PromoCell®

Long-term cultivation of dendritic and TRP1-positive cells using serum- and BPE-free Melanocyte Growth Medium M3 (prf)

Application note

Our optimized, clinically upgradable medium ensures excellent growth performance for primary and adult skin cells, outperforming competitor formulations.

Background

Melanocytes are a specialized cell type that produces melanin, the pigment responsible for skin, hair, and eye color.[2] They originate from neural crest precursors during embryonic development, migrating to the basal layer of the epidermis. However, melanocytes are also found in hair follicles, the uveal tract of the eye, and the inner ear.[2–4] Melanogenesis, one of the key functions of melanocytes, involves a cascade of enzymatic reactions.

Tyrosinase is the rate-limiting enzyme that catalyzes the initial oxidation of tyrosine to L-DOPA (3,4-dihydroxyphenylalanine) and subsequently to dopaquinone.[2] Additional enzymes involved in melanogenesis include tyrosinase-related protein 1 (TYRP1) and dopachrome tautomerase (DCT).[2,4] The expression of TYRP1 (also known as TRP1) is commonly used as a marker for identifying and characterizing melanocytes.[4]

Melanocytes function as part of the melanin unit, which is the functional unit of skin pigmentation. Each melanin unit consists of a single melanocyte and approximately 30–40 surrounding keratinocytes in the epidermis.[2,5] Within these units, melanocytes transfer melanin-containing organelles called melanosomes to neighboring keratinocytes, creating a protective layer of pigment over the nuclei of these cells.[2]

Disruption of melanocyte function or distribution can lead to pigmentation disorders. For example, selective destruction of melanocytes leads to vitiligo, a pigmentation disorder that is challenging to treat.[6] The autoimmune destruction of melanocytes in individuals with vitiligo highlights the complex interplay between melanocytes and the immune system.[6] Albinism is another pigmentation disorder that results from genetic defects in melanin synthesis pathways. This defect leads to reduced or absent pigmentation and increased susceptibility to UV-induced skin damage and skin cancer.[7] Melanocytes are commonly used to study the mechanisms underlying pigmentation disorders and to develop therapies to restore melanogenesis.[8]

Melanocytes are also widely used in melanoma research. The transformation from normal melanocytes to malignant melanoma cells involves complex molecular alterations that affect the cell cycle, apoptosis, and cell invasion.[9] Understanding the differences between normal melanocytes and melanoma cells can help researchers identify potential therapeutic targets for melanoma.

Additionally, understanding the molecular differences between normal melanocytes and melanoma cells offers insights into the early stages of melanoma development and identifies potential biomarkers for early detection.[10]

Melanocyte cultures are also used in the cosmetic industry for dermatological testing of products. Melanocytes are widely used to test formulations that are designed to modulate pigmentation or protect against UV exposure.[11] Regulatory agencies increasingly require *in vitro* testing using human-derived cells before approving new cosmetic and pharmaceutical products, making reliable melanocyte culture systems essential for preclinical studies.[12,13] Emerging applications of melanocytes in regenerative medicine include tissue engineering for the development of cell-based therapies and bioengineered skin grafts that maintain natural pigmentation patterns.[8]

Despite their importance in various research fields, melanocytes can be challenging to culture *in vitro*. They have a slow proliferation rate, which poses practical challenges for experiments that require large cell numbers and creates the need for the inclusion of various growth factors in the cell culture medium.[14]

To address these challenges, we introduce our Melanocyte Growth Medium M3 that provides the following advantages:

- **Serum-free, BPE-free, and PMA-free** formulation that is free of cholera toxin
- **Eliminates the need for ECM coating** and supports adult melanocytes without the requirement for additional growth factors, such as endothelin-3 (ET3)
- **Available in a phenol red-free (prf) variant,** making it suitable for regulatory and GMP environments, thereby supporting research transitioning towards clinical applications
- **Supports at least 15 population doublings** for both juvenile and adult normal human epidermal melanocytes (NHEM)
- **Enables serum-free cell isolation** from juvenile and adult donors
- **Supports stable TRP1 expression** (melanocyte-specific marker) during long-term culture, as confirmed via FACS analysis

