MatTek CORPORATION

Human Epidermal Keratinocytes Data Sheet

Product Description

Normal Human Epidermal Keratinocytes (NHEKs) are isolated from the epidermis of neonatal foreskin (NHEK-CRY-NEO) or adult (breast or abdominal) skin (NHEK-CRY-AD) and cryopreserved. NHEKs exhibit cobblestone morphology in monolayer culture (**Figure 1**). NHEKs are characterized by positive staining for cytokeratin 14 (**Figure 2**). The cells are guaranteed to provide a minimum of 15 population doublings when handled according to the protocol below. Cells test negative for mycoplasma, bacteria, yeast, other fungi, Hepatitis B, Hepatitis C, and HIV-1.

Intended Use

When used with NHEK Growth Medium (NHEK-GM), NHEKs provide an ideal serum-free culture system to study cellular differentiation, cytoskeletal organization, wound healing, and toxicity testing. NHEKs require the use of NHEK-GM for optimal expansion in monolayer culture.

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

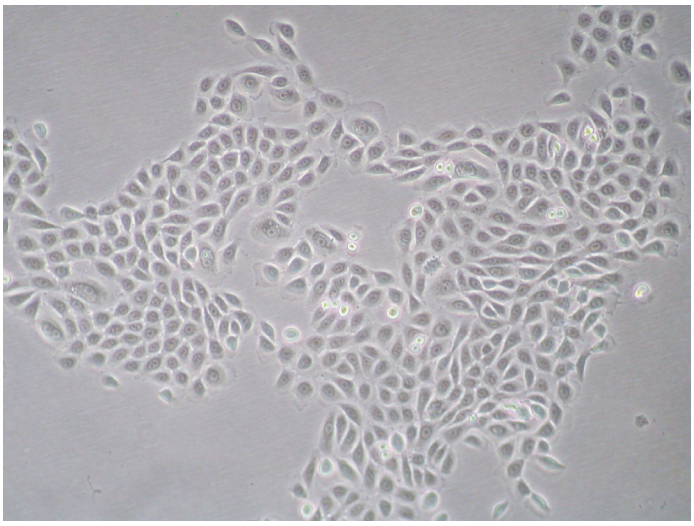

Figure 1. Phase contrast image of NHEKs in monolayer culture, 10X.

Format

NHEKs are provided frozen in 1 mL of cryopreservation medium. Each vial contains ≥500K viable cells.

Storage & Handling

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

Directions for Use

Prepare NHEK-Growth Medium (NHEK-GM)

1. Thaw the growth supplement (NHEK-GS) at room temperature in the dark for 30 minutes or until the supplement is completely thawed. Be sure that all components are in solution and that no precipitate is visible.
2. Aseptically transfer the entire contents of one growth supplement vial into one 500 mL bottle of basal medium (NHEK-BM).
3. Cap the bottle and swirl to mix.

Note: NHEK-BM supplemented with NHEK-GS is referred to as NHEK-GM. NHEK-GM has an expiration date of one month when stored at 2-8°C. NHEK-GM is antibiotic-free. Antibiotics are not required, however they may be added. If desired, up to 50 µg/mL Gentamycin (Gibco cat# 15750-060) and 50 ng/mL Fungizone (Gibco cat# 15920-018) may be used without compromising cell growth or function.

Thawing and Seeding of Cryopreserved Cells

1. The recommended seeding density for NHEKs is 2500 cells/cm^2.
2. Aseptically vent any nitrogen from cryopreserved vials in a biosafety cabinet prior to thawing. 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 remains, remove the vial and wipe with 70% ethanol before opening in a biosafety cabinet.
3. Transfer cells from the cryovial(s) into a sterile conical tube containing 20 mL of NHEK-GM at room temperature. Cap and gently swirl to ensure even distribution of cells.

Note: MatTek Serum-Free Normal Human Epidermal Keratinocyte Growth Medium (NHEK-GM), consisting of NHEK Basal Medium and NHEK Growth Supplement, is specially designed to support the growth of MatTek Normal Human Keratinocytes (NHEKs). Use of NHEK-GM (NHEK-BM supplemented with NHEK-GS) is strongly recommended for culturing MatTek NHEKs.

