




CELLular
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iCell® Cardiomyocytes
User's Guide



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CDI does not in any way guarantee or represent that you will obtain satisfactory results from using iCell Cardiomyocytes as described herein. The only warranties provided to you are included in the Limited Warranty enclosed with this guide. You assume all risk in connection with your use of iCell Cardiomyocytes.

Conditions of Use

iCell Cardiomyocytes are for life science research use only and subject to the use restrictions contained in Appendix A. You are responsible for understanding and performing the protocols described within. CDI does not guarantee any results you may achieve. These protocols are provided as CDI’s recommendations based on its use and experience with iCell Cardiomyocytes.

Origin

iCell Cardiomyocytes are manufactured in the United States of America.

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Before You Begin

- Immediately transfer the frozen vials to liquid nitrogen storage.
- Read this entire iCell® Cardiomyocytes User's Guide before handling or using iCell Cardiomyocytes.
- iCell Cardiomyocytes are for life science research use only. See Appendix A for more information and other restrictions.
- A Material Safety Data Sheet (MSDS) for dimethyl sulfoxide (DMSO), in which iCell Cardiomyocytes are frozen, is available online at www.cellulardynamics.com/lit/ or on request from Cellular Dynamics International. Only technically qualified individuals experienced in handling DMSO and human biological materials should access, use, or handle iCell Cardiomyocytes.

Notes

Chapter 1. Introduction

Cellular Dynamics International's (CDI) iCell Cardiomyocytes are highly purified, human cardiomyocytes derived from induced pluripotent stem (iPS) cells through CDI's proprietary differentiation and purification protocols. iCell Cardiomyocytes are a mixture of spontaneously electrically active atrial, nodal, and ventricular-like myocytes with typical biochemical, electrophysiological, and mechanical characteristics and expected responses upon exposure to exogenous agents. Thus, these cells provide a reliable source of human cardiomyocytes suitable for use in targeted drug discovery, toxicity testing, and other life science research.

When thawed and plated with iCell Cardiomyocytes Plating Medium and maintained in iCell Cardiomyocytes Maintenance Medium as instructed in this User's Guide, iCell Cardiomyocytes will begin to beat spontaneously within 24 - 48 hours. When plated at appropriate densities, iCell Cardiomyocytes also will form electrically connected syncytial layers that beat in synchrony. A wash step at 48 hours post-thaw using the Maintenance Medium will remove non-adhered cells, leaving a population of plated, electrically and mechanically active cardiomyocytes that are ready for use.

iCell Cardiomyocytes Maintenance Medium is antibiotic-free and has been specially formulated to maintain the health and function of the cardiomyocytes while limiting the proliferation of the small percentage of non-cardiomyocyte cells. iCell Cardiomyocytes therefore can be maintained in culture for at least 2 weeks in the Maintenance Medium without appreciable loss of purity, enabling longer term studies. Thus, the combination of CDI's purification process and adherence to the procedures described in this User's Guide makes additional use of antibiotics unnecessary.

Components Supplied by Cellular Dynamics

Notes

Item	Catalog Number
iCell Cardiomyocytes*	(1 unit) CMC-100-110-001 ⁺
	(5 unit) CMC-100-110-005 ⁺
	(1 unit) CMC-100-010-001 [#]
	(5 unit) CMC-100-010-005 [#]
iCell Cardiomyocytes Plating Medium*	(1 unit) CMM-100-110-001
	(5 unit) CMM-100-110-005
iCell Cardiomyocytes Maintenance Medium*	(1 unit) CMM-100-120-001
	(5 unit) CMM-100-120-005
iCell Cardiomyocytes User's Guide Also available online at www.cellulardynamics.com/lit/	
Certificate of Analysis Also available online at www.cellulardynamics.com/coa/	
Certificate of Origin	

* Material Safety Data Sheets available online at www.cellulardynamics.com/lit/

+ Contains monomeric red fluorescent protein (RFP) expressed under control of the endogenous Myh6 promoter

No RFP expression

Required Equipment and Consumables

Item	Vendor	Catalog Number
Equipment		
Liquid Nitrogen Storage Unit	Multiple Vendors	
Biological Safety Cabinet with UV Lamp	Multiple Vendors	
Tabletop Centrifuge	Multiple Vendors	
37 °C Water Bath	Multiple Vendors	
Cell Culture Incubator	Multiple Vendors	
Hemocytometer or Automated Cell Counter	Multiple Vendors	
Phase Contrast Microscope	Multiple Vendors	
Optional Equipment		
Floating Microcentrifuge Tube Rack	Multiple Vendors	
Consumables		
Pipettors and Pipettes	Multiple Vendors	
50 ml Centrifuge Tubes	Multiple Vendors	
Suitable Cell Culture Vessels (sterile, TC grade)	Multiple Vendors	
15 mm Coverslips - Electrophysiology Applications	Warner Instrument Corp.	64-0703
Trypan Blue	Gibco	15250
Gelatin, Type A, Cell Culture Tested	Sigma-Aldrich	G1890
Distilled Water	Multiple Vendors	

