Keratinocytes isolated in Keratinocyte Growth Medium 3

PromoCell®

Instruction Manual

Product	Size	Catalog Number
Normal Human Epidermal Keratinocytes (NHEK) GM3 juvenile foreskin, single donor	500,000 cryopreserved cells 500,000 proliferating cells	C-12011 C-12012
Normal Human Epidermal Keratinocytes (NHEK) GM3 juvenile foreskin, pooled	500,000 cryopreserved cells 500,000 proliferating cells	C-12015 C-12017
Normal Human Epidermal Keratinocytes (NHEK) GM3 adult, single donor	500,000 cryopreserved cells 500,000 proliferating cells	C-12013 C-12014
Normal Human Epidermal Keratinocytes (NHEK) GM3 adult, pooled	500,000 cryopreserved cells 500,000 proliferating cells	C-12016 C-12018

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. We offer a range of Normal Human Epidermal Keratinocytes (NHEK) from single donors or pooled donors produced at our 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.

NHEK GM3 are isolated in our optimized BPE- and serum-free Keratinocyte Growth Medium 3. This animal-extract free environment favours the growth of a rapidly proliferating cell population with a homogenous cobble stone morphology. Shortly after isolation, all Normal Human Epidermal Keratinocytes are cryopreserved using our our proprietary,defined, animal-component free, and protein-free cryopreservation medium, CryoSFM. Thawing and seeding results in passage 2. Each cryovial 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 in our hands and shipped as growing cultures.

Quality Control

We perform rigid quality control tests for each lot of Normal Human Epidermal Keratinocytes. The cells are tested for cell morphology, adherence rate, and cell viability. Furthermore, each lot is characterized by flow cytometric analysis for the cell-type specific marker cytokeratin (see page 5). Growth performance is tested through multiple passages up to 15 population doublings (PD) under culture conditions without antibiotics or 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 must be processed immediately (see page 3).

Protocol for Cryopreserved Cells

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

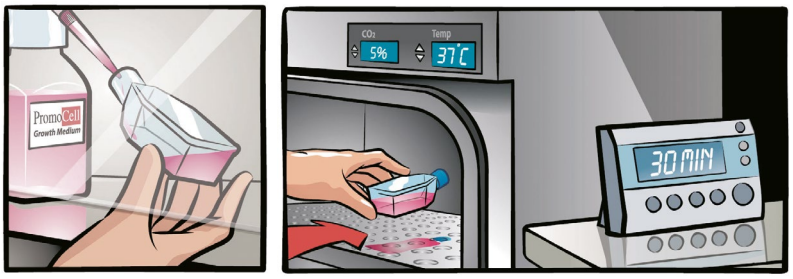
Note: Storage at -80°C is not sufficient for cell preservation and causes irreversible cell damage.

Use aseptic techniques and a laminar flow bench.

1

Prepare the medium and the culture vessel

Calculate the required culture surface area according to the recommended plating density of 5.000 cells per cm^2 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

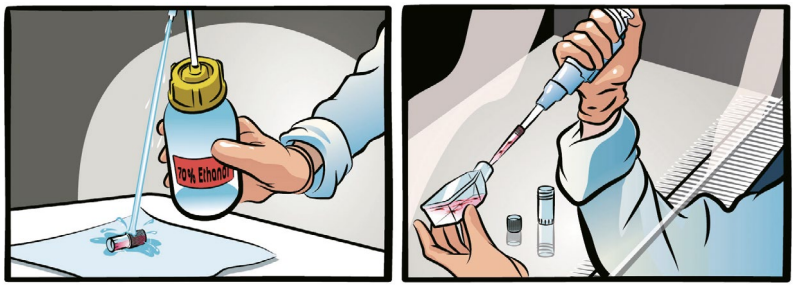
Thaw the cells

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 retighten. Immerse the vial in a water bath (37°C) up to the height of 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 ≥50% confluence and avoid a confluence >80% during subsequent cell cultivation.

