PrimaPure™

Human Pulmonary Artery Endothelial Cells (HPAEC)

amsbio

Catalog #	Description/Content	Amount
PH30205A	HPAEC, Adult	>500,000 cells
PH30205AK	HPAEC, Adult Complete System	1 Kit*
*Each kit contains an ampoule of cryopreserved HPAEC (PH30205A), 500 ml of Endothelial Cell Growth Medium (PM211500), and a Subculture Reagent Kit (PR090100K).		

Related Products	Catalog #
Endothelial Cell Growth Medium, 500 ml	PM211500
HEPES Buffered Saline Solution (HBSS), 100 ml	PR062100
Trpsin/EDTA, 100 ml	PR070100
Trypsin Neutralizing Solution, 100 ml	PR080100
Subculture Reagent Kit, including100 ml each of HBSS, Trpsin/EDTA, and Trpsin Neutralizing Solution	PR090100K
GenePORTER 2 Transfection Reagent, 0.75 ml	T202007
GeneSilencer siRNA Transfection Reagent, 200 reactions	T500750

Storage:	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 Pulmonary Artery Endothelial Cells (HPAEC) are isolated from normal human pulmonary arteries. They are cryopreserved at second passage and can be cultured and propagated at least 15 population doublings. HPAEC possess an array of enzymatic activities[1,2]. They respond to a wide range of vasoactive substances[3], commensurate with their control of blood pressure and blood pH *in vivo* . HPAEC have been used for the study of vascular permeability[4] and inflammatory responses[5]. HPAEC in co-culture with HPASMC have been used as a model for pulmonary angiopathy[6].

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 HPAEC

A. PREPARING CELL CULTURE FLASKS FOR CULTURING HPAEC

1. Take the Endothelial Cell Growth Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile hood.
2. Pipette 15 ml of Endothelial Cell Growth Medium* to a T-75 flask.

* Keep the medium to surface area ratio at 1ml per 5 cm^2.

For example,
7.5 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.

B. THAWING AND PLATING HPAEC

1. Remove the cryopreserved vial of HPAEC from the liquid nitrogen storage tank using proper protection for your eyes and hands.
2. Turn the vial cap a quarter turn to release any liquid nitrogen that may be trapped in the threads, then re-tighten the cap.
3. Thaw the cells quickly by placing the lower half of the vial in a 37°C water bath for 1minute.
4. Take the vial out of the water bath and wipe dry.
5. Decontaminate the vial exterior with 70% alcohol in a sterile Biological Safety Cabinet.
6. Remove the vial cap carefully. Do not touch the rim of the cap or the vial.
7. 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.
8. Pipette the cell suspension (1ml) from the vial into the T-75 flask containing 15 ml of Endothelial Cell Growth Medium.
9. Cap the flask and rock gently to evenly distribute the cells.
10. 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.
11. Change to fresh Endothelial Cell Growth Medium after 24 hours or overnight to remove all traces of DMSO.

12. Change Endothelial Cell Growth Medium every other day until the cells reach 60% confluent.
13. Double the Endothelial Cell Growth Medium volume when the culture is >60% confluent or for weekend feedings.
14. Subculture the cells when the HPAEC reach 80% confluent.

III. Subculturing HPAEC

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

1. Take the Endothelial Cell Growth Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile hood.
2. Pipette 30ml of Endothelial Cell Growth Medium to a T-175 flask (to be used in Section III C Step 15.)

C. SUBCULTURING HPAEC

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

1. Remove the medium from culture flasks by aspiration.
2. Wash the monolayer of cells with HBSS and remove the solution by aspiration.
3. Pipette 5 ml of Trypsin/EDTA Solution into the T-75 flask. Rock the flask gently to ensure the solution covers all the cells.
4. Remove 4.5 ml of the solution immediately.
5. Re-cap the flask tightly and monitor the trypsinization progress at room temperature under an inverted microscope. It usually takes about 1 minute for the cells to become rounded but still attached to the flask. (When rounded cells detach by itself without hitting, it means the cells are over trypsinized.)
6. 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.
7. Pipette 5 ml of Trypsin Neutralizing Solution to the flask to inhibit further tryptic activity.
8. Transfer the cell suspension from the flask to a 50 ml sterile conical tube.
9. Rinse the flask with an additional 5 ml of Trypsin Neutralizing Solution and transfer the solution into the same conical tube.
10. Examine the T-75 flask under a microscope. If there are >20% cells left in the flask, repeat Steps 2-9.
11. Centrifuge the conical tube at 220 x g for 5 minutes to pellet the cells.
12. Aspirate the supernatant from the tube without disturbing the cell pellet.
13. Flick the tip of the conical tube with your finger to loosen the cell pellet.
14. Resuspend the cells in 5 ml of Endothelial Cell Growth Medium by gently pipetting the cells to break up the clumps.
15. 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

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4. Downie, G.H. et al, Am. J. Respir. Cell. Mol. Biol. 7:58 (1992).
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