel. A seeding density of 6,000-7,000 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 residual DMSO in the culture. It is also important that cells are plated in fibronectin-coated culture vessels to promote cell attachment.

6. Replace the cap or lid of the culture vessel and gently rock the vessel to distribute the cells evenly. Loosen cap, if necessary, to allow gas exchange.

7. Return the culture vessel to the incubator.

8. For best results, do not disturb the culture for at least 16 hours after the culture has been initiated. Refresh culture medium the next day to remove residual DMSO and unattached cells.

Maintenance of Culture:

1. Refresh supplemented culture medium the next morning after establishing a culture from cryopreserved cells.

2. Change the medium every three days thereafter, until the culture is approximately 70% confluent.

3. Once the culture reaches 70% confluency, change medium every other day until the culture is approximately 90% confluent.

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Subculture:

1. Subculture when the culture reaches 90% confluency.

2. Prepare fibronectin-coated culture vessels (2 µg/cm^2) one day before subculture.

3. Warm complete medium, trypsin/EDTA solution (T/E Sol.), T/E neutralization solution (TNS), and DPBS (Ca^{++}- and Mg^{++}-free) to room temperature. We do not recommend warming reagents and medium in a 37°C water bath prior to use.

Note: *DPBS, trypsin/EDTA solution & trypsin neutralization solution are included in the "Primary Cells Detach Kit provided by Innoprot (Cat. Nº P60305).*

4. Rinse the cells with DPBS.

5. Add 10 ml of DPBS and then 1 ml of T/E solution into flask (in the case of a T-75 flask). Gently rock the flask to ensure complete coverage of cells by T/E solution. Incubate the flask in a 37°C incubator for 1 to 2 minutes or until cells completely round up. Use a microscope to monitor the change in cell morphology.

6. During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum (FBS).

7. Transfer T/E solution from the flask to the 50 ml centrifuge tube (a small percent of cells may detach) and continue to incubate the flask at 37°C for another 1 minute (no solution in the flask at this moment).

8. At the end of incubation, gently tap the side of the flask to dislodge cells from the surface. Check under a microscope to make sure that all cells detach.

9. Add 5 ml of TNS solution to the flask and transfer detached cells to the 50 ml centrifuge tube. Rinse the flask with another 5 ml of TNS to collect the residual cells.

10. Examine the flask under a microscope for a successful cell harvest by looking at the number of cells being left behind; there should be less than 5%.

11. Centrifuge the 50 ml centrifuge tube at 1000 rpm for 5 minutes. Resuspend cells in culture medium.

12. Count and plate cells in a new fibronectin-coated culture vessel with the recommended cell density.

Caution: *Handling human derived products is potentially bioharzadous. Although each cell strain testes negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions mush 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].*

[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).

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