Porcine Lens Epithelial Cells (PLEpiC)

Catalog Number: P6550

Cell Specification

The cells that make up the mammalian lens consist of two 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. The normal development of the lens of the eye involves the progressive differentiation and maturation of the lens epithelial cells. As these cells migrate from the equatorial region of the lens into the interior of the lens, they produce the transparent crystallins, elongate to form lens fiber cells, and lose their nuclei and other organelles [1]. The causes of lens epithelial cell differentiation are not well understood; however, some progress has been made in determining the underlying molecular and cellular processes of lens epithelial cell differentiation. This process can be promoted by growth factors present in the ocular fluids [2]. Some growth factors, such as epidermal growth factor, promote mitosis; others, such as basic fibroblast growth factor, insulin growth factor, and insulin, promote cell migration and differentiation [3].

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

Recommended Medium

It is recommended to use Epithelial Cell Medium (EpiCM, Cat. No. 4101) for the culturing of PLEpiC *in vitro*.

Product Use

PLEpiC 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]. Wagner, L. M. and Takemoto, D. J. (2001) PKCa and PKC? overexpression causes lentoid body formation in the N/N 1003A rabbit lens epithelial cell line. *Molecular Vision* 7: 138-144.

[2]. Lang, R. A. (1999) Which factors stimulate lens fiber cell differentiation in vivo? *Invest Opthalmol Vis Sci* 40:3075-8.

[3]. Leenders, W. P., van Genesen, S. T., Schoenmakers, J. G., van Zoelen, E. J., Lubsen, N. H. (1997) Synergism between temporally distinct growth factors: bFGF, insulin and lens cell differentiation. *Mech Dev*. 67:193-201.

Instruction for culturing cells

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 poly-L-lysine coated flask (2 µg/cm^2, T-75 flask is recommended) and leave the flask in incubator for 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 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, and transfer it to a sterile field. Rinse the vial with 70% ethanol, and then wipe to remove excess. 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^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 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 caps 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. A healthy culture will display polygonal shaped, sheets of contiguous cells and the cell number will be doubled after two to three days in culture.

Maintenance of Culture:

1. Change the medium to fresh supplemented medium the next morning after establishing a culture from cryopreserved cells. For subsequent subcultures, change medium 48 hours after establishing the subculture.
2. Change the medium every other day thereafter, until the culture is approximately 50% confluent.
3. Once the culture reaches 50% confluence, change medium every day until the culture is approximately 80% confluent.

Subculture:

1. Subculture the cells when they are over 90% confluent.
2. Prepare Poly-L-Lysine coated flasks (2 μg/cm^2) one day before subculture.
3. Warm medium, trypsin/EDTA solution (T/E, cat. no. 0103), trypsin neutralization solution (TNS, cat. no. 0113), and DPBS to **room temperature**. We do not recommend warming the reagents and medium at 37°C waterbath prior to use.
4. Rinse the cells with DPBS.
5. Add 10 ml of DPBS first and then 2 ml of trypsin/EDTA solution into flask (in the case of T-75 flask); gently rock the flask to make sure cells are covered by trypsin/EDTA solution; incubate the flask at 37°C incubator for 1 to 2 minutes or until cells are completely rounded up (monitored with inverted microscope). During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum (FBS, cat. no. 0500); transfer trypsin/EDTA solution from the flask to the 50 ml centrifuge tube (a few percent of cells may detached); continue incubate the flask at 37°C for 1 minutes (no solution in the flask at this moment); at the end of trypsinization, one hand hold one side of flask and the other hand gently tap the other side of the flask to detach cells from attachment; check the flask under inverted microscope to make sure all cells are detached, add 5 ml of trypsin neutralization solution to the flask and transfer detached cells to the 50 ml centrifuge tube; add another 5 ml of TNS to harvest the residue cells and transfer it to the 50 ml centrifuge tube. Examine the flask under inverted microscope to make sure the cell harvesting is successful by looking at the number of cells left behind. There should be less than 5%.
   Note: Use ScienCell Research Laboratories' trypsin/EDTA solution that is optimized to minimize the killing of the cells by over trypsinization.
6. Centrifuge the 50 ml centrifuge tube (harvested cell suspension) at 1000 rpm (*Beckman Coulter* Allegra 6R centrifuge or similar) for 5 min; re-suspend cells in growth medium.
7. Count cells and plate cells in a new, Poly-L-Lysine coated flask with cell density as recommended.

Caution: Handling animal derived products is potentially biohazardous. Proper precautions must be taken to avoid inadvertent exposure. 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).