Datasheet for SV40-immortalized Human gallbladder epithelium cells

Catalog number:SL421

Description

Organism: Homo sapiens, human
Immortalization Method: Transduction with lentiviruses carrying SV40
Tissue: Gallbladder
Cell Type: epithelium cells
Gender: female
Morphology: epithelial
Growth Properties: adherent
Antibiotic used for selection: Puromycin . (It is not necessary to maintain the antibiotic selection during cell culture.)
Longevity: >25 PDLs post-thaw
DNA Profile (STR):
TPOX :8,12
D13S317:9,11
vWA:14,18
D5S818:10,11
FGA:24,25
D3S1358:15,16
D22S1045:15,16
D8S1179:12,14
D16S539:11,11
CSF1PO:12,14
Penta D:10,12
AMEL:X,X
TH01:9,9.3
D7S820:8,12
D21S11:30,30
D18S51:15,20
Penta E:11,15

Complete Growth Medium:

The base medium for this cell line is Epithelial Cell basal Medium (Sciencell,Cat. No.4101). To make the complete growth medium,add 10 ml of fetal bovine serum (FBS, Cat. No. 0010), 5 ml of epithelial cell growth supplement(EpiCGS, Cat. No. 4152), and 5 ml of penicillin/streptomycin solution (P/S, Cat. No. 0503).

Safety instructions:

To ensure safety, protective gloves, clothing, and a face mask should be worn when handling frozen vials. Some leakage may occur in the vial during storage. The liquid nitrogen will be converted to gas upon thawing. Due to the nature of nitrogen gas, pressure may build within the vial and possibly result in the vial exploding or losing its cap. This may cause flying debris.

Recovery of Frozen Cells

To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If storage of the frozen culture is necessary upon arrival, store the vial in liquid nitrogen vapor phase and **NOT** at -80°C. Storage at -80°C will result in loss of viability.

1. Prepare a 25-cm^2 or a 75-cm^2 culture flask containing the recommended complete culture medium. Prior to the addition of the vial contents, the vessel containing the growth medium should be placed in the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6) and to avoid excessive alkalinity of the medium during recovery of the cells.
2. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep cap out of the water. Thawing should be rapid (approximately 2 minutes).
3. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in Or spraying with 70% ethanol. All operations from this point on should be carried out under strict aseptic conditions.
4. Transfer the vial contents to a centrifuge tube containing 9.0 ml of complete culture medium and centrifuge the cell suspension at approximately 200 x g for 5 minutes.
5. Discard the supernatant and resuspend the cells in fresh growth medium. Count the cells and seed new culture flasks at a density of 5,000 viable cells per cm^2.
6. Incubate the culture at 37° C in a suitable incubator.
7. A 5% CO2/95% air atmosphere is recommended if using the medium described on this product sheet.

Handling Procedure for Flask Cultures

Receiving Flask Cultures

The flask was seeded with cells, incubated, and completely filled with medium to prevent loss of cells during shipping. Upon receipt, visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).

1. **If the cells are still attached**, aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO2 in air atmosphere until they are ready to be subcultured.

2. **If the cells are not attached**, aseptically remove the entire contents of the flask and centrifuge at 200 x g for 5 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm^2 flask. Incubate at 37°C in a 5% CO2 in air atmosphere until cells are ready to be cultured.

Subculturing Procedure

Protocol:
1. Passage cells when the culture has reached approximately 80% confluence.
2. Warm both the Trypsin-EDTA and the complete growth medium to 37°C prior to use with the cells.
3. For each flask, carefully aspirate the spent media without disturbing the monolayer.
4. Rinse the cell layer one time with 3 to 5 mL D-PBS to remove residual medium.
5. Add prewarmed trypsin-EDTA solution (1 to 2 mL for every 25 cm2) to each flask.
6. Gently rock each flask to ensure complete coverage of the trypsin-EDTA solution over the cells, and then aspirate the excess fluid off of the monolayer.
7. Observe the cells under the microscope. When the cells pull away from each other and round up (typically within 3 to 5 minutes), remove the flask from the microscope and gently tap it from several sides to promote detachment of the cells from the flask surface.
8. When the majority of cells appear to have detached, quickly add an equal volume of the complete growth medium to each flask. Gently pipette or swirl the culture to ensure all of the trypsin-EDTA solution has been neutralized.
9. Transfer the dissociated cells to a sterile centrifuge tube and set aside while processing any remaining cells in the culture flask.
10. Add 3 to 5 mL PBS to the tissue culture flask to collect any additional cells that might have been left behind.
11. Transfer the cells suspension to the centrifuge tube containing the trypsin-EDTA- dissociated cells.
12. Repeat steps 10 and 11 as needed until all cells have been collected from the flask.
13. Centrifuge the cells at 200 x g for 5 minutes.
14. Aspirate dissociation solution from the cell pellet and resuspend the cells in 2 to 8 mL fresh, prewarmed, complete growth medium.
15. Count the cells and seed new culture flasks at a density of 5,000 viable cells per cm2.
16. Place newly seeded flasks in a 37°C, 5% CO2 incubator for at least 24 to 48 hours before processing the cells further.

Cell seeding density: 5,000 viable cells per cm^2
Medium renewal: every 2 to 3 days

Cryopreservation Medium

90% complete growth medium plus 10% DMSO
Store in liquid nitrogen vapor. Avoid immersing vials into liquid nitrogen.

Product QC: >95% viability before freezing. All cells were tested and found to be free of mycoplasma, bacteria, viruses, and other toxins. The cells were also observed to express SV40 and the cell-specific surface markers.

Citation of product: If use of this item results in a publication, please use this information: SV40-immortalized Human gallbladder epithelium cells (SL421, GeneCopoeia, Inc., Rockville, MD).

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