

## Instruction Manual

Product	Size	Catalog Number
Normal Human Epidermal Keratinocytes (NHEK) juvenile foreskin, single donor	500,000 cryopreserved cells	C-12001
	500,000 proliferating cells	C-12002
Normal Human Epidermal Keratinocytes (NHEK) juvenile foreskin, pooled	500,000 cryopreserved cells	C-12005
	500,000 proliferating cell	C-12007
Normal Human Epidermal Keratinocytes (NHEK) adult, single donor	500,000 cryopreserved cells	C-12003
	500,000 proliferating cells	C-12004
Normal Human Epidermal Keratinocytes (NHEK) adult, pooled	500,000 cryopreserved cells	C-12006
	500,000 proliferating cells	C-12008

### Product Description

Epidermal keratinocytes represent the major cell type of the epidermis, making up about 90% of the cells. They originate in the *stratum basale* and undergo gradual differentiation including profound morphological changes during their shift to the *stratum corneum*. In the *stratum corneum*, the final barrier-layer of the skin, keratinocytes are found as nucleus-free, flat, and highly keratinized squamous cells. PromoCell offers a range of Normal Human Epidermal Keratinocytes (NHEK) from single donors or pooled donors produced at PromoCell's cell culture facility. The cells are isolated from juvenile foreskin or from adult normal human tissue from different locations, e.g. face, breast, abdomen, and thighs.

Shortly after isolation, all PromoCell Normal Human Epidermal Keratinocytes are cryopreserved at passage 2 (P2) using PromoCell's proprietary, serum-free freezing medium, Cryo-SFM. Each cryo vial contains more than 500,000 viable cells after thawing.

Proliferating cell cultures are made from 500,000 cryopreserved cells that have been thawed and cultured for three days at PromoCell.

### Quality Control

Rigid quality control tests are performed for each lot of PromoCell Normal Human Epidermal Keratinocytes.

They are tested for cell morphology, adherence rate, and cell viability. Furthermore, immunohistochemical tests for the cell-type specific marker cytokeratin are carried out for each lot (see page 5). Growth performance is tested through multiple passages up to 15 population doublings (PD) under culture conditions without antibiotics and antimycotics.

In addition, all cells have been tested for the absence of HIV-1, HIV-2, HBV, HCV, HTLV-1, HTLV-2 and microbial contaminants (fungi, bacteria, and mycoplasma). A detailed certificate of analysis (CoA) for each lot can be downloaded at: [www.promocell.com/coa](http://www.promocell.com/coa)

### Intended Use

PromoCell Normal Human Epidermal Keratinocytes are for *in vitro* research use only and not for diagnostic or therapeutic procedures.

### Warning

Although tested negative for HIV-1, HIV-2, HBV, HCV, HTLV-1 and HTLV-2, the cells – like all products of human origin – should be handled as potentially infectious. No test procedure can completely guarantee the absence of infectious agents.

### Follow appropriate safety precautions!

*After delivery, start immediately with the protocol for cryopreserved cells (see page 2) or the protocol for proliferating cells (see page 3).*

*Start immediately after delivery.  
Use aseptic techniques and a laminar flow bench.*

### Protocol for Cryopreserved Cells

**Straight after arrival, store the cryopreserved cells in liquid nitrogen, or seed them immediately.**

**Note:** Storage at  $-80^{\circ}\text{C}$  is not sufficient for cell preservation and causes irreversible cell damage.

#### 1. Prepare the medium

Calculate the needed culture surface area according to the plating density (see page 5) and the lot-specific cell numbers stated on the certificate of analysis. Fill the appropriate volume of PromoCell Growth Medium (at least 9 ml per vial of cells) in cell culture vessels. Place the vessels in an incubator ( $37^{\circ}\text{C}$ , 5%  $\text{CO}_2$ ) for 30 minutes.



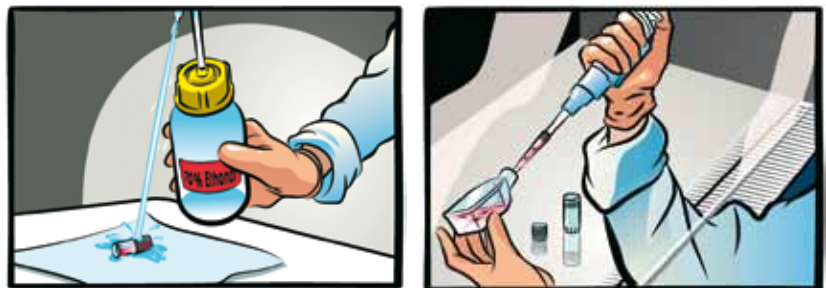
#### 2. Thaw the cells

Remove the cryovial from the liquid nitrogen container and immediately place it on dry ice - even for short transportation. Under a laminar flow bench, briefly twist the cap a quarter turn to relieve pressure, then re-tighten. Immerse the vial into a water bath ( $37^{\circ}\text{C}$ ) just up to the screw cap for 2 minutes. Ensure that no water enters the thread of the screw cap.



#### 3. Disinfect the vial and seed the cells

Thoroughly rinse the cryovial with 70% ethanol under a laminar flow bench. Then, aspirate the excess ethanol from the thread area of the screw cap. Open the vial and transfer the cells to a cell culture vessel containing the prewarmed medium from step 1.



#### 4. Incubate the cells

Place the vessel in an incubator ( $37^{\circ}\text{C}$ , 5%  $\text{CO}_2$ ) for cell attachment. Replace the medium after 16 – 24 hours. The cells should be subcultured, according to the subcultivation protocol (see page 4), once they have reached 70 – 90% confluency.



