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iCell® Cardiomyocytes^2

User's Guide

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Conditions of Use

iCell Cardiomyocytes^2 are for life science research use only and subject to the use restrictions as 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^2.

Origin

iCell Cardiomyocytes^2 are manufactured in the United States of America.

Copyright Notice

Revision History

Version 0.4: August 2016
Version 0.3: September 2015
Version 0.2: July 2015
Version 0.1: March 2015

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Table of Contents

Before You Begin

- Immediately transfer the frozen vials to liquid nitrogen storage.
- Read this entire iCell® Cardiomyocytes^{2} User's Guide before handling or using iCell Cardiomyocytes^{2}.
- iCell Cardiomyocytes^{2} are for life science research use only. See Appendix A for more information and other restrictions.
- A Safety Data Sheet (SDS) for dimethyl sulfoxide (DMSO), in which iCell Cardiomyocytes^{2} 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^{2}.

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Chapter 1. Introduction

Cellular Dynamics International's (CDI) iCell Cardiomyocytes^2 are highly purified, human cardiomyocytes derived from induced pluripotent stem (iPS) cells using CDI's proprietary differentiation and purification protocols. iCell Cardiomyocytes^2 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^2 will begin to beat spontaneously within 24 hours. When seeded at appropriate densities, iCell Cardiomyocytes^2 also will form electrically connected syncytial layers that beat in synchrony and can be used for electrophysiology interrogation via such techniques as microelectrode array and impedance measurements on day 4 post- thawing.

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^2 therefore can be maintained in culture for at least 14 days 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.

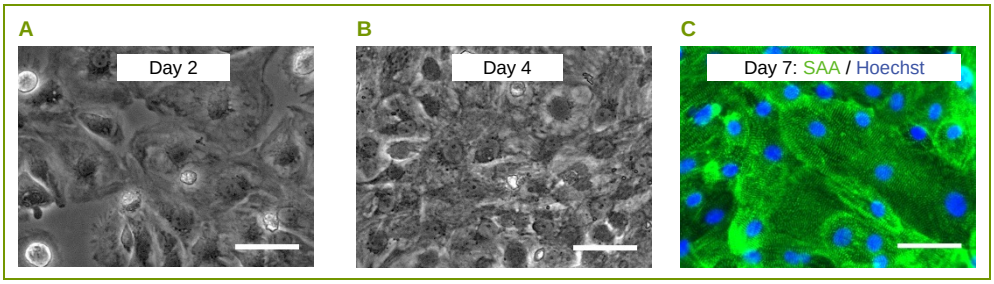

Figure 1: iCell Cardiomyocytes^2 Represent a Highly Pure Population of Human Myocytes
These images show iCell Cardiomyocytes^2 at days 2, 4 and 7 post-plating. iCell Cardiomyocytes^2 form a syncytial layer in culture within 4 days post-plating and show the appropriate cardiac markers as demonstrated by immunocytochemistry: sarcomeric alpha-actinin (SAA, green) and Hoechst (blue). Scale bar = 50 µm.

Components Supplied by Cellular Dynamics

Item	Catalog Number
iCell Cardiomyocytes^{2} †	CMC-100-012-000.5 (0.5 unit) CMC-100-012-001 (1 unit)
iCell Cardiomyocytes Plating Medium†	CMM-100-110-001 (1 unit) CMM-100-110-005 (5 unit)
iCell Cardiomyocytes Maintenance Medium†	CMM-100-120-001 (1 unit) CMM-100-120-005 (5 unit)
iCell Cardiomyocytes^{2} User's Guide†	
Certificate of Testing#	
Certificate of Origin If required for shipping purposes	

† Safety Data Sheets and User's Guide available online at www.cellulardynamics.com/lit/

Available online at www.cellulardynamics.com/cot/

Required Equipment and Consumables

Item	Vendor	Catalog Number
Equipment		
37°C Water Bath	Multiple Vendors	
Biological Safety Cabinet with UV Lamp	Multiple Vendors	
Cell Culture Incubator	Multiple Vendors	
Hemocytometer or Automated Cell Counter*	Multiple Vendors	
Liquid Nitrogen Storage Unit	Multiple Vendors	
Phase Contrast Microscope	Multiple Vendors	
Pipettors	Multiple Vendors	
Tabletop Centrifuge	Multiple Vendors	
Consumables		
50 ml Centrifuge Tubes	Multiple Vendors	
Cell Culture Vessels, Sterile, TC Grade	Multiple Vendors	
Gelatin,Type A, Cell Culture Tested	Multiple Vendors	
Pipettes	Multiple Vendors	
Sterile Distilled Water	Multiple Vendors	
Trypan Blue	Gibco	15250

* Ensure automated cell counter is appropriately calibrated before use.

