MatTek CORPORATION

Human Corneal Epithelial Cells Data Sheet

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.

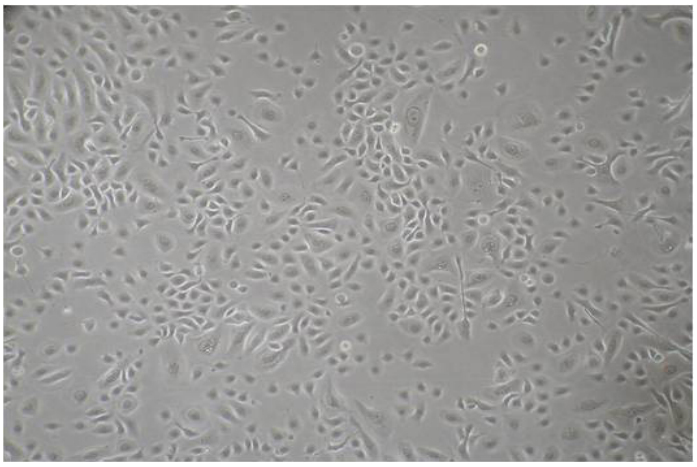

Figure 1. Phase contrast image of NHCE cells in monolayer culture, 100X magnification.

Format

NHCE cells are provided frozen in 2 mL of cryopreservation medium. Each vial contains ≥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 supplements (NHCE-GS and NHCE-GS1) at room temperature for 30 minutes or until completely thawed. Be sure that all components are in solution and no precipitate is visible.
2. Aseptically transfer the contents of both growth supplement vials into one 500 mL bottle of basal medium (NHEK-BM). Rinse NHCE-GS1 vial with medium to ensure complete transfer.
3. Cap the bottle and swirl to mix.

Note: NHEK-BM supplemented with NHCE-GS and NHCE-GS1 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°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 6,000 cells/cm^2.
2. Prior to thawing cells, prepare a conical tube with 10 ml of NHCE-GM at room temperature.
3. Aseptically vent any nitrogen from cryovial in a biosafety cabinet prior to thawing by briefly loosening and re-tightening the cap. Quickly thaw the cryovial in a 37°C

Table 1. NHCE Product Information			
Product	Catalog #	Size	Storage
Normal Human Corneal Epithelial Cells	NHCE-CRY	≥5x10^5 cells (1 mL)	Liquid Nitrogen Vapor
NHCE Growth Medium (NHEK-BM + NHCE-GS + GS1)	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 Growth Supplement-1	NHCE-GS1	50 µl	≤-15°C
NHCE Coating Mixture	NHCE-CM	10 mL	2-8°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.

4. Transfer cells from the cryovial(s) into an empty sterile 50 mL conical tube. Slowly add 1 ml of NHCE-GM into the 50 mL tube and shake gently. Add additional 1 ml aliquots of NHCE-GM and shake gently until the cells are resuspended in 5 mL medium; then add the rest of the medium (5 ml) and centrifuge at 100 X g for 5 min to pellet the cells.

5. Aspirate the supernatant and add 25 mL (1 mL/3.3 cm^2 culture surface) of NHCE-GM at room temperature. Cap and gently swirl to ensure even distribution of cells.

6. 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% CO_2, 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 cm^2 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 4-5 days after plating).

2. Prepare sufficient Accutase® cell detachment solution and DPBS.

3. Aspirate the medium from the culture vessel and rinse with DPBS (0.2 mL/5 cm^2).

4. Add Accutase cell detachment solution (0.3 mL/5 cm^2 culture area) and gently rock to ensure complete coverage. Incubate at 37°C for 10 min or until cells completely round up.

5. Continue incubation until all the cells can be detached by gentle agitation of the vessel. To avoid damage to the cells, keep the Accutase cell detachment solution exposure to the minimum time necessary.

6. After the cells have been detached collect the cells in a conical tube.

7. Rinse the culture vessel with DPBS (0.2 mL/5 cm^2 culture area) and combine with the cell suspension.

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

9. Resuspend the pellet in fresh, warm NHCE-GM. Coat new culture vessel with NHCE-CM and seed cells at a density of 6,000 cells/cm^2.

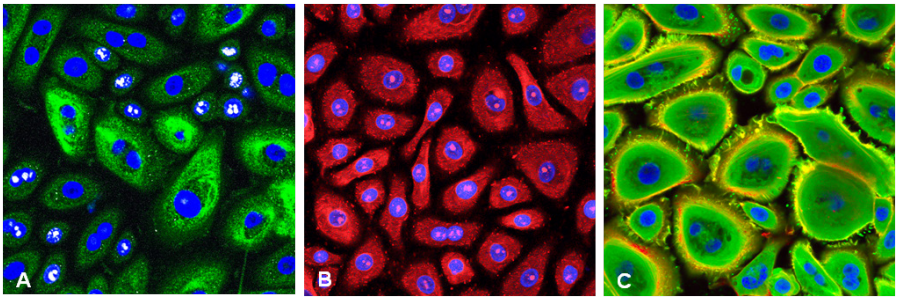

Figure 2. Immunohistochemical analysis of NHCE cells at passage 3. A. Cytokeratins 3/12 (green) and Ki67 (white). B. CK15 (red) C. ALDH-3A1 (green) and phalloidin (red). 60x, DAPI (blue).

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