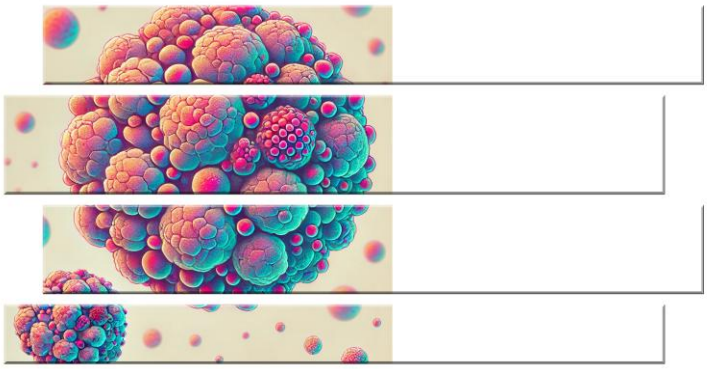

TriDix Bio
INNOVATING DRUG DISCOVERY

PRODUCT DATA SHEET

Primary Epidermal Keratinocytes; Neonatal (HEKn)

SKU: TDC-P1202

Product Details

Catalog Number: TDC-P1202

Organism: *Homo Sapiens*, Human

Cell Type: keratinocyte

Tissue: Skin

Age: Neonate

Gender: Male

Clinical Information: Healthy (with no known disease phenotypes)

Package Size: 5 x 10^5 cells/vial

Passage Number: P1

Growth Properties: Adherent

Associated Media: Keratinocyte Growth Medium (Cat. # TDM-1016)

Storage Conditions & Shipment

Product Format/Shipped: Cryopreserved / Dry ice

Storage: Vapor phase of liquid nitrogen

Safety Precaution

PLEASE READ BEFORE HANDLING ANY FROZEN VIALS. *Please wear appropriate Personal*

Description

Primary Epidermal Keratinocytes neonatal (HEKn) are primary cells that exhibit a rounded, cobblestone-like appearance. Isolated from neonatal foreskin, HEKn serve as an optimal cell system for cultivating keratinocytes under serum-free conditions. These cells are cryopreserved at the first passage to maintain maximum viability and plating efficiency. HEKn can be utilized in a wide range of research areas, including toxicology, wound healing, skin cancer, response to UV radiation, psoriasis, eczema, viral infections, gene delivery systems, and cellular differentiation.

Product Data

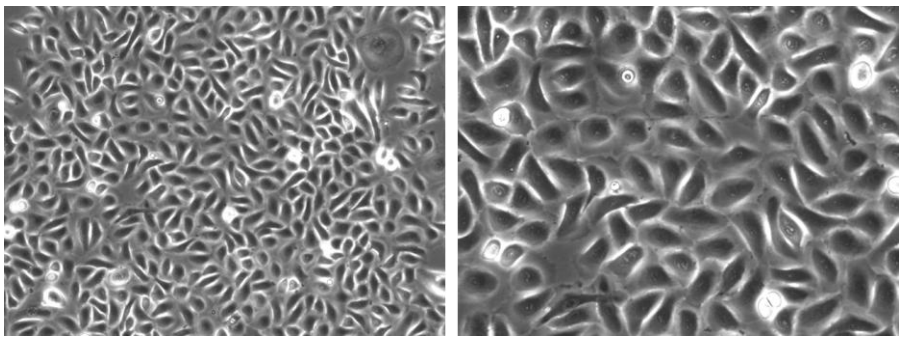

Figure 1: Phase contrast images of Primary Epidermal Keratinocytes neonatal (HEKn), Karetinocytes were grew in Keratinocyte Growth Medium (Cat. # TDM-1016). The images above show the morphology and cellular arrangement of the keratinocytes. The cells exhibit typical polygonal shapes with well-defined.borders and distinct nucleus visibility.

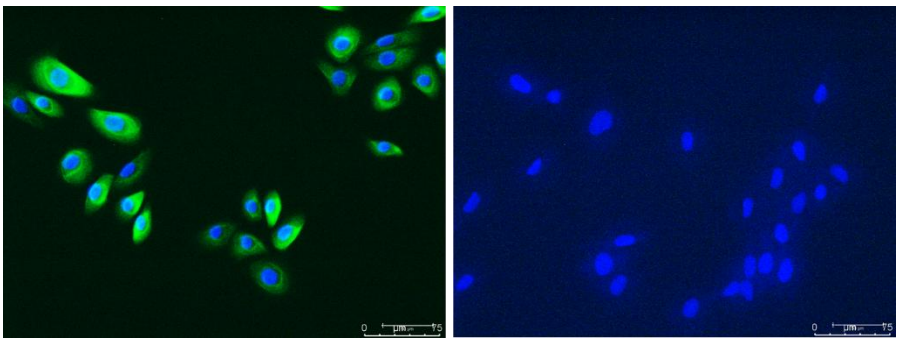

Figure 2, Staining of HEKn with Cytokeratin and Vimentin: Cytoplasmic cytokeratin is shown in green; nuclei were counterstained with Dapi and are shown in blue (Left). Staining of keratinocytes with anti-vimentin: no staining was observed with this antibody. Nuclei are shown in blue (Right). Scale bar=75um.

