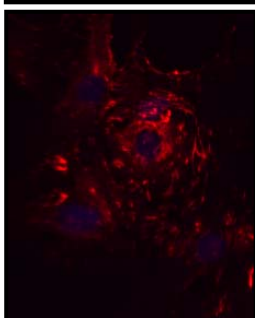


CARDIAC CELL SYSTEM INNOPROFILE™ RAT CARDIAC FIBROBLASTS



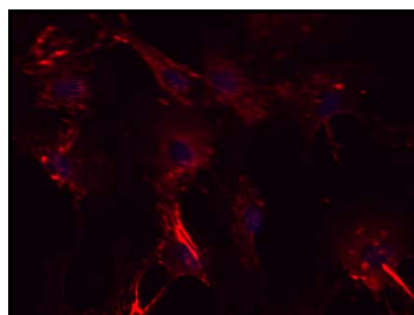
Product Type:	Cryo-preserved Cardiac Fibroblasts
Catalog Number:	P10402
Source:	Rat Heart Tissue (Sprague Dawley)
Number of Cells:	5 x 10 ⁵ Cells / vial (1ml)
Storage:	Liquid Nitrogen

Rat cardiac fibroblasts (RCF) are isolated from adult Sprague Dawley rat heart tissue. RCF are cryopreserved from passage one culture. RCF are guaranteed to further expand for 10 population doublings at the conditions recommended in the instructions provided by Innoprot.

The cardiac fibroblasts (CF) are the principal cell type of the heart. They provide structural support for cardiac myocytes and are responsible for extracellular matrix synthesis, which are involved in processes like inflammatory stimuli and can contribute to accelerate heart diseases. Cardiac Fibroblasts are an important cellular component of myocardial responses to injury and the source of paracrine growth factors. Their proliferation and synthesis of matrix is essential for scar formation at sites of myocardial infarction, cardiac fibrosis, and is often complicated by cardiac hypertrophy. CF culture has been widely used as a model to study the cardiac matrix remodeled by physiological (exercise) and pathological (hypertension) stressors.

Recommended Medium

- Fibroblast Medium
(Reference: P60108)



Product Characterization

Immunofluorescent method

- Fibronectin

The cells test negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi

Product Use

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in vitro diagnostic or clinical procedures

INSTRUCTIONS FOR CULTURING CELLS

IMPORTANT: Cryopreserved cells are very delicate. Thaw the vial in a 37 °C waterbath and return them to culture as quickly as possible with minimal handling!

Set up culture after receiving the order:

1. Prepare a poly-L-lysine coated flask (2 $\mu\text{g}/\text{cm}^2$, T-25 flask is recommended). Leave the flask in incubator overnight (minimum one hour at 37°C incubator).
2. Prepare complete medium: decontaminate the external surfaces of medium and medium supplements with 70% ethanol and transfer them to sterile field. Aseptically open each supplement tube and add them to the basal medium with a pipette. Rinse each tube with medium to recover the entire volume.
3. Rinse the poly-L-lysine coated flask with sterile water twice and add the appropriate amount of medium to the vessel (4 ml/T-25 or 200 μl per well for M96) and allow the flask to equilibrate in 37°C, 5% CO₂ incubator for at least 30 min.
4. Place the vial in a 37°C waterbath, hold and rotate the vial gently until the contents are completely thawed. Remove the vial from the waterbath immediately, wipe it dry, rinse the vial with 70% ethanol and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads with fingers. Using 1 ml eppendorf pipette gently resuspend the contents of the vial.
5. Dispense the contents of the vial into the equilibrated, poly-L-lysine coated culture vessels. A seeding density of 10,000 cells/cm² is recommended. *Note: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of DMSO residue in the culture. It is also important that fibroblasts are plated in poly-L-lysine coated culture vessels that promote cell attachment.*
6. Replace the cap or cover, and gently rock the vessel to distribute the cells evenly. Loosen cap if necessary to permit gas exchange.
7. Return the culture vessels to the incubator.
8. For best result, do not disturb the culture for at least 16 hours after the culture has been initiated. Change the growth medium the next day to remove the residual DMSO and unattached cells, then every other day thereafter. A healthy culture will display stellate or spindle-shaped cell morphology, nongranular cytoplasm, and the cell number will be double after two to three days in culture.

Maintenance of Culture:

1. Change the medium to fresh supplemented medium the next morning after establishing a culture from cryopreserved cells. For subsequent subcultures, change medium 48 hours after establishing the subculture.
2. Change the medium every other day thereafter, until the culture is approximately 50% confluent.
3. Once the culture reaches 50% confluence, change medium every day until the culture is approximately 80% confluent.

Subculture:

1. Subculture the cells when they are over 90% confluent.
2. Prepare poly-L-lysine coated cell culture flasks.
3. Warm medium, trypsin/EDTA solution, trypsin neutralization solution, and DPBS to room temperature. We do not recommend warming the reagents and medium at 37°C waterbath prior to use.
4. Rinse the cells with DPBS.
5. Incubate cells with 10 ml of trypsin/EDTA solution (in the case of T-75 flask) until 80% of cells are rounded up (monitored with microscope). Add 10 ml of trypsin neutralization solution to the digestion immediately and gently rock the culture vessel.
6. Harvest and transfer released cells into a 50 ml centrifuge tube. Rinse the flask with another 10 ml of growth medium to collect the residue cells. Examine the flask under microscope to make sure the harvesting is successful by looking at the number of cells left behind. There should be less than 5%.
7. Centrifuge the harvested cell suspension at 1000 rpm for 5 min and resuspend cells in growth medium.
8. Count cells and plate them in a new, poly-L-lysine coated flask with cell density as recommended.

Caution: Handling human derived products is potentially biohazardous. Although each cell strain testes negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions must be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].

- [1]. Grizzle, W. E., and Polt, S. S. (1988) Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues. *J Tissue Culture Methods*. 11(4).