Cellular Dynamics international

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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Edition

Version 1, December 2009
Version 1.1, September 2010

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Before You Begin

- Read the entire iCell Cardiomyocytes User's Guide before handling or using iCell Cardiomyocytes.
- iCell Cardiomyocytes are for life science research use only. Please see Appendix A for more information.
- Material Safety Data Sheets (MSDS) for the dimethyl sulfoxide (DMSO), in which iCell Cardiomyocytes are frozen, are included with this shipment and available on request from Cellular Dynamics. Only technically qualified individuals experienced in handling DMSO and human biological materials should have access to, use, or handle iCell Cardiomyocytes.

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. The cells express monomeric red fluorescent protein (mRFP) and blasticidin resistance, both under the control of the alpha-myosin heavy chain (Myh6) promoter that allows simultaneous cardiomyocyte purification and identification. iCell Cardiomyocytes are a mixture of spontaneously electrically active atrial, nodal, and ventricular-like myocytes that possess typical electrophysiological characteristics and exhibit 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 set forth in this User's Guide, iCell Cardiomyocytes will begin to beat spontaneously within 24 to 48 hours. When plated at appropriate densities, iCell Cardiomyoctyes also will form electrically connected syncytial layers that beat in synchrony. A wash step at 48 hours post-thaw with iCell Cardiomyocytes Maintenance Medium will remove non-adhered cells and leave a population of plated, electrically and mechanically active cardiomyocytes that are ready for use.

iCell Cardiomyocytes Maintenance Medium has been specially formulated to limit the proliferation of the small percentage of non-cardiomyocyte cells while maintaining the health and function of the cardiomyocytes. iCell Cardiomyocytes can be maintained in culture for up to two weeks in iCell Cardiomyocytes 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 within this User's Guide makes additional use of antibiotics unnecessary.

Components Supplied by Cellular Dynamics

Item	Description	Catalog Number
iCell Cardiomyocytes	As ordered	(1X) CMC-100-110-001 (5X) CMC-100-110-005
iCell Cardiomyocytes Plating Medium	Cellular Dynamics	(1X) CMM-100-110-001 (5X) CMM-100-110-005
iCell Cardiomyocytes Maintenance Medium	Cellular Dynamics	(1X) CMM-100-210-001 (5X) CMM-100-210-005
iCell Cardiomyocytes User's Guide	One booklet. Also available online at www.cellulardynamics.com	NA
Certificate of Analysis	One certificate. Also available online at www.cellulardynamics.com/coa	NA

Required Equipment and Consumables

Item	Vendor	Catalog Number
Equipment		
Liquid Nitrogen Storage Unit	Multiple Vendors	NA
Biological Safety Cabinet with UV Lamp	Multiple Vendors	NA
Desktop Centrifuge	Multiple Vendors	NA
37°C Water Bath	Multiple vendors	NA
Cell Culture Incubator	Multiple vendors	NA
Consumables		
Pipettors and Pipettes	Multiple Vendors	NA
15 mL Centrifuge Tubes	Multiple vendors	NA
6-well Cell Culture Plates	Nunc	140675
12-well Cell Culture Plates	Corning/Costar	3513
96-well Cell Culture Plates	Corning/Costar	3603
15mm Coverslips - Electrophysiology applications	Warner Instrument Corp	64-0703
Trypan Blue	Gibco	15250
D-PBS	Invitrogen	14190, 14040
Gelatin, Type A, Cell Culture Tested	Sigma-Aldrich	G1890
Distilled Water	Multiple Vendors	NA
100 mm Tissue Culture Dish	Multiple Vendors	NA
Tergazyme Solution	Multiple Vendors	NA
Hemocytometer or Automated Cell Counter	Multiple Vendors	NA
Dissecting Scope	Multiple Vendors	NA