Technical Support and Training

CDI's Technical Support Scientists have the necessary laboratory and analytical experience to respond to your inquiries. In addition, hands-on training is available (www.cellulardynamics.com/training/) or watch our Handling iCell Cardiomyocytes Training Video (www.cellulardynamics.com/cm_handling/). In-lab training may be available upon request.

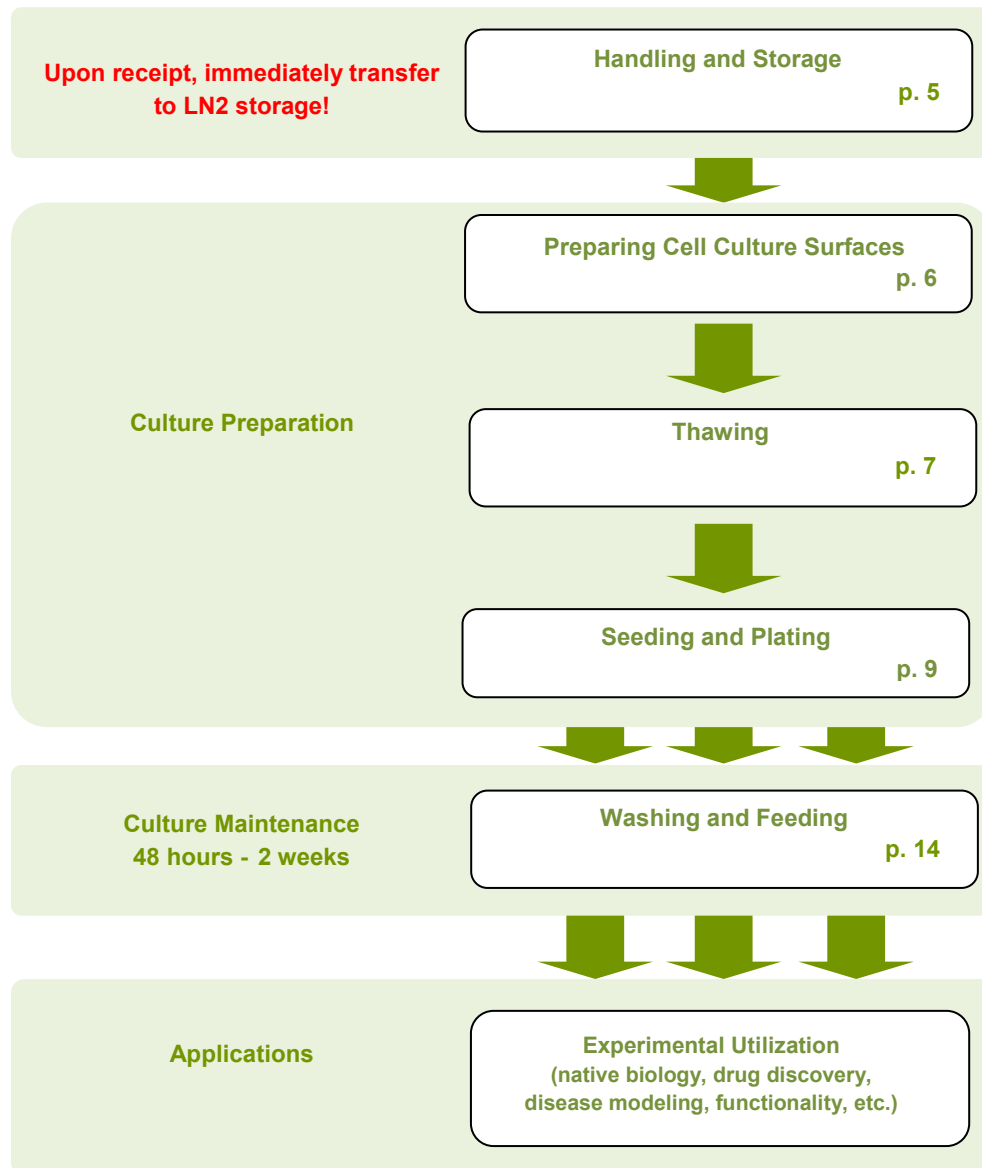
Telephone (877) 310-6688 x5 (US toll-free) / (608) 310-5100 x5
Monday - Friday, 8:30 am - 5:00 pm US Central Time

Fax (608) 310-5101

Email support@cellulardynamics.com

Workflow Diagram

Notes



Chapter 2. Handling and Storage

Handling iCell Cardiomyocytes

iCell Cardiomyocytes are provided as cryopreserved single-cell suspensions in 1 ml cryovials. Upon receipt, directly transfer the cryobox containing the iCell Cardiomyocytes to the vapor phase of liquid nitrogen storage. We strongly recommend transferring the entire cryobox into the storage racks. Avoid transferring individual vials.



