PrimaPure™

Porcine Pulmonary Artery Smooth Muscle Cells (PPASMC)

Genlantis
A division of Gene Therapy Systems, Inc.

Catalog #	Description/Content	Amount
PP35205	PPASMC	>500,000 cells
PP35205K	PPASMC Complete System	1 Kit*
*Each kit contains an ampoule of cryopreserved PPASMC (PP35205), 500 ml of Porcine Smooth Muscle Cell Growth Medium (PMP311500), and a Subculture Reagent Kit (PR090100K).		

Related Products	Catalog #
Porcine Smooth Muscle Cell Growth Medium, 500 ml	PMP311500
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

Porcine large artery smooth muscle cells are derived from tunica intima and tunica media of healthy, fibrous plaque-free porcine arteries. PPASMC are cryopreserved at first passage and can be cultured and propagated at least 16 population doublings. Smooth muscle cells have been found in fatty streaks of early arteriosclerosis. Proliferation of the smooth muscle cells is considered a key event in the development of advanced lesions[1]. Porcine smooth muscle cells serve as an alternative for the investigations of cardiac disease and arteriosclerosis[1-3]. It can be used in co-culture investigation with species-matched porcine artery endothelial cells.

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 PPASMC

A. PREPARING CELL CULTURE FLASKS FOR CULTURING PPASMC

1. Take the Porcine Smooth Muscle Cell Growth Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile hood.
2. Pipette 20 ml of Porcine Smooth Muscle Cell Growth Medium* into a T-75 flask.
   Note: Keep the medium to surface area ratio at 1 ml per 5 cm^2. E.g., 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 PPASMC

1. Remove the cryopreserved PPASMC vial from the liquid nitrogen storage tank using proper protection for your eyes and hands.
2. Turn the cap of the vial a quarter turn to release any liquid nitrogen that may be trapped in the threads, then retighten the cap.
3. Thaw the cells quickly by placing the lower half of the vial in a 37°C water bath for 1-2 minutes.
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 19 ml of Porcine Smooth Muscle 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 the Porcine Smooth Muscle Cell Growth Medium after 24 hours or overnight to remove all traces of DMSO.
12. Change Porcine Smooth Muscle Cell Growth Medium every other day until the cells reach 60% confluent.
13. Double the Porcine Smooth Muscle Cell Growth Medium volume when the culture is >60% confluent or for weekend

feedings.

14. Subculture the cells when the PPASMC reach 80% confluent.

III. Subculturing PPASMC

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 in the dark 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 Smooth Muscle Cell Growth Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile hood.
2. Pipette 40 ml of Porcine Smooth Muscle Cell Growth Medium to a T-175 flask. (To be used in Step 16.)

C. SUBCULTURING PPASMC

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 ml of the solution immediately.
5. Replace the cap tightly and monitor the trypsinization progress at room temperature under an inverted microscope. It usually takes about 2 to 5 minutes for the cells to become rounded.
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 Porcine Smooth Muscle Growth Medium 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 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 200 x g for 5 minutes to pellet the cells.
12. Remove the conical tube. Aspirate the supernatant.
13. Flick the tip of the conical tube with your finger to loosen the pellet.
14. Resuspend the cells in 2 ml of Porcine Smooth Muscle Growth Medium by gently pipetting the cells to break up the clumps.
15. Count the cells with a hemocytometer or cell counter. Inoculate at 15,000 cells per cm2 for rapid growth, or at 10,000 cells per cm2 for regular subculturing.
16. Transfer the 2 ml cell suspension to a T-175 flask containing 35 ml of Porcine Smooth Muscle Growth Medium and do a 1 to 6 split thereafter.

IV. Differentiating PPASMC

A. SEEDING PPASMC FOR DIFFERENTIATION

1. Seed PPASMC in the desired format at 15,000 per cm^2. Follow instructions in Section IV C.
2. Change to Porcine Smooth Muscle Differentiation Medium the next day.

B. DIFFERENTIATING PPASMC TO EXPRESS CONTRACTILE PROTEIN

1. Remove growth medium from culture tissue ware by aspiration. Do not allow cells to dry during medium changes.
1. Add the appropriate volume of Porcine Smooth Muscle Differentiation Medium.
2. Incubate cell in a 37°C, 5% CO_2 humidified incubator in the Porcine Smooth Muscle Differentiation Medium.
3. Change to fresh Porcine Smooth Muscle Differentiation Medium every other day.
4. PPASMC are in growth arrest and smooth muscle □-actin is expressed in 10 days.

REFERENCES

1. Ross, R.N., Nature 362:801 (1993).
2. Stary, H.C., Arteriosclerosis 3:471a (1983).
3. McGill, H.C. Jr., Arteriosclerosis 4:443 (1984).

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