Technical Support

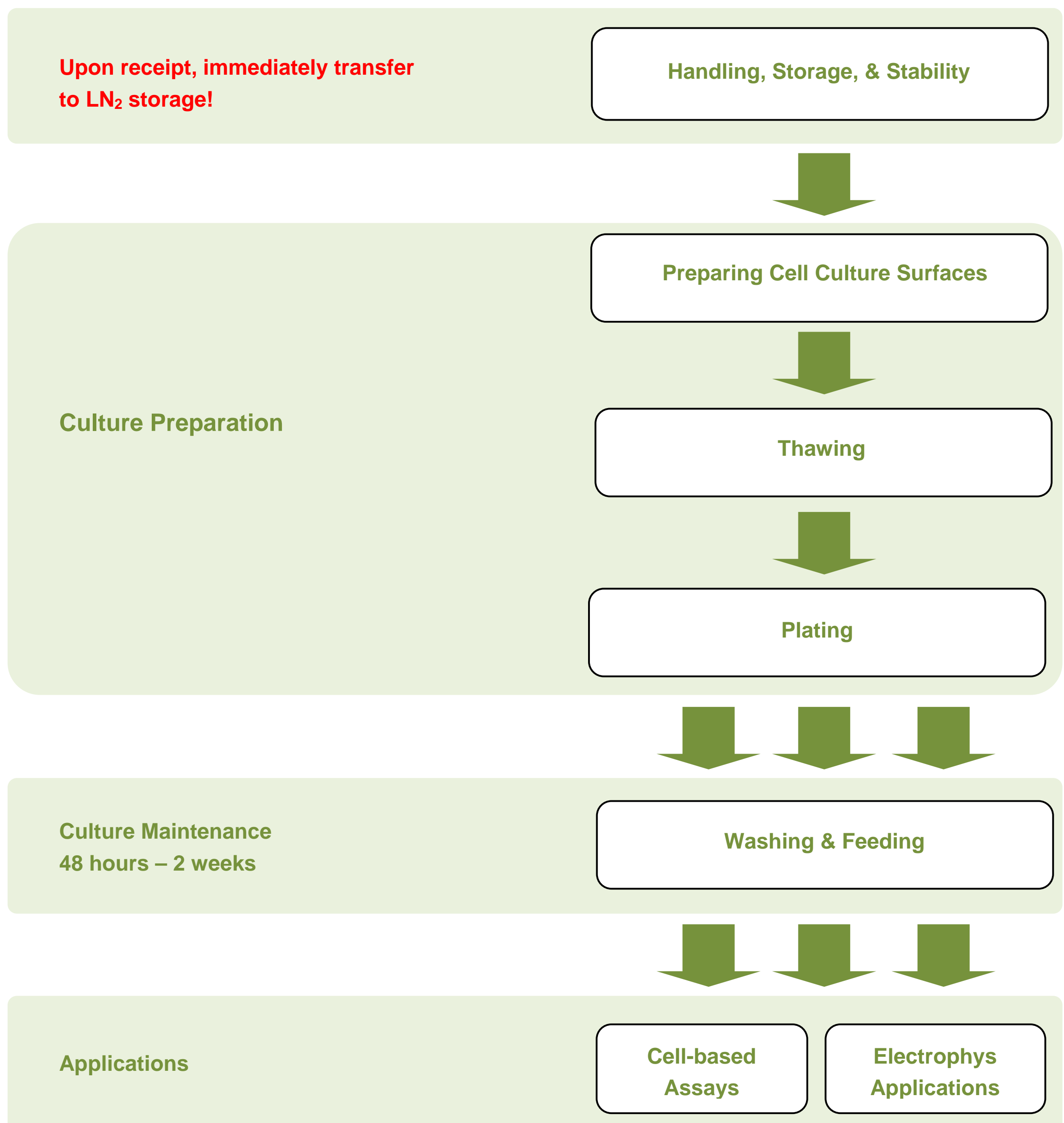
Cellular Dynamics' Technical Support scientists have the necessary laboratory and analytical experience to respond to your inquiries.

Telephone (877) 310-6688X5 (US toll-free) / (608) 310-5100X5
Monday - Friday, 8:30am to 5:00pm US Central Time

Fax (608) 310-5101

Email support@cellulardynamics.com

Workflow Diagram



Chapter 2. Handling, Storage, and Stability

Handling iCell Cardiomyocytes

iCell Cardiomyocytes are provided as cryopreserved single-cell suspensions in 1 mL cryovials. When received, ***immediately transfer the frozen vials to liquid nitrogen storage***, preferably storing the vials in the vapor phase of the liquid nitrogen storage unit.

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. We strongly recommend transferring the entire cryobox containing iCell Cardiomyocytes into liquid nitrogen storage. Avoid transferring individual vials.