It is critical to maintain cryopreserved iCell Cardiomyocytes at a stable temperature. Minimize exposure of cryopreserved iCell Cardiomyocytes to ambient temperature when transferring vials to liquid nitrogen storage.

Handling iCell Cardiomyocytes Media

iCell Cardiomyocytes Plating Medium and iCell Cardiomyocytes Maintenance Medium are shipped frozen on dry ice. Store at -20°C before use and 4°C for up to 2 weeks after thaw. If media will be used for longer than two weeks, aliquot and freeze again after the initial thaw. Do not subject media to more than a single refreeze and thaw cycle.

Chapter 3. Preparing Cell Culture Surfaces

iCell Cardiomyocytes will plate and function on a variety of substrates. Freshly prepared gelatin, collagen, PDL-laminin, and fibronectin have been shown to support attachment, viability, and function of iCell Cardiomyocytes with similar efficiencies. Coating plates with a 0.1% gelatin solution is an economical, simple, and recommended method for preparing cell culture plates for culturing iCell Cardiomyocytes.

CDI provides application notes that recommend assay-specific substrates. See www.cellulardynamics.com/lit/ for a list of available application notes for iCell Cardiomyocytes. Regardless of the substrate of choice, prepare plating surfaces before thawing iCell Cardiomyocytes.

Preparing 0.1% (w/v) Gelatin Solution

1. Prepare the bottles for mixing the 0.1% gelatin solution.
 - Do not use bottles that have previously contained or been exposed to detergent.
 - Acid-wash new bottles before use.
 - Use only high-purity, 18M Ω sterile water for washing bottles.
2. Measure 500 mg of gelatin (Type A, powder, cell culture tested) and add this amount to each bottle.
3. Add 500 ml of sterile water to each bottle.
4. Sterilize the gelatin solution by autoclaving using liquid cycle.

Note: 0.1% gelatin solution can be filter sterilized, but it will be necessary to heat the solution to 60°C to ensure that the gelatin has completely dissolved before filtering. Ensure that the final volume is 500 ml before filtering.

The sterile 0.1% gelatin solution is stable for 3 months when prepared as described above and stored at room temperature.

Preparing the Plate or Coverslip Surface

1. Select the culture dish appropriate for your experimental use, i.e. 96-well plate, 6-well plate, or 15 mm coverslips in each well of a 12-well plate, etc.
2. Add gelatin:
 - **96-well plate:** Pipette 100 μ l of 0.1% gelatin solution into each well.
 - **6-well plate:** Pipette 2 ml of 0.1% gelatin solution into each well.
 - **15 mm coverslip in each well of a 12-well plate:** Add 1 ml of 0.1% gelatin to each well to cover coverslips with gelatin solution.
3. Incubate in 37°C cell culture incubator for at least 1 hour.
4. Aspirate gelatin solution immediately before addition of cell suspension.

Chapter 4. Thawing iCell Cardiomyocytes and Media

Thawing iCell Cardiomyocytes Plating Medium

Thaw iCell Cardiomyocytes Plating Medium overnight at 4 °C in preparation for next-day thawing of the iCell Cardiomyocytes. Any unused portion of iCell Cardiomyocytes Plating Medium can be stored at 4 °C for 2 weeks.

Note: In the remainder of this chapter, iCell Cardiomyocytes Plating Medium is referred to as Plating Medium.

Remove iCell Cardiomyocytes Plating Medium from 4 °C and place at room temperature for 2 - 4 hours before thawing cells. Ensure enough Plating Medium is thawed to allow for proper dilution. Calculate the amount of Plating Medium necessary to thaw for each unit of cardiomyocytes as follows:

$$\text{Plating Medium to Thaw (ml)} = \frac{6 \times 10^6 \text{ Cells} \times \text{Viability} \times \text{Plating Efficiency}}{\text{Target Plating Density (cells/ml)}}$$

Viability and Plating Efficiency are batch specific and listed on the Certificate of Analysis. Plating Efficiency and Target Plating Density are further defined in Chapter 5.

