



## Human Intrahepatic Biliary Epithelial Cells (HIBEpiC)

Catalog Number: 5100

### Cell Specification

Intrahepatic biliary epithelial cells (IBEpiC) line the intrahepatic biliary tree, a complex three-dimensional network of tubular conduits of different diameters within the liver. They comprise only 3 – 5% of the total population of liver cells, yet produce as much as 40% of the daily output of bile. A number of studies have demonstrated that IBEpiC play an important role in maintaining, modifying and augmenting the composition of canalicular bile by a coordinated series of hormone-regulated secretory and absorptive processes [1]. IBEpiC also appear to have active immunologic roles in both innate and adaptive immune responses; for example, IBECs secrete chemokines and cytokines and serve to localize the immune response by expressing critical cell adhesion molecule [2]. Under basal conditions, IBEpiC are thought to be mitotically dormant. However, IBEpiC proliferate markedly in response to a number of perturbations [3].

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

### Product Use

HIBEpiC 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 is needed for experiments.

### Shipping

Dry ice.

### Reference

- [1] Roberts, S. K. and LaRusso, N. F. (1994) Pathobiology of biliary epithelia. *Curr. Opin. Gastroenterol.* 10:526-533.
- [2] Reynoso-Paz, S., Coppel, R. L., Mackay, I. R., Bass, N. M., Ansari, A. A. and Gershwin, M. E. (1999) The immunology of bile and biliary epithelium. *Hepatology* 30:351-357.
- [3] Lunz III, J. G., Contrucci, S., Ruppert, K., Nurase, N., Fung, J. J., Starzl, T. E. and Demetris, A. J. (2001) Replicative senescence of biliary epithelial cells precedes bile duct loss in chronic liver allograft rejection. *Am. J. Pathol.* 158:1379-1390.

## **Instruction for culturing cells**

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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. 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 pipet gently resuspends the contents of the vial.
2. Dispense the contents of the vial into the equilibrated, poly-L-lysine coated culture vessels. A seeding density of 7,500 cells/cm<sup>2</sup> 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 HIBEpiC are plated in poly-L-lysine coated culture vessels that promote epithelial cell attachment and growth.*
3. Replace the cap or cover, and gently rock the vessel to distribute the cells evenly. Loosen caps if necessary to permit gas exchange.
4. Return the culture vessels to the incubator.
5. 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 health culture will display polygonal, sheet 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 80% 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°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 centrifuge tube. Rinse the flask with another 10l 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 biohazardous. Although each cell strain testes negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, 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 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).