

PrimaPure™



A division of Gene Therapy Systems, Inc.

Rat Cardiac Fibroblasts (RCF)

Catalog #	Description/Content	Amount
SR30605A	Rat Cardiac Fibroblasts (RCF)	>500,000 cells
SR30605AK	Rat Cardiac Fibroblast (RCF) Complete System	1 Kit*
*Each Complete System contains an ampoule of cryopreserved RCF (SR30605A), 500 ml of Rat Fibroblast Growth Medium (PMR116500), and a Subculture Reagent Kit (PR090100K).		

Related Products	Catalog #
Rat Fibroblast Growth Medium, 500 ml	PMR116500
Rat Fibroblast Basal Medium, 500 ml	PMR115500
Rat Fibroblast Growth Supplements	PMR116GS
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

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.
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INTRODUCTION

PrimaPure™ Rat Cardiac Fibroblasts (RCF) are derived from the heart tissue of normal Sprague Dawley rats. They are cryopreserved at second passage and can be cultured and propagated at least 8 population doublings. Rat Cardiac Fibroblasts are known to play key roles in the development of cardiac disease and hypertrophy¹. The regulation of fibroblast growth after acute myocardial infarction was determined in RCF². Cardiac fibroblasts were examined as a potential source of pro-inflammatory cytokine IL-6 synthesis which had been implicated in the pathophysiology of heart disease³. RCF have also been used to study the autocrine/paracrine capability mediated by vasodilator such as adrenomedullin⁴. RCF are used to investigate their role in local hormone metabolism⁵. Nitric oxide (NO) production in RCF decreases cAMP accumulation largely by the cGMP-mediated activation of PDE2⁶. IL-1 beta-induced inducible NO synthase expression can trigger NO-dependent apoptosis in adult RCF⁷.

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 Flasks For Culturing of RCF

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

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

1. Remove the cryopreserved vial of RCF from the liquid N₂ storage tank. Use proper protection for 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 Fibroblast 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 Rat Fibroblast Growth Medium after 24 hours or overnight to remove all traces of DMSO.
12. Change Rat Fibroblast Growth Medium every other day until the cells reach 60% confluent.
13. Double the Rat Fibroblast Growth Medium volume when the culture is >60% confluent or for weekend feedings.
14. Subculture the cells when the RCF reach 80% confluent.

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IV. Subculturing Rat Cardiac Fibroblasts

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 RCF

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

C. SUBCULTURING RCF.

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 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. 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.

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 2 ml of Rat Fibroblast 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.

REFERENCES

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