Thawing iCell Cardiomyocytes

Maintain iCell Cardiomyocytes in liquid nitrogen until immediately before thawing to ensure maximal performance of the cells. Complete the following steps of the thawing procedure in a time-efficient manner to facilitate optimal iCell Cardiomyocytes viability and performance.

Note: Thaw no more than three vials of iCell Cardiomyocytes at one time.

1. Remove the frozen iCell Cardiomyocytes cryovial from the liquid nitrogen storage tank.

Note: If necessary, cryovials can be placed on dry ice for up to 10 minutes before thawing.

2. Place the vial in a 37 °C water bath for 4 minutes (avoid submerging the cap) and hold stationary (no swirling). Use of a floating microcentrifuge tube rack is recommended.



Precise timing of the placement is critical to maximizing viable cell recovery.

3. Immediately remove the cryovial from the water bath following the 4 minute incubation, spray with 70% ethanol, and place into the biological safety cabinet.
4. Gently transfer the iCell Cardiomyocytes cryovial contents to a 50 ml centrifuge tube using a 1 ml pipettor.

Note: Use of a 50 ml centrifuge tube facilitates suitable mixing to minimize osmotic shock and increase cardiomyocyte viability.



Avoid repeated pipetting of the thawed iCell Cardiomyocytes cell suspension.

5. Rinse the empty iCell Cardiomyocytes cryovial with 1 ml of room temperature Plating Medium to recover residual cells from the vial. Transfer the Plating Medium rinse from the cryovial to the thawed cardiomyocytes by adding the rinse drop-wise over 90 seconds (i.e. one drop every 4 - 5 seconds) to the 50 ml centrifuge tube while gently swirling the tube after addition of each drop.



Drop-wise addition of Plating Medium to the iCell Cardiomyocytes cell suspension is critical to ensure maximum viability and attachment of the cells once plated. It is recommended that the first ml of media added to the cell suspension is added evenly over ~90 seconds, i.e. one drop every 4 - 5 seconds. Following the addition of each drop, the suspension is mixed thoroughly by gently swirling the tube. Refer to our Handling iCell Cardiomyocytes Training Video (www.cellulardynamics.com/cm_handling/).

6. Add 8 ml of room temperature Plating Medium to the 50 ml centrifuge tube. Add the first 1 ml in a drop-wise fashion over 30 - 60 seconds. Then add the remaining 7 ml over the next ~30 seconds.



It is critical to slowly add the 8 ml of room temperature Plating Medium to the thawed cardiomyocytes to ensure maximum viability and attachment. Refer to our Handling iCell Cardiomyocytes Training Video (www.cellulardynamics.com/cm_handling/).

7. Gently mix the contents of the 50 ml centrifuge tube by inverting 2 - 3 times. Gentle inversion is critical to ensure maximum viability.

The cell suspension is now ready for cell counting and seeding.

Accurate assessment of viable cell number and seeding of correct cell numbers is critical for the subsequent steps of this protocol. Following counting, be sure to invert the thawed cardiomyocyte suspension 2 - 3 times to ensure an even cardiomyocyte distribution before plating.

Note: You can thaw up to three iCell Cardiomyocytes vials at a time. When thawing multiple vials, combine the thawed contents of the cryovials before adding the rinse and final volume of Plating Medium. Follow the timing outlined in steps 4 and 5. For example, if pooling three cryovials, add each 1 ml of rinse over 90 seconds (270 seconds total).

Chapter 5. Plating iCell Cardiomyocytes

iCell Cardiomyocytes are provided as a highly pure, single-cell suspension of cryopreserved cardiomyocytes. Following the protocols described in this User's Guide, you can expect a percentage of the seeded, viable cardiomyocytes to attach to the cell culture plates, a metric we refer to as *Plating Efficiency*. *Plating Efficiency* is calculated from the following characteristics of the thawed iCell Cardiomyocytes single-cell suspension:

Viable Cell Count	The total number of viable cells received from cell counting analysis of the single-cell suspension immediately following thaw.
Viable Cell Density	The number of viable cells/ml received from cell counting analysis of the single-cell suspension immediately following thaw.
Seeded Cell Count	The total number of viable cells added to the cell culture well.
Plated Cell Count	The number of viable cells that have firmly attached to the cell culture well after 48 hours.
Plating Efficiency	$\text{Plating Efficiency} = \frac{\text{Plated Cell Count}}{\text{Seeded Cell Count}} \times 100$ <p>Example: If 600,000 cells were seeded, and 350,000 plated cells were recovered after 48 hours in culture, the <i>Plating Efficiency</i> for that particular lot of cells would be calculated as follows:</p> $58.3\% = \frac{350,000 \text{ Plated Cells}}{600,000 \text{ Cells Seeded}} \times 100$ <p>Note: <i>Plating Efficiency is a quality control metric that is determined for each lot of iCell Cardiomyocytes and is found on the Certificate of Analysis provided with each shipment. Using the lot number, you can also look up your Certificate of Analysis on the Cellular Dynamics website (www.cellulardynamics.com/coa).</i></p>



Because iCell Cardiomyocytes are non-proliferative, you may need to initially seed a higher number of cardiomyocytes to achieve the same appearance as cultures of proliferating cells.

