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Product Information

Human Epidermal Melanocytes-light (HEM-l)

Catalog Number	10HU-164	Cell Number	0.5 million cells/vial
Species	*Homo sapiens*	Storage Temperature	Liquid Nitrogen

Description

Melanocytes are melanin-producing neural crest-derived cells located in the bottom layer (the stratum basale) of the skin's epidermis, the middle layer of the eye (the uvea), the inner ear, vaginal epithelium, meninges, bones, and heart [1] . Melanocytes, which are derived from the neural crest, are unique in that they produce eu-/pheo-melanin pigments in unique membrane-bound organelles termed melanosomes, which can be divided into four stages depending on their degree of maturation [2]. Dysregulation of melanocyte migration, proliferation, or survival during embryonic development thus causes congenital disorders in those tissues as seen in Tietz syndrome, Waardenburg syndrome, and piebaldism [3]. In the bottom layer of skin epidermis, melanocytes synthesize and transfer dark-colored melanin to surrounding keratinocytes to give skin pigmentation. Melanin also blocks UV-B light to protect the hypodermis from solar exposure-induced photodamage. Progress in culture techniques, along with an improved understanding of melanocyte biology, has led to a successful culture system to model melanomas, inner ear homeostasis, vitiligo, and mitochondrial dysfunction in Duchenne Muscular Dystrophy [4].

iXCells Biotechnologies provides high quality Human Epidermal Melanocytes-light (HEM-l), which are isolated from neonatal human skin and cryopreserved at P1, with >0.5 million cells in each vial. HEM-l express fibronectin and NGF-receptor (p75). They are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast, and fungi and can further expand no more than 3 passages in Melanocyte Growth Medium (Cat# MD-0049) under the condition suggested by iXCells Biotechnologies. Prolonged culture process may decrease the purity.

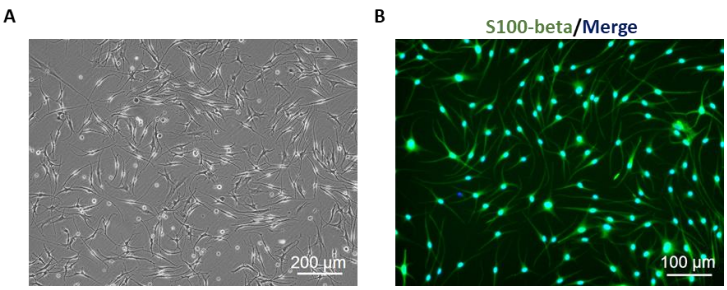

Figure 1. Human Epidermal Melanocytes-light (HEM-l). **(A)** Phase contrast image of HEM-I. **(B)** Immunofluorescence staining with antibodies against S100-beta.

Product Details

Tissue	Neonatal human skin
Package Size	0.5 million cells/vial
Passage Number	P1
Shipped	Cryopreserved
Storage	Liquid nitrogen
Growth Properties	Adherent
Media	Melanocyte Growth Medium (Cat# MD-0049)

Protocols

Pre-coated culture vessels with Poly-L-Lysine

1. Dilute poly-L-Lysine till 0.01% using tissue culture grade water.
2. Pre-coat desired culture vessel using 0.01% poly-L-Lysine overnight at 37°C incubator (eg.10 mL/T75 flask).
3. Rinse the poly-L-Lysine-coated vessel twice with sterile water and then add complete medium into the culture vessel to get the culture vessel ready (15 mL/T75 flask).

Thawing of Frozen Cells

1. Upon receipt of the frozen cells, it is recommended to thaw the cells and initiate the culture immediately in order to retain the highest cell viability.
2. To thaw the cells, put the vial in 37°C water bath with gentle agitation for 1-2 minutes. Keep the cap out of water to minimize the risk of contamination.
3. Pipette the cells into a 15 mL conical tube with 5 mL pre-warmed **Melanocyte Growth Medium** (Cat# MD-0049).
4. Centrifuge at 1,000 rpm (~220 g) for 5 minutes under room temperature.
5. Remove the supernatant and resuspend the cells in fresh culture medium.
6. Culture the cells in poly-L-Lysine pre-coated T75 flask. Change the medium every other day until cells reach 80-90% confluence.

Safety Precaution: *it is highly recommended that protective gloves and clothing should be used when handling frozen vials.*

Standard Culture Procedure

1. Human Epidermal Melanocytes-light (HEM-l) can be cultured in **Melanocyte Growth Medium** (Cat# MD-0049).
2. When cells reach ~80-90% confluence, remove the medium, and wash once with sterile PBS (5 mL/T75 flask).
3. Add 3 mL of 0.05% Trypsin-EDTA to the flask and incubate for 1 minute at 37°C. Neutralize the enzyme by adding 2-3 volumes of melanocyte growth medium.
4. Centrifuge 1,000 rpm (~220 g) for 5 minutes and resuspend the cells in desired volume of medium.
5. Seed new culture vessels at 5 × 10^3 cells/cm^2. Change the medium every other day until cells reach 80-90% confluence.

Reference

[1] McGrath JA, Eady RAJ, Pope FM. (2004). "Anatomy and Organization of Human Skin". In Burns T, Breathnach S, Cox N, Griffiths C. *Rook's Textbook of Dermatology* (7th ed.). Blackwell Publishing. p. 4190.
[2] Yuji Yamaguchi and Vincent J. Hearing (2014) "Melanocytes and Their Diseases". Cold Spring Harb Perspect Med. 2014 May; 4(5): a017046
[3] Juying Li, Jun S. Song, Robert J. A. Bell, Thanh-Nga T. Tran, Rizwan Haq, Huifei Liu, Kevin T. Love, Robert Langer, Daniel G. Anderson, Lionel Larue, and David E. Fisher (2012) " YY1 Regulates Melanocyte Development and Function by Cooperating with MITF" PLoS Genet. 8(5): e1002688
[4] M Y Hsu, M Herlyn. (1996) "Cultivation of normal human epidermal melanocytes" Methods Mol Med. 2:9-20.

Disclaimers

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