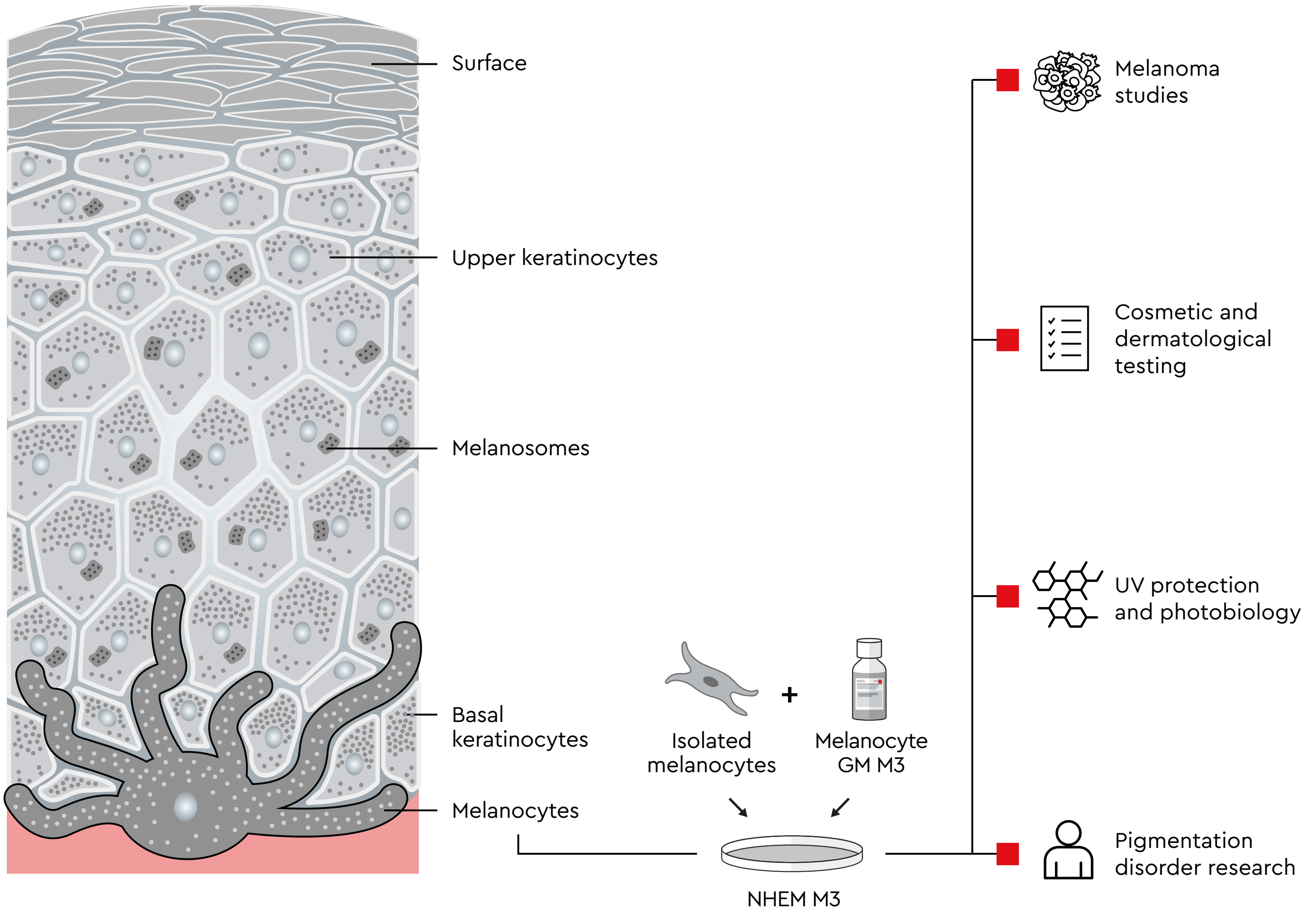

Fig. 1: Melanin distribution across the epidermis: *The dispersion of melanin in the epidermis is mediated through the formation of epidermal-melanin units. The level of melanin is higher in the basal layers, regardless of the skin phototype. In lighter skins (lower phototypes), the level of melanin is low in the basal layer. In darker skins (higher phototypes), the level of melanin is high in the basal layer, and melanin is also present in the immediate suprabasal layers. Adapted from Bento-Lopes et al., 2023.[1] Isolated melanocytes cultured in Melanocyte Growth Medium M3 (C-24310) or Melanocyte Growth Medium M3 (prf) (C-24311) serve as valuable models for diverse scientific applications, including melanoma research and studies on pigmentation disorders.*

