

## HCE-T Cells | 305255

### General information

#### Description

HCE-T is an SV40-transformed human corneal epithelial cell line derived from primary human corneal epithelium. The line was established by infection with a recombinant SV40–adenovirus hybrid vector (Ad–SV40), enabling stable expression of SV40 large T antigen and continuous proliferation. The original characterization specifically aimed to generate a continuously growing corneal epithelial cell line without shedding free viral particles.

In culture, HCE-T cells show typical epithelial “cobblestone” morphology and grow as adherent monolayers. Ultrastructural epithelial features such as desmosomes and apical microvilli have been reported, and the cells have been described as producing a cornea-associated 64 kD keratin. Under suitable differentiation conditions (e.g., air–liquid interface culture on collagen), HCE-T cells can form multilayered, stratified structures and develop measurable barrier properties, supporting their use in ocular surface research.

HCE-T cells are widely used to study corneal epithelial barrier function, permeability and formulation effects, migration/repair-related processes, and cellular responses to inflammatory or irritant stimuli. However, transporter expression patterns and differentiation-marker profiles can differ from native human cornea and from primary limbal/corneal epithelial systems. Therefore, HCE-T is best suited for mechanistic and comparative *in vitro* studies, while direct quantitative extrapolation to *in vivo* human corneal absorption or corneal differentiation biology should be performed with caution.

**Organism** Human

**Tissue** Eye, cornea, epithelium

**Synonyms** HCET, Human Corneal Epithelial cells-Transformed, HCE, SV40-HCEC

### Characteristics

**Age** 49 years

**Gender** Female

**Ethnicity** Japanese

**Morphology** Epithelial

**Cell type** Epithelial cell

**Growth properties** Adherent

### Regulatory Data

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<b>Citation</b>	HCE-T (Cytion catalog number 305255)
<b>Biosafety level</b>	1
<b>NCBI_TaxID</b>	9606
<b>CellosaurusAccession</b>	CVCL_1272
<b>GMO Status</b>	GMO-S1: This human corneal epithelial cell line (HCE-T) contains an SV40 early-region construct (RSV-T / pRSV-T vector), enabling immortalization. The insert is stably integrated into primary human corneal epithelial cells. This classification applies only within Germany and may differ elsewhere.

## Biomolecular Data

<b>Viruses</b>	Transformant: plasmid RSV-T (pRSV-T). This plasmid is an SV40 ori-construct containing the SV40 early region genes and the Rous sarcoma virus long terminal repeat.
<b>Products</b>	Keratin (64kD)

## Handling

<b>Culture Medium</b>	DMEM:Ham's F12 (1:1), w: 3.1 g/L Glucose, w: 2.5 mM L-Glutamine, w: 15 mM HEPES, w: 0.5 mM Sodium pyruvate, w: 1.2 g/L NaHCO <sub>3</sub> (Cytion article number 820400a)
<b>Supplements</b>	Supplement the medium with 5% FBS, 1% ITS (0.625 mg/mL human insulin, 0.625 mg/mL human transferrin, 0.625 microgram/mL sodium selenite, 0.535 mg/mL linoleic acid, 125 mg/mL BSA) and 10 ng/mL human EGF
<b>Dissociation Reagent</b>	Accutase
<b>Subculturing</b>	Remove the old medium from the adherent cells and wash them with PBS that lacks calcium and magnesium. For T25 flasks, use 3-5 ml of PBS, and for T75 flasks, use 5-10 ml. Then, cover the cells completely with Accutase, using 1-2 ml for T25 flasks and 2.5 ml for T75 flasks. Let the cells incubate at room temperature for 8-10 minutes to detach them. After incubation, gently mix the cells with 10 ml of medium to resuspend them, then centrifuge at 300xg for 3 minutes. Discard the supernatant, resuspend the cells in fresh medium, and transfer them into new flasks that already contain fresh medium.
<b>Freeze medium</b>	As a cryopreservation medium, we use complete growth medium (including FBS) + 10% DMSO for adequate post-thaw viability, or CM-1 (Cytion catalog number 800100), which includes optimized osmoprotectants and metabolic stabilizers to enhance recovery and reduce cryo-induced stress.

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### Thawing and Culturing Cells

1. Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit.
2. Upon receipt, either store the cryovial immediately at temperatures below  $-150^{\circ}\text{C}$  to ensure the preservation of cellular integrity, or proceed to step 3 if immediate culturing is required.
3. For immediate culturing, swiftly thaw the vial by immersing it in a  $37^{\circ}\text{C}$  water bath with clean water and an antimicrobial agent, agitating gently for 40-60 seconds until a small ice clump remains.
4. Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
5. Carefully open the disinfected vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of room-temperature culture medium, mixing gently.
6. Centrifuge the mixture at  $300 \times g$  for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
7. Gently resuspend the cell pellet in 10 ml of fresh culture medium. For adherent cells, divide the suspension between two T25 culture flasks; for suspension cultures, transfer all the medium into one T25 flask to promote effective cell interaction and growth.
8. Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

### Incubation Atmosphere

$37^{\circ}\text{C}$ , 5%  $\text{CO}_2$ , humidified atmosphere.

### Shipping Conditions

Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately  $-78^{\circ}\text{C}$  throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage.

### Storage Conditions

For long-term preservation, place vials in vapor-phase liquid nitrogen at about  $-150$  to  $-196^{\circ}\text{C}$ . Storage at  $-80^{\circ}\text{C}$  is acceptable only as a short interim step before transfer to liquid nitrogen.

## Quality Control & Molecular Analysis

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**Sterility**

Mycoplasma contamination is excluded using both PCR-based assays and luminescence-based mycoplasma detection methods.

To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections.