

# Protocol for CardioCluster Creation

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## Method Article

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# Abstract

Cellular therapy to treat heart failure is an ongoing focus of intense research, but with limited progress. Engineered augmentation of established cellular effectors overcomes impediments, enhancing reparative activity. Such 'next generation' implementation includes delivery of combinatorial cell populations working together synergistically. Concurrent isolation and expansion of three distinct cardiac-derived interstitial cell types from human heart tissue prompted design of a 3D structure that maximizes cellular interaction, allows for defined cell ratios, controls size, enables injectability, and minimizes cell loss. Here we describe a method wherein, mesenchymal stem cells (MSCs), endothelial progenitor cells (EPCs) and c-Kit<sup>+</sup> cardiac interstitial cells (cCICs) when cultured together spontaneously form scaffold-free 3D microenvironments termed CardioClusters.

## Introduction

## Reagents

96 well, ultra-low attachment multiwell round-bottom plates (Corning, catalog #CLS7007)

## Equipment

## Procedure

### CardioCluster Formation

CardioClusters are formed using 96 well, ultra-low attachment multiwell round-bottom plates (Corning, catalog #CLS7007) in a two-step process.

Step 1 generates the inner core composed of cCICs and MSCs in a 1:2 ratio. The inner core of cCICs and MSCs is seeded in 100  $\mu$ L/well MSC media for 24 hours at 37°C in 5% CO<sub>2</sub> incubator.

Step 2 forms the outer EPC layer using a cell number equal to the number of cells used to create the central core. The EPCs are added in an additional 50  $\mu$ L/well MSC media and incubated at 37°C in 5% CO<sub>2</sub> for an additional 24 hours until CardioCluster 3D structure has formed.

The radius of a CardioCluster is approximately 150  $\mu$ m, composed of a total of 400 $\pm$ 100 cells.

## Troubleshooting

## Time Taken

48 hours

## **Anticipated Results**

The formation of a CardioCluster approximately 150  $\mu\text{m}$  in diameter, composed of a total of  $400\pm 100$  cells.

## **References**

## **Acknowledgements**