The near 100% purity of iCell Cardiomyocytes in combination with the minimal outgrowth of contaminating cells when cultured in iCell Cardiomyocytes Maintenance Medium allows highly pure cultures to be maintained for at least 2 weeks.

Note: In the remainder of this chapter, iCell Cardiomyocytes Plating Medium is referred to as Plating Medium and iCell Cardiomyocytes Maintenance Medium as Maintenance Medium.

Determining Viable Cell Density

Quantification of viable cells is performed using an automated cell counter or a hemocytometer (using trypan blue exclusion to identify viable cells). This step is required to determine the *Viable Cell Density* (in cells/ml) of the thawed iCell Cardiomyocytes cell suspension. *Viable Cell Density* and *Viable Cell Count* are related by the following equation:

$$\text{Viable Cell Density} = \frac{\text{Viable Cell Count}}{\text{Cell Suspension Volume (ml)}}$$

Note: If your application requires higher cell densities, simply pellet iCell Cardiomyocytes using a tabletop centrifuge at 180 x g for 5 minutes, remove the necessary amount of *Plating Medium* to achieve the desired density, and gently resuspend the iCell Cardiomyocytes. Note that over-pipetting could reduce cell viability.

Plating iCell Cardiomyocytes for Cell-based Assays

iCell Cardiomyocytes perform well in many cell-based assays, including cell viability, apoptosis, and mitochondrial function. The optimal density of plated cardiomyocytes per unit of surface area can be assay dependent and must be determined empirically based on the intended use. However, a density of ~47,000 - 63,000 cardiomyocytes / cm² provides a beating syncytium and is a good place to start for most cell-based assays. The following table provides the desired cell number per well and seeding volume per well for a variety of common cell culture plates / vessels.

Note: This table provides a guide for syncytial formation only. See the application notes available online at www.cellulardynamics.com/lit/ for assay-specific densities and seeding volumes.

Culture Vessel	Growth Area (cm ²)	Recommended Cell Number	Recommended Seeding Volume (ml)
96-well plate	0.32	15 - 20x10 ³	0.1
24-well plate	1.9	90 - 120x10 ³	0.6
12-well plate	3.8	180 - 240x10 ³	1.2
6-well plate	9.5	450 - 600x10 ³	3
T25 flask	25	1.2 - 1.6x10 ⁶	8

Table 1: Summary of Recommended Volumes and Measures

All volumes and measures are *per well*, if applicable.

The number of viable cells that attach is dependent on the *Plating Efficiency* of the cell lot. *Plating Efficiency* is measured for each lot of iCell Cardiomyocytes and is found on the Certificate of Analysis provided with each lot/shipment. Using the lot number, you can also look up your Certificate of Analysis on the Cellular Dynamics website (www.cellulardynamics.com/coa/).

Plating in 96-well Plates for Cell-based Assay Applications

The recommended plated density for performing cell-based assays in 96-well plates using iCell Cardiomyocytes is 15,000 to 20,000 plated cardiomyocytes per well. However as mentioned in the previous section, the optimal plated density is dependent on the biology in question as well as the sensitivity of the assay and must be determined empirically.

The following procedure describes how to plate 15,000 cells/well in a 96-well plate assuming a Seeding Volume of 100 μ l per well, a *Plating Efficiency* of 45%, a post-thaw *Viable Cell Density* of 0.4×10^6 cells/ml, and an actual post-thaw cell volume of 9.5 ml (0.5 ml was used to attain the *Viable Cell Density*).

This same procedure can be used to plate cells in other culture dish formats by substituting the appropriate recommended cell numbers and seeding volumes.