Handling iCell Cardiomyocytes Media

iCell Cardiomyocytes Plating Medium and iCell Cardiomyocytes Maintenance Medium are shipped frozen on dry ice. When received, ***immediately transfer frozen media to -20°C storage***.

iCell Cardiomyocytes Plating Medium and iCell Cardiomyocytes Maintenance Medium should be stored at 4°C after thawing and should be used within two weeks.

Chapter 3. Preparing Cell Culture Surfaces

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

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 prior to use.
   - Use only high-purity, 18MΩ sterile water for washing the bottles.
2. Using a calibrated balance, measure 500 mg of gelatin (Type A, powder, cell culture tested, Sigma-Aldrich G1890), and add this amount to each bottle.
3. Using a graduated cylinder, add 500 mL of sterile water to each bottle.
4. Autoclave sterilize for 30 minutes on liquid cycle.

   Note: *0.1% gelatin solution may be filter sterilized, but it will be necessary to heat the solution to 60˚C to ensure that the gelatin has completely dissolved prior to filtering. Ensure that the final volume is 500 mL prior to filtration.*

Sterile 0.1% gelatin solution prepared as described above and stored at room temperature is stable for up to 3 months.

Preparing the Plate or Coverslip Surface

1. Select the culture dish appropriate for your experimental use: 96-well plate, 6-well plate, or 15 mm coverslips in each well of a 12-well plate.
2. Add gelatin:
   - ***96-well plate:*** Pipette 100 µL 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 prior to 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 the iCell Cardiomyocytes Plating Medium can be stored at 4°C for up to two weeks. The iCell Cardiomyocytes Plating Medium should be maintained at 4°C for use in the thawing protocol below.

Thawing iCell Cardiomyocytes

Maintain iCell Cardiomyocytes in liquid nitrogen until immediately prior to 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 and immerse the vial (avoid submerging the cap) in a 37°C water bath.

   Note: *After removal from liquid nitrogen storage, cryovials may be placed on dry ice for up to 10 minutes prior to thawing.*

2. <u>Gently</u> swirl the cryovial in the water bath until the cryopreserved cell suspension is fully thawed (avoid submerging the cap in the water bath).
3. When the cryopreserved cell suspension is fully thawed, immediately remove the cryovial from the water bath, spray it with 70% ethanol, and place it in a biological safety cabinet.
4. Gently transfer the iCell Cardiomyocytes cryovial contents to a 15 mL centrifuge tube using a 1 mL pipettor.

Avoid repeated pipetting of the thawed iCell Cardiomyocytes cell suspension.

5. Rinse the empty iCell Cardiomyocytes cryovial with 1 mL of cold iCell Cardiomyocytes Plating Medium to recover residual cells from the vial and add, drop-wise, with a 1 mL pipettor to the 15 mL centrifuge tube while <u>gently</u> swirling the tube.

Drop-wise addition of the iCell Cardiomyocytes Plating Medium to the iCell Cardiomyocytes cell suspension is <u>critical</u> to ensure maximum viability and attachment of the cells once plated.

6. Add 1 mL of cold iCell Cardiomyocytes Plating Medium drop-wise to the 15 mL centrifuge tube, and then slowly add the remaining 7 mL.

It is <u>critical</u> to add the 8 mL of cold iCell Cardiomyocytes Plating Medium slowly to ensure maximum viability and attachment of the cells once plated.

7. Gently mix the contents of the 15 mL centrifuge tube by inverting it 2 - 3 times.

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

Note*: You can thaw up to three iCell Cardiomyocytes vials at one time. However, each vial must be thawed according to the outlined procedure (that is, 9 mL of iCell Cardiomyocytes Plating Medium for each vial (1 mL for transferring residual cells and 8 mL for dilution). Once diluted, you can pool the cells for plating according to the specific needs of the user.*

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 viable cardiomyocytes to plate and spread on the cell culture plates. Throughout this User's Guide, we refer to this metric 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 the thawing protocol.
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	$Plating\ Efficiency = \frac{Plated\ Cell\ Count}{Seeded\ Cell\ Count}\ x\ 100$ ***Example:*** If 600,000 cells were seeded, and 350,000 plated cells were recovered after 2 days in culture, the *Plating Efficiency* for that particular lot of cells would be calculated as: $58.3\% = \frac{350{,}000\ cells\ plated}{600{,}000\ cells\ seeded}\ x\ 100$ *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 lot/ shipment. (Using the lot number, you can also look up your Certificate of Analysis on the Cellular Dynamics website at www.cellulardynamics.com/coa)*

Since iCell Cardiomyocytes are non-proliferative, it is critical to determine the density at which to seed the cell culture plates to achieve the proper plated cell density for your intended application. As such, you may need to initially seed a higher number of cardiomyocytes (relative to dividing cell types) in order to achieve the same appearance as cultures plated with a proliferative cell type.