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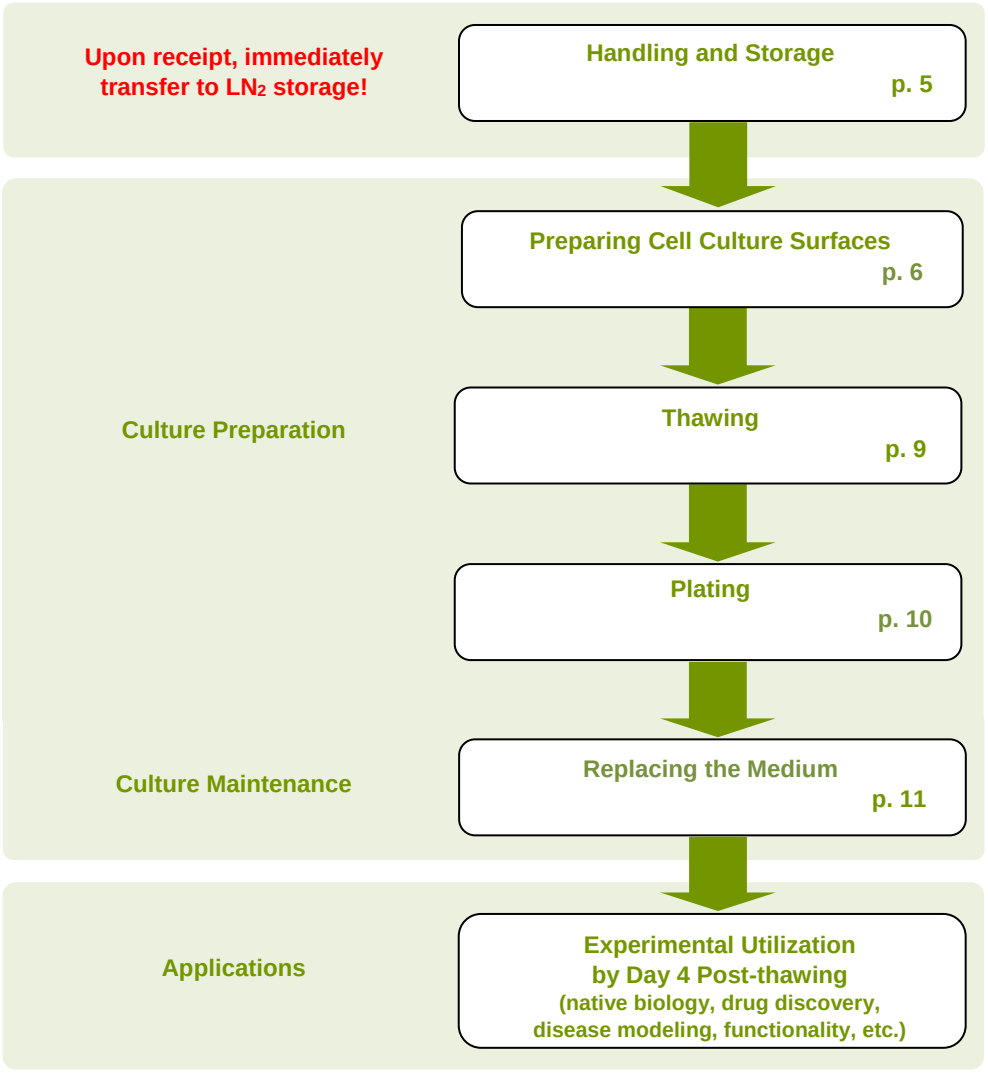
Technical Support and Training

CDI's Technical Support Scientists have the necessary laboratory and analytical experience to respond to your inquiries. In-lab training may be available upon request.

Telephone	(877) 320-6688 (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



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Chapter 2. Handling and Storage

Handling iCell Cardiomyocytes²

iCell Cardiomyocytes² are provided as cryopreserved single-cell suspensions in 1.5 ml cryovials. Upon receipt, directly transfer the cryobox containing iCell Cardiomyocytes² to the vapor phase of a liquid nitrogen storage dewar. CDI strongly recommends transferring the entire cryobox into the storage rack to avoid transferring individual vials.

