Melanocytes

PromoCell®

Instruction manual

Product	Size	Catalog number
Normal Human Epidermal Melanocytes (NHEM) juvenile foreskin	500,000 cryopreserved cells 500,000 proliferating cells	C-12400 C-12450
Normal Human Epidermal Melanocytes (NHEM) juvenile foreskin cultured in M3 Medium	500,000 cryopreserved cells 500,000 proliferating cells	C-12422 C-12472
Normal Human Epidermal Melanocytes (NHEM) adult donor, cultured in M3 Medium	500,000 cryopreserved cells 500,000 proliferating cells	C-12413 C-12463

Product description

Epidermal melanocytes represent 5–10% of the cells in the epidermis. Located in the stratum basale with extensions to the suprabasal layers, they are specialized in the production of melanin. This protein is responsible for the pigmentation of the skin, hair and eyes and exerts a protective effect on adjacent cells by shielding them from the harmful effects of UV radiation.

We offer a range of Normal Human Epidermal Melanocytes (NHEM) produced at our cell culture facility from donors with lightly, moderately and darkly pigmented skin (detailed pigmentation-related donor-information available on request).

The cells are isolated from juvenile foreskin or adult skin from different locations, e.g. face, breast, abdomen and thighs, using either the serum free, PMA (Phorbol Myristate Acetate) containing Melanocyte Growth Medium or optimized serum-free, BPE-free and PMA-free Melanocyte Growth Medium M3. Since PMA is a tumor-promoting mitogen, which can interfere with experimental approaches, we recommend using Melanocytes isolated and cultured in Melanocyte Growth Medium M3. Shortly after isolation, all our Normal Human Epidermal Melanocytes are cryopreserved using our proprietary, defined, animal-component free, and protein-free cryopreservation medium, Cryo-SFM. Each cryovial contains more than 500,000 viable cells after thawing. Thawing and seeding results in passage 2.

Proliferating cell cultures are made from 500,000 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 Melanocytes. The cells are tested for cell morphology, adherence rate and cell viability. Furthermore, immunohistochemical tests for the cell-type specific marker Mel-5 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 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

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

Cell culture

Since our Melanocyte Growth Medium M3 enables serum-, BPE- and PMA-free cultivation of NHEM without any cell culture plastic coating, we point out that the cells in this medium require special culture conditions. To maintain the cells in a robust adherent pro-proliferative phase, we recommend passage of the cells at 70–90% confluency. According to the literature, it is known that high cell densities of NHEM can promote 3D spheroid growth.[1] Therefore, hyperconfluency should be avoided if possible.

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.

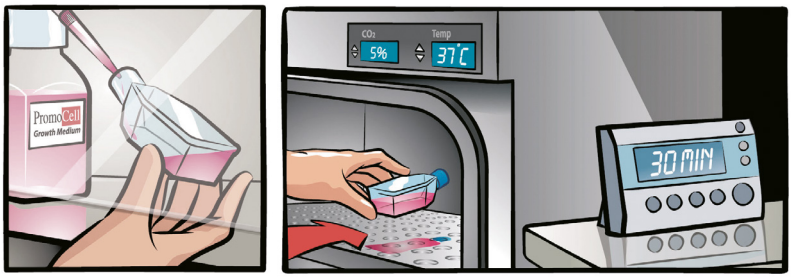
Note: Storage at -80°C is not sufficient for cell preservation and causes irreversible cell damage.

Use aseptic techniques and a laminar flow bench.

1

Prepare the medium

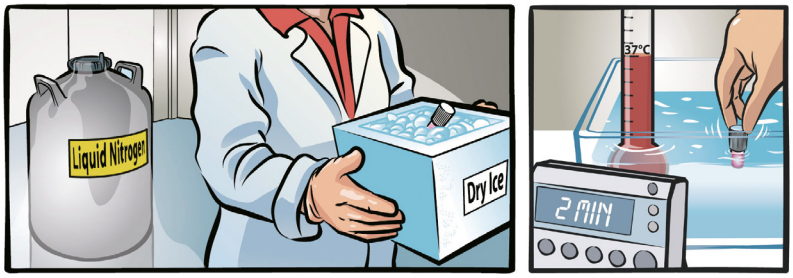
Calculate the required culture surface area according to the plating density (see page 5) and the lot-specific cell numbers stated on the certificate of analysis. Fill the appropriate volume of PromoCell Growth Medium (at least 9 ml per vial of cells) in cell culture vessels. Place the vessels in an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