The combination of the high cardiomyocyte purity of iCell Cardiomyocytes and the ability to minimize contaminating cell outgrowth when cultured in the iCell Cardiomyocytes Maintenance Medium facilitates the maintenance of a high cardiomyocyte purity culture with consistent confluence for up to 2 weeks in culture.

Determining Viable Cell Count

Quantification of viable cells is performed using an automated cell counter or a hemocytometer (using Trypan blue exclusion to identify viable cells) 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:

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

Note: *If necessary, you can further dilute the cell suspension to achieve the desired cell density with iCell Cardiomyocytes Plating Medium to ensure maximal Plating Efficiency.*

If the Viable Cell Density obtained from the cell counting procedure is too low for your application, pellet the iCell Cardiomyocytes in a tabletop centrifuge at 400 X g for 8 minutes and resuspend in iCell Cardiomyocytes Plating Medium to the appropriate density, taking care to gently resuspend the cell pellet with the minimal required pipetting.

Plating iCell Cardiomyocytes for Cell-based Assays

iCell Cardiomyocytes perform well in many cell-based assays, including cell viability, apoptosis, and mitochondrial function assays. The optimal plated cardiomyocyte density is dependent on the sensitivity of the assay and must be determined empirically based on the intended use of the cells. The actual number of viable cells that attach is dependent on the *Plating Efficiency* of the cell batch. *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 at www.cellulardynamics.com/coa.)

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

The recommended plated cell density for performing cell-based assays in 96-well plates using iCell Cardiomyocytes is 10,000 to 20,000 plated cells per well. The optimal plated cardiomyocyte density is dependent on the sensitivity of the assay and must be determined empirically based on the intended use of the cells.

The following procedure describes how to plate 15,000 cells/well in a 96-well plate assuming a plating volume of 100μL per well, a *Plating Efficiency* of 45%, a post-thaw viable iCell Cardiomyocytes density of 0.5 X10^6 cells/mL, and an actual post-thaw cell volume of 9.5mL (0.5mL was used to attain the *Viable Cell Count*).

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 your Certificate of Analysis on the Cellular Dynamics website at www.cellulardynamics.com/coa. (For purposes of this example, assume the *Plating Efficiency* is 45%.)

   b. Obtain the *Viable Cell Density* (cells/mL) from the "Determining Viable Cell Count" section earlier in this chapter. (For purposes of this example, assume the Viable Cell Density is 0.5 X 10^6 cells/mL.)

   c. Calculate the *Target Plating Density*.

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

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

   $$0.15x106\ cells/mL = \frac{15{,}000\ cells\ per\ well}{0.1\ mL}$$

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

   $$Total\ Plating\ Volume = \frac{Actual\ Volume\ (mL)\ x\ Actual\ Density\ (cells/mL)\ x\ Plating\ Efficiency}{Target\ Plating\ Density\ (cells/mL)}$$

   where *Actual Volume* and *Actual Density* are the post-thaw suspension volume (after cell counting) and *Viable Cell Density*, respectively. Thus, the *Total Plating Volume* is

   $$14.25\ mL = \frac{9.5\ mL\ x\ 0.5x10^6\ \ cells/mL\ x\ 0.45}{0.15x10^6\ \ cells/mL}$$

   e. Add sufficient iCell Cardiomyocytes 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.

