Human Epidermal Keratinocytes

300692

GENERAL INFORMATION

Description

Normal Human Epidermal Keratinocytes (NHEK) are primary cells isolated from adult skin. They are used to build reconstructed epidermis for animal-free skin irritation testing and to study UV protection, atopic dermatitis and wound healing.
Under organotypic culture conditions, NHEK stratify into a multilayered, differentiated epidermis, the basis of reconstructed human epidermis models. Combined with dermal fibroblasts such as NHDF-Ad, they also form full-thickness skin equivalents for sensitization and wound healing studies.

Tissue	Skin (epidermis)
Disease	Normal

Applications

Reconstructed human epidermis for in vitro skin irritation testing (OECD TG 439 framework); Validation of reconstructed epidermis models for skin irritation testing; Reconstructed human skin with Langerhans-like cells for skin sensitization; Atopic dermatitis: IL-17-dependent keratinocyte cytokine responses; UVB photoprotection: oxidative stress, DNA damage and inflammasome activation

Synonyms

- NHEK
- HKER-D
- Human keratinocytes
- dermis adult
- provitro 1210512

DONOR

Donor age	Adult
Ethnicity	Lot-specific (see CoA)

CLASSIFICATION

Organism	Human
Sex	Lot-specific
Growth properties	Adherent
Biosafety level	1

CHARACTERISTICS

Morphology	Polygonal (epithelial)
Cell type	Primary keratinocytes
Tumourigenic	No

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HANDLING AND CULTURE

Incubation atmosphere	37°C, 5% CO_2, saturated humidity.
Dissociation reagent	Subculture reagents as recommended on the certificate of analysis

Subculturing

Subculture the cells at about 75 % confluence. Denser cultures undergo irreversible contact inhibition and stop proliferating. Use the subculture reagents recommended on the certificate of analysis.

Seeding density	More than 6,000 cells/cm²
Fluid renewal	24 hours after seeding, then every 48 to 72 hours
Post-thaw recovery	Post-thaw viability ≥ 70 % viable cells. More than 80 % confluence within the expected recovery period.

Thawing and culturing

Thaw the cryovial quickly in a 37 °C water bath, swirling gently. Just before the last ice has melted, move the vial to a laminar flow hood and decontaminate its outer surface.
Transfer the cell suspension into a 15 ml centrifuge tube, add 10 ml of keratinocyte growth medium and centrifuge at 250 × g for 5 minutes.
Remove the supernatant and resuspend the cell pellet in 10 ml of keratinocyte growth medium. Check cell number and viability.
Divide the suspension between two 25 cm² culture flasks (5 ml each). Using two flasks reduces the risk of losing all cells if a passage fails.
Incubate at 37 °C, 5 % CO_2 and saturated humidity, with the screw caps opened by half a turn for gas exchange.
Change the medium after 24 hours to remove unattached cells, then every 48 to 72 hours, using pre-warmed medium only.
Subculture the cells at about 75 % confluence. Denser cultures undergo irreversible contact inhibition and stop proliferating. Use the subculture reagents recommended on the certificate of analysis.
Recommended seeding density: more than 6,000 cells/cm².

QUALITY CONTROL AND CRYOPRESERVATION

Sterility

Mycoplasma contamination is excluded by PCR.
To ensure there is no bacterial, fungal or yeast contamination, cultures are subjected to visual inspection.

Freeze medium	As a cryopreservation medium, we use complete growth medium (including FBS) + 10% DMSO for adequate post-thaw viability, or CM-1 (Cytion catalog number 800100), which includes optimized osmoprotectants and metabolic stabilizers to enhance recovery and reduce cryo-induced stress.

Storage conditions

For long-term preservation, store vials in liquid nitrogen at about −150 to −196 °C. Storage at −80 °C is acceptable for up to one week only.

Shipping conditions

Cryopreserved primary cells are shipped on dry ice. On receipt, check the cryovial for damage and transfer it without delay to appropriate storage.

EXPRESSION AND SUSCEPTIBILITY

Protein expression

Pan-cytokeratin$^+$, vimentin$^-$

IDENTIFIERS

Citation

Human Epidermal Keratinocytes (Cytion catalog number 300692)

NCBI TaxID	9606

LEGAL AND SAFETY

Safety precautions

When planning to store a cryovial in liquid nitrogen for future thawing, it is mandatory to adhere to stringent safety measures. Appropriate protective gloves and clothing are essential, and the use of a face mask or safety goggles is required during the transfer of frozen samples to or from the liquid nitrogen tank. This is to mitigate the risk of injury from potential cryovial explosions upon removal, which can result in the projection of sharp fragments. Primary cells are of human origin, and no known test procedure can ensure the complete absence of infectious agents. Handle all products as potentially infectious.

Warranty

At Cytion, we stand behind the quality and viability of our products.
We guarantee that our cells will be viable at the start of culture for
60 days after shipment from our facilities.
We assure that all materials conform to the specifications described in the
Certificate of Analysis and/or Product Sheet.
For full details, please refer to our
Warranty Statement.

Material Transfer Agreement required	Yes

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