# Keratinocytes

PromoCell®

## Instruction Manual

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

### 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, cryopreserved cells should be stored in liquid nitrogen or seeded directly (see page 2). Proliferating cells have to be processed immediately (see page 3).*

*Use aseptic techniques and a laminar flow bench.*

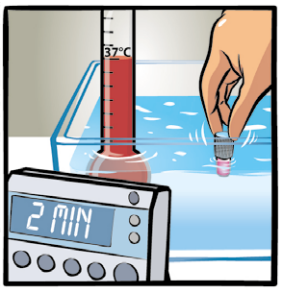
## Protocol for Cryopreserved Cells

**Straight after arrival, store the cryopreserved cells in liquid nitrogen, or seed them immediately.**
**Note:** Storage at -80°C is not sufficient for cell preservation and causes irreversible cell damage.

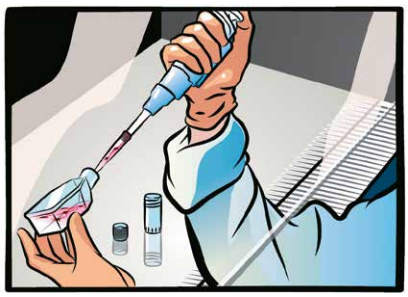
1. **Prepare the medium**
   Calculate the needed culture surface area according to the plating density (see page 5) and the lot-specific cell numbers stated on the certificate of analysis. Fill the appropriate volume of PromoCell Growth Medium (at least 9 ml per vial of cells) in cell culture vessels. Place the vessels in an incubator (37°C, 5% $CO_2$) for 30 minutes.



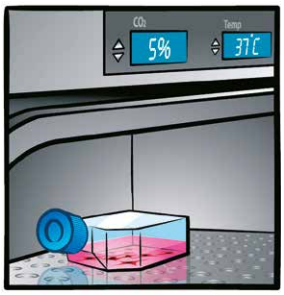
2. **Thaw the cells**
   Remove the cryovial from the liquid nitrogen container and immediately place it on dry ice – even for short transportation. Under a laminar flow bench, briefly twist the cap a quarter turn to relieve pressure, then re-tighten. Immerse the vial into a water bath (37°C) just up 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°C, 5% $CO_2$) for cell attachment. Replace the medium after 16–24 hours and every two to three days thereafter. The cells should be subcultured, according to the subcultivation protocol (see page 4), once they have reached 70–90% confluency.




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

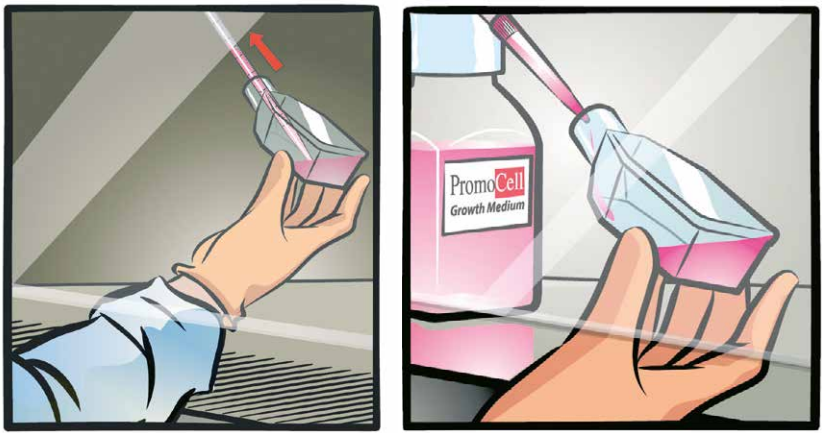
## Protocol for Proliferating Cells

**1. Incubate the cells**

Unpack the culture vessel, do not open the cap, and immediately place it in an incubator (37°C, 5% $CO_2$) for 3 hours to allow the cells to recover from the transportation.

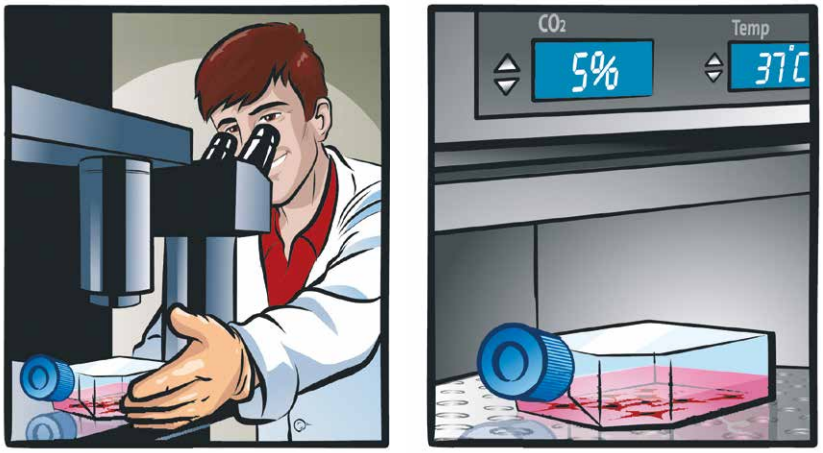

**2. Replace the transport medium**

Carefully open the vessel, rinse the inner side of the cap 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 cap half a turn and place the vessel in an incubator (37°C, 5% $CO_2$). Change the medium every two or three days. The cells should be subcultured, according to the subcultivation protocol (see page 4), once they have reached >70% confluency.

