



Product Description

Normal Human Corneal Epithelial (NHCE) cells are isolated from the progenitor-rich limbal region of human corneas and cryopreserved. NHCE cells exhibit cobblestone morphology in monolayer culture (**Figure 1**). NHCE cells are characterized by positive staining for cytokeratins 3/12 and 15, and aldehyde dehydrogenase ALDH-3A1 (**Figure 2**). Cells are guaranteed to provide a minimum of 3 passages when handled according to the protocol below. Cells test negative for Hepatitis B, Hepatitis C, HIV-1, mycoplasma, bacteria, yeast, and fungi.

Intended Use

NHCE cells require the use of NHCE Growth Medium (NHCE-GM) and coating mixture (NHCE-CM) for optimal expansion in monolayer culture. When used with NHCE-GM, NHCE cells provide an ideal serum-free culture system to study cell-cell and cell-matrix interactions, gene regulation, cellular differentiation, cytoskeletal organization, wound healing, and cellular toxicity.

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

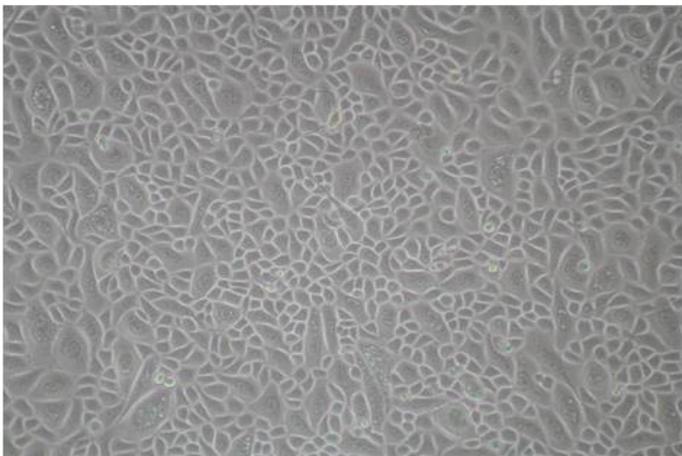


Figure 1. Phase contrast image of NHCE cells in monolayer culture, 100X magnification.

Format

NHCE cells are provided frozen in 2 mL of cryopreservation medium. Each vial contains $\geq 500K$ viable cells.

Storage & Handling

Upon receipt, immediately transfer the cryovial(s) into the vapor phase of a liquid nitrogen storage dewar. Cells are stable for ≥ 2 years when stored under these conditions. See **Table 1** for storage conditions.

Directions for Use

Preparation of culture surface using NHCE-Coating Mixture (NHCE-CM)

1. Warm enough NHCE-CM to room temperature to coat the entire culture surface to be inoculated (0.2 mL/cm^2).
2. Coat the culture surface with NHCE-CM just prior to use. Add NHCE-CM solution to coat the entire surface, and withdraw after 30 seconds. NHCE-CM can be reused.

Preparation of NHCE-Growth Medium (NHCE-GM)

1. Thaw the growth supplement (NHCE-GS) at room temperature for 30 minutes or until completely thawed.
2. Aseptically transfer the contents of one vial of growth supplement into one 500 mL bottle of basal medium (NHEK-BM). Label the bottle "NHCE-GM."
3. Cap the bottle and swirl to mix.

Note: NHEK-BM supplemented with NHCE-GS is referred to as NHCE-GM. Protect NHCE-GM from light exposure. NHCE-GM has an expiration date of 2 weeks when stored at $2-8^\circ\text{C}$. NHCE-GM is antibiotic-free. Use of antibiotics may compromise NHCE cell growth or function, and is not recommended.

Thawing and seeding of Cryopreserved Cells

1. The recommended seeding density for NHCE cells is $4,000 \text{ cells/cm}^2$.
2. Aseptically vent the cryovial in a biosafety cabinet prior to thawing by briefly loosening and re-tightening the cap. Quickly thaw the cryovial in a 37°C water bath, being careful not to submerge the cap. Watch the cryovial closely. When the last sliver of ice melts, remove the vial and wipe with 70% ethanol before opening in a biosafety cabinet.
3. Transfer cells from the cryovial(s) into a sterile 50 mL conical tube containing 10 mL of NHCE-GM at room temperature. Centrifuge at $100 \times g$ for 5 min to pellet the