Applications

1. Toxicology
2. Wound healing
3. Epithelial function and disease
4. Skin Regeneration and Tissue Engineering
5. Inflammation and Immune Response
6. Photodamage and UV radiation

Ordering Information

Product	Catalog Number
Human Epidermal Keratinocytes, neonatal (HEKn)	TDC-P1202
Keratinocyte Growth Medium	TDM-1016
Keratinocyte Culture Supplement	TDM-1016A

Protocols

1. Recovering HEKn

1) Coat the plates with Collagen I at least one hour at RT.
2) Wash the plates twice with PBS before dry in the hood with lid open.
3) Remove one vial of from liquid nitrogen storage and thaw the cells by gentle agitation in a 37°C water bath.
   Note: To reduce the possibility of contamination, keep the O-ring and cap out of the water.
4) Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol.
   Note: Thawing should be rapid (approximately 1 minute).
5) Add the appropriate volume of Keratinocyte Growth Medium [volume = (1 mL x number of flasks to be seeded) into a sterile conical tube.
6) Using a sterile pipette, transfer the cells from the cryovial to the conical tube. Gently pipette the cells to homogenize the suspension. Do not centrifuge.
7) Count the cells with trypan blue. Seed the cells at density of 2500 cells/cm^2.
8) Place the seeded culture flasks in the incubator at 37°C, 5% CO2 atmosphere and incubate for at least 24 hours before processing the cells further.

2. Culturing HEKn

1) Passage HEKn when the culture has reached approximately 70% to 80% confluence.
2) For each flask, carefully aspirate the media and rinse the cells one time with 3 to 5 mL DPBS.
3) Add pre-warmed trypsin-EDTA solution (1 to 2 mL for every 25 cm^2) to each flask. Gently rock each flask to ensure complete coverage of the trypsin-EDTA solution over the cells, and then aspirate the excess fluid off of the monolayer.
4) Observe the cells under the microscope. When the cells pull away from each other and round up (typically within 3 to 6 minutes), remove the flask from the microscope and gently tap it from several sides to promote detachment of the cells from the flask surface.
   Note: If cells are difficult to detach, incubate each flask containing cells and the trypsin-EDTA solution at 37°C to facilitate dispersal.
5) When the majority of cells appear to have detached, quickly add an equal volume of the Neutralizing Solution. Gently pipette or swirl the culture to ensure all of the trypsin-EDTA solution has been neutralized.
6) Transfer the dissociated cells to a sterile centrifuge tube. Add 3 to 5 mL DPBS to the tissue culture flask to collect any additional cells that might have been left behind.
7) Transfer the cell and DPBS suspension to the centrifuge tube and centrifuge the cells at 150 x g for 3 to 5 minutes.
8) Aspirate neutralized dissociation solution from the cell pellet and resuspend the cells in 2 to 8 mL fresh, pre-warmed, Keratinocyte Growth Medium.
9) Count the cells and seed new culture flasks at a density of 2,500 to 5,000 cells per cm2. Place newly seeded flasks in a 37°C, 5% CO2 incubator for at least 24 to 48 hours before processing the cells further.

3. Maintenaning HEKn

1) Before beginning, pre-warm Keratinocyte Growth Medium in a 37°C water bath. If using a small volume of medium (50 mL or less), warm only the volume needed in a sterile conical tube. Avoid warming Keratinocyte Growth Medium multiple times.
2) 24 hours after seeding, remove the cells from the incubator and view each flask under the microscope to determine percent cellular confluence. Carefully remove the spent media without disturbing the monolayer.
3) Add 5 mL of fresh, pre-warmed Keratinocyte Growth Medium per 25 cm2 of surface area and return the flasks to the incubator. After 24 to 48 hours, view each flask under the microscope to determine percent cellular confluence.
4) If not ready to passage, repeat steps 3 and 4 as described above. When cultures have reached approximately 80% confluence, and are actively proliferating (many mitotic figures are visible), it is time to subculture. Keratinocytes will begin to terminally differentiate once they become 100% confluent.

Disclaimers

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