1. Prepare the thawed cell suspension for plating into a 96-well plate.
 - a. Obtain the *Plating Efficiency* from the Certificate of Analysis. Alternatively, you can use the lot number to look up specific Certificate of Analyses on the Cellular Dynamics website (www.cellulardynamics.com/coal/). (For purposes of this example, assume the *Plating Efficiency* is 45%.)
 - b. Obtain the *Viable Cell Density* (cells/ml) from the “Determining Viable Cell Density” section earlier in this chapter. (For purposes of this example, assume the *Viable Cell Density* is 0.4×10^6 cells/ml.)
 - c. Calculate the *Target Plating Density*.

$$\text{Target Plating Density} = \frac{\text{Desired Cell Number per Well}}{\text{Seeding Volume per Well (ml)}}$$

In this example, the desired number of plated cells per well is 15,000, and the suggested seeding volume is 100 μ l. Thus, the *Target Plating Density* is

$$0.15 \times 10^6 \text{ cells/ml} = \frac{15,000 \text{ cells per well}}{0.1 \text{ ml}}$$

- d. Calculate the *Total Plating Volume* necessary to bring the thawed cell suspension to the *Target Plating Density*.

$$\text{Total Plating Volume} = \frac{\text{Actual Volume (ml)} \times \text{Viable Cell Density (cells/ml)} \times \text{Plating Efficiency}}{\text{Target Plating Density (cells/ml)}}$$

where *Actual Volume* is the post-thaw suspension volume after cell counting. Thus, the *Total Plating Volume* is

$$11.4 \text{ ml} = \frac{9.5 \text{ ml} \times 0.4 \times 10^6 \text{ cells/ml} \times 0.45}{0.15 \times 10^6 \text{ cells/ml}}$$

- e. Add sufficient Plating Medium to the thawed cell suspension to achieve the calculated *Total Plating Volume*.

2. Aliquot the *Total Plating Volume* into one or more 96-well plates.
 - a. Obtain gelatin-coated 96-well plate(s). For more information, see Chapter 3, Preparing Cell Culture Surfaces.
 - b. After aspirating the gelatin from the wells, gently mix the cell suspension and dispense seeding volume aliquots of cell suspension (i.e. 100 μ l) to each well of the 96-well plate using a multichannel pipettor.
 - c. Place plate in cell culture incubator at 37°C, 7% CO₂.

Media exchange and recommended washing procedure before assaying the cells are described in Chapter 6, Maintaining iCell Cardiomyocytes.

Expected Plating Densities

The images in Figure 1 illustrate the expected coverage that can be obtained by following the provided plating instructions. iCell cardiomyocytes were added to a 96-well plate at the indicated seeding densities to obtain the desired plating densities. The cells were allowed to recover for 48 hours and then labeled with Calcein-acetoxymethylester (calcein-AM), a non-fluorescent, cell permeant compound that is converted by intracellular esterases into the cell-impermeant fluorescent dye calcein. Here the dye is used to specifically demonstrate cell viability, to indicate their homogenous distribution, and to provide morphological information regarding the expected substrate coverage. The left column of images shows an entire well while the right column shows a close-up image from that well. Note that the cells have not been plated long enough to form syncytia.

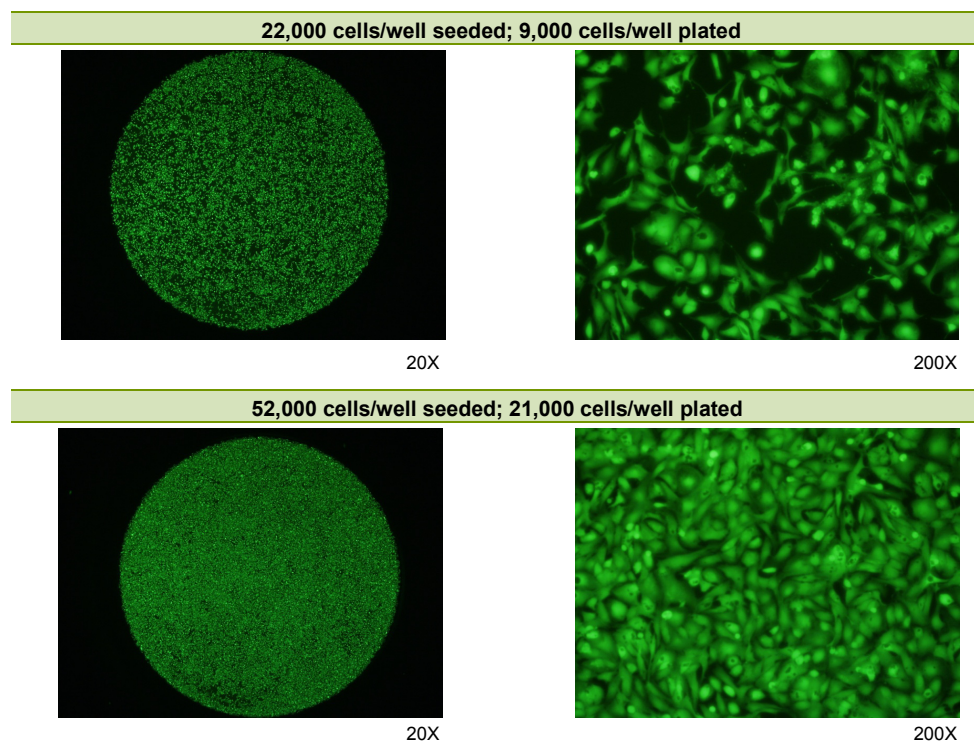


Figure 1: Examples of iCell Cardiomyocytes Plating Density

The above images illustrate the expected density of calcein-positive cells after 48 hours of incubation. These iCell Cardiomyocytes had a Plating Efficiency of ~40%.

