Product description

Immortalized Human Vaginal Epithelial Cell (HVEC) line derived from primary normal human epithelial cells isolated from premenopausal vaginal hysterectomy tissue, obtained from a healthy female donor.

Name: Human Vaginal Epithelial Cell (HVEC) line

Alternate name: HVEC E6/E7/TERT cells (V428)

Organism: Human

Tissue: Vaginal epithelium

Model: Immortalized cell line

Production details: Primary normal human epithelial cells were isolated from premenopausal vaginal hysterectomy tissue, obtained from a woman who did not have cancer. Cells were grown in keratinocyte serum-free medium (KSFM) (Gibco) on plastic and passaged with a 1:4 split using trypsin-EDTA. Early-passage cells were doubly transduced with retroviruses expressing human papillomavirus 16 (HPV-16) E6/E7 and the reverse transcriptase component of telomerase, hTERT, and selected with 50 µg of the antibiotic G418 per ml (Sigma). The E6/E7/TERT cells (V428) had high levels of telomerase and became immortal without a crisis, whereas normal un-transduced cells senesced around passage 9.

Growth properties: Adherent

CRISPR Edited: No

Biosafety level: 2

Contributor(s)

Inventor: Aloysius Klingelhutz
Institute: The University of Iowa

Properties

Product format: Frozen

Unpacking and storage:

1. Check all containers for leakage or breakage.
2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

Recommended medium: Keratinocyte serum-free medium (KSFM) (Gibco™), supplemented with 25 ug/ml bovine pituitary extract (BPE) and 0.16 ng/ml epidermal growth factor (EGF) and a 2% final volume of penicillin-streptomycin-amphotericin B.

Do not keep media for more than 1 month past adding supplements. Do not freeze and thaw medium.

Change media every other day. The cells will not do well if they are left over the weekend without a media change or if they are left to grow to greater than 95% confluency.

Subculture: Split 1:4 every 4-5 days

Culture conditions: 37.0°C ± 1.0°C incubator with 5.0% ± 1.0% CO_2

Cryopreservation: Freeze in K-SFM Freezing Media with 10% DMS0 and 15 % FBS. Approximately 3 vials per 80% confluent 10 cm plate. Slow freeze (30 minutes at 4°C, 1 hour at -20°C, overnight at -80, transfer liquid nitrogen vapor phase). Do not leave cells in -80 C for more than a week or they will begin to lose viability.

Handling instructions

1. Please ensure that vials are frozen when received, and store at **<-130 °C long term**. When removing frozen cells from storage, it is important to minimize exposure to room temperature (15 - 25°C). If not proceeding directly to thawing, place the cells on dry ice or in a liquid nitrogen container.
2. **Do not thaw at room temperature.** To thaw, swirl the vial quickly in a 37 °C water bath with O-ring and cap above the water to avoid contamination. Remove from the water bath with a small ice pellet remaining (this should not take more than 2 minutes) and wipe the exterior with 70% ethanol or isopropanol before transferring to a biosafety cabinet. Further steps should be conducted under aseptic conditions.
3. We strongly recommend that the volume of cell suspension is measured at this point, and a 20 uL aliquot be removed for a **viable cell count** using trypan blue or similar dye. This ensures that provided cells are viable, and the cell count can be used to determine volume of growth medium to be added to the cell suspension.
4. Transfer using a 5 mL pipette, to 5 mL PBS in a 15 mL tube to dilute the DMSO and FBS used in the freeze media. If this is not removed, the cells will differentiate and die.
5. Mix gently and spin at 1,200 RPM for 5 minutes.
6. Carefully aspirate the supernatant and resuspend in 10 mL media, gently mix, and plate in pre-labelled tissue culture plates.
7. On the next day, observe cells and note how well they have attached and spread. There will be some cells floating in media. Media change with 10 mL fresh medium.
8. Cells are passaged when they are 70-90% confluent.
   Subculture routine: Aspirate medium and wash plate with (1X) PBS (without calcium or magnesium). Add 4 ml (0.05%) Trypsin/EDTA and incubate at 37°C for 5-10 minutes. Add 4 ml PBS-A containing 2% FBS to inactivate the trypsin. Transfer cells to a sterile 15ml tube and centrifuge at 1200 rpm for 5min. Carefully aspirate supernatant taking care not to disturb the pellet and resuspend in K-SFM.

References

- Peterson et al. 2005. ASM Journals. Infect Immun. 2005 Apr;73(4):2164–2174. PMID: 15784559.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: Human Vaginal Epithelial Cell (HVEC) line, was invented by Aloysius Klingelhutz (CancerTools.org #153753).

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