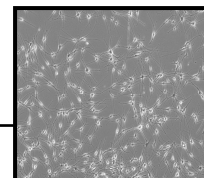


Clonetics™ Melanocyte Cell Systems



Introduction

Clonetics™ Melanocyte Cell Systems contain either Normal Human Epidermal Neonatal Melanocytes (NHEM-neo) or Normal Human Adult Melanocytes (NHEM-Ad) and optimized media for their growth. Each system can quickly generate NHEM cultures for the study of cellular differentiation, pigmentation (melanogenesis), viral-induced transformation, antigen expression and cell adhesion. Clonetics™ Melanocyte Cell Systems are convenient and easy to use, allowing the researcher to focus on results. NHEM cultures are typically cryopreserved following second passage after isolation.

Melanocyte cultures are tested for purity through immunofluorescent labeling of Mel-5 (gp75/TRP-1) and for functional performance through confirmation of L-dopa conversion activity.

Clonetics™ Cells, Medium and Reagents are quality tested together and guaranteed to give optimum performance as a complete cell system.

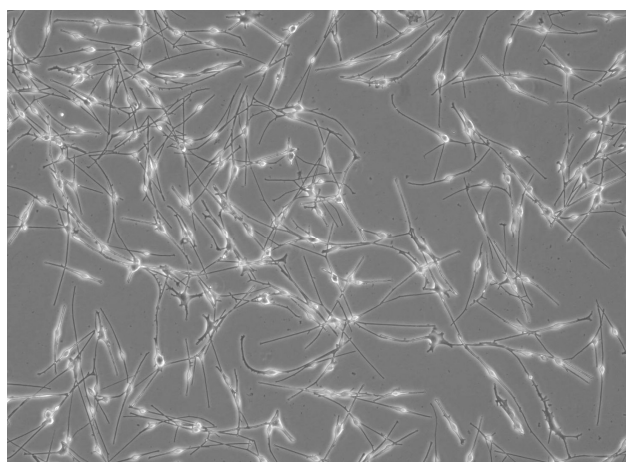
Cell System Components (Need to be purchased separately)

- One Melanocyte Cell Product (Cryopreserved or Proliferating)
- One Melanocyte Medium BulletKit™ - 500 ml Clonetics™ MGM™-4 BulletKit™ (CC-3249) contains one 500 ml bottle of Melanocyte Cell Basal Medium-4 (MBM™-4) and the following growth supplements: CaCl₂, 1.0 ml; BPE, 2.0 ml; rhFGF-B, 1.0 ml; rh-Insulin, 1.0 ml; Hydrocortisone, 0.5 ml; PMA, 0.5 ml; GA-1000, 0.5 ml; FBS, 2.5 ml
- Endothelin-3 (ET-3) for NHEM-Ad (must be purchased separately)
- Subculturing reagents must be purchased separately.

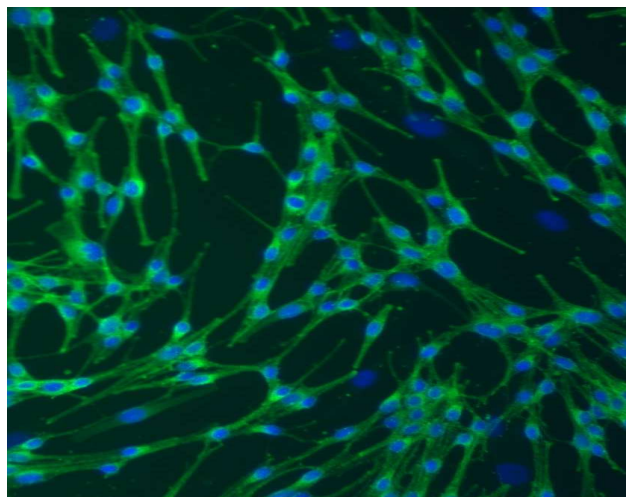
Characterization of Cells

Routine characterization of NHEM includes:

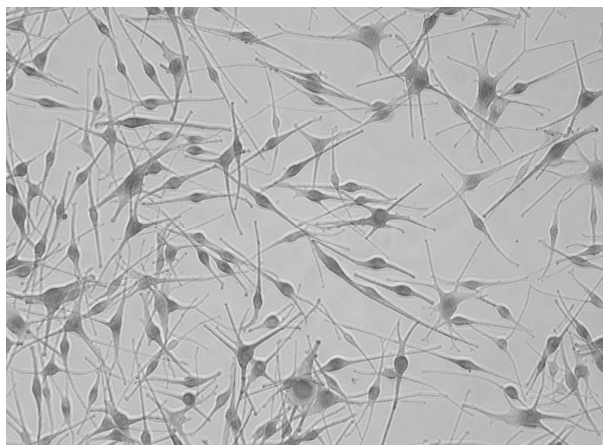
1. Morphological observation throughout serial passage
2. Mel-5 immunofluorescent labeling
3. L-Dopa conversion to melanin



Normal healthy melanocytes have multi- or bi-polar processes with small cell bodies.



Normal Human Melanocytes labeled with anti-Mel-5. Mel-5 (Ta99) is specific for gp75 expressed in melanosomes of human pigmented melanocytes (Bhawan J. 1997, Burkhalter A. 1997).



