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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 this guide. 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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Revision History

Document ID: X1000
Version 1.0: December 2017

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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 Safety Data Sheet (SDS) 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.
Chapter 1. Introduction

Cellular Dynamics International’s (CDI) iCell Cardiomyocytes are highly purified, human cardiomyocytes derived from induced pluripotent stem (iPS) cells using 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-plating 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

<table>
<thead>
<tr>
<th>Item</th>
<th>Catalog Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>iCell Cardiomyocytes Kit, 01434</td>
<td>R1007</td>
</tr>
<tr>
<td>• iCell Cardiomyocytes, 01434</td>
<td>C1006 (≥4.0 x 10^6 viable cells)</td>
</tr>
<tr>
<td>• iCell Cardiomyocytes Plating Medium</td>
<td>M1001 (30 ml)</td>
</tr>
<tr>
<td>• iCell Cardiomyocytes Maintenance Medium</td>
<td>2 x M1003 (100 ml)</td>
</tr>
<tr>
<td>• iCell Cardiomyocytes User’s Guide</td>
<td>X1000</td>
</tr>
<tr>
<td>iCell Cardiomyocytes Kit, 11713</td>
<td>R1105</td>
</tr>
<tr>
<td>• iCell Cardiomyocytes, 11713</td>
<td>C1105 (≥1.0 x 10^6 viable cells)</td>
</tr>
<tr>
<td>• iCell Cardiomyocytes Plating Medium</td>
<td>M1001 (30 ml)</td>
</tr>
<tr>
<td>• iCell Cardiomyocytes Maintenance Medium</td>
<td>M1003 (100 ml)</td>
</tr>
<tr>
<td>• iCell Cardiomyocytes User’s Guide</td>
<td>X1000</td>
</tr>
</tbody>
</table>

**Certificate of Analysis**

Certification of Origin

If required for shipping purposes

1 These products were formerly known by these names and/or catalog numbers:
- iCell Cardiomyocytes, 01434 = iCell Cardiomyocytes (Cat No. CMC-100-110-001, CMC-100-110-005, CMC-100-010-000.5, CMC-100-010-001, or CMC-100-010-005)
- iCell Cardiomyocytes Plating Medium = iCell Cardiomyocytes Plating Medium (Cat. No. CMM-100-110-001 or CMM-100-110-005)
- iCell Cardiomyocytes Maintenance Medium = iCell Cardiomyocytes Maintenance Medium (Cat. No. CMM-100-120-001 or CMM-100-120-005)

2 Safety Data Sheets and User’s Guide available online: www.cellulardynamics.com/lit/

3 Available online: www.cellulardynamics.com/coa/

### Required Equipment and Consumables

<table>
<thead>
<tr>
<th>Item</th>
<th>Vendor</th>
<th>Catalog Number</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Equipment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37°C Water Bath</td>
<td>Multiple Vendors</td>
<td></td>
</tr>
<tr>
<td>Biological Safety Cabinet with UV Lamp</td>
<td>Multiple Vendors</td>
<td></td>
</tr>
<tr>
<td>Cell Culture Incubator</td>
<td>Multiple Vendors</td>
<td></td>
</tr>
<tr>
<td>Hemocytometer or Automated Cell Counter</td>
<td>Multiple Vendors</td>
<td></td>
</tr>
<tr>
<td>Liquid Nitrogen Storage Unit</td>
<td>Multiple Vendors</td>
<td></td>
</tr>
<tr>
<td>Phase Contrast Microscope</td>
<td>Multiple Vendors</td>
<td></td>
</tr>
<tr>
<td>Pipettors</td>
<td>Multiple Vendors</td>
<td></td>
</tr>
<tr>
<td>Tabletop Centrifuge</td>
<td>Multiple Vendors</td>
<td></td>
</tr>
<tr>
<td><strong>Consumables</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1% Gelatin in Water</td>
<td>STEMCELL Technologies</td>
<td>07903</td>
</tr>
<tr>
<td>24-well Flat-bottom Plate, TC-treated, Costar</td>
<td>STEMCELL Technologies</td>
<td>38017</td>
</tr>
<tr>
<td>6-well Flat-bottom Plate, TC-treated, Costar</td>
<td>STEMCELL Technologies</td>
<td>38015</td>
</tr>
<tr>
<td>96-well Flat-bottom Microplate, TC-treated, Falcon</td>
<td>STEMCELL Technologies</td>
<td>38022</td>
</tr>
<tr>
<td>Conical Tubes, 50 ml, Falcon (Centrifuge Tubes)</td>
<td>STEMCELL Technologies</td>
<td>38010</td>
</tr>
<tr>
<td>Serological Pipettes, 1, 2, 5, 10, 25 ml</td>
<td>STEMCELL Technologies</td>
<td>38001, 38002, 38003, 38004, 38005</td>
</tr>
</tbody>
</table>
### Technical Support, Knowledge Base, and Training