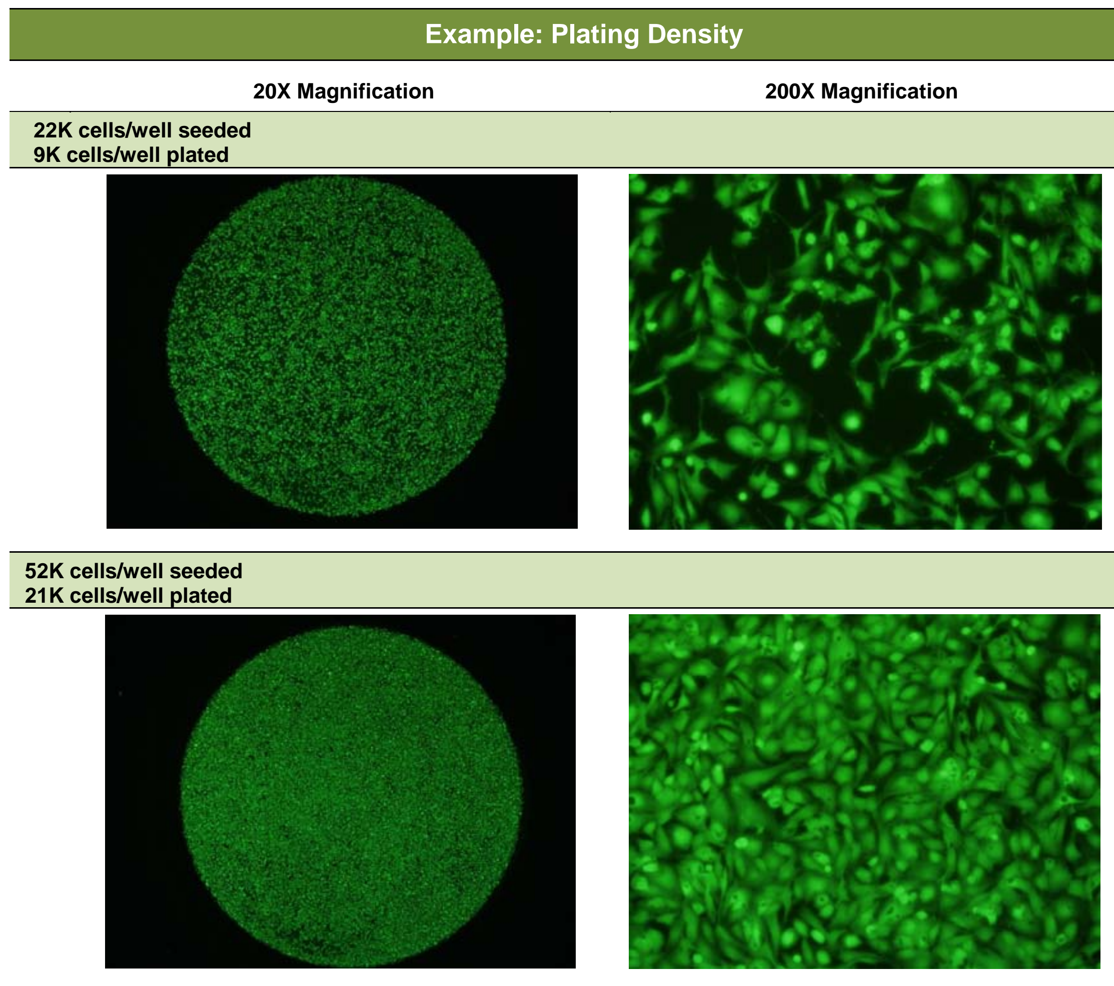
   b. After aspirating the gelatin from the wells, gently mix the cell suspension and dispense *Seeding Volume* aliquots of cell suspension (i.e. 100µL) to each well of the 96-well plate using a multichannel pipettor.

   c. Place plate in cell culture incubator at 37°C, 7%CO_2.

Media exchange and recommended washing procedure prior to assaying the cells is described in Chapter 6, Maintaining iCell Cardiomyocytes and specifically for cell-based assays in Chapter 7, Applications in Cell-based Assays.

Expected Plating Densities

The images below 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-hand column of images shows an entire well while the right hand column of images shows a close-up image from that well. Note that the cells have not been plated long enough to form syncitia.



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 Electrophysiology Applications

Electrophysiological techniques such as conventional whole-cell patchclamp 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 20,000 to 40,000 cardiomyocytes in 2 mL iCell Cardiomyocytes Plating Medium into each well. Do not allow the coverslips to dry out prior to cell addition.
3. Incubate for at least two days in a standard cell culture incubator at 37°C, 7% CO_2.
4. Remove non-adhered cells and debris by rinsing with iCell Cardiomyocytes Maintenance Medium 48 hours after the initial plating step.
5. Feed the plated cardiomyocytes by exchanging the iCell Cardiomyocytes Maintenance Medium every 2 – 3 days following removal of non-adherent cells and debris.

