FUJIFILM Cellular Dynamics

Quick Guide

iCell® Cardiomyocytes², *01434*

1.25 x 10^6 viable cells/vial

***NOTE:* This protocol is only applicable for the 1.25x10^6 size of iCell® Cardiomyocytes², 01434.**

Handling and Storage

Upon receipt, immediately transfer the cryovial(s) to liquid nitrogen storage.

Store medium at -20ºC until use.

Preparing Cell Culture Surfaces

For glass coverslips for immunocytochemistry or electrophsiological applications, see the iCell Cardiomyocytes Application Protocols available online at www.fujifilmcdi.com/product-literature/.

1. Add the volume of 0.1% gelatin solution specified in the table below. Scale volumes accordingly.

Culture Vessel	Surface Area (cm²)	0.1% Gelatin Solution Volume (ml)
6-well Cell Culture Plate	9.6	3
96-well Cell Culture Plate	0.32	0.1

2. Incubate culture vessels at 37ºC for at least 1 hour.
3. Aspirate gelatin solution immediately before adding the cell suspension

Preparing the Plating and Maintenance Medium

1. Thaw medium overnight at 4ºC.
2. Store at 4ºC for up to 2 weeks.
3. Store single use aliquots at -20ºC; avoid multiple freeze and thaw cycles.

Thawing the Cells

1. Equilibrate Plating Medium at room temperature.
2. Thaw iCell Cardiomyocytes² cryovial in a 37°C water bath for 2 minutes. Clean with 70% ethanol.
3. Transfer contents to sterile 50 ml centrifuge tube.
4. Rinse vial with 650 µl of Plating Medium. Transfer rinse drop-wise over 90 seconds to the 50 ml centrifuge tube while gently swirling.
5. Slowly add 1 ml of Plating Medium to the 50 ml centrifuge tube. Add this drop-wise over 40 - 60 seconds while gently swirling.
6. Gently pipette the cell suspension to mix 2 times to ensure maximum viability. Avoid vigorous shaking or vortexing.

Plating the Cells

1. Dilute the cell suspension with Plating 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.

Culture Vessel	Surface Area (cm²)	Plating Volume (ml)	Cell Number (156,000 cells/cm²)	Cell Density (cells/ml)
6-well Cell Culture Plate	9.6	3	~1,500,000	~500,000
96-well Cell Culture Plate	0.32	0.1	~50,000	~500,000

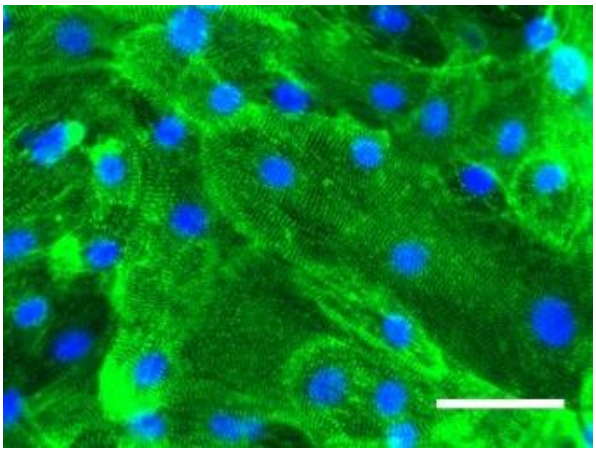

Figure 1: iCell Cardiomyocytes², 01434
Day 7 post-plating: Sarcomeric alpha-actinin (SAA, green) and Hoechst (blue)

Table 1: Required Consumables for Preparing Cell Culture Surfaces

Component	Vendor
Cell Culture Vessels, Sterile, TC Grade	Multiple Vendors
0.1% Gelatin in Water	STEMCELL Technologies 07903
0.4% Trypan Blue Solution	Gibco 15250

Contacting Technical Support

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2. Gently pipette the cell suspension 2 times to mix.
3. The recommended plating density for syncytial formation is 156,000 viable cells/cm^2.
4. Remove a sample to confirm viability using a hemocytometer.
5. Aspirate the gelatin solution from the pre-coated vessel.
6. Invert the cell suspension 2 - 3 times to mix.
7. Dispense the cells into the cell culture vessel.
8. Culture the cells at 37°C, 5% CO_2 for **4 hours**.

Maintaining the Cells

1. Equilibrate Maintenance Medium in a 37ºC water bath prior to use.
2. Four (4) hours post-plating, perform 100% medium change.
3. Replace the Maintenance Medium every other day.
4. Culture the cells at 37°C, 5% CO_2.

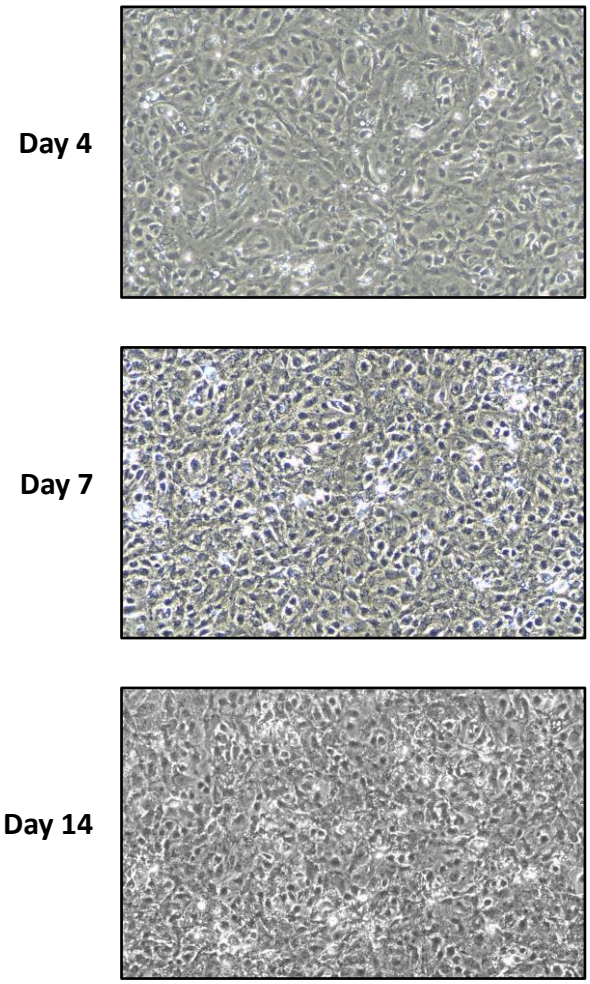

Figure 2: iCell Cardiomyocytes[2]
A stable, contracting syncytium is formed relatively quickly and cell viability remains high throughout 14 days of culture.

Revision History
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