It is critical to maintain cryopreserved iCell Cardiomyocytes² at a stable temperature. Minimize exposure of cryopreserved iCell Cardiomyocytes² 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. Upon receipt, store iCell Cardiomyocytes media at -20°C until ready for use and at 4°C for up to 2 weeks post-thawing. If media will be used for longer than 2 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

Notes

iCell Cardiomyocytes² will plate and function on a variety of substrates including freshly prepared gelatin and fibronectin, which have been shown to support attachment, viability, and function of iCell Cardiomyocytes² with similar efficiencies.

CDI provides application protocols and notes that recommend assay-specific substrates. See www.cellulardynamics.com/lit/ for a list of available application protocols for iCell Cardiomyocytes². Regardless of the substrate of choice, prepare plating surfaces before thawing iCell Cardiomyocytes².

Preparing 0.1% (w/v) Gelatin Solution

1. Prepare 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, 18 MΩ sterile water for washing bottles.
2. Prepare 0.1% gelatin solution by dissolving 500 mg of gelatin (Type A, powder, cell culture tested) in 500 ml of sterile water in each bottle.
3. Sterilize the gelatin solution by autoclaving using liquid cycle. The sterile 0.1% gelatin solution is stable for 3 months when prepared as described above and stored at room temperature.

   Note: *Alternatively, use commercially available 0.1% gelatin in water (Stem Cell Technologies, Cat. No. 07903).*

Preparing the Gelatin Cell Culture Vessel

1. Select the cell culture vessel appropriate for your experimental use. Add the volume of 0.1% gelatin solution specified in the table below. Scale volumes appropriately for other vessel formats.

Culture Vessel	Surface Area (cm^2)	Volume of 0.1% Gelatin Solution (ml)
12-well Cell Culture Plate	3.8	1.2
96-well Cell Culture Plate	0.32	0.1

Table 1: Summary of Useful Volumes and Measures
*All volumes and measures are **per well**, if applicable.*

Note: *For glass coverslips for immunocytochemistry or electrophysiological applications, see the iCell Cardiomyocytes Application Protocols available online at www.cellulardynamics.com/lit/.*

2. Incubate the vessel(s) in a 37°C cell culture incubator for at least 1 hour.
3. Aspirate gelatin solution immediately before addition of the cell suspension.

Do not allow the gelatin-coated surface to dry.

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Chapter 4. Thawing Media

iCell Cardiomyocytes Plating Medium (Plating Medium) and iCell Cardiomyocytes Maintenance Medium (Maintenance Medium) have been specially formulated to maximize the cell viability and recovery at thaw, and to maintain the health and function of iCell Cardiomyocytes^{2} in culture over time, respectively. Thaw and store the media as follows:

1. 24 hours before use, thaw the media overnight at 4°C.
2. Prepare aliquots of media and store at 4°C for up to 2 weeks.

   Note: *The medium aliquots can be stored at -20˚C. Do not thaw and refreeze the medium aliquots multiple times.*

Chapter 5. Thawing iCell Cardiomyocytes²

Notes

Maintain iCell Cardiomyocytes² 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 Cardiomyocytes² viability and performance.

Note: *Thaw no more than 3 vials of iCell* Cardiomyocytes² *at one time.*

1. Equilibrate the Plating Medium at room temperature before thawing iCell Cardiomyocytes².
2. Remove the iCell Cardiomyocytes² cryovial from the liquid nitrogen storage tank.

   Note: *If necessary, place cryovials on dry ice for up to 60 minutes before thawing.*
3. Immerse the cryovial in a 37°C water bath for 3 minutes (avoid submerging the cap), holding the tube stationary (no swirling). Use of a floating microcentrifuge tube rack is recommended.

   

   Precise timing is critical to maximizing viable cell recovery.
4. Immediately remove the cryovial from the water bath, spray with 70% ethanol, and place into the biological safety cabinet.
5. Gently transfer the iCell Cardiomyocytes² cryovial contents to a sterile 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 Cardiomyocytes² cell suspension.
6. Rinse the empty iCell Cardiomyocytes² cryovial with 1 ml of room temperature Plating Medium to recover any residual cells from the vial. Transfer the 1 ml of Plating Medium rinse from the cryovial drop-wise over 90 seconds (i.e. 1 drop every 4 - 5 seconds) to the 50 ml centrifuge tube containing the iCell Cardiomyocytes² cell suspension. Gently swirl the tube while adding the medium to mix the solution completely and minimize the osmotic shock on the thawed cells.