Protocol

Protocol for thawing and subcultivation of melanocytes

This protocol describes the thawing and serum- and BPE-free expansion of primary melanocytes from cryopreserved cells. Our qualified NHEM are positive for TRP1 (Mel-5) staining and can be cultured in Melanocyte Growth Medium M3 (with or without phenol red) for at least 15 population doublings. NHEM from juvenile foreskin have a mean population doubling time of ≤50 hours during the first three passages when cultured in Melanocyte Growth Medium M3.

I. Thawing protocol

Materials

- Normal Human Epidermal Melanocytes (NHEM) from juvenile foreskin in M3 Medium C-12422, or Normal Human Epidermal Melanocytes (NHEM) from an adult donor in M3 Medium C-12413
- 70% EtOH
- Tissue culture treated cell culture vessel (e.g., Corning®)
- Centrifuge tube
- Melanocyte Growth Medium M3 (C-24310) or Melanocyte Growth Medium M3 (prf) (C-24311)
- 37°C water bath

1

Prepare Melanocyte Growth Medium M3

Thaw the SupplementMix at 15–25°C. Aseptically mix the supplement solution by carefully pipetting up and down. Then transfer the entire content of the supplement to 500 ml of growth medium.

2

Adjust the medium to room temperature and fill the cell culture vessel with medium

Prewarm only an aliquot of the complete medium and keep the remaining medium refrigerated at 4–8°C. We recommend pre-warming the medium in a cell culture vessel at 37°C and 5% CO_2 to allow the medium to reach physiological pH and temperature in the incubator. Use 180 µl medium per cm^2 of the cell culture vessel surface.

3

Thaw the melanocytes

Prepare a centrifuge vial with Melanocyte Growth Medium M3 under the laminar flow bench. We recommend diluting one cryovial (1 ml) in at least 9 ml of growth medium (room temperature). Remove the cryovial from liquid nitrogen and transport it on dry ice. Under a laminar flow bench, release the vial pressure by briefly twisting the cap counterclockwise by a quarter turn and then retightening it. Allow the cell suspension to thaw in a water bath at 37°C for 2 minutes. Rinse the vial with 70% EtOH and place it under a laminar flow bench. Aspirate the EtOH from the threads of the screw cap. Carefully open the cryovial and transfer the cell suspension to a centrifuge tube containing medium. Mix the cell suspension with the growth medium by slowly pipetting up and down.

Note: Our cryopreserved cells are frozen in Cryo-SFM Plus (C-29920), which contains DMSO. Work quickly to prevent prolonged incubation of the cell suspension in Cryo-SFM Plus. Melanocyte Growth Medium M3 contains no FCS or BPE to protect cryo-sensitive cells. To reduce DMSO concentration, dilute the thawed cell suspension at least 1:10 with growth medium.

4

Count the cells and plate them

Take an aliquot of the cell suspension from the centrifuge tube, count the cells using your standard method, and centrifuge the cell suspension for 2 minutes at 300 x g. Aspirate the supernatant, and resuspend the cells in at least 1 ml of growth medium. Calculate the volume of cell suspension needed to reach a seeding density of 5,000 cells per cm^2. Plate the cells in the prepared vessel container from step 2. From the time of thawing and throughout cell culture, the cells will appear brownish or dark in color (Figure 2).

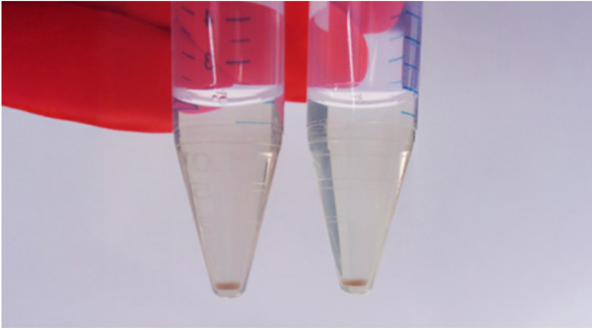

Fig. 2: Brownish or dark cell pellets indicate pigment melanocytes. *NHEM-f M3 are derived from human foreskin and cultured in Melanocyte Growth Medium M3 medium. One cell vial was thawed and seeded at a density of 5,000 cells per cm^2 in two different culture media: Melanocyte Growth Medium M3 and Melanocyte Growth Medium M3 (prf). Cells were subcultured and reseeded at a density of 5,000 cells per cm^2. After reaching >15 population doublings, single cells were generated using Accutase detachment and centrifugation at 300 x g for 2 minutes. The color of cell pellets was documented using digital photography. The brownish color of the cell pellets indicates melanin production in functionally active pigment cells in both media. Donor characteristics: male, 5 years old, Caucasian, light pigmentation.*

