Rev. 1

Rat Pulmonary Alveolar Epithelial Cells (RPAEpiC)

Catalog #R3200

Cell Specification

Pulmonary alveolar epithelial cells, 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 lung surface. They contain aquaporins and exhibit the highest osmotic water permeability of any mammalian cell type. Type II cells, which cover 2-5% of the internal lung surface, produce, secrete, and recycle pulmonary surfactant [2]. The currently accepted hypothesis is that Type II cells maintain pulmonary fluid homeostasis by regulating active Na+ transport in the lungs, whereas Type I cells are "inert" cells that provide only a barrier function. A recent study, however, suggests that Type I cells may also regulate ion and fluid transport [3].

RPAEpiC from ScienCell Research Laboratories are isolated from rat lung. RPAEpiC are cryopreserved at P0 and delivered frozen. Each vial contains >1 x 10^6 cells in 1 ml volume. RPAEpiC are characterized by immunofluorescence with antibodies specific to cytokeratin-18. RPAEpiC are negative for, mycoplasma, bacteria, yeast, and fungi. RPAEpiC are guaranteed to further culture under the conditions provided by ScienCell Research Laboratories. *This cell type, however, is not recommended for expanding or long term cultures since the cells would differentiate to 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 RPAEpiC *in vitro.*

Product Use

RPAEpiC 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: S42-6.

[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: 1966-71.

Instructions for culturing cells

Caution: Cryopreserved primary cells are very delicate. Thaw the vial in a 37ºC 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: ScienCell primary cells must be cultured in a 37ºC, 5% CO_2 incubator. Cells are only warranted if ScienCell media and reagents are used and the recommended protocols are followed. *RPAEpiC should not be subcultured beyond their initial plating.*

Initiating the culture:

Note: ScienCell primary cells must be cultured in a 37ºC, 5% CO_2 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 plate (2 µg/cm^2 is recommended). For example, add 2 ml of sterile water to one well of a 6-well plate and then add 20µl of poly-L-lysine stock solution (1 mg/ml, Cat. #0403). Leave the plate in a 37ºC 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 tubes with medium to recover the entire volume.
3. Rinse the poly-L-lysine-coated culture vessel twice with sterile water and then add the volume of complete medium recommended in Table 1 or Table 2. Leave the vessel in the sterile field and proceed to thaw the cryopreserved cells.
4. Place the frozen vial in a 37ºC 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. Carefully remove the cap without touching the interior threads and gently resuspend the cell suspension. A seeding density of 10,000-15,000 cells/cm^2 is recommended depending on your experiments. We recommend following Table 1 for seeding RPAEpiC onto 6-well, 12-well, or 24-well plates.

Table 1
Recommended cell suspension volume per vial using a 6-well, 12-well, or 24 well format

Well format	Surface area/well (approx. values)	Volume of media/well	Volume of cell suspension from vial/well	# of wells/vial

6-well	9.6 cm^2	3.0 ml	150 µl	6 wells
12-well	3.9 cm^2	2.0 ml	60 µl	15 wells
24-well	1.9 cm^2	1.0 ml	30 µl	30 wells

7. 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.
8. Return the culture vessel to the incubator.
9. For best results, do not disturb the culture for at least 16 hours after the culture has been initiated. Refresh culture medium the next day to remove residual DMSO and unattached cells.
10. Use cells promptly for experiments.

It is not recommended that RPAEpiC be subcultured beyond their initial plating 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 animal-derived products is potentially biohazardous. 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.