Plating for Microelectrode Assay (MEA) Recording

The procedure for plating iCell Cardiomyocytes onto microelectrode array (MEA) plates is a multi-step process consisting of pre-plating freshly thawed cells, preparing MEA plates, and plating onto the prepared plates. The detailed procedure below is specific for the multi-channel 6-well plate but is suitable for single-well plates as well.

1. Thaw iCell Cardiomyocytes. For more information, see Chapter 4, Thawing iCell Cardiomyocytes and Media.
2. Obtain gelatin-coated 6-well plate(s). For more information, see Chapter 3, Preparing Cell Culture Surfaces.
3. Divide the final volume of cell suspension in iCell Cardiomyocytes Plating Medium equally into individual wells of the 6-well plate, adding 2 – 5 mL final volume per well. Each well should be allocated no more than 1.5 million seeded cardiomyocytes.
4. Incubate in a standard cell culture incubator at 37°C, 7% CO_2 for 24 hours.
5. Remove non-adhered cells and debris by rinsing with 2 mL iCell Cardiomyocytes Maintenance Medium, twice.
6. Incubate for an additional 48 hours in 3 mL of iCell Cardiomyocytes Maintenance Medium in a standard cell culture incubator at 37°C, 7% CO_2.

7. Prepare the 6-well MEA chip.

   a. Place a dry 6-well MEA chip into a sterile 100 mm tissue culture dish or equivalent.

   b. Add 3 mL of sterile water to the bottom of the dish to prevent substrate evaporation. Do not allow water into the wells of the MEA chip.

   c. Place a 2 µL bead of fibronectin (1:20 in D-PBS; 50 µg/mL) or equivalent substrate over recording electrode area of each well of the MEA chip.

      Note: *If a bead fails to form in any well, use a different MEA chip.*

   d. Incubate the MEA chip at least 60 minutes in a standard cell culture incubator at 37°C.

8. Collect cardiomyocytes from the 6-well plate.

   a. Aspirate media from each well of the 6-well plate containing the iCell Cardiomyocytes.

   b. Rinse cells with 1 - 2 mL D-PBS, and aspirate D-PBS.

   c. Add 1 mL 0.25% trypsin-EDTA to each well.

      Cells will begin to lift off the plate within 1 - 2 minutes.

   d. Add 3 mL iCell Cardiomyocytes Maintenance Medium to each well to quench trypsin.

   e. Gently triturate the cardiomyocytes 5 - 8 times to complete the detachment process and to break apart remaining cell clumps.

   f. Transfer trypsin / iCell Cardiomyocytes Maintenance Medium suspension to a 15 mL centrifuge tube.

   g. Rinse each well with 2 mL iCell Cardiomyocytes Maintenance Medium, and add to the same 15 mL tube.

   h. Pellet cells with desktop centrifuge for 5 minutes at 180 x *g*.

   i. Aspirate the supernatant, being careful not to disturb the cardiomyocyte pellet. Resuspend cell pellet in 5 mL iCell Cardiomyocytes Maintenance Medium.

   j. Count the cells using a hemacytometer.

   k. Suspend cells in iCell Cardiomyocytes Maintenance Medium to a final concentration of 15,000 cells/µL.

   l. If necessary, pellet cells with desktop centrifuge for 5 minutes at 180 x *g* and resuspend the cells in iCell Cardiomyocytes Maintenance Medium to a final volume of 15,000 cells/µL.

9. Plate the iCell Cardiomyoctyes onto the MEA chip.

   a. Remove fibronectin-coated MEA chip from incubator and aspirate fibronectin or equivalent substrate from each well.

b. Under a dissecting scope, place a bead of the iCell Cardiomyocytes suspension over the recording electrode area of each well of the MEA chip.
   - Use approximately 2 μL for each array on a 6-well chip.
   - Use approximately 10 μL for the array on a single-well chip.

   Note: *If a bead fails to form in any well, use a different MEA chip.*

c. After seeding all wells, incubate the MEA chip in a standard cell culture incubator at 37°C for 1 - 2 hours. Confirm cell attachment at this point by observing the MEA wells under the microscope.

d. Gently add 100 μL of iCell Cardiomyocytes Maintenance Medium to each well of the MEA chip. Move quickly enough so other beads do not dry up but slow enough so as to not disturb the plated cells. Media can be added to the MEA chip in the biosafety cabinet or the dissecting hood.

e. Gently add 400 μL of iCell Cardiomyocytes Maintenance Medium to each well for a final volume of 500 μL per well.

f. Incubate in a standard cell culture incubator at 37°C, 7% CO_2.