II. Culture protocol

Materials

- Melanocyte Growth Medium M3 (C-24310) or Melanocyte Growth Medium M3 (prf) (C-24311)
- Culture vessel (e.g., Easy Grip from Corning)
- Accutase solution (C-41310) or alternative Trypsin/EDTA from DetachKit (C-41210)
- HEPES Buffered Saline Solution (C-40000) or other physiological buffer (e.g., Dulbecco's PBS w/o Ca^{++}/Mg^{++} [C-40232])

1

Let the cells grow

Change the medium every 2–3 days. We recommend making medium changes on Mondays, Wednesdays, and Fridays. Use 180 µl of medium per cm^2 of growth area for each medium change. Melanocytes grown in Melanocytes Growth Medium M3 show typical dendrites (Figure 3).

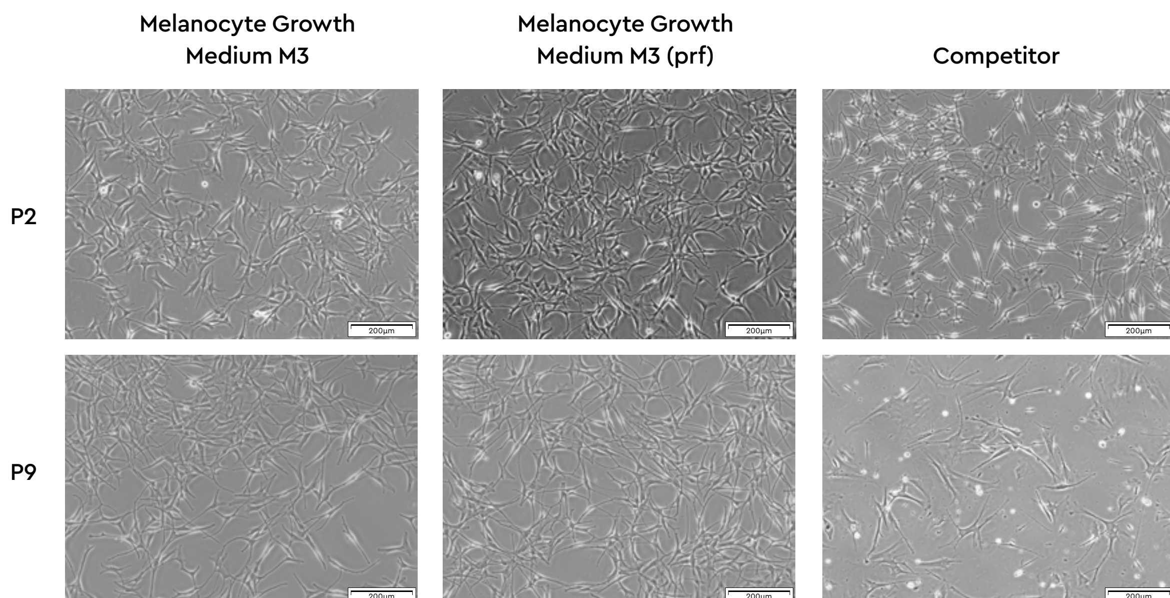

Fig. 3: Cell morphology of NHEM-f M3 in different media. *NHEM-f M3 from the same donor and vial were thawed and seeded at a density of 5,000 cells per cm^2 in three different culture media: Melanocyte Growth Medium M3, Melanocyte Growth Medium M3 (prf), and competitor medium. Cells were passaged when they reached 70–90% confluence and reseeded at a density of 5,000 cells per cm^2. Imaging using transmitted light microscopy shows morphology at passage 2 (P2) and passage 9 (P9). Microscopy 10x magnification, scale bar = 200 µm. Donor characteristics: male, 5 years old, Caucasian, light pigmentation.*