2

Thaw the cells

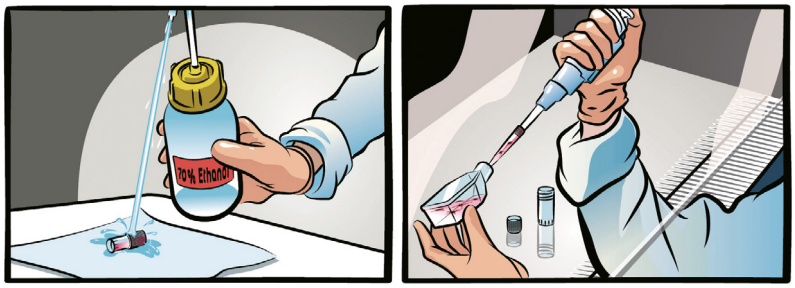
Remove the cryovial from the liquid nitrogen container and immediately place it on dry ice – even for short transportation. Under a laminar flow bench, briefly twist the cap a quarter turn to relieve pressure, then retighten. Immerse the vial in a water bath (37°C) up to the height of the screw cap for 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 pre-warmed 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. We strongly recommend to avoid 100% confluency to keep the cells in a adherent pro-proliferative phase.

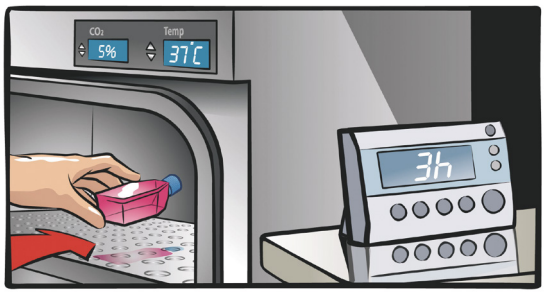
Protocol for proliferating cells

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

1

Incubate the cells

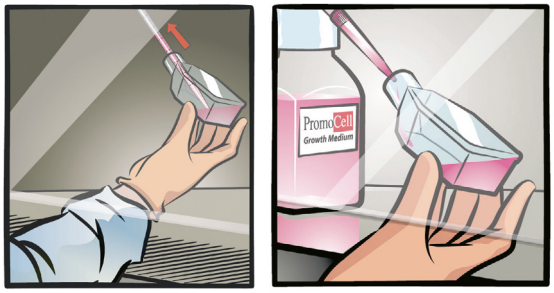
Unpack the culture vessel, do not open the cap, and immediately place it in an incubator (37°C, 5% CO_2) for 3 hours to allow the cells to recover from transportation.



2

Replace the transport medium

Carefully open the vessel, rinse the inner side of the cap with 70% ethanol, and 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). The cells should be subcultured according to the subcultivation protocol (see page 4) once they have reached 70–90% confluency. We strongly recommend to avoid 100% confluency to keep the cells in a adherent pro-proliferative phase.

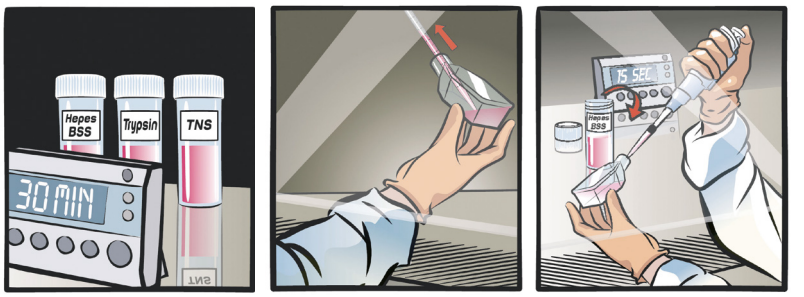
Subcultivation protocol

Use aseptic techniques and a laminar flow bench.

1

Prepare the reagents and wash the cells

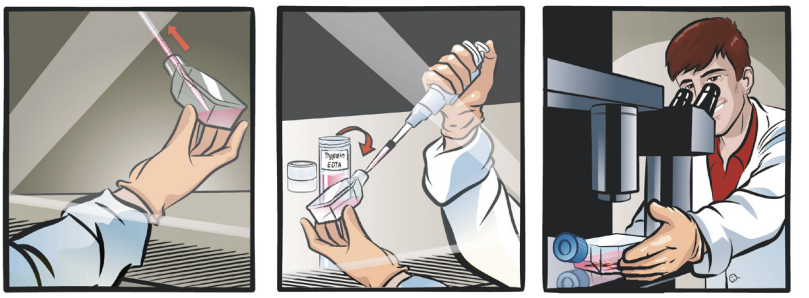
Place the PromoCell DetachKit at room temperature for at least 30 minutes to adjust the temperature of the reagents. Carefully aspirate the medium from the culture vessel. Add 100 µl Hepes BSS Solution per cm^2 of vessel surface to wash the cells and agitate the vessel carefully for 15 seconds.



