PrimaPure™

Human Epidermal Melanocytes (HEM), Cryopreserved

Genlantis
A division of Gene Therapy Systems, Inc.

Catalog #	Description/Content	Amount
PH10405A	HEM Cells, Cryopreserved	>500,000 cells
PH10405AK	HEM Cells, Complete System*	1 Kit
*Each kit contains an ampoule of cryopreserved HEM (PH10405A), 500 ml of Melanocyte Growth Medium (PM135500), and a Subculture Reagent Kit (PR090100K).		

Related Products	Catalog #
Melanocyte Growth Medium	PM135500
Subculture Reagent Kit, including100 ml each of HBSS, Trypsin/EDTA, and Trypsin Neutralizing Solution	PR090100K
GenePORTER® 2 Transfection Reagent, 0.75 ml	T202007
GeneSilencer® siRNA Transfection Reagent, 200 reactions	T500750

Storage:	**Store cells vials in liquid nitrogen immediately upon arrival.** Store the growth medium at 4°C in the dark immediately upon arrival. Store the Subculture Reagent Kit at -20°C upon arrival and store the reagents at 4°C upon thawing.

INTRODUCTION

Human Epidermal Melanocytes (HEM) are primary cells isolated from normal human neonatal foreskin. HEM are cryopreserved at second passage and can be cultured and propagated at least 12 population doublings. These epidermal melanocytes produce melanin, thus a useful cell model in the study of hyperpigmentation through accentuated melanocyte proliferation and differentiation[1,2], as well as progression of melanocytic neoplasia[3,4].

MATERIALS AND METHODS

I. Preparation for Culturing

1. Make sure your Class II Biological Safety Cabinet, with HEPA filtered laminar airflow, is in proper working condition.
2. Clean the Biological Safety Cabinet with 70% alcohol to ensure it is sterile.
3. Turn the Biological Safety Cabinet blower on for 10 min. before cell culture work.
4. Make sure all serological pipettes, pipette tips, and reagent solutions are sterile.
5. Follow the standard sterilization technique and safety rules:
   a. Do not pipette with mouth.
   b. Always wear gloves and safety glasses when working with human cells even though all the strains have been tested negative for HIV, Hepatitis B and Hepatitis C.
   c. Handle all cell culture work in a sterile hood.

II. Culturing HEM

A. **Preparing Cell Culture Flasks for Culturing HEM.**

1. Decontaminate a bottle of Melanocyte Growth Medium with 70% alcohol in a sterile hood.
2. Pipette 15 ml of Melanocyte Growth Medium into a T-75 flask.
   NOTE: Keep the medium to surface area ratio at 1 ml per 5 cm^2, for example 5 ml for a T-25 flask (or 60 mm tissue culture dish), or 15 ml for a T-75 flask (or a 100 mm tissue culture dish).

B. **Thawing and Plating HEM**

3. Remove the cryopreserved vial of HTEpC from the liquid nitrogen storage tank using proper protection for your eyes and hands.
4. Turn the vial cap a quarter turn to release any liquid nitrogen that may be trapped in the threads, then re-tighten the cap.
5. Thaw the cells quickly by placing the lower half of the vial in a 37°C water bath for 1 minute.
6. Take the vial out of the water bath and wipe dry.
7. Decontaminate the vial exterior with 70% alcohol in a sterile Biological Safety Cabinet.
8. Remove the vial cap carefully. Do not touch the rim of the cap or the vial.
9. Resuspend the cells in the vial by gently pipetting the cells 5 times with a 2 ml pipette. Be careful not to pipette too vigorously as to cause foaming.
10. Pipette the cell suspension (1ml) from the vial into the T-75 flask containing 15 ml of Melanocyte Growth Medium.
11. Cap the flask and rock gently to evenly distribute cells.
12. Place the T-75 flask in a 37°C, 5% CO_2 humidified incubator. Loosen the cap to allow gas exchange. For best results, do not disturb the culture for 24 hours after inoculation.
13. Change to fresh Melanocyte Growth Medium after 24 hours or overnight to remove all traces of DMSO.

14. Change to fresh Melanocyte Growth Medium every other day until the cells reach 45% confluent.
15. Double the Melanocyte Growth Medium volume when the culture is >60% confluent or for weekend feedings.
16. Subculture the cells when the HEM reaches 80% confluent.

III. Subculturing HEM

A. Preparing Subculture Reagents

1. Remove the Subculture Reagent Kit from the -20°C freezer and thaw overnight in a refrigerator.
2. Make sure all the subculture reagents are thawed. Swirl each bottle gently several times to form homogeneous solutions.
3. Store all the subculture reagents at 4°C for future use. The activity of Trypsin/EDTA Solution will be stable for 2 weeks when stored at 4°C.
4. Aliquot Trypsin/EDTA solution and store the unused portion at -20°C if only portion of the Trypsin/EDTA is needed.

B. Preparing Culture Flask

5. Take the Melanocyte Growth Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile hood.
6. Pipette 40 ml of Melanocyte Growth Medium to a T-175 flask (to be used in Section III C Step 17).

C. Subculturing HEM

NOTE: Trypsinize Cells at Room Temperature**. Do Not Warm Any Reagents to 37°C.**

7. Remove the medium from culture flasks by aspiration.
8. Wash the monolayer of cells with HBSS and remove the solution by aspiration.
9. Pipette 8 ml of Trypsin/EDTA Solution into the T-75 flask. Rock the flask gently to ensure the solution covers all the cells.
10. Recap the flask tightly and monitor the trypsinization progress at room temperature under an inverted microscope. . It usually takes about 2 to 4 minutes for the cells to become rounded. The cells may not become completely round during the trypsinization and some cells may maintain some processes even though they are loosened from the culture surface.
11. Release the rounded cells from the culture surface by hitting the side of the flask against your palm until most of the cells are detached.
12. Pipette 5 ml of Trypsin Neutralizing Solution to the flask to inhibit further tryptic activity.
13. Transfer the cell suspension from the flask to a 50 ml sterile conical tube.
14. Rinse the flask with an additional 5 ml of Trypsin Neutralizing Solution and transfer the solution into the same conical tube.
15. Examine the T-75 flask under a microscope. If there are >20% cells left in the flask, repeat Steps 8-14.
16. Centrifuge the conical tube at 220 x g for 5 minutes to pellet the cells.
17. Aspirate the supernatant from the tube without disturbing the cell pellet.
18. Flick the tip of the conical tube with your finger to loosen the cell pellet.
19. Resuspend the cells in 2 ml of Melanocyte Growth Medium by gently pipetting the cells to break up the clumps.
20. Count the cells with a hemocytometer or cell counter. Inoculate at 10,000 cells per cm^2 for rapid growth, or at 6,000 cells per cm^2 for regular subculturing.

REFERENCES

1. Jimbow, K. et al, J. Invest. Dermatol. 78:108 (1982).
2. Imokawa, G. et al, Arch. Dermatol. Res. 278:352 (1986).
3. Herlyn, M. et al, Lab. Invest. 56:461(1987)
4. Simon, H., Cancer Res. 56:3112 (1996).

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