Plating iCell Cardiomyocytes for Manual Patch Clamp Applications

Electrophysiological techniques such as conventional whole-cell voltage clamp and microelectrode array (MEA) methodologies are excellent techniques for measuring the electrical behavior of cardiomyocytes and their reactions to exogenously applied agents at the single cell and population level, respectively. iCell Cardiomyocytes are fully functional human cardiomyocytes that express cardiac Na^+ , Ca^{2+} , and K^+ channels in their native environment. Thus the electrical activity and controllable in vitro environmental conditions of the iCell Cardiomyocytes test system provides an ideal cardiac model for drug discovery and detection of potential compound toxicity.

Plating for Conventional Patch Clamp Recording

1. Obtain 12-well plate(s) containing gelatin-coated coverslips. For more information, see Chapter 3, Preparing Cell Culture Surfaces.
2. Seed 40,000 - 80,000 viable cardiomyocytes in 2 ml of Plating Medium into each well. Do not allow the coverslips to dry before cell addition.
3. Incubate for at least 2 days in a standard cell culture incubator at 37°C, 7% CO_2 .
4. Remove non-adhered cells and debris by rinsing with Maintenance Medium 48 hours after the initial plating step.
5. Feed the plated cardiomyocytes by exchanging the Maintenance Medium every other day following removal of non-adherent cells and debris.

Chapter 6. Maintaining iCell Cardiomyocytes

iCell Cardiomyocytes are shipped cryopreserved, at high purity. The cells maintain a high purity for at least 2 weeks after plating if plated in the iCell Cardiomyocytes Plating Medium and maintained in iCell Cardiomyocytes Maintenance Medium as recommended.

Note: In the remainder of this chapter, iCell Cardiomyocytes Plating Medium is referred to as Plating Medium and iCell Cardiomyocytes Maintenance Medium as Maintenance Medium.



Thaw the Maintenance Medium at 4°C overnight before its use. This medium is stable for 2 weeks when stored at 4°C. Aliquoting the media into single use volumes post-thaw and subsequent storage at 4°C in tightly sealed containers will minimize pH changes.

1. One day before use, thaw the Maintenance Medium overnight at 4°C.
2. Immediately before use, warm the Maintenance Medium in a 37°C water bath.
3. 48 hours after plating, gently wash off the non-adherent cells and debris by pipetting the medium up and down approximately 5 times while gently washing the surface of the plate with a single- or multi-channel pipette or serological pipette.

Note: Alternatively, aspirate the Plating Medium to remove the non-adherent cells and perform 2 washes with Maintenance Medium changes of appropriate volume, gently washing the surface of the plate with each medium change.

4. Aspirate the Plating Medium in the well and replace with the appropriate volume of Maintenance Medium. Be careful not to touch or disrupt the adhered cardiomyocytes. Recommended volumes are the same as those listed Chapter 5, Seeding Volumes.
5. Exchange media every other day after plating. Culture iCell Cardiomyocytes in a standard cell culture incubator at 37°C, 7% CO₂.

Chapter 7. Applications in Cell-based Assays

iCell Cardiomyocytes can be used for cell-based assays. See the application notes available online at www.cellulardynamics.com/lit/. CDI has tested iCell Cardiomyocytes with a variety of assay platforms including the following:

Item	Vendor	Catalog Number
CellTiter-Glo Luminescent Cell Viability Assay	Promega	G7570
MultiTox-Fluor Multiplex Cytotoxicity Assay	Promega	G9200
MultiTox-Glo Multiplex Cytotoxicity Assay	Promega	G9270
CellTiter-Fluor Cell Viability Assay	Promega	G6080
Caspase-Glo 3/7 Assay	Promega	G8090
CyQUANT Cell Proliferation Assay	Invitrogen	C7026
JC-10 Mitochondrial Membrane Potential Assay	ABD Bioquest	22800



We recommend performing cell-based assays no earlier than 48 hours after plating. The wash step at 48 hours to remove non-attached cells and debris before the start of the assay is critical for assay performance. Typically cell-based assays are performed after the formation of a beating syncytium.

The results shown below demonstrate the function of iCell Cardiomyocytes in general viability, apoptosis, and mitochondrial membrane potential assays.

