



## Porcine Corneal Epithelial Cells (PCEpiC)

Catalog #P6510

### Cell Specification

The cornea is a unique tissue due to its transparency and avascularity. The cornea consists of three distinct cell layers: the outer epithelium, the central stroma composed of interconnected keratocytes, and the inner endothelium. The corneal epithelium plays a role in the innate immune response by sensing the presence of pathogens and providing signals to activate the corneal defense system [1]. Corneal epithelial cells also express a high level of aldehyde dehydrogenase to protect them against UV- and 4-hydroxynonenal-induced cellular damage [2]. The renewal of corneal epithelium is regulated by a host of cytokines, including epidermal growth factor, which activates their cognate receptors in the deeper layers of the epithelium [3]. Cultured corneal epithelial cells can be an alternative to animal testing in toxicology studies. They are also suitable for understanding the highly integrated balance between the processes of corneal epithelial proliferation, differentiation, and cell death.

PCEpiC from ScienCell Research Laboratories are isolated from porcine cornea. PCEpiC are cryopreserved at passage one and delivered frozen. Each vial contains  $>5 \times 10^5$  cells in 1 ml volume. PCEpiC are characterized by their cobble stone morphology in serum-free culture and immunofluorescence with antibodies specific to cytokeratin-18. PCEpiC are negative for mycoplasma, bacteria, yeast, and fungi. PCEpiC are guaranteed to further expand for 10 population doublings under the conditions provided by ScienCell Research Laboratories.

### Recommended Medium

It is recommended to use Corneal Epithelial Cell Medium-C (CEpiCM-C, Cat. #6511) for culturing PCEpiC *in vitro*.

### Product Use

PCEpiC 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] Zhang J, Wu XY, Yu FS. (2005) "Inflammatory response of corneal epithelial cells to pseudomonas aeruginosa infection." *Curr Eye Res.* 30: 527-34.
- [2] Pappa A, Brown D, Koutalos Y, Degregori J, White C, Vasiliou V. (2005) "Human aldehyde dehydrogenase 3A1 inhibits proliferation and promotes survival of human corneal epithelial cells." *J Biol Chem.* 280: 27998-8006.
- [3] Lu L, Reinach PS, Kao WW. (2001) "Corneal epithelial wound healing." *Exp Biol Med (Maywood).* 226: 653-64.

## **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.

### **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.
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 90-95% 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  $5,000 \text{ cells}/\text{cm}^2$  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 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.