

PrimaPure™



A division of Gene Therapy Systems, Inc.

Human Tracheal Epithelial Cells (HTEpC), Cryopreserved

Catalog #	Description/Content	Amount	Related Products	Catalog #
PH50405A	HTEpC	>500,000 cells	Bronchial/Tracheal Epithelial Cells Growth Medium	PM511500
PH50205K	HTEpC Complete System*	1 Kit	Bronchial/Tracheal Epithelial Cells Basal Medium	PM510500
*Each kit contains an ampoule of cryopreserved HTEpC (PH50405A), 500 ml of Bronchial/Tracheal Epithelial Cells Growth Medium (PM511500), and a Subculture Reagent Kit (PR090100K).			Bronchial/Tracheal Epithelial Cells Growth Supplement	PM511GS
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.			Human Bronchial Epithelial Cells (HBEpC), >500,000 cells	PH50205A
			Human Bronchial Epithelial Cells (HBEpC), Complete System	PH50205AK
			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

INTRODUCTION

Human Bronchial/Tracheal Epithelial Cells (HBEpC/HTEpC) are derived from the surface epithelium of normal human bronchi/trachea. They are cryopreserved at first passage and can be cultured and propagated at least 16 population doublings. Airway epithelial cells are targets in asthma, viral infection, and fibrotic lung disease. These cells have been used to study the regulation of intracellular pH¹, the stimulant effect of IL-1b on airway epithelial cell growth², and the effect of human rhinovirus infection in terms of upregulation of ICAM-1 expression on epithelial cells³.

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 HBEpC

A. Preparing Cell Culture Flasks for Culturing HTEpC.

1. Decontaminate a bottle of Tracheal Epithelial Cell Growth Medium with 70% alcohol in a sterile hood.
2. Pipette 15 ml of Tracheal Epithelial Cell Growth Medium into a T-75 flask.
NOTE: Keep the medium to surface area ratio at 1 ml per 5 cm², 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 HTEpC

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 Tracheal Epithelial Cell Growth Medium.
11. Cap the flask and rock gently to evenly distribute cells.
12. Place the T-75 flask in a 37°C, 5% CO₂ 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 Tracheal Epithelial Cell Growth Medium after 24 hours or overnight to remove all traces of DMSO.

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

III. Subculturing HTEpC

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 Tracheal Epithelial Cell Growth Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile hood.
6. Pipette 30 ml of Tracheal Epithelial Cell Growth Medium to a T-175 flask (to be used in Section III C Step 17).

C. Subculturing HTEpC

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. Do this for 60 seconds. Do not over trypsinize, or the cells could be irreversibly damaged.
11. Aspirate Trypsin/EDTA
12. Re-cap the flask and place in a 37° incubator for 1.5 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 30 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 8-16.
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 Tracheal Epithelial Cell Growth Medium by gently pipetting the cells to break up the clumps.
23. Count the cells with a hemocytometer or cell counter. Inoculate at 7,500 cells per cm² for rapid growth, or at 5,000 cells per cm² for regular subculturing.

REFERENCES

1. Poulsen, J.H. *et al.* (1996) *J Physiol.-Abs.* **432** (3).
2. Murlas, C.G. *et al.* (1997) *Lung* **175**: 117-126.
3. Terajima, M. *et al.* (1997) *Lung Cell & Molec. Phys* **273** (4):749.

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