FUJIFILM Cellular Dynamics

Quick Guide

iCell® Cardiac Fibroblasts

Handling and Storage

Upon receipt, immediately transfer components to the proper storage temperature.

Component	Storage Temperature
iCell® Cardiac Fibroblasts Cryovial	Vapor Phase of Liquid Nitrogen
iCell Cardiomyocytes Maintenance Medium	-20°C

iCell Cardiac Fibroblasts can be co-cultured with iCell Cardiomyocytes or iCell Cardiomyocytes[2] (see Table 2 for a list of companion products). When establishing a co-culture, both cell types are combined directly from thaw. For co-culture thawing and plating directions, refer to the relevant iCell Cardiac Fibroblasts Application Protocol (see Table 3).

Preparing Cell Culture Surfaces

The recommended ECM for 2D culture of iCell Cardiac Fibroblasts is Vitronectin (VTN-N).

1. Thaw frozen stock solution of vitronectin to room temperature.
2. Dilute vitronectin in DPBS to 2.5 µg/ml. Mix by inversion and do not vortex.
3. Dispense vitronectin solution into cell culture vessel according to the table below.

Culture Vessel	VTN-N solution
12-well Cell Culture Plate	1 ml
96-well Cell Culture Plate	100 µl

*All volumes and measures are **per well.***

4. Incubate at room temperature for at least 1 hour.
5. Store coated vessels at 4°C for up to 1 week.

Preparing the Medium

1. Thaw iCell Cardiomyocytes Maintenance Medium overnight at 4°C.
2. Store at 4°C for up to 2 weeks.
3. Equilibrate medium to room temperature before each use.

Thawing the Cells

1. Thaw iCell Cardiac Fibroblasts in a 37°C water bath for 3 minutes.
2. Clean cryovial with 70% ethanol and transfer contents to a sterile 50 ml tube.
3. Rinse cryovial with 500 µl of medium and slowly transfer content drop-wise over 30 seconds to the 50 ml centrifuge tube while gently swirling.
4. Slowly add 1 ml of medium drop-wise to the 50 ml centrifuge tube while gently swirling to mix.
5. Gently pipette the cell suspension to mix 2 times to ensure maximum viability.

Avoid vigorous shaking or vortexing of the cells.

6. The final volume of cell suspension is 2 ml.

Plating the Cells

1. Equilibrate the plates at room temperature for at least 1 hour.
2. Dilute the cell suspension with iCell Cardiomyocytes Maintenance Medium to obtain the desired cell plating density using the total viable cells from the Certificate of Analysis. See table below for plating density examples.

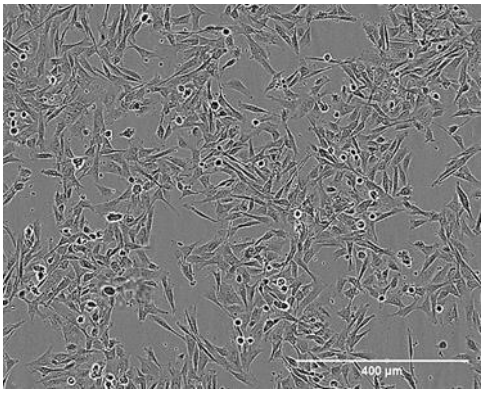

Figure 1: iCell Cardiac Fibroblasts, 01434
10X image of iCell Cardiac Fibroblasts at 24 hours post-plating (78k viable cells/cm^2).

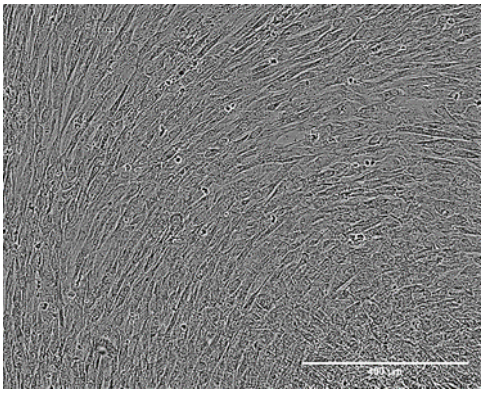

Figure 2: iCell Cardiac Fibroblasts, 01434
10X image of iCell Cardiac Fibroblasts at Day 4 post-plating (78k viable cells/cm^2).

Table 1: Required Consumables

Component	Vendor	Catalog #
Culture Vessels, Sterile, TC Grade	Multiple Vendors	
Vitronectin[1]	Thermo Fisher	A14700
Dulbecco's Phosphate Buffered Saline without Ca2+ and Mg2+ (D-PBS)	Thermo Fisher	14190

[1] *Reconstitute according to manufacturer's recommendations.*

Table 2: Companion iCell Products

Product	Catalog #
iCell Cardiomyocytes, 01434	C1006, C1056
iCell Cardiomyocytes, 11713	C1106, C1105
iCell Cardiomyocytes[2], 01434	C1016, C1058

Culture Vessel	Surface Area	Plating Volume	Cell Number	Cell Density (Viable cells/ml)
12-well Cell Culture Plate	3.8 cm^2	1.5 ml	380,000	250,000
96-well Cell Culture Plate	0.32 cm^2	100 µl	25,000	250,000

*All volumes and measures are **per well.***

***Note:** Optimal plating density may vary by application. See Table 3 for iCell Cardiac Fibroblasts Application Protocols.*

3. Gently pipette the cells suspension two times to mix.
4. Remove the vitronectin coating from the cell culture vessel.
5. Dispense the cells into the cell culture vessel.
6. Culture the cells at 37°C, 5% CO_2.

Maintaining the Cells

1. Equilibrate medium to room temperature prior to use.
2. Replace medium every other day.
3. Culture the cells at 37°C, 5% CO_2.

*Do **NOT** passage the cells. Use for co-culture experiments within 4-5 days post-thaw.*

Table 3: Co-Culture Application Protocols

Application Protocols[1]
1. Culturing and Assaying Calcium Transients of 3D Cardiac Tri-Culture Microtissues
2. Evaluating iCell Cardiomyocytes² Co-culture with iCell Cardiac Fibroblasts on the FLEXcyte 96 System

[1] *Available at fujifilmcdi.com*

Contacting Technical Support

Email: fcdi-support@fujifilm.com

Phone: 1-877-320-6688

Revision History
Document ID: X1052
Version 1.0: August 2023