



**Human Amniotic Epithelial Cells  
(HAEpiC)  
Catalog #7110**

**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. As a 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. In addition, AEC express mRNA coding for dopamine receptors and dopamine transporters [1]. They also express neuronal and glial cell markers, 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 membranes. HAEpiC are cryopreserved at passage one and delivered frozen. Each vial contains  $>5 \times 10^5$  cells in 1 ml volume. HAEpiC are characterized by immunofluorescence with antibodies specific to cytokeratin-18 and/or cytokeratin-19. HAEpiC are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi. HAEpiC are guaranteed to further culture under the conditions provided by ScienCell Research Laboratories.

**Recommended Medium**

It is recommended to use Epithelial Cell Medium (EpiCM, Cat. #4101) for the culturing of HAEpiC *in vitro*.

**Product Use**

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

**Storage**

Upon receiving, directly and immediately transfer the cells from dry ice to liquid nitrogen and keep the cells in liquid nitrogen until they are needed for experiments.

**Shipping**

Dry ice.

**References**

- [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 MA, 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.

## Instructions for culturing cells

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**Caution:** Cryopreserved cells are very delicate. Thaw the vial in a 37°C water bath and return the cells 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.*

### Initiating the culture:

**Note:** ScienCell primary cells must be cultured in a 37°C, 5% CO<sub>2</sub> incubator. Cells are only warranted if ScienCell media and reagents are used and the recommended protocols are followed.

1. Prepare a poly-L-lysine-coated culture vessel (2 µg/cm<sup>2</sup>, T-75 flask is recommended). To obtain a 2 µg/cm<sup>2</sup> poly-L-lysine-coated culture vessel, add 10 ml of sterile water to a T-75 flask and then add 15 µl of poly-L-lysine stock solution (10 mg/ml, Cat. #0413). Leave the vessel in a 37°C incubator overnight (or for a minimum of one hour).
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. Rinse the poly-L-lysine-coated vessel twice with sterile water and then add 20 ml of complete medium. 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, poly-L-lysine-coated culture vessel.

*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 poly-L-lysine-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. Do not disturb the culture for at least 16 hours after initiation. Refresh culture medium the next day to remove residual DMSO and unattached cells.

### Maintaining the culture:

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

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2. Change the medium every three days, 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.

### **Subculturing:**

1. Subculture when the culture reaches 90% confluency.
2. Prepare poly-L-lysine-coated culture vessels ( $2 \mu\text{g}/\text{cm}^2$ ) one day before subculture.
3. Warm complete medium, trypsin/EDTA solution, 0.05% (T/E, Cat. #0183), T/E neutralization solution (TNS, Cat. #0113), and DPBS ( $\text{Ca}^{++}$ - and  $\text{Mg}^{++}$ -free, Cat. #0303) to **room temperature**. We do not recommend warming reagents and medium in a  $37^\circ\text{C}$  water bath prior to use.
4. Rinse the cells with DPBS.
5. Add 5 ml DPBS and 5 ml 0.05% T/E solution (Cat. #0183) into flask (in the case of a T-75 flask). Gently rock the flask to ensure complete coverage of cells by T/E solution. Use a microscope to monitor the change in cell morphology.

**Note:** We recommend using ScienCell 0.05% T/E solution which is optimized to minimize cell damage due to over trypsinization. If 0.25% T/E solution (Cat. #0103) is used, then 9 ml of DPBS and 1 ml of 0.25% T/E solution should be used.

**Caution:** Do NOT use undiluted trypsin when subculturing primary cells.

6. During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum (FBS, Cat. #0500).
7. Once cells completely round up, 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  $37^\circ\text{C}$  for another minute (no solution in the flask at this time).
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. Gently resuspend cells in culture medium.
12. Count and plate cells in a new poly-L-lysine-coated culture vessel with the recommended cell density. A seeding density of 7,000-8,000 cells/ $\text{cm}^2$  is recommended.

**Note:** We do not recommend cryopreservation of primary cells by the end user. Refreezing cells may damage them and affect cell performance. ScienCell does not guarantee primary cells cryopreserved by the end user.

*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 with these materials.*

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*Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].*

[1] Grizzle WE, Polt S. (1988) "Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues." *J Tissue Cult Methods*. 11: 191-9.