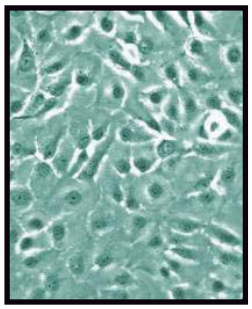
REF: P10957

REPRODUCTIVE CELL SYSTEM INNOPROFILE™

HUMAN AMNIOTIC EPITHELIAL CELLS



Product Type:	Cryo-preserved Amniotic Epithelial Cells
Catalog Number:	P10957
Source:	Amniotic membrane
Number of Cells:	5 x 10^5 Cells / vial (1ml)
Storage:	Liquid Nitrogen

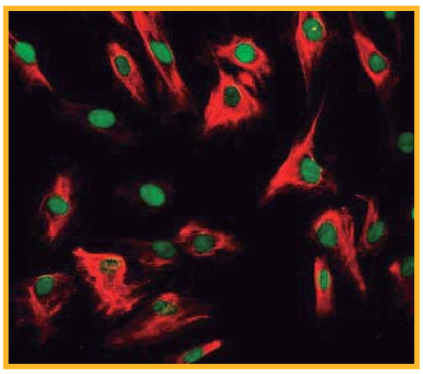
Human Amniotic Epithelial Cells (HAEpiC) provided by Innoprot are isolated from human amniotic membrane. AEpiC are cryopreserved on primary culture after purification and delivered frozen. HAEpiC are guaranteed to further culture under the conditions described in this technical sheet.

Human amniotic membrane is composed of an epithelial cell layer, a basement membrane and an avascular matrix. The amniotic epithelial cells (AEC) are formed from epiblasts on the 8th day after fertilization. A probable result of their embryonic origin, AEC lack major histocompatibility complex antigens and have been used for allotranplantation to treat patients with lysosomal diseases. Studies have shown that AEC have multiple functions such as synthesis and release of acetylcholine and catecholamine as well as expressing mRNA coding for dopamine receptors and dopamine transporter. They express neuronal and glial cell marks and produce basic fibroblast growth factor, hepatocyte growth factor and transforming growth factor-beta.

Recommended Medium

- Epithelial Cell Medium

(Reference: P60106)



Product Characterization

Immunofluorescent method

- Cytokeratin-18
- Cytokeratin-19
- Vimentin

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!

Note: HAEpiC are not expected to proliferate many times in culture. Experiments should be well organized before thawing the cells. It is recommended that HAEpiC are used for experiments at earliest passage after initial plating with minimal expansion. If subculture is inevitable, follow the instructions below with special care and it is recommended that the cells only be subcultured once.

Set up culture after receiving the order:

1. Prepare a collagen type I-coated flask following manufacturer instructions. Leave the flask in incubator overnight (minimum four hours at 37°C incubator).

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. Rinse the collagen-coated flask with sterile water twice and add 20 ml of complete medium to the flask. Leave the flask in the hood and go to thaw the cells.

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 re-suspend the contents of the vial.

5. Dispense the contents of the vial into the equilibrated, poly-L-lysine coated culture vessels. Seeding density of 7,000 - 8,000 cells/cm^2.

   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 smooth muscle cells are plated in poly-L-lysine coated flask that promotes cell attachment and growth.

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

7. Return the culture vessels to the incubator.

8. For best result, 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.

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.

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

Subculture:

1. Subculture when the culture reaches 90% confluency.

2. Prepare collagen-coated culture vessels one day before subculture.

3. Warm complete medium, trypsin/EDTA solution (T/E), TNS 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. N° P60305).*

4. Rinse the cells with DPBS.

5. Add 8 ml of DPBS and then 2 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.

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 to 2 minutes (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 5ml 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 collagen-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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