   

   Drop-wise addition of Plating Medium to the cell suspension is critical to minimize osmotic shock and ensure maximum viability and attachment of the cells to the plating substrate. See the Handling iCell Cardiomyocytes Training Video available online at www.cellulardynamics.com/cm_handling/ as a reference.

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7. Slowly add 8 ml (3.5 ml for the 0.5 unit size) of room temperature Plating Medium to the 50 ml centrifuge tube. Add the first 1 ml drop-wise over 30 - 60 seconds. Then add the remaining volume over the next ~30 seconds. Gently swirl the centrifuge tube while adding the medium.

It is critical to add the Plating Medium slowly to ensure maximum viability and attachment of the cells once plated. See the Handling iCell Cardiomyocytes Training Video available online at www.cellulardynamics.com/cm_handling/ as a reference.

8. Gently mix the contents of the 50 ml centrifuge tube by inverting 2 - 3 times. Gentle mixing is critical to ensure maximum viability. Avoid vigorous shaking or vortexing of the cell suspension.

***Note**: Thaw up to 3 vials of iCell Cardiomyocytes² at one time. Once thawed, you can pool the contents of the vials before adding the rinse and final volume of Plating Medium. Follow the timing outlined in steps* 6 *and 7. For example, if pooling 3 vials, add each 1 ml of rinse over 90 seconds (270 seconds total).*

Chapter 6. Plating iCell Cardiomyocytes²

Notes

The recommended seeding density for iCell Cardiomyocytes² in standard cell culture plates is 156,000 viable cells/cm^2. For application-specific plating instructions, see the Application Protocols available online at www.cellulardynamics.com/lit/.

1. Obtain the number of viable cells/vial and viability from the Certificate of Testing.
2. Invert the thawed iCell Cardiomyocytes² cell suspension 2 - 3 times to ensure an even cardiomyocyte distribution before performing the cell count.
3. Remove a sample of cells to confirm viability using a hemocytometer (using trypan blue exclusion to identify viable cells) or an automated cell counter.
4. Calculate the final volume of room temperature Plating Medium needed to obtain a desired cell plating density (i.e. 5 x 10^5 cells/ml) using the number of viable cells/vial from the Certificate of Testing. See Table 2 below for examples.

   Note: *If your application requires higher cell densities, centrifuge iCell Cardiomyocytes² at 180 x g for 5 minutes, remove the necessary amount of the Plating Medium to achieve the desired density, and gently resuspend the iCell Cardiomyocytes² pellet. Note that over-pipetting could reduce cell viability.*

Culture Vessel	Surface Area (cm^2)	Plating Volume (ml)	Cell Number (1.56 x 10^5 cells/cm^2)
12-well Cell Culture Plate	3.8	1.2	593 x 10^3
96-well Cell Culture Plate	0.32	0.1	50 x 10^3

Table 2: Summary of Recommended Volumes and Measures
This table provides a guide for syncytial formation only. All volumes and measures are ***per well*** *unless otherwise indicated.*

5. Aspirate the gelatin solution from the pre-coated cell culture vessel(s).
6. Invert the cell suspension 2 - 3 times and immediately dispense the cell suspension into the pre-coated cell culture vessel(s).
7. Culture iCell Cardiomyocytes² in a cell culture incubator at 37°C, 5% CO_2 **for 4 hours**. Proceed immediately to Chapter 7, Maintaining iCell Cardiomyocytes².

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Chapter 7. Maintaining iCell Cardiomyocytes^2

1. Immediately before use, equilibrate iCell Cardiomyocytes Maintenance Medium (Maintenance Medium) in a 37°C water bath.
2. 4 hours post-plating iCell Cardiomyocytes^2, aspirate the Plating Medium using a pipettor and replace with the appropriate volume of Maintenance Medium. Be careful not to touch or disrupt the adhered cardiomyocytes.

   Note: *Avoid excessive washing at this stage as it could affect cell adherence.*
3. Replace the Maintenance Medium every other day.
4. Culture iCell Cardiomyocytes^2 in a cell culture incubator at 37°C, 5% CO_2.

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