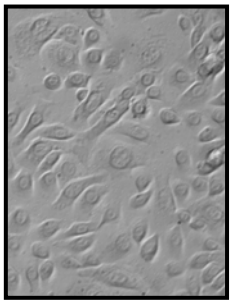
REF: P10831

OCULAR CELL SYSTEM INNOPROFILE™

PORCINE CORNEAL EPITHELIAL CELLS



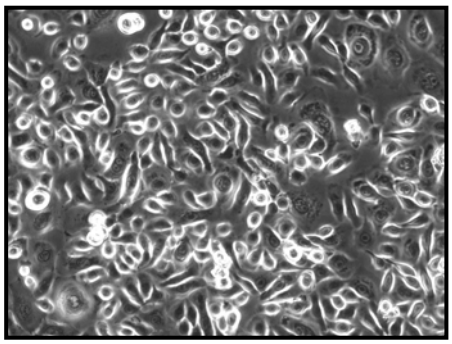
Product Type:	Cryo-preserved Corneal Epithelial Cells
Catalog Number:	P10831
Source:	Porcine Cornea
Number of Cells:	5 x 10^5 Cells / vial (1ml)
Storage:	Liquid Nitrogen

PCEpiC from Innoprot are isolated from porcine cornea. PCEpiC are cryopreserved from passage one culture. PCEpiC are guaranteed to further expand for 10 population doublings at the condition provided in the technical sheet.

The cornea is a unique tissue for two reasons, the transparency function and the synthesis of most of its proteins because the cornea is an avascular tissue. The cornea consists of three distinct cell layers, the outer epithelium, the inner endothelium and the central stroma. The corneal epithelium plays a role in the innate immune response by sensing the presence of pathogens and providing signals that activate the corneal defense system. It also highly expresses aldehyde dehydrogenase to protect against UV- and 4-hydroxynonenal-induced cellular damage. Instead of testing products in the eyes of animals, cultured corneal epithelial cells could be used for toxicology tests in in vitro models and for studying the molecular mechanisms involved in regulating human corneal epithelial cell differentiation.

Recommended Medium

- Corneal Epithelial Cell Medium (Reference: P60131)



Product Characterization

Immunofluorescent method

- Cytokeratin-18
- Cytokeratin-19

The cells test negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi

Product Use

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in vitro diagnostic or clinical procedures

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INSTRUCTIONS FOR CULTURING CELLS

IMPORTANT: 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/cm2, 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 epithelial 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. 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 70% confluent.

3. Once the culture reaches 70% confluency, change medium every other day until the culture is approximately 90% confluent.

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Subculture:

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, Trysin Neutralization Solution (TNS), and DPBS (Ca^{++}-and Mg^{++}-free) to room temperature. We do not recommend warming reagents and medium in a 37ºC water bath prior to use.

4. Rinse the cells with DPBS.

5. Add 5ml of DPBS and then 5ml 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 37ºC incubator for 3 to 5 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).

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 37ºC 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%.

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 human derived products is potentially bioharzadous. Although each cell strain testes negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions mush 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 human 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).

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