10. For optimal conditions, perform MEA recordings 3 – 5 days after plating on MEA chip.

    Note: *Monolayers may be suitable for recording on later days, but the cardiomyocytes may begin to pile up on top of each other at these later dates.*

Cleaning the MEA Chip

1. Rinse the MEA chip with sterile water.
2. Clean the chip with 1% Tergazyme solution for 15 minutes – 1 hour.
3. Rinse the chip with sterile water to remove any remaining Tergazyme solution.
4. Repeat steps 1 – 3 until all debris is removed. All debris must be cleaned from the plate prior to adding alcohol. Failure to do so will fix the debris to the MEA chip.
5. Fill wells with 70% ethanol and let soak for a minimum of 1 hour to a maximum of 24 hours.
6. UV radiate the MEA chip in a Biological Safety Cabinet or other sterile environment for 30 minutes.
7. Aspirate ethanol and allow to dry in a Biological Safety Cabinet for 2 days. The plates must be completely dry for substrate coating to bead.

Chapter 6. Maintaining iCell Cardiomyocytes

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

Thaw iCell Cardiomyocytes Maintenance Medium at 4°C overnight prior to its use. This medium is stable for two weeks when stored at 4°C.

1. One day prior to use, thaw the iCell Cardiomyocytes Maintenance Medium overnight at 4°C.
2. Immediately prior to use, warm the iCell Cardiomyocytes 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 media 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 iCell Cardiomyocytes Plating Medium to remove the non-adherent cells and perform two washes with iCell Cardiomyocytes Maintenance Medium changes of appropriate volume, gently washing the surface of the plate with each medium change.*

4. Aspirate the iCell Cardiomyocytes Plating Medium in the well and replace with the appropriate volume of iCell Cardiomyocytes Maintenance Medium. Recommended volumes are:
   - 100 μL/well for 96-well plates
   - 3 mL/well for 6-well plates
   - 0.5 mL/well for single-well MEA plates or 1 mL/well for 6-well MEA plates
5. Exchange media every other day for up to 2 weeks after plating. iCell Cardiomyocytes should be cultured in a standard cell culture incubator at 37°C, 7% CO_2.

Chapter 7. Applications in Cell-based Assays

iCell Cardiomyocytes can be used for cell-based cytotoxicity, apoptosis, and mitochondrial membrane potential assays. CDI has tested iCell Cardiomyocytes with a variety of assay platforms, including:

Item	Vendor	Catalog
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

Cell-based Assay Methods

The recommended plating density for iCell Cardiomyocytes is 10,000 to 20,000 plated cells per well of a 96-well plate. The optimal density is dependent on the sensitivity of the assay and must be determined empirically based on the intended use of the cells. The actual number of plated cells that attach is dependent on the *Plating Efficiency* of the cell batch. *Plating Efficiency* is measured for each lot of iCell Cardiomyocytes and this information 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 at www.cellulardynamics.com/coa.)

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 prior to the start of the assay may improve assay performance.

iCell Cardiomyocytes express monomeric RFP, which may interfere with certain fluorescence-based assays.

1. After 48 hours, gently pipette the iCell Cardiomyocytes Plating Medium in the wells up and down approximately 5 times to wash the non-adherent cells off the plate.

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

2. Aspirate the iCell Cardiomyocytes Plating Medium and replace with 90 μL iCell Cardiomyocytes Maintenance Medium.

3. Dilute test compounds to 10X maximum test concentration in iCell Cardiomyocytes Maintenance Medium.

4. Make serial dilution series from the 10X maximum test concentration for each compound in iCell Cardiomyocytes Maintenance Medium.

   ***Note**: If the test compounds are dissolved in a non-aqueous solvent, such as DMSO, the serial dilution series should be made in iCell Cardiomyocytes Maintenance Medium containing the same concentration of solvent as the 10X maximum desired test concentration solution.*

   iCell Cardiomyocytes are tolerant to 1% DMSO for at least one week if cultured in iCell Cardiomyocytes Maintenance Medium. Therefore, if the compound(s) to be tested are dissolved in DMSO, it is important to ensure the maximum concentration of the 10X test compound dilutions do not exceed 10% DMSO.