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## Protocol for Proliferating Cells

### 1. Incubate the cells

Unpack the culture vessel, do not open the lid, and immediately place it in an incubator (37°C, 5% CO<sub>2</sub>) for 3 hours to allow the cells to recover from the transportation.



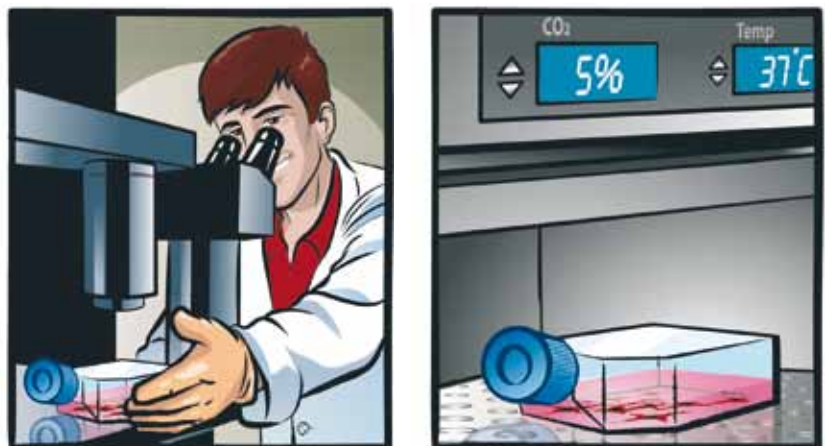
### 2. Replace the transport medium

Carefully open the vessel, rinse the inner side of the lid with 70% ethanol, and let air dry. Aspirate the transport medium from the vessel. Add 10 ml of the appropriate PromoCell Cell Growth Medium.



### 3. Check and incubate the cells

Check the cell density. Open the lid half a turn and place the vessel in an incubator (37°C, 5% CO<sub>2</sub>). The cells should be subcultured according to the subcultivation protocol (see page 4) once they have reached 70–90% confluency.





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## Subcultivation Protocol

### 1. Prepare the reagents and wash the cells

Place the PromoCell DetachKit at room temperature for at least 30 minutes to adjust the temperature of the reagents. Carefully aspirate the medium from the culture vessel. Add 100  $\mu$ l Hepes BSS Solution per  $\text{cm}^2$  of vessel surface to wash the cells and agitate the vessel carefully for 15 seconds.



### 2. Detach the cells

Carefully aspirate the Hepes BSS from the culture vessel. Add 100  $\mu$ l Trypsin/EDTA Solution per  $\text{cm}^2$  of vessel surface. Note: We recommend detaching the cells at room temperature. Close the vessel and examine the cells under a microscope. When the cells start to detach, gently tap the side of the vessel to loosen the remaining cells.



### 3. Neutralize the trypsin and harvest the cells

Add 100  $\mu$ l Trypsin Neutralization Solution per  $\text{cm}^2$  of vessel surface and gently agitate. Carefully aspirate the cell suspension and transfer it to a centrifugation tube. Spin down the cells for 3 minutes at 220 x g.



### 4. Incubate the cells

Discard the supernatant (step 1), add 1 ml of the appropriate PromoCell Cell Growth Medium (step 2), and resuspend the cells by carefully pipetting up and down. Plate the cells according to the recommended seeding density in new cell culture vessels containing PromoCell Cell Growth Medium pre-warmed to 37°C. Place the vessels in an incubator (37°C, 5%  $\text{CO}_2$ ).



## Specifications

Product	Recommended Culture Media*	Plating Density	Passage after Thawing	Marker	Population Doublings
Normal Human Epidermal Keratinocytes (NHEK), juvenile foreskin, single donor	C-20011	5,000 cells per cm <sup>2</sup>	P2	Cytokeratin <sup>+</sup>	> 15
Normal Human Epidermal Keratinocytes (NHEK), juvenile foreskin, pooled	C-20011	5,000 cells per cm <sup>2</sup>	P2	Cytokeratin <sup>+</sup>	> 15
Normal Human Epidermal Keratinocytes (NHEK), adult, single donor	C-20011	5,000 cells per cm <sup>2</sup>	P2	Cytokeratin <sup>+</sup>	> 15
Normal Human Epidermal Keratinocytes (NHEK), adult, pooled	C-20011	5,000 cells per cm <sup>2</sup>	P2	Cytokeratin <sup>+</sup>	> 15

## Related Products

Product	Size	Catalog Number
Keratinocyte Growth Medium 2 (Ready-to-use)	500 ml	C-20011
Keratinocyte Growth Medium 2 Kit	500 ml	C-20111
Keratinocyte Basal Medium 2	500 ml	C-20211
Keratinocyte Basal Medium 2, phenol red-free	500 ml	C-20216
Keratinocyte Growth Medium 2 SupplementMix	for 500 ml	C-39016
Keratinocyte Growth Medium 2 SupplementPack	for 500 ml	C-39011
DetachKit	30 ml 125 ml 250 ml	C-41200 C-41210 C-41220
Cryo-SFM	30 ml 125 ml	C-29910 C-29912
NHEK.f single donor Pellet	1 million cells per pellet	C-14001
NHEK adult, single donor Pellet	1 million cells per pellet	C-14002
NHEK.f pooled Pellet	1 million cells per pellet	C-14003
NHEK adult pooled Pellet	1 million cells per pellet	C-14004

\*The catalog numbers in this table are for media in ready-to-use packaging.

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