Porcine Lens Epithelial Cells (PLEpiC)
Catalog #P6550

Cell Specification

The mammalian lens consist of two cell types, lens fiber cells which form the bulk of the lens, and a monolayer of epithelial cells that cover the anterior surface of the fibers. Lens epithelial cells are responsible for homeostasis regulation of the lens, including electrolyte and fluid transport [1]. Under normal development, lens epithelial cells progressively differentiate and mature. Lens epithelial cells then migrate from the equatorial region into the interior of the lens to produce transparent crystallins, elongate to form lens fiber cells, and eventually lose their nuclei and other organelles [2]. Previous studies suggested that lens epithelial cell differentiation and lens polarization are regulated by growth factors present in the ocular fluids [3], such as epidermal growth factor, basic fibroblast growth factor, insulin growth factor, and insulin [4].

PLEpiC from ScienCell Research Laboratories are isolated from porcine lens. PLEpiC are cryopreserved at P0 and delivered frozen. Each vial contains >5 x 10^5 cells in 1 ml volume. PLEpiC are characterized by immunofluorescence with antibodies specific to cytokeratin-18, -19, and fibronectin. PLEpiC are negative for mycoplasma, bacteria, yeast, and fungi. PLEpiC are guaranteed to further expand for 5 population doublings under the condition provided by ScienCell Research Laboratories.

Recommended Medium

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

Product Use

PLEpiC 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] Candia OA. (2004) “Electrolyte and fluid transport across corneal, conjunctival and lens epithelia.” *Exp Eye Res*. 78: 527-35.
[2] Wagner LM, Takemoto DJ. (2001) “PKCa and PKC? overexpression causes lentoid body formation in the N/N 1003A rabbit lens epithelial cell line.” *Molecular Vision*. 7: 138-44.
[3] Lang RA. (1999) “Which factors stimulate lens fiber cell differentiation in vivo?” *Invest Opthalmol Vis Sci*. 40: 3075-8.
[4] Leenders WP, van Genesen ST, Schoenmakers JG, van Zoelen EJ, Lubsen NH. (1997) “Synergism between temporally distinct growth factors: bFGF, insulin and lens cell differentiation.” *Mech Dev*. 67: 193-201.

Instructions for culturing cells

Caution: Cryopreserved 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!

Initiating the culture:

1. Prepare a poly-L-lysine-coated culture vessel (2 μg/cm^2, T-75 flask is recommended). 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 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 tube with medium to recover the entire volume.
3. Rinse the poly-L-lysine-coated vessel twice with sterile water and then add 15 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 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. A seeding density of 5,000 cells/cm^2 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. 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, then every other day thereafter.

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, until the culture is approximately 50% confluent.
3. Once the culture reaches 50% confluency, change medium every other day until the culture is approximately 80% confluent.

Subculturing:

1. Subculture when the culture reaches 90% confluency or above.
2. Prepare poly-L-lysine-coated culture vessels (2 μg/cm^2) one day before subculture.
3. Warm complete medium, trypsin/EDTA solution (T/E, Cat. #0103), T/E neutralization solution (TNS, Cat. #0113), and DPBS (Ca^{++}- and Mg^{++}-free, Cat. #0303) to **room temperature**. We do not recommend warming reagents and medium in a 37°C water bath prior to use.
4. Rinse the cells with DPBS.
5. Add 10 ml of DPBS and then 2 ml of T/E solution into flask (in the case of a T-75 flask). Gently rock the flask to ensure complete coverage of cells by T/E solution. Incubate the flask in a 37°C incubator for 1 to 2 minutes or until cells completely round up. Use a microscope to monitor the change in cell morphology.
6. During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum (FBS, Cat. #0500).
7. 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°C for another 1 to 2 minutes (no solution in the flask at this moment).
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%.

    Note: Use ScienCell T/E solution that is optimized to minimize cell damages due to over trypsinization.

11. Centrifuge the 50 ml centrifuge tube at 1000 rpm for 5 minutes. Resuspend cells in culture medium.
12. Count and plate cells in a new poly-L-lysine-coated culture vessel with the recommended cell density.

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.