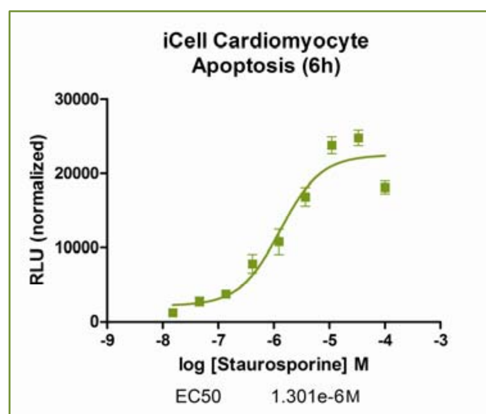
5. Add 10 μl of each test compound dilution to triplicate wells in 96-well plate.

6. Perform desired cell-based assay of choice after suitable compound exposure duration.

Example Cell-based Assay Results

iCell Cardiomyocytes are well-suited to measure compound cytotoxicity in cell-based assays. These human cells exhibit spontaneous contractile activity in culture, are provided at a high level of cardiomyocyte purity, and do not divide in culture. These qualities provide the researcher with a cell type possessing typical cardiomyocyte characteristics and exhibiting expected responses upon exposure to exogenous agents, thus providing a reliable source of human cardiomyocytes suitable for use in targeted drug discovery, toxicity testing, and life science research. The results shown below demonstrate iCell Cardiomyocytes function in apoptosis, general viability, and mitochondrial membrane potential assays.

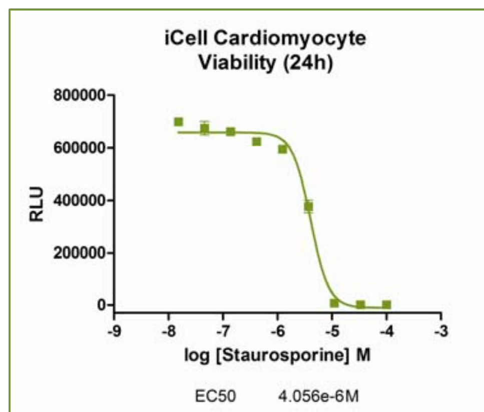
iCell Cardiomyocytes Apoptosis Assay



iCell Cardiomyocytes with a *Plating Efficiency* of 40% were seeded at 41K viable cells/well and plated for 48 hours in 0.1% gelatin-coated 96-well plates prior to 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 μM. Cell viability was measured using the Caspase-glo 3/7 Assay (Promega, G8091) after 6 hours of

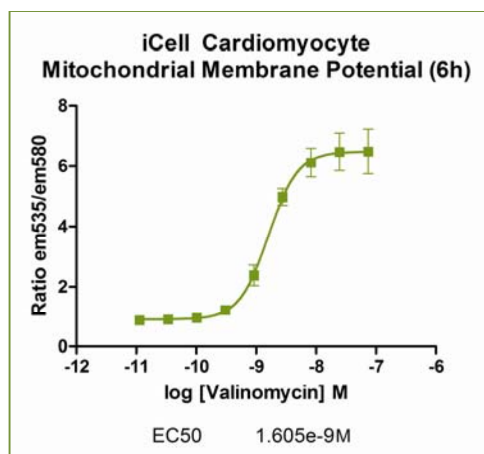
staurosporine treatment using a Tecan GENios Pro microplate reader (1 second/well integration time). Error bars represent standard deviation of triplicate wells.

iCell Cardiomyocytes Viability Assay



iCell Cardiomyocytes with a *Plating Efficiency* of 40% were seeded at 41K viable cells/well and plated for 48 hours in 0.1% gelatin-coated 96-well plates prior to 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 μM. 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). Error bars represent standard deviation of the mean of triplicate wells.

iCell Cardiomyocytes Mitochondrial Membrane Potential Assay



iCell Cardiomyocytes with a *Plating Efficiency* of 40% were seeded at 41K viable cells/well and plated for 24 hours in 0.1% gelatin-coated 96-well plates prior to washing away unattached cells. A 3-fold serial dilution series of valinomycin (Fluka, 94675) diluted in iCell Cardiomyocytes Maintenance Medium was applied to triplicate wells per concentration. Valinomycin concentrations ranged from 11 pM to 74 nM. Cell viability was measured using

the JC-10 Assay (ABD Bioquest, 22800) after 6 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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