Human Choroid Plexus Epithelial Cells (CPEpiC)

Catalog Number: 1310

Cell Specification

The choroid plexus epithelial cells (CPEpiC) are the structural basis, form a selective barrier between the nervous system and the blood, and secrete cerebrospinal fluid into the ventricles of the brain [1]. CPEpiC express inward-rectifying anion channels which are thought to have an important role in the secretion of cerebrospinal fluid [2]. CPEpiC synthesize and secrete many growth factors and other peptides that are of trophic benefit following injury to regions of the cerebroventricular system. For example, several growth factors are upregulated in choroid plexus after ischemic and traumatic insults to the central nervous system. The presence of numerous types of growth factor receptors in choroid plexus allows growth factor mediation of recovery processes by autocrine and paracrine mechanisms [3]. CPEpiC culture has been widely used to evaluate their morphological and physiological properties, and its capacity to serve as an *in vitro* model to access the cellular mechanisms of organic cation transport across the cerebrospinal fluid-blood barrier.

HCPEpiC from ScienCell Research Laboratories are isolated from human choroid plexus. HCPEpiC are cryopreserved immediately after purification and delivered frozen. Each vial contains >5 x 10^5 cells in 1 ml volume. HCPEpiC are characterized by immunofluorescent method with antibodies to cytokeratin-18, -19 and vimentin. HCPEpiC are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi. HCPEpiC are guaranteed to further expand for 15 population doublings in the conditions specified by ScienCell Research Laboratories.

Recommended Medium

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

Product Use

HCPEpiC are for research use only. They are not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Storage

Transfer cells directly and immediately from dry ice to liquid nitrogen upon receiving and keep the cells in liquid nitrogen until cell culture is needed for experiments.

Shipping

Dry ice.

Reference

[1] Segal, M. B. (1993). Extracellular and cerebrospinal fluid. *Journal of Inherited and Metabolic Disorder* **16**, 617-638.

[2] Speake, T., Kajita, H., Smith, C. P. and Brown, P. D. (2002) Inward-rectifying anion channels are expressed in the epithelial cells of choroid plexus isolated from ClC-2 'knock-out' mice. *J Physiol* 539(Pt 2):385-90.

[3] Johanson CE, Palm DE, Primiano MJ, McMillan PN, Chan P, Knuckey NW, Stopa EG. (2000) Choroid plexus recovery after transient forebrain ischemia: role of growth factors and other repair mechanisms. *Cell Mol Neurobiol* 20(2):197-216.

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 ordering:

1. Prepare a poly-L-lysine coated flask (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, ScienCell cat. no. 0413). Leave the flask in incubator 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, rinse the vial with 70% ethanol and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads with fingers. Using a 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 cap 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 double 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 cell culture flasks.
3. Warm medium, trypsin/EDTA solution, trypsin neutralization solution, and DPBS to **room temperature**. We do not recommend warming the reagents and medium at 37^{o}C waterbath prior to use.
4. Rinse the cells with DPBS.
5. Incubate cells with 10 ml of trypsin/EDTA solution (in the case of T-75 flask) until 80% of cells are rounded up (monitored with microscope). Add 10 ml of trypsin neutralization solution to the digestion immediately and gently rock the culture vessel.
   Note: Use ScienCell Research Laboratories' trypsin/EDTA solution that is optimized to minimize the killing of the cells by over trypsinization.
6. Harvest and transfer released cells into a 50 ml centrifuge tube. Rinse the flask with another 10 ml of growth medium to collect the residue cells. Examine the flask under microscope to make sure the harvesting is successful by looking at the number of cells left behind. There should be less than 5%.
7. Centrifuge the harvested cell suspension at 1000 rpm for 5 min and resuspend cells in growth medium.
8. Count cells and plate them in a new, poly-L-lysine coated flask with cell density as recommended.

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).