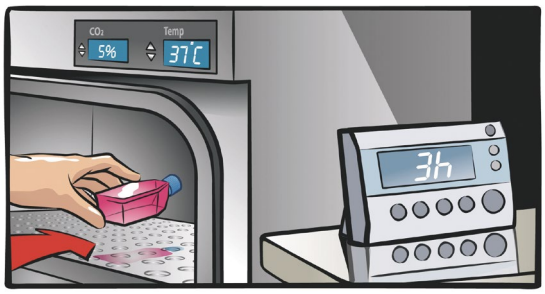
Protocol for Proliferating Cells

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

1

Incubate the cells

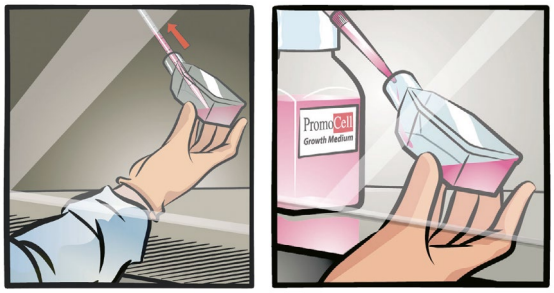
Unpack the culture vessel, do not open the cap, and immediately place it in an incubator (37°C, 5% CO_2) for 3 hours to allow the cells to recover from transportation.



2

Replace the transport medium

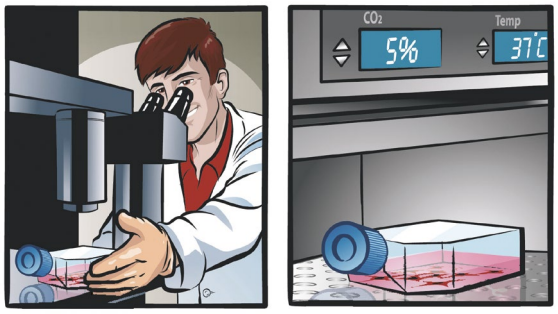
Carefully open the vessel, rinse the inner side of the cap with 70% ethanol, and leave to dry. Aspirate the transport medium from the vessel. Add 10 ml of the appropriate PromoCell Cell Growth Medium.



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.



Subcultivation Protocol

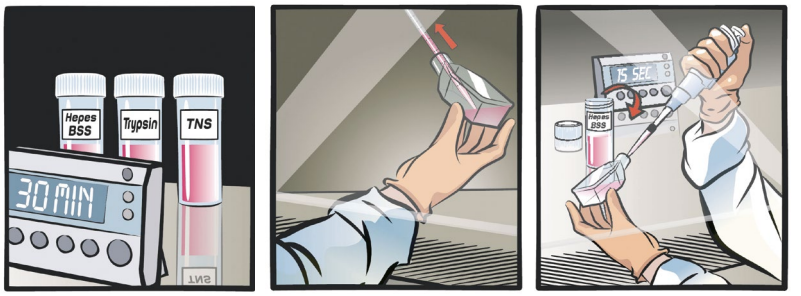
We strongly recommend avoiding contact inhibition of NHEK cultured in Keratinocyte Growth Medium 3. To achieve maximum proliferative potential, subcultivate the cells when reaching ≥50% confluence and avoid a confluence >80% during subsequent cell cultivation. Rapidly proliferating cells can be subcultured with time intervals – for example thaw the cells on Thursday, first split after 4 days on Monday, second split after 4 days on Friday and 3rd split after 5 days on Wednesday.

Use aseptic techniques and a laminar flow bench.

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.

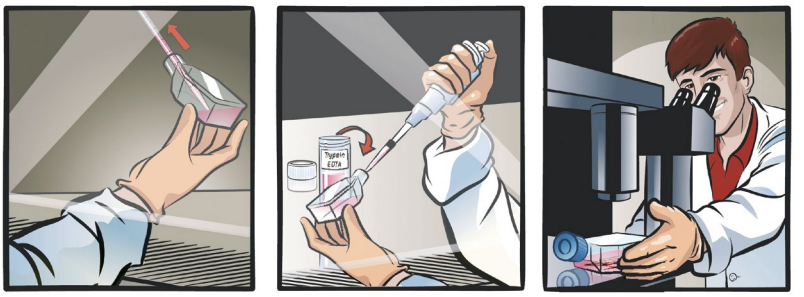


2

Detach the cells

Carefully aspirate the Hepes BSS from the culture vessel. Add 100 µl Trypsin/EDTA Solution per cm² of vessel surface. Incubate the cells for 4 minutes at 37°C in an incubator. Examine the cells under a microscope. When the cells start to detach, gently tap the side of the vessel to loosen the remaining cells.