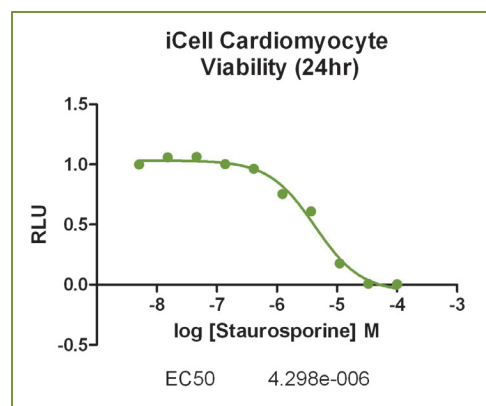


Figure 2: iCell Cardiomyocytes Viability Assay

iCell Cardiomyocytes with a Plating Efficiency of 56% were seeded at 34,000 viable cells/well (~19,000 plated cells/well) and plated for 48 hours in 0.1% gelatin-coated 96-well plates before washing away unattached cells. A 3-fold serial dilution series of staurosporine (AG Scientific, S-1016) diluted in iCell Cardiomyocytes Maintenance Medium was applied to triplicate wells per concentration. Staurosporine concentrations ranged from 15 nM to 100 μ M. Cell viability was measured using the CellTiter-Glo Luminescent Cell Viability Assay (Promega, G7570) after 24 hours of staurosporine treatment using a Tecan GENios Pro microplate reader (1 second/well integration time).

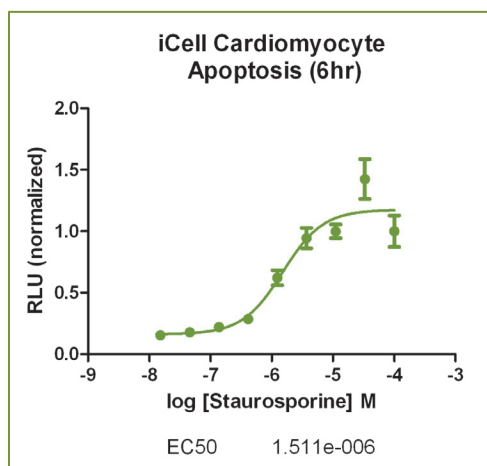


Figure 3: iCell Cardiomyocytes Apoptosis Assay

iCell Cardiomyocytes with a Plating Efficiency of 56% were seeded at 34,000 viable cells/well (~19,000 plated cells/well) and plated for 48 hours in 0.1% gelatin-coated 96-well plates before washing away unattached cells. A 3-fold serial dilution series of staurosporine (AG Scientific, S-1016) diluted in *iCell Cardiomyocytes Maintenance Medium* was applied to triplicate wells per concentration. Staurosporine concentrations ranged from 15 nM to 100 μ M. Caspase activity was measured using the Caspase-Glo 3/7 Assay (Promega, G8091) after 6 hours of staurosporine treatment using a Tecan GENios Pro microplate reader (1 second/well integration time). Error bars represent standard deviation of triplicate wells.

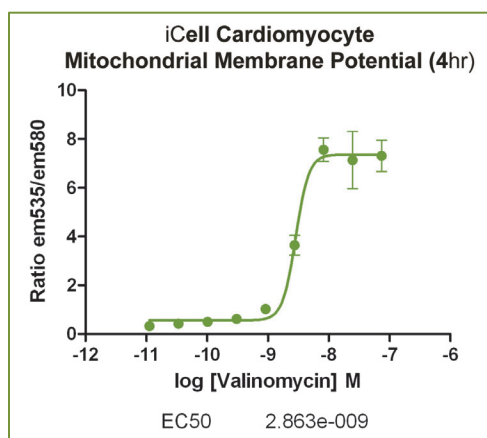


Figure 4: iCell Cardiomyocytes Mitochondrial Membrane Potential Assay

iCell Cardiomyocytes with a Plating Efficiency of 56% were seeded at 34,000 viable cells/well (~19,000 plated cells/well) and plated for 48 hours in 0.1% gelatin-coated 96-well plates before washing away unattached cells. Cells were cultured for another 96 hours with Maintenance Medium before mitochondrial membrane potential assay. A 3-fold serial dilution series of valinomycin (Fluka, 94675) diluted in Maintenance Medium was applied to triplicate wells per concentration. Valinomycin concentrations ranged from 11 μ M to 74 nM. Mitochondrial membrane potential was measured using the JC-10 Assay (ABD Bioquest, 22800) after 4 hours of valinomycin treatment with a Tecan GENios Pro microplate reader (ex490/em535 and ex490/em580 filters). Error bars represent standard deviation of triplicate wells.

Appendices

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Notes

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