2

Detach the cells

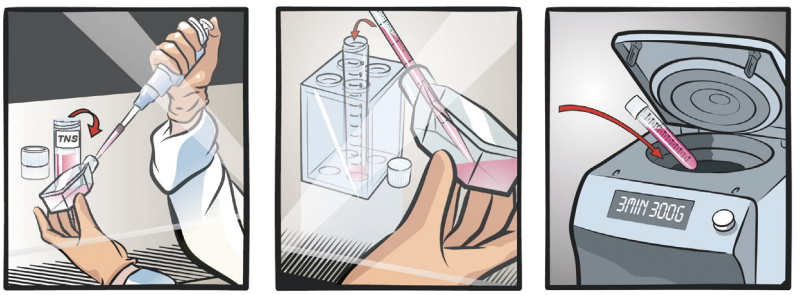
Carefully aspirate the Hepes BSS from the culture vessel. Add 100 µl Trypsin/EDTA Solution per cm^2 of vessel surface. Note: We recommend detaching the cells at room temperature. Close the vessel and examine the cells under a microscope. When the cells start to detach, gently tap the side of the vessel to loosen the remaining cells. Melanocytes are sensitive to longer trypzinization times. Avoid Trypsin/ EDTA incubation time longer than 5 minutes.



3

Neutralize the trypsin and harvest the cells

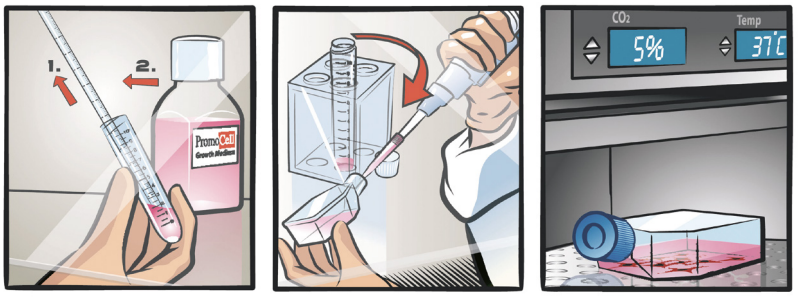
Add 100 µl Trypsin Neutralization Solution per cm^2 of vessel surface and gently agitate. Carefully aspirate the cell suspension and transfer it to a centrifugation tube. Spin down the cells for 3 minutes at 300 x g.



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% CO_2).



Specifications

Product	Recommended culture media*	Plating density	Passage after thawing	Marker	Population doublings
Normal Human Epidermal Melanocytes (NHEM), juvenile foreskin	C-24010	5,000 – 10,000 cells per cm^2	P2	Mel-5^+	> 15
Normal Human Epidermal Melanocytes (NHEM), juvenile foreskin, cultured in M3 medium	C-24310	5,000 – 10,000 cells per cm^2	P2	Mel-5^+	> 15
Normal Human Epidermal Melanocytes (NHEM), adult donor, cultured in M3 medium	C-24310	5,000 – 10,000 cells per cm^2	P2	Mel-5^+	> 15

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

Related products

Product	Size	Catalog number
Melanocyte Growth Medium (Ready-to-use)	500 ml	C-24010
Melanocyte Growth Medium Kit	500 ml	C-24110
Melanocyte Basal Medium	500 ml	C-24210
Melanocyte Basal Medium, phenol red-free	500 ml	C-24215
Melanocyte Growth Medium SupplementMix	for 500 ml	C-39415
Melanocyte Growth Medium SupplementPack	for 500 ml	C-39410
Melanocyte Growth Medium M3 (ready-to-use)	500 ml	C-24310
DetachKit	30 ml 125 ml 250 ml	C-41200 C-41210 C-41220
Cryo-SFM	30 ml 125 ml	C-29910 C-29912
NHEM.f Pellet	1 million cells per pellet	C-14040
NHEM.f M3 Pellet	1 million cells per pellet	C-14044
NHEM M3 adult Pellet	1 million cells per pellet	C-14045

References

1. Lin, S.-J., et al. (2005). "Formation of melanocyte spheroids on chitosan-coated surface." Biomaterials 26(12): 1413–1422

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12/2023