



## Human Pulmonary Alveolar Epithelial Cells (RPAEpiC)

Catalog Number: R3200

### 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 Na<sup>+</sup>-, K<sup>+</sup>-ATPase and amiloride-sensitive epithelial Na<sup>+</sup> channels. The currently accepted hypothesis is that Type II cells maintain pulmonary fluid homeostasis by regulating active Na<sup>+</sup> transport in the lungs, whereas Type I cells are "inert" cells that provide solely a barrier function, rather than having active functions. Recent study indicate that Type I cells are also important in regulating ion and fluid transport [3].

RPAEpiC from ScienCell Research Laboratories are isolated from neonate day 2 rat lung tissue. RPAEpiC are cryopreserved at primary culture and delivered frozen. Each vial contains >1 x 10<sup>6</sup> cells in 1 ml volume. RPAEpiC are characterized by immunofluorescent method with antibodies CK-18, -19, and vimentin. HPAEpiC are negative for, mycoplasma, bacteria, yeast and fungi. RPAEpiC are guaranteed to further culture at the conditions provided by ScienCell Research Laboratories. *However, this cell type is not recommend 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. No. 3201) for the culturing of RPAEpiC *in vitro*.

### Product Use

RPAEpiC 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 needed for experiments.

### Shipping

Dry ice.

### Reference

- [1] Crapo, J. D., Young, S. L., Fram, E. K., Pinkerton, K. E., Barry, B. E., & Crapo, R. O. (1983) Morphometric characteristics of cells in the alveolar region of mammalian lungs. *Am. Rev. Respir. Dis.* 128:S42-S46.
- [2] Wright, J. R. & Dobbs, L. G. (1991) Regulation of pulmonary surfactant secretion and clearance. *Annu. Rev. Physiol.* 53:395-414.
- [3] Johnson, M. D., Widdicombe, J. H., Allen, L., Barbry, P. and Dobbs, L. G. (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..

# Instruction for culturing cells

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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 order:

1. Prepare a poly-L-lysine coated flask ( $2 \mu\text{g}/\text{cm}^2$ , T-75 flask is recommended). Add 10 ml of sterile water to a T-75 flask and then add 15  $\mu\text{l}$  of poly-L-lysine stock solution (10 mg/ml, ScienCell cat. no. 0413). Leave the flask in incubator overnight (minimum one hour 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 poly-L-lysine 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 1 ml eppendorf pipette gently resuspend the contents of the vial.
5. Dispense the contents of the vial into the equilibrated, poly-L-lysine coated culture vessels. A seeding density of 5,000 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 DMSO residue in the culture. It is also important that cells are plated in poly-L-lysine coated culture vessels that promote cell attachment.*
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. Change the medium to fresh supplemented medium the next morning after establishing a culture from cryopreserved cells.
2. Change the medium every two to three days thereafter.

***It is not recommended that RPAEpiC be subcultured beyond their initial plating.***

*Caution: Handling animal derived products is potentially biohazardous. Always wear gloves and safety glasses when working these materials. Never mouth pipette. We recommend following the universal procedures for handling products of animal 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).