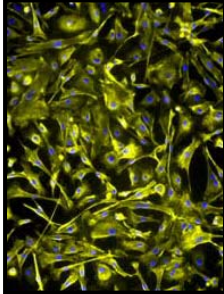


## GASTROINTESTINAL CELL SYSTEM INNOPROFILE™ HUMAN PRIMARY MESOTHELIAL CELLS



<b>Product Type:</b>	Cryo-preserved Mesothelial Cells
<b>Catalog Number:</b>	P10770
<b>Source:</b>	Human Mesentery Tissue
<b>Number of Cells:</b>	1 x 10 <sup>6</sup> Cells / vial (1ml)
<b>Storage:</b>	Liquid Nitrogen

Human Primary Mesothelial Cells (HMTc) provided by Innoprot have been isolated from human mesentery tissue. HMTc are cryopreserved as primary culture and delivered frozen. HMTc are guaranteed to further expand for 10 population doublings in the conditions provided in the technical sheet.

The mesentery is the double layer of peritoneum that suspends the jejunum and ileum from the posterior wall of the abdomen. Its meaning, however, is frequently extended to include double layers of peritoneum connecting various components of the abdominal cavity. Mesentery tissue is composed of an extensive monolayer of specialized cells that line the body's serous cavities. The main purpose of these cells is to produce a lubricating fluid that is released between layers, providing a slippery, non-adhesive, and protective surface to facilitate intracoelomic movement. Mesothelial cells enable researchers to study them and their implications in inflammatory response, metabolic diseases, ovarian cancer metastasis or peritoneal dialysis.

### **Recommended Medium**

- Mesothelial Cell Medium Kit  
(Innoprot Ref# P60167)

### **Pathogens Analysis**

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

### **Preservation**

- Freeze Medium: Cryostor CS10  
(Innoprot Ref # 210102)
- Storage Temperature: Liquid nitrogen vapour phase.

### **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

## 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. Use or prepare a collagen coated flasks (one T-75 flask or two T-25 flasks are recommended) following provider's instructions.
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. Add complete medium to the flask/s. 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, collagen coated culture vessels. A seeding density of approximately 15,000-20,000 cells/cm<sup>2</sup> is recommended to initiate the cell culture.

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 collagen coated culture vessels that promote cell attachment and growth.

6. Replace the cap or cover of flask, 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. Change the medium to fresh supplemented 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% confluence, change medium every other day until the culture is approximately 90% confluent.

**Subculture:**

1. Subculture the cells when they are over 90% confluent.
3. Warm medium, trypsin/EDTA solution (T/E Solution), trypsin neutralization solution (TNS), and DPBS to room temperature. We do not recommend warming the reagents and medium at 37° C waterbath prior to use.

Note: DPBS, trypsin/EDTA solution & trypsin neutralization solution (TNS) are included in the “Primary Cells Detach Kit provided by Innoprot (Cat. N° P60305).

4. Rinse the cells with DPBS.
5. Add 8 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); 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%.

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, collagen 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).

株式会社ケー・エー・シー

試薬事業部

〒661-0978 兵庫県尼崎市久々知西町2丁目1-20

(お問合せ先)

TEL : 06-6435-9747 FAX : 06-6435-9748

URL : <http://www.kacnet.co.jp/>

E-mail : [cs-info@kacnet.co.jp](mailto:cs-info@kacnet.co.jp)