2

Detach the cells when they reach 70–90% confluence

Once the cells have reached 70–90% confluence, they can be passaged. Carefully aspirate the culture medium. Add 150 µl of HEPES BSS (optional Dulbecco's PBS w/o Ca^{++}/Mg^{++}) per cm^2 of the vessel surface to wash the cells and agitate the vessel carefully for 15 seconds. Aspirate the HEPES BSS (or PBS buffer) and cover the cells with 50 µl Accutase solution (or trypsin) per cm^2. Transfer the vessel to an incubator for 4 minutes.

Note: Since our Melanocyte Growth Medium M3 enables serum-, BPE- and PMA-free cultivation of NHEM without the need for coating, the cells in this medium are sensitive to hyperconfluence. To maintain the cells in a robust adherent proliferative phase, we recommend passing the cells at 70–90% confluency. According to the literature, high cell densities of NHEM can promote 3D spheroid growth.[15] Therefore, hyperconfluency should be avoided whenever possible.

3

Harvest the detached cells

Check the round-up process of the cells under a microscope. Tap the side of the culture vessel to enhance the cell dissolution from the plastic.

Resuspend the cells and pipette the cell suspension into a centrifuge tube containing an appropriate volume of growth medium. Rinse the culture vessel with HEPES BSS (or PBS) and pool the cell suspension into the centrifuge tube. Place the culture vessel under a microscope and verify that all cells have been harvested. Centrifuge the cell suspension at 300 x g for 2 minutes. Inhibition of the enzymatic reaction is not required if Accutase solution is used.

Note: If trypsin is used instead of Accutase solution, when cells are detached, add 50 µl of Trypsin Neutralization Solution per cm^2 and gently agitate.

4

Resuspend the cell pellet

Discard the supernatant and resuspend the cell pellet carefully by pipetting up and down in an appropriate volume of Melanocyte Growth Medium M3.

5

Count the cells using your standard method

Our PromoCell qualified NHEM-f M3 reach 15 population doublings in approximately 3 weeks of culture (Figure 4).

