CELL
APPLICATIONS, INC.

General Instructions for Culturing Rat Cardiomyocytes (RCm)

Be sure to wear face protection mask and gloves when retrieving cryovials from the liquid nitrogen storage tank. The dramatic temperature change from the tank to the room could cause any trapped liquid nitrogen in the cryovials to burst and cause injury.

Open all the packages immediately upon arrival and examine each component for shipping damage. Notify Cell Applications, Inc. or your distributor immediately if there is any problem.

I. STORAGE

A. CULTURE FLASKS (R357-25, -75)
CULTURE PLATES (R357-6W, R357-96W)
CULTURE INSERTS (R357-6i, R357-12W, R357-24i)

1. Examine under a microscope to check if all the cells are attached to the bottom of the flask. If not, notify CAI or your distributor immediately.
2. Decontaminate the exterior of the cell culture flask with 70% alcohol.
3. Place the sealed flask in a 37°C, 5% CO_2 humidified incubator for 2 hours as shipped.
4. In a sterile Biological Safety Cabinet, open the cap of the flask very slowly and carefully.
5. Remove the Transport Medium by aspiration. Add fresh Growth Medium: 5 ml for a T-25 flask and 15 ml for a T-75 flask.
6. Place the flask in a 37°C, 5% CO_2 humidified incubator with loosened cap to allow gas exchange.
7. Change medium every other day.

B. GROWTH MEDIUM (R313-500)

Store the Growth Medium at 4°C in the dark immediately upon arrival.

II. PREPARATION FOR CULTURING

1. Make sure the 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 protective lab gear (lab coat, gloves, safety glasses, etc.) when working with cell cultures.
   c. Handle all cell culture work in a sterile hood.

III. CULTURING RCm

A. FLASKS AND PLATES

1. In a sterile Biological Safety Cabinet
   Flasks: open the cap of flasks very slowly and carefully
   Plates: remove the seal very slowly and carefully
2. Remove the Transport Medium by aspiration. Add fresh Growth Medium:
   5 ml for a T-25 flask and 15 ml for a T-75 flask.
   3 ml for 6 well, 2 ml for 12 well, 1 ml for 24 well
3. Place the flask in a 37°C, 5% CO_2 humidified incubator with loosened cap to allow gas exchange.
4. Change medium every other day.

B. INSERTS*

1. In a sterile Biological Safety Cabinet, open the cap of transport jar very slowly and carefully.
2. Prepare well plates for accommodate the inserts by adding fresh Growth Medium to each well: 3 ml for 6 well, 2 ml for 12 well, 1 ml for 24 well
3. Remove most of the Transport Medium by aspiration.
4. Pick up each inserts with sterile forceps and transfer to each well and immediately add Growth Medium to each insert to make the level of Growth Medium inside and outside insert equal.
5. Place the flask in a 37°C, 5% CO_2 humidified incubator with loosened cap to allow gas exchange.
6. Change medium every other day.

* Do not let cells in the inserts dry up during transfer.