Human Esophageal Epithelial Cells (HEEC)

Catalog Number: 2700

Cell Specification

The human esophagus is lined by a non-keratinizing, moist stratified squamous epithelium whose apical cell membranes and intercellular junctional complexes combine to produce an effective permeability barrier against the influx of luminal content [1]. In particular, the barrier created by these structures limits exposure of the surface cells' basolateral cell membranes and entire membrane of cells of the deeper layers to the wide swings in osmolality occurring regularly within the esophageal lumen [2]. Histologically, the esophageal epithelium consists of two zones, the basal and differentiated zones. Cellular proliferation is limited to the basal zone, and cells are thought to migrate from this area towards the esophageal lumen. Migration is associated with the initiation of differentiation and the sequential expression of differentiation markers [3]. The availability of human esophageal epithelial cell culture provides an excellent in vitro model in the study of the physiology of esophageal epithelium and the mechanisms of the esophageal carcinogenesis.

HEEC from ScienCell Research Laboratories are isolated from human esophagus. HEEC are cryopreserved on passage one culture and delivered frozen. Each vial contains >5 x 10^5 cells in 1 ml volume. HEEC are characterized by immunofluorescent method with antibodies to cytokeratine-8, -18 and -19. HEEC are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi. HEEC are guaranteed to further expand for 15 population doublings in the condition provided by ScienCell Research Laboratories.

Recommended Medium

It is recommended to use keratinocyte medium (KM, Cat. No. 2101) for the culturing of HEEC *in vitro*.

Product Use

HEEC 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 needed for experiments.

Shipping

Dry ice.

Reference

[1]. Orlando, RC. (2000) Gastroesophageal Reflux Disease: Offensive Factors and Tissue Resistance. New York: Dekker.

[2]. Orlando, G.S., Tobey, N.A., Wang, P., Abdulnour-Nakhoul, S. and Orlando, R.C. (2002) Regulatory volume decrease in human esophageal epithelial cells. Am J Physiol Gastrointest Liver Physiol 283: G932-G937.

[3]. Jankowski, J., Hopwood, D., Dover, R. and Wormsley, K.G. (1993). Development and growth of normal, metaplastic and dysplastic oesophageal mucosa: biological markers of neoplasia. *Eur. J. Gastroenterol. Hepatol.* 5:235-246.

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 order:

1. Prepare a poly-L-lysine coated flask (2 μg/cm^2, T-75 flask is recommended) and 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 poly-L-lysine 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 1 ml eppendorf pipette gently resuspend the contents of the vial.
5. Dispense the contents of the vial into the above flask in step 3. 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 HEEC are plated in poly-L-lysine coated culture vessels that promote the cell attachment.
6. Replace the cap, and gently rock the flask to distribute the cells evenly. Loosen caps if necessary to permit gas exchange and culture the cells in 37°C, 5% CO_2 incubator.
7. 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 cobblestone morphology, nongranular cytoplasm, 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 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) for 5 – 7 min at 37^{o}C or until cells are completely rounded up (monitored with inverted microscope). Remove trypsin/EDTA solution and further incubate the cells at 37^{o}C for 1 min. With one hand hold the flask, gently tap the edge of flask with the other hand to release cells from the culture. Add 10 ml of trypsin neutralization solution to flask.
   Note: Use ScienCell Research Laboratories' trypsin/EDTA solution that is optimized to minimize the killing of the cells by over trypsinization.
6. Transfer the 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).