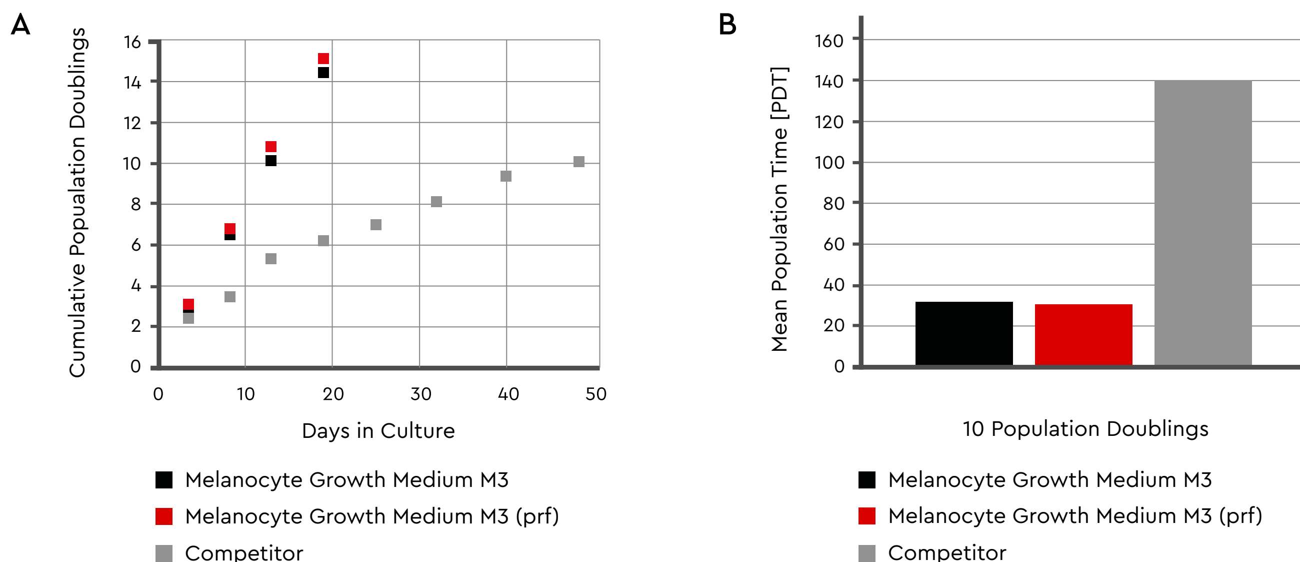

Fig. 4: Growth of NHEM-f M3 in different media. *One vial of NHEM-f M3 was thawed and seeded at a density of 5,000 cells per cm^2 in three different culture media: Melanocyte Growth Medium M3, Melanocyte Growth Medium M3 (prf), and competitor medium. Cells were passaged when they reached 70–90% confluence and then reseeded at a density of 5,000 cells/cm^2. Cell counts at each passage were calculated using MUSE Cell Analyzer® (Cytek). As a starting population, 98,000 cells were used, and population doublings were cumulated for each subcultivation step.* ***A)*** *Plot showing days needed to reach 15 population doublings. Cells cultured in our medium reached 15 population doublings after four passages in less than 20 days, whereas cells in competitor medium were cultured for 48 days to reach 10 population doublings.* ***B)*** *Mean population time after reaching 10 population doublings during simultaneous cultivation. Donor characteristics: male, 5 years old, Caucasian, light pigmentation.*

6

Reseed the cells in a culture vessel

Use a seeding density of 5,000 viable cells per cm².

7

Change medium every 2–3 days

Use Melanocyte Growth Medium M3 for serial expansion of the cells. We recommend using the cells in early passage for experiments.

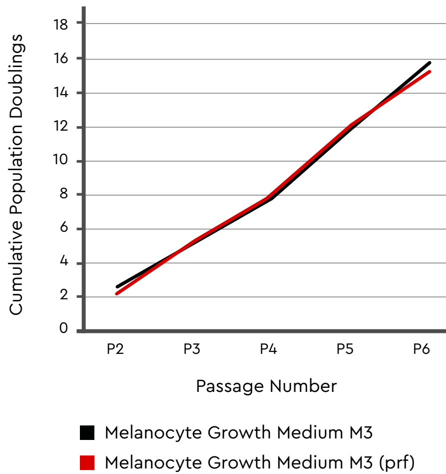

Fig. 5: Adult melanocytes can grow for at least 15 population doublings in serum-free medium with or without phenol red. *One vial of NHEM adult M3 derived from human skin labia was thawed and seeded at a density of 5,000 cells per cm² in two different culture media: Melanocyte Growth Medium M3 and Melanocyte Growth Medium M3 (prf). Cells were passaged when they reached 70–90% confluence and then reseeded at a density of 5,000 cells per cm². Cell counts at each passage were calculated using MUSE Cell Analyzer® (Cytek). As a starting population, 98,000 cells were used, and population doublings were cumulated for each subcultivation step. Donor characteristics: female, 26 years old, Caucasian, light pigmentation.*

8

Characterization of functional melanocytes

Our NHEM are confirmed by flow cytometry to have over 80% positive TRP1 (Mel-5) cells (Figure 6). TRP1 levels correlate with melanin content. NHEM grown in Melanocyte Growth Medium M3 (with or without phenol red) consistently show a TRP1-positive cell population at each passage. Melanin production can also be observed as brownish to dark cell pellets after harvesting and centrifugation of viable single cells (see Figure 2).

