Product Description

Normal Human Melanocytes (NHMs) are available from single donors isolated from the epidermis of neonatal foreskin (NHM-CRY-NEO) or adult (breast or abdominal) skin (NHM-CRY-AD) and cryopreserved at passage 3. NHMs exhibit dendritic morphology in monolayer culture (Figure 1), are 3D-certified, and when cultured according to MatTek protocols, should expand for at least 2 passages. Each NHM lot is characterized by positive staining for the melanocyte specific marker, Mel-5 (Figure 2). NHMs are available from Caucasian, Black, or Hispanic donors. NHM cells test negative for mycoplasma, bacteria, yeast and other fungi, and Hepatitis B, Hepatitis C, and HIV-1.

Intended Use

When used with NHM-GM growth medium, NHM cells provide an ideal cell-culture system to study melanogenesis, UV effects, cellular differentiation, and cosmetic efficacy and toxicology. NHMs require the use of the optimized medium (Cat#: NHM-GM) for optimal expansion in monolayer culture.

This product is for research use only. It is not intended for human or animal therapeutic or diagnostic use.

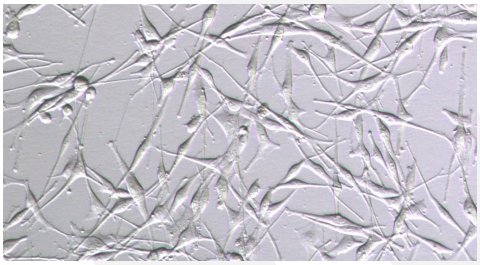

Figure 1. Phase contrast microscopy of NHMs in monolayer culture, passage 3, 100x.

Format

NHMs are provided cryopreserved in 1 mL of cryopreservation medium. Each vial contains ≥500K viable cells. Upon thawing, the cells are guaranteed to be ≥75% viable (as determined by trypan blue exclusion) and are sufficient to seed a 90 cm2 culture surface when handled according to the protocol below.

Storage & Handling

Upon receipt, transfer the cryovial(s) immediately into liquid nitrogen and store in vapor phase. Cells will be stable for more than two years if stored in the vapor phase of liquid nitrogen. See Table 1 for reagent storage conditions.

Directions for Use:

Prepare NHM-Growth Medium (NHM-GM)

1. Thaw growth supplement (part# NHM-GS) at room temperature for 30 min or until the supplement is completely thawed; make sure that all components are in solution and that no precipitate is visible.
2. Transfer the entire contents of the vial to 500 ml of basal medium (part# NHM-BM) using sterile technique in a laminar flow hood. NHM-BM supplemented with NHM-GS is referred to as NHM-GM.
3. Cap the bottle of NHM-GM and swirl to mix. After supplementing, the NHM-GM is stable for 1 month if stored at 4°C in the dark.

Thawing and Seeding of Cryopreserved Cells

1. The recommended seeding density for NHMs is 4,200 cells/cm^2.
2. Aseptically vent any nitrogen from cryopreserved vials in a biosafety cabinet prior to thawing the vials in the water bath. Remove the cryovial from storage and quickly thaw the cryovial in a 37°C water bath being careful not to submerge the entire vial. Watch the cryovial closely; when the last sliver of ice melts, remove it, and wipe with 70% ethanol before opening.
3. Transfer cells from the cryovial into a 15 mL conical tube containing 10 mL of NHM-GM medium warmed to room temperature. Centrifuge at 100 X g for 5 minutes to pellet the cells.
4. Carefully aspirate the supernatant and add 15 ml (1 mL per 5 cm^2) of NHM-GM warmed to room temperature for seeding the culture vessel(s).
5. Gently transfer the cell suspension into the culture vessel(s). Carefully rock the culture flask(s) to evenly distribute the cells and place in 37°C, 5% CO_2, and 95% humidified

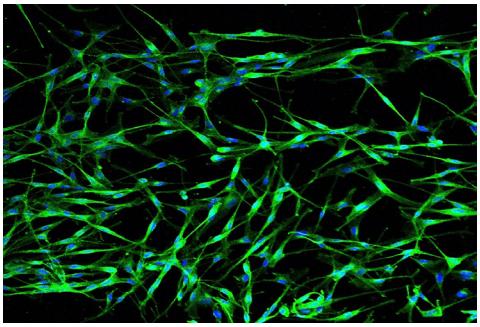

Figure 2. Immunohistochemical analysis of NHM cells at passage 3. MEL-5 (green), DAPI (blue), 100x.

Maintenance of Cell Culture

1. Change the NHM-GM growth medium every 48 hours using 1 mL per 5 cm^2.
2. Avoid repeated warming and cooling of the NHM-GM medium. Transfer and warm only the required volume in a sterile secondary container.
3. When changing the media, take care not to pipet medium directly onto the growing monolayer as this will cause cell detachment. Slowly add medium by pipetting against the side of the culture vessel.

Subculture Procedure

1. Subculture the cells when they are 70-80% confluent and contain many mitotic figures (after 4-5 days).
2. Prepare sufficient Trypsin (0.025%)/EDTA (0.265 mM) in Ca^{2+}/Mg^{2+}-free PBS (DPBS), DPBS, and Soybean Trypsin inhibitor (STI, 0.25 mg/mL) in DPBS at room temperature (0.35 mL per 5 cm^2).
3. Aspirate the medium from the culture vessel and rinse twice with DPBS (0.1 mL per 5 cm^2). In this and subsequent steps until the trypsinization process is complete, take care not to pipet solutions directly onto the monolayer of cells.
4. Add Trypsin/EDTA solution and gently rock to insure complete coverage of cells. Immediately aspirate the trypsin solution.
5. Observe the cells under the microscope. When the tips of the dendrites have started to round up and shorten (typically 2-3 min), add the diluted STI to the vessel and rinse the vessel surface to collect any remaining cells.
6. Transfer the suspension to a centrifuge tube. Rinse the culture vessel with DPBS (0.1 mL per 5 cm^2) and transfer the rinse solution to the centrifuge tube containing the cell suspension.
7. Take a small aliquot for cell counting and centrifuge the cell suspension at 100 X g for 5 minutes.
8. Re-suspend the pellet in fresh, warm NHM-GM medium. Seed the cells in a new culture vessel at 4,200 cells/cm^2.

Table 1. Reagents for use with NHM-CRY

Product	Size	Catalog Number	Storage
Normal Human Melanocytes	$5x10^5$	NHM-CRY	Liquid Nitrogen
Normal Human Melanocyte Growth Medium Kit	500 mL	NHM-GM	2-8°C
Normal Human Melanocyte Basal Medium	500 mL	NHM-BM	2-8°C
Normal Human Melanocyte Growth Supplement	10 mL	NHM-GS	≤-15°C

Note: MatTek Normal Human Melanocyte Medium (NHM-GM), consisting of NHM Basal Medium and NHM Growth Supplement, is specially designed to support the growth of MatTek Normal Human Melanocytes (NHMs). Use of NHM-GM (NHM-BM supplemented with NHM-GS) is strongly recommended for culturing MatTek NHM-CRY cells.

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