NHEM that have converted L-dopa into dopa-melanin. Conversion of L-dopa to dopa-melanin requires the activity of tyrosinase enzyme (Aberdam 1998, Tsuboi T. 1998).

Performance

Recommended seeding density for subculture	10,000 cells/cm ²
Typical time from subculture to confluent monolayer	9 - 14 days
Population doublings possible using Clonetics™ System	For Information Only. Review COA for details.

References

1. Aberdam E, Bertolotto C, Sviderskaya E, de Thillot V, Hemesath T, Fisher D, Bennett D, Ortonne J, Ballotti R. Involvement of microphthalmia in the inhibition of melanocyte lineage differentiation and of melanogenesis by agouti signal protein. *J Biol Chem* 1998; 31:19560-19565.
2. Bhawan J, Mel-5: a novel antibody for differential diagnosis of epidermal pigmented lesions of the skin in paraffin-embedded sections. *Melanoma Res.* 1997; 7(1):43-48.
3. Dupin E, Glavieux C, Vaigot P, Le Douarin NM. Endothelin 3 induces reversion of melanocytes to glia through a neural crest-derived glial-melanocytic progenitor. *Proc Natl Acad Sci USA.* 2000; 97(14):7882-7.
4. Lahav R, Ziller C, Dupin E, Le Douarin NM. Endothelin 3 promotes neural crest cell proliferation and mediates a vast increase in melanocyte number in culture. *Devel Bio.* 1996; 93(9): 3892-7.
5. Tsuboi T, Kondoh H, Hiratsuka J, Mishima Y. Enhanced Melanogenesis induced by tyrosinase gene-transfer increases boron-uptake and killing effect of boron neutron capture therapy for amelanotic melanoma. *Pigment Cell Res.* 1998; 11:275-282.

Quality Control

Melanocyte Cell Systems undergo extensive Quality Control testing*.

Melanocyte testing routinely includes:

- Negative results for HIV-1, Hepatitis-B, Hepatitis-C.
- Immunofluorescent labeling of Mel-5 (gp75/TRP-1) to ensure purity.
- Verification of melanocyte conversion of L-dopa into dopa-melanin to ensure functional activity.
- Morphology and proliferative capacity throughout serial passage after recovery from cryopreservation.

Clonetics™ MGM™-4 is formulated for optimal growth of melanocytes. MGM™-4 SingleQuots™ testing routinely includes:

- Verification of melanocyte conversion of L-dopa into dopa-melanin to ensure support of functional activity.
- Promotion of melanocyte morphology and proliferative capacity throughout serial passage after recovery from cryopreservation.

* For detailed information concerning QC testing and release specifications, please refer to the Certificate of Analysis (COA). COAs for each cell lot are shipped with each order. COAs for all other products are available upon request.

Ordering Information

Cryopreserved Cells

CC-2504	NHEM-neo	≥500,000 cells
CC-2586	NHEM-Ad	≥500,000 cells

Proliferating formats are available. Contact Scientific Support or refer to the Lonza website for details.

Cell Culture Media

CC-3249	MGM™-4 BulletKit™, MBM™-4 plus SingleQuots™ of Growth Supplements	500 ml
CC-3250	MBM™-4, Melanocyte Cell Basal Medium	500 ml
CC-4435	MGM™-4 SingleQuots™, Formulates MBM™-4 to MGM™-4	
CC-4510	Endothelin-3 (NHEM-Ad only)	130 µg

Subculturing Reagents

17-516F	Phosphate Buffered Saline (PBS)	500 ml
CC-5012	Trypsin/EDTA Solution	100 ml
17-711E	Versene® (EDTA) 0.02%	100 ml
CC-5002	Trypsin Neutralizing Solution (TNS)	100 ml

When placing an order or for technical service, please refer to the product numbers and descriptions listed above. For a complete listing of all Clonetics™ Products, refer to the Lonza website or the current Lonza catalog. To obtain a catalog, additional information or technical service you may contact Lonza by web, e-mail, telephone, fax or mail.

Product Warranty

CULTURES HAVE A FINITE LIFESPAN IN VITRO. Lonza guarantees the performance of its cells only if Clonetics™ Media and Subculture Reagents are used exclusively, and the recommend protocols are followed. The performance of cells is not guaranteed if any modifications are made to the complete Cell System. Cryopreserved NHEM are assured to be viable and functional when thawed and maintained properly.

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or in vitro procedures.

WARNING: CLONETICS™ AND POIETICS™ PRODUCTS CONTAIN HUMAN SOURCE MATERIAL, TREAT AS POTENTIALLY INFECTIOUS. Each donor is tested and found non-reactive by an FDA approved method for the presence of HIV-1, Hepatitis B Virus and Hepatitis C Virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV, Hepatitis B Virus, and Hepatitis C Virus. Testing can not offer complete assurance that HIV-1, Hepatitis B Virus, and Hepatitis C Virus are absent. All human sourced products should be handled at the Biological Safety Level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH Manual, [Biosafety in Microbiological and Biomedical Laboratories](#), 5th Edition. If you require further information, please contact your site Safety Officer or Scientific Support.