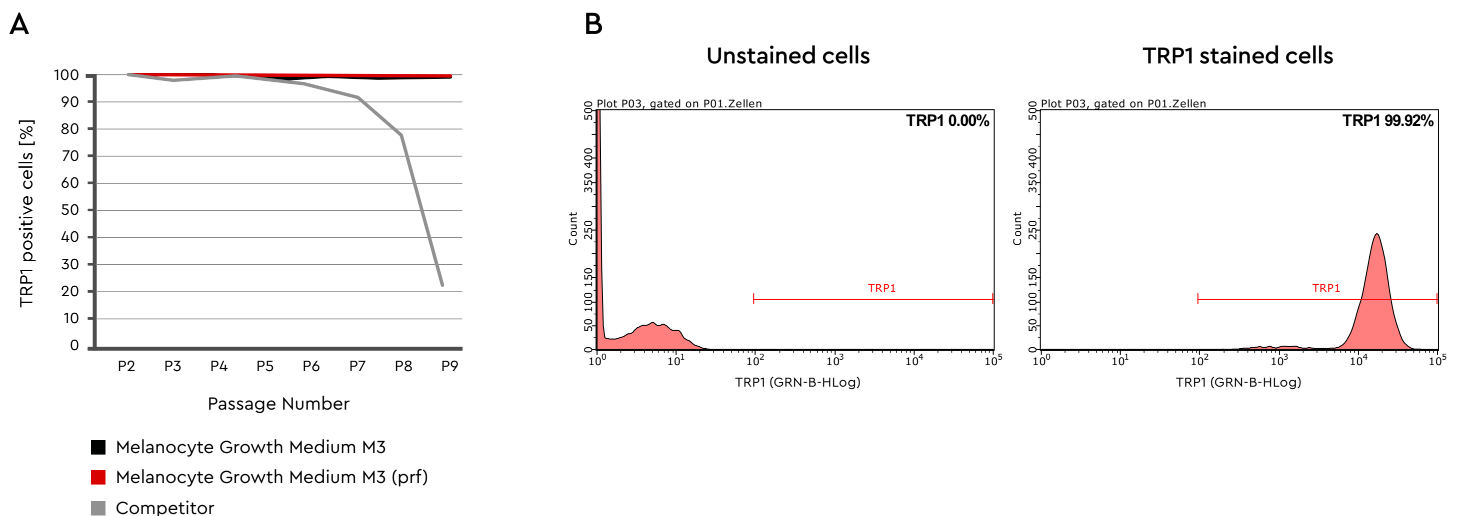

Fig. 6: Identification of melanocyte-specific marker TRP1 in NHEM during long-term culture. *One vial of NHEM-f M3 was thawed and seeded at a density of 5,000 cells per cm² in three different culture media: Melanocyte Growth Medium M3, Melanocyte Growth Medium M3 (prf), and competitor medium. Cells were subcultivated with Accutase detachment, and flow cytometry analysis for TRP1 (Mel-5) was performed using an aliquot of fixed cells (150,000 per sample) at each passage. Unstained fixed cells were used as a TRP1-negative control for gating.* ***A)*** *Percentage of TRP1-positive cells at each passage during long-term culture.* ***B)*** *Representative flow cytometry plot showing TRP1 expression in NHEM-f M3 cultured in Melanocyte Growth Medium M3 (prf) for more than 15 population doublings.* ***Left panel:*** *unstained cells used to gate for TRP1-negative cells.* ***Right panel:*** *anti-TRP1-stained cells can be distinguished from TRP1-negative cells. Donor characteristics: male, 5 years old, Caucasian, light pigmentation.*

Trademark references

MUSE Cell Analyzer® is a registered trademark of Cytek® Biosciences. Corning® is a registered trademark of Corning® Incorporated.

Products

Product	Size	Catalog number
Melanocyte Growth Medium M3 (Ready-to-use) – Includes Basal Medium and SupplementMix	500 ml	C-24310
Melanocyte Growth Medium M3, phenol red-free (Ready-to-use) – Includes Basal Medium and SupplementMix	500 ml	C-24311
Melanocyte Growth Medium M3 SupplementMix	For 500 ml	C-39430
Melanocyte Basal Medium M3	500 ml	C-24410
Normal Human Epidermal Melanocytes (NHEM) juvenile foreskin	≥ 500,000 cells	C-12400
Normal Human Epidermal Melanocytes (NHEM) juvenile foreskin, cultured in Melanocyte Growth Medium M3	≥ 500,000 cells	C-12422
Normal Human Epidermal Melanocytes (NHEM) adult donor, cultured in Melanocyte Growth Medium M3	≥ 500,000 cells	C-12413
Accutase solution, primary human cell culture tested	100 ml	C-41310
Dulbecco's PBS, ready-to-use without Ca2+/Mg2+	500 ml	C-40232
HEPES Buffered Saline Solution	30 ml	C-40000

Additional products

Product	Size	Catalog number
Easy Grip Tissue Culture Dish (60 × 15 mm)	-	353004 (Corning)
Trypsin from DetachKit (contains HEPES-BSS, Trpysin/EDTA, and Trypsin-Neutralization Solution)	3x 125 ml	C-41210

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