Human Corneal Epithelial Cells Data Sheet

Table 1. Reagents for use with NHCE Cells			
Product	Catalog Number	Size	Storage
Normal Human Corneal Epithelial Cells	NHCE-CRY	≥5x10 ⁵ cells (2 mL)	Liquid Nitrogen Vapor
NHCE Growth Medium (NHCE-BM + NHCE-GS)	NHCE-GM	500 mL	2-8°C
NHEK Basal Medium	NHEK-BM	500 mL	2-8°C
NHCE Growth Supplement	NHCE-GS	5 mL	≤-15°C
NHCE Coating Mixture	NHCE-CM	10 mL	2-8°C

cells.

4. Aspirate the supernatant and add 25 mL (1 mL/5 cm² culture surface) of NHCE-GM at room temperature. Cap and gently swirl to ensure even distribution of cells.

5. Gently transfer the cell suspension into the culture vessel(s). Carefully rock the culture vessel(s) to evenly distribute the cells and place in a 37°C, 5% CO₂, humidified incubator.

Maintenance of Monolayer Cell Cultures

1. Change the growth medium 24 hours after initial plating and every 48 hours thereafter using 1 mL of pre-warmed media for every 5 cm² culture area.

2. Avoid repeated warming and cooling of the NHCE-GM. Warm only the volume of medium needed.

Subculturing of Cells

1. Subculture the cells when they are 70-80% confluent and contain many mitotic figures (generally 4-6 days after plating).

2. Prepare sufficient Trypsin (0.025%)/EDTA (0.265 mM) solution in Ca²⁺/Mg²⁺-free PBS (DPBS), and Soybean Trypsin Inhibitor solution (STI, 0.25 mg/mL) in DPBS at room temperature (0.2 mL/5 cm² culture area).

3. Aspirate the medium from the culture vessel and rinse

twice with DPBS (0.2 mL/5 cm²).

4. Add Trypsin/EDTA solution (0.2 mL/5 cm² culture area) and gently rock to ensure complete coverage. Incubate at 37°C for 5 min or until cells completely round up.

5. Continue trypsinization until ~80% of the cells can be detached by gentle agitation of the vessel. Then, rap the vessel against a hard surface 3-4 times to release remaining cells. To avoid damage to the cells, keep the trypsin exposure to the minimum time necessary.

6. After the cells have been detached, add STI solution (0.2 mL/5 cm² culture area) to the vessel and collect the cells in a conical tube.

7. Rinse the culture vessel with DPBS (0.2 mL/5 cm² culture area) and combine with the cell suspension.

8. Take a small aliquot for cell counting. Centrifuge the remaining suspension at 100 X g for 5 minutes.

9. Resuspend the cell pellet in fresh, warm NHCE-GM. Coat a new culture vessel with NHCE-CM and seed cells at a density of 4,000 cells/cm².

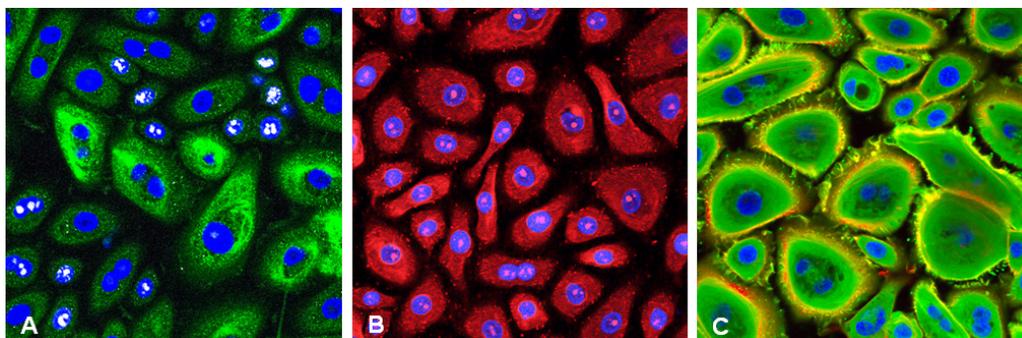


Figure 2. Immunohistochemical analysis of NHCE cells at passage 3. A. Cytokeratins 3/12 (green) and Ki67 (white). B. CK15 (red) C. ALDH-3A1 (green) and phalloidin (red). 60x, DAPI (blue).