Table 1. Reagents for use with NHEK cells

Product	Catalog Number	Size	Storage
Normal Human Epidermal Keratinocytes (Neonatal)	NHEK-CRY-NEO	≥5x10^5 cells (1 mL)	Liquid Nitrogen Vapor
Normal Human Epidermal Keratinocytes (Adult)	NHEK-CRY-AD		
NHEK Growth Medium (NHEK-BM + NHEK-GS)	NHEK-GM		2-8°C
NHEK Basal Medium	NHEK-BM	500 mL	2-8°C
NHEK Growth Supplement	NHEK-GS	5 mL	≤-20°C

4. Gently transfer the cell suspension into the culture vessel(s). Carefully rock the culture vessel(s) to evenly distribute the cells and place in 37°C, 5% CO_2 humidified incubator.

Maintenance of Monolayer Cell Cultures

1. Change the NHEK-GM 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 NHEK-GM. Transfer and warm only the required volume of medium into a sterile secondary container.

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 Trypsin (0.025%)/EDTA (0.265 mM) solution in Ca^{2+}/Mg^{2+}-free PBS (DPBS), and Soybean Trypsin inhibitor solution (STI, 0.25 mg/mL) in DPBS at room temperature (0.35 mL per 5 cm^2).
3. Aspirate the medium from the culture vessel and rinse twice with DPBS (0.33 mL per 5 cm^2).
4. Add Trypsin/EDTA solution and gently rock to ensure complete coverage of cells. Incubate at room temperature for 10-15 min or until cells completely round up.
5. Continue trypsinization until most cells (~80%) can be detached by gentle agitation of the vessel. Once ~80% of the cells have detached, rap the vessel against the edge of a hard surface 3-4 times to release remaining cells.
6. After the cells have been detached, add STI solution (0.2 mL/5cm^2) to the vessel and collect the cells in a conical tube.
7. Rinse the culture vessel with DPBS (0.33 mL per 5 cm^2) and transfer the rinse solution to the conical tube containing 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 NHEK-GM. Seed cells in a new culture vessel at a density of 2,500 cells/cm^2.

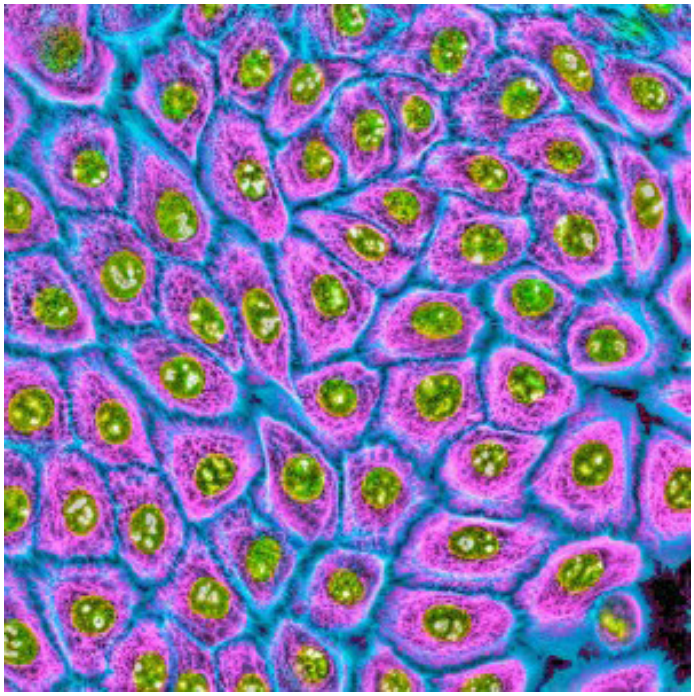

Figure 2. Primary Human Epidermal Keratinocytes cultured in NHEK-GM were stained for Keratin 14 (pink), Phalloidin (blue) and Ki67 (yellow).

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