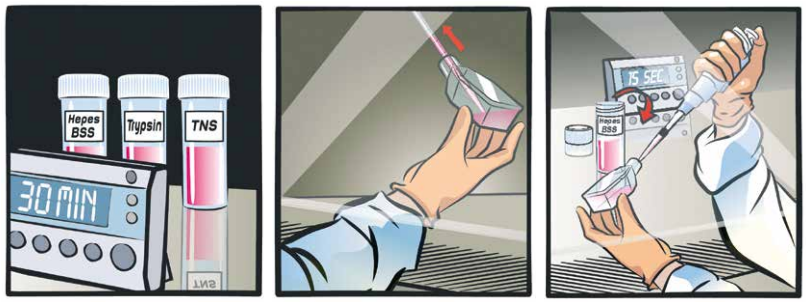


*Use aseptic techniques and a laminar flow bench.*

## Subcultivation Protocol

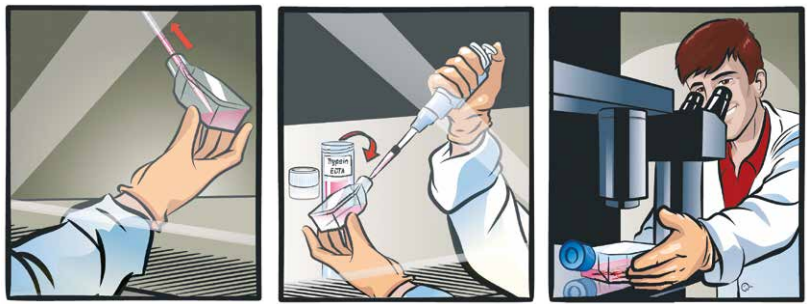
**1. Prepare the reagents and wash the cells**

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 µl Hepes BSS Solution per cm² of vessel surface to wash the cells and agitate the vessel carefully for 15 seconds.



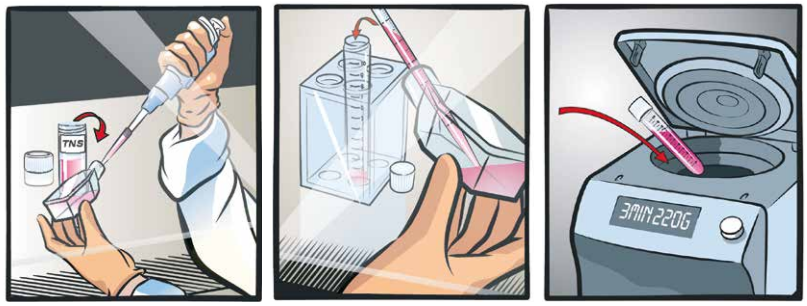

**2. Detach the cells**

Carefully aspirate the Hepes BSS from the culture vessel. Add 100 µl Trypsin/EDTA Solution per cm² 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.



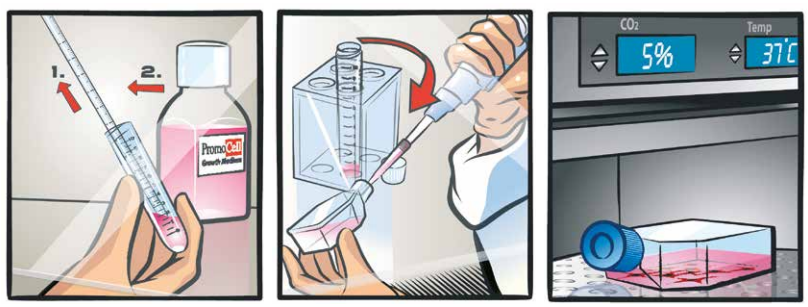
**3. Neutralize the trypsin and harvest the cells**

Add 100 µl Trypsin Neutralization Solution per cm² 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 prewarmed PromoCell Growth Medium. Place the vessels in an incubator (37°C, 5% $CO_2$) and change the media every two or three days.



## 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 cm² | P2 | Cytokeratin⁺ | > 15 |
| Normal Human Epidermal Keratinocytes (NHEK), juvenile foreskin, pooled | C-20011 | 5,000 cells per cm² | P2 | Cytokeratin⁺ | > 15 |
| Normal Human Epidermal Keratinocytes (NHEK), adult, single donor | C-20011 | 5,000 cells per cm² | P2 | Cytokeratin⁺ | > 15 |
| Normal Human Epidermal Keratinocytes (NHEK), adult, pooled | C-20011 | 5,000 cells per cm² | P2 | Cytokeratin⁺ | > 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<br>125 ml<br>250 ml | C-41200<br>C-41210<br>C-41220 |
| Cryo-SFM | 30 ml<br>125 ml | C-29910<br>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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