



## Human Pulmonary Alveolar Epithelial Cells-adult (HPAEpiC-a)

Catalog #3240

### Cell Specification

Pulmonary alveolar epithelial cells (PAEpiC), comprised of alveolar type I and type II epithelial cells, line more than 99% of the internal surface area of the lung [1]. Type I cells are large squamous cells whose thin cytoplasmic extensions cover >95% of the internal surface area. They contain aquaporins and exhibit the highest osmotic water permeability of any mammalian cell type. Type II cells, which cover 2-5% of the surface area, produce, secrete, and recycle pulmonary surfactant [2]. Type II cells contain  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase and amiloride-sensitive epithelial  $\text{Na}^+$  channels. The currently accepted hypothesis is that Type II cells maintain pulmonary fluid homeostasis by regulating active  $\text{Na}^+$  transport in the lungs, whereas Type I cells are "inert" cells that provide solely a barrier function, rather than having active functions. Recent studies indicate that Type I cells are also important in regulating ion and fluid transport [3].

HPAEpiC-a from ScienCell Research Laboratories are isolated from human adult lung tissue. HPAEpiC-a are cryopreserved at P0 and delivered frozen. Each vial contains  $>1 \times 10^6$  cells in 1 ml volume. HPAEpiC-a are characterized by immunofluorescence with antibodies specific to cytokeratin-18, and/or cytokeratin-19. HPAEpiC-a are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi. HPAEpiC-a are guaranteed to further culture under the conditions provided by ScienCell Research Laboratories; *however, HPAEpiC-a are not recommended for expanding or long-term cultures since the cells would differentiate to become type I alveolar epithelial cells immediately after plating and type I alveolar epithelial cells do not proliferate in culture.*

### Recommended Medium

It is recommended to use Alveolar Epithelial Cell Medium (AEpiCM, Cat. #3201) for culturing HPAEpiC-a *in vitro*.

### Product Use

HPAEpiC-a 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] Crapo JD, Young SL, Fram EK, Pinkerton KE, Barry BE, Crapo RO. (1983) "Morphometric characteristics of cells in the alveolar region of mammalian lungs." *Am. Rev. Respir. Dis.* 128: S42-S46.
- [2] Wright JR, Dobbs LG. (1991) "Regulation of pulmonary surfactant secretion and clearance." *Annu. Rev. Physiol.* 53: 395-414.
- [3] Johnson MD, Widdicombe JH, Allen L, Barbry P, Dobbs LG. (2002) "Alveolar epithelial type I cells contain transport proteins and transport sodium, supporting an active role for type I cells in regulation of lung liquid homeostasis." *Proc. Natl. Acad. Sci. USA.* 99(4): 1966-1971.

## **Instructions for culturing primary cells**

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**Caution:** Cryopreserved primary 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! Do not centrifuge the cells after thawing as this can damage the cells.

*Note: HPAEpiC-a are very sensitive cells and do not proliferate in culture. Experiments should be well organized before thawing the cells and cells should be used as soon as possible for experiments. Cells should be plated directly onto the vessel that will be used for experiments, as we do not recommend subculturing these cells.*

### **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. A seeding density of 15,000-20,000 cells/cm<sup>2</sup> 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 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.

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**Maintaining the culture:**

1. Refresh supplemented culture medium the next morning after establishing a culture from cryopreserved cells.
2. Change the medium every three days thereafter.

***HPAEpiC-a are not recommended to be subcultured because this cell type will terminally differentiate in long-term cultures.***

**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. 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.