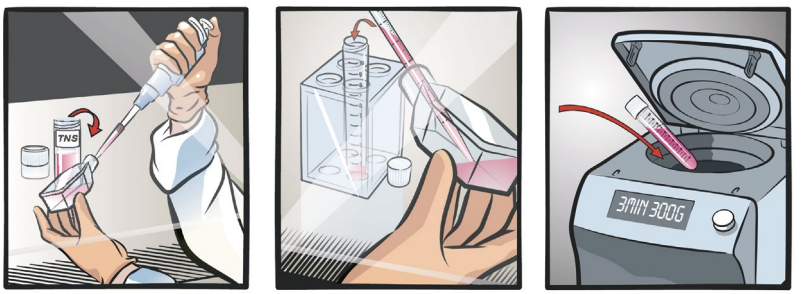
Note: Do not overtrypsinize the cells (≥6 minutes) because this will damage them and result in a reduced growth rate.



3

Neutralize the trypsin and harvest the cells

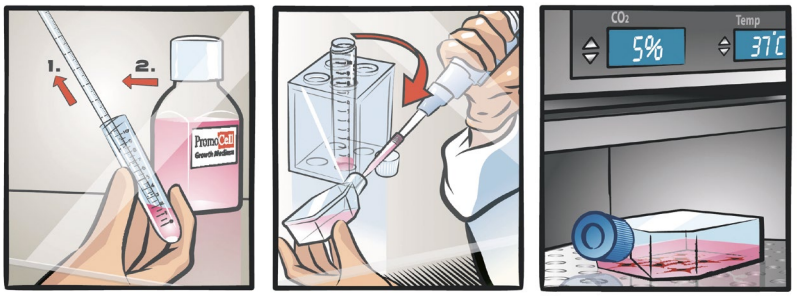
Add 100 µl Trypsin Neutralization Solution per cm² of vessel surface and gently agitate. Resuspend the cells and pipet the cell suspension in a centrifuge tube with appropriate volume of Keratinocyte Growth Medium 3. Rinse the culture vessel with Hepes BSS and pool the cell suspension into the centrifuge tube. Place the culture vessel under a microscope to check if all the keratinocytes are harvested. Remaining cells should be <5 %. Spin down the cells for 3 minutes at 300 x g.



4

Incubate the cells

Discard the supernatant, add 1 ml of the appropriate Keratinocyte Growth Medium 3, and resuspend the cells by carefully pipetting up and down. Count the cells by your method. Plate the cells according to the recommended seeding density of 5.000 cells per cm^2 in new cell culture vessels containing prewarmed Keratinocyte Growth Medium 3. 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	Marker
Normal Human Epidermal Keratinocytes (NHEK), GM3 juvenile foreskin, single donor	C-20021	5,000 cells per cm^2	P2	Cytokeratin$^+$	> 15
Normal Human Epidermal Keratinocytes (NHEK), GM3 juvenile foreskin, pooled	C-20021	5,000 cells per cm^2	P2	Cytokeratin$^+$	> 15
Normal Human Epidermal Keratinocytes (NHEK), GM3 adult, single donor	C-20021	5,000 cells per cm^2	P2	Cytokeratin$^+$	> 15
Normal Human Epidermal Keratinocytes (NHEK), GM3 adult, pooled	C-20021	5,000 cells per cm^2	P2	Cytokeratin$^+$	> 15

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

Related Products

Product	Size	Catalog Num-
Keratinocyte Growth Medium 3 (Ready-to-use)	500 ml	C-20021
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

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