**Description**

Organism: *Homo sapiens*, human  
Tissue: eye, cornea  
Cell Type: epithelial Adenovirus 12-SV40 hybrid transformed  
Gender: male  
Morphology: epithelial  
Growth Properties: adherent  
Isoenzymes:
- AK-1, 1  
- ES-D, 1  
- G6PD, A  
- GLO-I, 2  
- Me-2, 1-2  
- PGM1, 1  
- PGM3, 2  
DNA Profile:
- Amelogenin: X,Y  
- CSF1PO: 10,13  
- D13S317: 8,11  
- D16S539: 9,11  
- D5S818: 11,12  
- D7S820: 8,10  
- THO1: 6,9  
- TPOX: 10,11  
- vWA: 15,18  
Cytogenetic Analysis: aneuploid; Y chromosome is present

**SAFETY PRECAUTION**

ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

Refer to the Certificate of Analysis for batch-specific test results.

**Intended Use**

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

**Complete Growth Medium**

Keratinocyte-Serum Free Medium (Gibco 17005-042) 
Supplemented with frozen additives included (from GIBCO):  
1) 0.05 mg/ml bovine pituitary extract (BPE)  
2) 5 ng/ml epidermal growth factor (EGF).  
NOTE: Do not filter EGF  
And also supplemented with 500 ng/ml hydrocortisone and 0.005mg/ml insulin (not included).

**Citation of Strain**

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: HCE-2 [50.B1] (ATCC® CRL-11135™)

**Handling Procedure for Frozen Cells**

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary; it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).  
2. 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 of the operations from this point on should be carried out under strict aseptic conditions.  
3. It is recommended that the cryoprotective agent be removed immediately. Transfer the vial contents to a 15 mL centrifuge tube and dilute with the recommended complete culture medium. Centrifuge the cell suspension at approximately 125 x g for 5 to 10 minutes. Discard the supernatant and resuspend the cell pellet in an appropriate amount of fresh growth medium. **NOTE: Seed cells on flasks precoated with a mixture of 0.01 mg/ml fibronectin, 0.03 mg/mL bovine collagen type I and 0.01 mg/mL bovine serum albumin.**  
4. Transfer the cells to an appropriate size vessel. It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the growth medium be placed into the incubator for at least 15 minutes.
Incubate the culture at 37°C in a suitable incubator.

Discard supernatant and resuspend cells in fresh growth medium.

25 cm²

To remove trypsin-EDTA solution, transfer cell suspension to centrifuge tube and spin at 125 x g for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 mL of this medium and add to precoated 25 cm² flask. Incubate at 37°C in a 5% CO₂ atmosphere until cells are ready to be subcultured.

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. 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).

2. If the cells are still attached, aspirate 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 atmosphere until they are ready to be subcultured.

3. If the cells are not attached, aspirate the entire contents of the flask and centrifuge at 125 x g for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pellet of cells in 10 mL of this medium and add precoated 25 cm² flask. Incubate at 37°C in a 5% CO₂ atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Volumes are given for a 75 cm² flask. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes.

1. Remove and discard culture medium.

2. Briefly rinse the cell layer with 0.05% (w/v) Trypsin-0.53 mM EDTA solution.

3. Add 2.0 to 3.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually with 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

4. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting. (Or neutralize with medium containing 10% fetal bovine serum).

5. To remove trypsin-EDTA solution, transfer cell suspension to centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes.

6. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of cell suspension to new culture vessels. Seed cells on flasks precoated with a mixture of 0.01 mg/mL fibronectin, 0.03 mg/mL bovine collagen type I and 0.01 mg/mL bovine serum albumin.

7. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:3 is recommended

Medium Renewal: Twice per week

Cryopreservation Medium

85% Growth Medium, 10% FBS, 5% DMSO

Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Comments

Keratinocyte Serum-Free medium is available from Gibco, Grand Island, New York.

References

References and other information relating to this product are available online at www.atcc.org.

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the Biosafety in Microbiological and Biomedical Laboratories from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

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Additional information on this culture is available on the ATCC web site at www.atcc.org.

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