PrimaPure™

Rat Aortic Smooth Muscle Cells (RAOSMC)

Catalog #	Description/Content	Amount
SR35405	Rat Aortic Smooth Muscle Cells (RAOSMC)	>500,000 cells
SR35405K	RAOSMC Complete System	1 Kit*
*Each Complete System contains an ampoule of cryopreserved RAOSMC (PB35405), 500 ml of Rat Smooth Muscle Cell Growth Medium (PMR311500), and a Subculture Reagent Kit (PR090100K).		

Related Products	Catalog #
Rat Smooth Muscle Cell Growth Medium, 500 ml	PMR311500
Rat Smooth Muscle Cell Basal Medium	PMR310500
Rat Smooth Muscle Cell Growth Supplements	PMR311GS
Subculture Reagent Kit, including100 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

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

PrimaPure™ Rat Aortic Smooth Muscle Cells (RAOSMC) are derived from the tunica intima and tunica media of healthy, fibrous plaque-free rat aorta. They are cryopreserved at second passage and can be cultured and propagated at least 16 population doublings. Increased arterial smooth muscle cell mass are found in the intimal lesion of atherosclerosis[1]. Rat aortic smooth muscle cells respond to various factors by cell proliferation[2] and hypertrophy[3], which are prominent indicators of atherosclerosis in vascular diseases. RAOSMC are well suited for the study of large vessel smooth muscle cell growth and differentiation[4,5] and serve as in vitro model in correlation with live rat models.

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 animal cells even though USDA has inspected all the animals.
   c. Handle all cell culture work in a sterile hood.

II. Preparing Culture Flasks

A. PREPARING FLASKS FOR CULTURING OF RAOSMC

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

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

III. Thawing and Plating RAOSMC

1. Remove the cryopreserved vial of RAOSMC from the liquid nitrogen storage tank. Use 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 (1ml) from the vial into the T-75 flask containing 15 ml of Rat 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% CO_2 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 Rat Smooth Muscle Cell Growth Medium every other day until the cells reach 60% confluent
12. Double the Rat Smooth Muscle Cell Growth Medium volume when the culture is >60% confluent or for weekend feedings.
13. Subculture the cells when the RAOSMC reach 80% confluent.

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IV. Subculturing Rat Aortic Smooth Muscle Cells

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 a portion of the Trypsin/EDTA is needed

B. PREPARING FLASKS FOR SUBCULTURING OF RAOSMC

1. Take the Rat Smooth Muscle Cell Growth Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile hood.
2. Pipette 35 ml of Rat Smooth Muscle Cell Growth Medium to a T-175 flask (to be used in Section IV C Step 15).

C. SUBCULTURING RAOSMC.

1. Remove the medium from culture flasks by aspiration.
2. Wash the monolayer of cells with HBSS and remove the solution by aspiration.

NOTE: Trypsinize Cells at Room Temperature. Do Not Warm Any Reagents to 37° C, or the Cells Will be Irreparably Damaged

3. Pipette 6 ml of Trypsin/EDTA Solution into the T-75 flask. Rock the flask gently to ensure the solution covers all the cells.
4. Remove 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 2-5 minutes for the cells to become rounded. The cells may not become completely round during trypsinization and some cells may maintain some processes even though they are loosened from the culture surface.

NOTE: Do Not Over-Trypsinize the Cells or They Could be Irreparably Damaged

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 Rat Smooth Muscle 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, into Attachment Factor Solution coated flask.

REFERENCES

1. Ross, R., (1993) *Nature* **362**:801.
2. Heldin, C.H., *et al.* (1985) *Mol. Cell. Endocrinol.* **39**:169.
3. Berk, B.C., *et al.* (1990) *J. Biol. Chem.* **265**:17334.
4. Owen, G.K., *et al.* (1986) *J. Cell Biol.* **102**: 343.
5. Skalli, O., *et al.* (1986) *J. Cell Biol.* **103**(6): 2787.

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