

Human Corneal Epithelial Cells (HCEC)

Catalog no. C018-5C

Quantity: $\geq 5 \times 10^5$ viable cells/vial

Product Description

HCEC are normal human corneal epithelial cells isolated from dissected limbal sections, the progenitor-rich region where the sclera and cornea join. Each vial of this product contains $\geq 5 \times 10^5$ viable cells that have been cryopreserved at the end of the secondary culture level (equivalent to end of first passage – p1) in a cryopreservation medium containing 10% DMSO. Upon thawing, the cells are guaranteed to be $\geq 70\%$ viable (as determined by trypan blue) and to be in sufficient number to seed $\geq 100 \text{ cm}^2$ of surface area when handled according to the protocol below. HCEC test negative for mycoplasma, bacteria, yeast, or other fungi, and viruses including Hepatitis B, Hepatitis C, and HIV-1. Each lot of HCEC is performance tested and guaranteed to reach 12 population doublings after thawing, when cultured in Keratinocyte SFM as described below. Each lot is further characterized by positive immunofluorescent staining for the corneal epithelial cell markers cytokeratin 15 (CK15) and p63 α .

Intended Use

Cryopreserved HCEC are intended for use by researchers investigating the molecular and biochemical bases of various normal and disease processes. **This product is for research use only. Not intended for human or animal therapeutic or diagnostic use.**

Storage and Stability

Cryopreserved HCEC should arrive frozen on dry ice or in a liquid nitrogen dry-shipper. If the cells are not to be used immediately, the user should prepare a space for storage of the vial in the vapor phase of a liquid nitrogen freezer. While wearing protective eyewear, gloves, and a laboratory coat, remove the vial from its shipping container and place immediately in the liquid nitrogen freezer. Cells should be stable for more than two years, if stored under the vapor phase of liquid nitrogen. See Table 1 for reagent storage conditions.

Caution

If handled improperly, some components of this product may present a health hazard. Take appropriate precautions when handling this product, including the wearing of protective clothing and eyewear. Dispose of the product properly.

Table 1 Reagent storage conditions

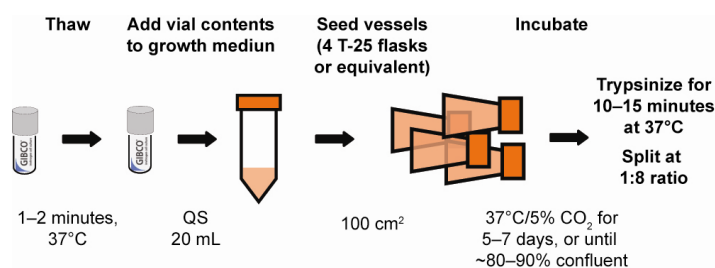
HCEC Serum-Free Culture Systems from Gibco®			
Description	Cat. no.	Size	Storage
Undefined			
Keratinocyte Serum Free Medium (SFM)*	17005-042	1 kit, 500 mL	4°C (in the dark), once supplemented
Defined			
Defined Keratinocyte Serum Free Medium (SFM)**	10744-019	1 kit, 500 mL	4°C (in the dark), once supplemented
Coating Matrix	R-011-K	1 kit, 50 mL	4°C
Related Reagents			
Antibiotic/Antimycotic (AA), 100X	15240-062	100 mL	4°C
TrypLE™ Express	12604-013	100 mL	Room Temperature
DPBS, Ca/Mg-Free	14190-144	500 mL	Room Temperature

* To guarantee optimal performance, we recommend Keratinocyte SFM as the growth medium for HCEC culture.
** Coating Matrix Kit is required for use with Defined Keratinocyte SFM.

Quick Start Protocol

Although we recommend following “Thawing and Seeding Cells” steps on page 2 and performing a viable cell count, HCEC can alternatively be seeded directly into tissue culture dishes or plastic in standard volumes of growth media as illustrated below in Figure 1. Using a split ratio of 1:8, HCEC can generally be passaged 2–4 times under these conditions.

Figure 1 Quick start workflow



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Preparing Growth Medium

1. Prepare supplemented Keratinocyte SFM by adding entire contents of the BPE and EGF supplement vials to a 500 mL bottle of Keratinocyte SFM basal medium. Mix gently. If desired, add 5.0 mL of 100X Antibiotic-Antimycotic (Cat. no. 15240-062) to the supplemented medium.
2. Store the supplemented medium at 4°C, protected from light. Supplemented medium is best used within 30 days of preparation.

Thawing and Seeding HCEC

1. Remove a vial of HCEC from liquid nitrogen storage, taking care to protect hands and eyes.
2. Dip the lower half of the vial into a 37°C water bath to thaw.
3. When the contents of the vial have thawed, wipe the outside of the vial with a disinfecting solution and transfer the vial to a biosafety hood.
4. Open the vial and gently resuspend the cells using a 1-mL pipette.
Note: HCEC can alternatively be seeded directly into tissue culture vessels according to the Quick Start protocol on page 1.
5. Take a 20- μ L aliquot of the cell suspension and add to 20 μ L of Trypan Blue stain (1:2 dilution, Cat. no. 15250-061).
6. Determine the concentration of viable cells using a hemocytometer or the Countess® Automated Cell Counter (Cat. no. C10227).
7. Dilute the cells appropriately in growth medium and seed the cells at **5,000 cells per cm²** into desired culture dishes or T-flasks.
8. Change the medium after 24 hours and then every 48 hours thereafter. Once the culture reaches 50% confluency, change the medium every 24 hours.

Note: HCEC typically take 5–7 days to reach confluency using these conditions.

Subculturing HCEC

The following protocol is designed for the subculture of one 25 cm² culture flask; adjust the volumes appropriately for multiple flasks or different sized vessels.

1. After the cells reach **~80–90% confluency** (see Figure 2, below), remove all of the culture medium from the flask by sterile aspiration.
2. Wash the cells once with 3 mL of Ca/Mg-Free DPBS.
3. Aspirate the DPBS, add 1 mL of TrypLE™ dissociation reagent, and incubate for 10–15 minutes at 37°C. Rock the flasks periodically during this process and monitor cell detachment.
4. After the cells have detached, add 3 mL of growth medium to the flasks and transfer the cell suspension to a conical tube. Wash the flask with an additional 3 mL of growth medium and add to the conical tube.
5. Centrifuge the cells at 180 \times g for 5 minutes at room temperature.
6. Carefully aspirate the medium from the cell pellet and resuspend the cells in an appropriate volume of growth medium (generally, 1–2 mL of medium per 25 cm² of cells harvested).
7. Count and seed the cells as described in “Thawing and Seeding HCEC”, Steps 5–8.

Phase Contrast Micrographs of HCEC

The image below shows the typical morphology of **~80–90% confluent** HCEC cultured in KSFM. It is important to subculture the cells when they are sub-confluent, because cells allowed to grow to confluency will have reduced mitotic index in subsequent cultures.

Figure 2 Typical morphology of ~80–90% confluent HCEC cultured in KSFM



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