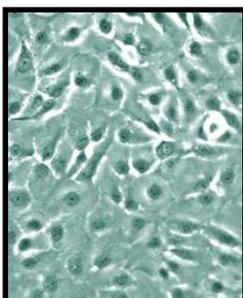


REPRODUCTIVE CELL SYSTEM INNOPROFILETM HUMAN AMNIOTIC EPITHELIAL CELLS



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 by ScienCell Research Laboratories from human amniotic membrane. HAEpiC are cryopreserved on primary culture after purification and delivered frozen. HAEpiC are guaranteed to further expand for 15 population doublings in the condition provided by ScienCell Research Laboratories.

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 allotransplantation 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. Human AEC

has been suggested as an appropriate human cell model for studying dopamine release and uptake processes, receptor signal transduction and exploring newly developed drugs acting at these receptors.

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

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. 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, and transfer it to a sterile field. Rinse the vial with 70% ethanol, and then wipe to remove excess. Remove the cap, being careful not to touch the interior threads with fingers. Using 1 ml eppendorf pipet gently resuspends the contents of the vial.
2. Dispense the contents of the vial into the equilibrated, poly-L-lysine coated culture vessels. A seeding density of 7,500 cells/cm² is recommended.
3. 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 HAEpiC are plated in poly-L-lysine coated culture vessels that promote the cell attachment growth.
4. Replace the cap or cover, and gently rock the vessel to distribute the cells evenly. Loosen caps if necessary to permit gas exchange.
5. Return the culture vessels to the incubator.
6. 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. A health culture will display polygonal, sheets of contiguous cells and the cell number will be doubled 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, poly-L-lysine coated flask with cell density as recommended.

Caution: Handling human derived products is potentially biohazardous. Although each cell strain testes 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].

- [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).