### Porcine Pulmonary Artery Endothelial Cells (PPAEC)

**INTRODUCTION**

Porcine large artery endothelial cells are derived from healthy, plaque free porcine. PPAEC are cryopreserved at first passage and can be cultured and propagated at least 16 population doublings. Arterial endothelial cells play critical roles in cardiac homeostasis. Insults to the endothelial cells have been linked to a variety of vascular diseases. Porcine artery endothelial cells have been used as a viable alternative for the study of artery endothelial function and metabolism, such as atherosclerosis, relaxin factor synthesis, antioxidant enzyme activities, etc. Co-culture of the artery endothelial cells with species-matched smooth muscle cells provides an ideal model for studying the interaction between these two cell types.

### 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 PPAEC

**A. PREPARING CELL CULTURE FLASKS FOR CULTURING PPAEC**

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

* Keep the medium to surface area ratio at 1 ml per 5 cm². For 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.

**B. THAWING AND PLATING PPAEC**

1. Remove the cryopreserved vial of PPAEC 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 1 minute.
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 (1 ml) from the vial into the T-75 flask containing 15 ml of Porcine 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
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11. Change to fresh Porcine Endothelial Cell Growth Medium after 24 hours or overnight to remove all traces of DMSO.
12. Double the Porcine Endothelial Cell Growth Medium every other day until the cells reach 60% confluent.
13. Change Porcine Endothelial Cell Growth Medium when the culture is >60% confluent or for weekend feedings.
14. Subculture the cells when the PPAEC reach 80% confluent.

III. Subculturing PPAEC

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 Porcine Endothelial Cell Growth Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile hood.
2. Pipette 35 ml of Porcine Endothelial Cell Growth Medium to a T-175 flask (to be used in Section III C Step 15).

C. SUBCULTURING PPAEC

Trypsinize Cells at Room Temperature. Do Not Warm Any Reagents to 37°C.
1. Remove the medium from culture flasks by aspiration.

REFERENCES

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