ScienCell™ Research Laboratories

Human Amniotic Epithelial Cells (HAEpiC)

Catalog Number: 7100

Cell Specification

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 [1]. They express neuronal and glial cell marks and produce basic fibroblast growth factor, hepatocyte growth factor and transforming growth factor-beta [2]. 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 [3].

HAEpiC from ScienCell Research Laboratories are isolated from human amniotic membrane. HAEpiC are cryopreserved at passage one and delivered frozen. Each vial contains >5 x 10^5 cells in 1 ml volume. HAEpiC are characterized by immunofluorescent method with antibodies to cytokeratin-18, -19 and vimentin. HAEpiC are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi. HAEpiC are guaranteed to further expand for 15 population doublings in the condition provided by ScienCell Research Laboratories.

Recommended Medium

It is recommended to use Epithelial Cell Medium (EpiCM, Cat. No. 4101) for the culturing of HAEpiC *in vitro*.

Product Use

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

Storage

Directly and immediately transfer cells from dry ice to liquid nitrogen upon receiving and keep the cells in liquid nitrogen until cell culture is needed for experiments.

Shipping

Dry ice.

Reference

[1] Sakuragawa, N., Elwan, M. A., Uchida, S., Fujii, T., Kawashima, K. (2001) Non-neuronal neurotransmitters and neurotrophic factors in amniotic epithelial cells: expression and function in humans and monkey. *Jpn J Pharmacol.* 85(1):20-3.

[2] Ishii, T., Ohsugi, K., Nakamura, S., Sato, K., Hashimoto, M., Mikoshiba, K., Sakuragawa, N. (1999) Gene expression of oligodendrocyte markers in human amniotic epithelial cells using neural cell-type-specific expression system. *Neurosci Lett.* 268(3):131-4.

[3] Elwan, M. A., Ishii, T., Sakuragawa, N. (2003) Characterization of the dopamine transporter gene expression and binding sites in cultured human amniotic epithelial cells. *Neurosci Lett.* 342(1-2):61-4.

Instruction for culturing cells

Caution: 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 ordering:

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/cm^2 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^{o}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. *Note: Use ScienCell Research Laboratories' trypsin/EDTA solution that is optimized to minimize the killing of the cells by over trypsinization.*
7. 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%.
8. Centrifuge the harvested cell suspension at 1000 rpm for 5 min and resuspend cells in growth medium.
9. 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 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).