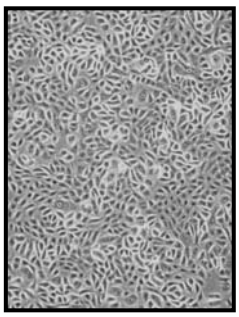
Innoprot
Speeding up drug discovery

REF: P10756

GASTROINTESTINAL CELL SYSTEM INNOPROFILE™
HUMAN SMALL INTESTINE EPITHELIAL CELLS



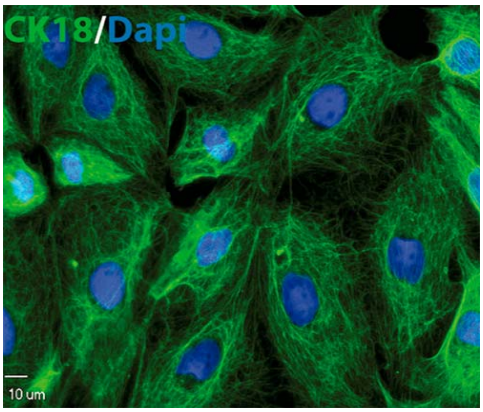
Product Type:	Cryo-preserved Epithelial Cells
Catalog Number:	P10756
Source:	Human Small Intestine
Number of Cells:	5 x 10^5 Cells / vial (1ml)
Storage:	Liquid Nitrogen

Human Small Intestine Epithelial Cells (HSIEpiC) provided by Innoprot have been isolated from human small intestine. HSIEpiC are cryopreserved at passage one and delivered frozen. HSIEpiC are guaranteed to further expand for 5 population doublings at the conditions provided in this data sheet.

Intestine epithelial cells play important functions in the maintenance of overall individual health. In addition to their primary roles in absorbing nutrients and in serving as a physical barrier between the outside world and the inside environment, intestine epithelial cells are crucial in maintaining homeostasis of the mammalian immune and endocrine system in the context of health and disease. A failing intestine epithelium can lead to various disorders such as hormonal imbalance, inflammatory bowel disease (IBD), weight gain, and diabetes. Thus insights into the mechanisms of and remedies for those disorders can be gained from in vitro systems offered by the availability of human intestine epithelial cells.

Recommended Medium

- Epithelial Cell Medium-Plus (Reference: P60106-PLUS)



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 a gelatin solution-coated T-25 flask following manufacturer instructions. Leave the flask in incubator overnight (minimum one hours 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 gelatin-coated 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 re-suspend the contents of the vial.

5. Dispense the contents of the vial into the equilibrated, gelatin coated T-25 flask.

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.

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 80% confluent.

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Subculture:

1. Subculture when the culture reaches 80% confluency.
2. Prepare gelatin-coated culture vessels a couple of hours before subculture.
3. Warm complete medium, trypsin/EDTA solution (T/E), TNS neutralization solution (TNS), and DPBS (Ca^{++}-and Mg^{++}-free) 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 8 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.
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 5ml 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 120 g for 5 minutes. Resuspend cells in culture medium.
12. Count and plate cells in a new gelatin-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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