

# PrimaPure™



A division of Gene Therapy Systems, Inc.

## Human Epidermal Keratinocytes (HEK)

Catalog #	Description/Content	Amount
PH10205A	HEK, Adult	>500,000 cells
PH10205F	HEK, Fetal	>500,000 cells
PH10205N	HEK, Neonatal	>500,000 cells
PH10205AK	HEK, Adult Complete System	1 Kit*
PH10205FK	HEK, Fetal Complete System	1 Kit*
PH10205NK	HEK, Neonatal Complete System	1 Kit*

\*Each kit contains an ampoule of cryopreserved HEK (PH10205A, PH10205F, or PH10205N), 500 ml of Keratinocyte Serum-Free Growth Medium (PM131500), and a Subculture Reagent Kit (PR090100K).

Related Products	Catalog #
Keratinocyte Serum-Free Growth Medium, 500 ml	PM131500
Keratinocyte Calcium-Free Growth Medium, 500 ml	PM133500
HEPES Buffered Saline Solution (HBSS), 100 ml	PR062100
Trypsin/EDTA, 100 ml	PR070100
Trypsin Neutralizing Solution, 100 ml	PR080100
Subculture Reagent Kit, including 100 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

<b>Storage:</b>	Store cryopreserved 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 Keratinocytes (HEK) are primary cells isolated from normal human neonatal foreskin or adult skin. They are cryopreserved at the end of primary culture and can be cultured and propagated at least 16 population doublings. HEK is a suitable *in vitro* model for dermal toxicology tests in screening new skin products<sup>1</sup>, and for developing dermal skin substitutes<sup>2</sup>. HEK has been used in numerous applications, including investigations of human epidermal development<sup>3</sup>, differentiation<sup>4</sup>, and cellular aspects of skin diseases<sup>5</sup>.

## 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.
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 1-2 minutes.
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 Keratinocyte Growth Medium.
11. Cap the flask and rock gently to evenly distribute the cells.
12. Place the T-75 flask in a 37°C, 5% CO<sub>2</sub> 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 Keratinocyte Growth Medium after 24 hours or overnight to remove all traces of DMSO.
14. Change Keratinocyte Growth Medium every other day until the cells reach 45% confluent.
15. Double the Keratinocyte Growth Medium volume when the culture is >45% confluent or for weekend feedings.
16. Subculture the cells when the HEK reach 60-80% confluent.

### II. Culturing HEK Cells

1. Take the Keratinocyte Growth Medium (Catalog PM131500 or PM133500) from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile hood.
2. Pipette 15 ml of Keratinocyte Growth Medium to a T-75 flask.  
**NOTE:** Keep medium to surface area ratio at 1ml per 5 cm<sup>2</sup>. Example: 5 ml for a T-25 flask or a 60 mm tissue culture dish; 15 ml for a T-75 flask or a 100 mm tissue culture dish.
3. Remove the cryopreserved vial of HEK from the liquid nitrogen storage tank. Use proper protection for your eyes and hands.

## Human Epidermal Keratinocytes (HEK) Manual

### III. Subculturing HEK Cells

1. Remove above solutions from the -20°C freezer and thaw overnight in a refrigerator.
2. Make sure all the reagents are thawed. Swirl each bottle gently several times to form homogeneous solutions.
3. Store all 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.
5. Take the Keratinocyte Growth Medium (Cat. No. PM131500 or Cat. No. PM133500) from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile hood.
6. Pipette 35ml of Keratinocyte Growth Medium to a T-175 flask (for later use in Step.22 below).
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.  
**NOTE: Trypsinize Cells at Room Temperature. Do Not Warm Any Reagents to 37°C.**
10. Re-cap the flask tightly and monitor the trypsinization progress at room temperature under an inverted microscope and over a 60 seconds period.
11. Aspirate the Trypsin/EDTA solution.
12. Re-cap the flask and place in a 37°C incubator for 2 minutes.
13. 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.
14. If the cells are not visually detached place the flask into the incubator for an additional thirty seconds, then repeat step 13.
15. Pipette 5 ml of Trypsin Neutralizing Solution to the flask to inhibit further tryptic activity.
16. Transfer the cell suspension from the flask to a 50 ml sterile conical tube.
17. Rinse the flask with an additional 5 ml of Trypsin Neutralizing Solution and transfer the solution into the same conical tube.
18. Examine the T-75 flask under a microscope. If there are >20% cells left in the flask, repeat Steps 9-15.
19. Centrifuge the conical tube at 220 x g for 5 minutes to pellet the cells.
20. Aspirate the supernatant from the tube without disturbing the cell pellet.
21. Flick the tip of the conical tube with your finger to loosen the cell pellet.
22. Resuspend the cells in 2 ml of Keratinocyte Growth Medium by gently pipetting the cells to break up the clumps. Transfer to 75 flask prepared in Step 6 above).
23. Count the cells with a hemocytometer or cell counter. Inoculate at 7,500 cells per cm<sup>2</sup> for rapid growth, or at 5,000 cells per cm<sup>2</sup> for regular subculturing.

### REFERENCES

1. Delco, V. et al, Alternative Methods in Toxicology, 3: 466 (1985).
2. Joyce, S.T. et al, J. Biomed. Mater. Res. 22:937 (1988).
3. Holbrook, K.A., Biochemistry and Physiology of the Skin, Vol. 1:64 (1983).
4. Fuchs, F.J., Cell Biol. 111 (No. 6, Pt. 2):2807 (1990).
5. Koizami, H. et al, British J. Dermatol. 134: 686 (1996).

### LICENSE

The purchase price paid for the PrimaPure™ cells and reagents grants end users a non-transferable, non-exclusive license to use the kit and/or its components for **internal research use only** as described in this manual; in particular, research use only excludes and without limitation, resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of Genlantis. Separate licenses are available for non-research use or applications. **The PrimaPure™ cells and reagents are not to be used for human diagnostic or included/used in any drug intended for human use.** Care and attention should be exercised in handling the product by following appropriate research lab practices.

Purchasers may refuse this license by returning the enclosed materials unused. By keeping or using the enclosed materials, you agree to be bound by the terms of this license. The laws of the State of California shall govern the interpretation and enforcement of the terms of this license.