CDI’s Technical Support Scientists have the necessary laboratory and analytical experience to respond to your inquiries. Our web-based Knowledge Base provides solutions for iCell related questions about plating and media, cell culture, general assay methods, and more. In addition, hands-on training is available or watch our Handling iCell Cardiomyocytes Training Video. 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

**Knowledge Base**  
www.cellulardynamics.com/knowledgebase/

**Hands-on Training**  
www.cellulardynamics.com/training/

**Training Video**  
www.cellulardynamics.com/cm_handling/

---

### Table of Materials

<table>
<thead>
<tr>
<th>Item</th>
<th>Vendor</th>
<th>Catalog Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterile Distilled Water</td>
<td>Multiple Vendors</td>
<td></td>
</tr>
<tr>
<td>Trypan Blue²</td>
<td>STEMCELL Technologies</td>
<td>07050</td>
</tr>
</tbody>
</table>

1. Ensure the automated cell counter is appropriately calibrated before use.
2. Similar products are available from multiple vendors.
Upon receipt, immediately transfer to LN2 storage!

Handling and Storage  p. 5

Preparing Cell Culture Surfaces  p. 6

Thawing  p. 7

Plating  p. 9

Replacing the Medium  p. 13

Experimental Utilization
(native biology, drug discovery, disease modeling, functionality, etc.)

Culture Preparation

Culture Maintenance

Applications
Chapter 2. Handling and Storage

Handling iCell Cardiomyocytes

iCell Cardiomyocytes are provided as cryopreserved single-cell suspensions in 1.5 ml cryovials. Upon receipt, directly transfer the cryobox containing iCell Cardiomyocytes 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 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. Upon receipt, store iCell Cardiomyocytes media at -20°C until ready for use and at 4°C for up to 2 weeks after thaw. 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.
iCell Cardiomyocytes will plate and function on a variety of substrates including gelatin and fibronectin, which have been shown to support attachment, viability, and function of iCell Cardiomyocytes with similar efficiencies. Coating plates with 0.1% gelatin in water is economical, simple, and recommended method for preparing cell culture plates for culturing iCell Cardiomyocytes.

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

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

```
<table>
<thead>
<tr>
<th>Culture Vessel</th>
<th>Surface Area (cm²)</th>
<th>Volume of 0.1% Gelatin in Water (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-well Cell Culture Plate</td>
<td>9.5</td>
<td>2</td>
</tr>
<tr>
<td>12-well Cell Culture Plate</td>
<td>3.8</td>
<td>1</td>
</tr>
<tr>
<td>24-well Cell Culture Plate</td>
<td>1.9</td>
<td>0.6</td>
</tr>
<tr>
<td>96-well Cell Culture Plate</td>
<td>0.32</td>
<td>0.1</td>
</tr>
<tr>
<td>384-well Cell Culture Plate</td>
<td>0.06</td>
<td>0.025</td>
</tr>
<tr>
<td>T25 Flask</td>
<td>25</td>
<td>8</td>
</tr>
</tbody>
</table>
```

*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 the 0.1% gelatin in water immediately before addition of the cell suspension.
Chapter 4. Thawing Medium and iCell Cardiomyocytes

Thawing iCell Cardiomyocytes Plating Medium

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

Remove Plating Medium from 4°C and equilibrate at room temperature before thawing iCell Cardiomyocytes. 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:

\[
\text{Plating Medium to Thaw (ml)} = \frac{\text{Viable Cells/Vial} \times \text{Plating Efficiency}}{\text{Target Plating Density (cells/ml)}}
\]

Viable Cells/Vial and Plating Efficiency are lot specific and listed on the Certificate of Analysis. Plating Efficiency and Target Plating Density are further defined in Chapter 5, Plating iCell Cardiomyocytes.

Thawing iCell Cardiomyocytes

Maintain iCell Cardiomyocytes in liquid nitrogen until immediately before thawing to ensure maximal performance of 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 3 vials of iCell Cardiomyocytes at one time.

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

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

2. Immerse the cryovial in a 37°C water bath for 4 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.

3. Immediately remove the cryovial from the water bath, spray with 70% ethanol, and place into the biological safety cabinet.

4. Gently transfer the iCell Cardiomyocytes 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 Cardiomyocytes cell suspension.
5. Rinse the empty iCell Cardiomyocytes cryovial with 1 ml of room temperature Plating Medium to recover any residual cells from the vial. Transfer the 1 ml 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 Cardiomyocytes 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/.

6. Slowly add 8 ml (3.5 ml for 0.5 unit size of cardiomyocytes) 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/.

7. 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 Cardiomyocytes 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 5 and 6. For example, if pooling 3 vials, 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:

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viable Cell Count</td>
<td>The total number of viable cells received from cell counting analysis of the single-cell suspension immediately following thaw.</td>
</tr>
<tr>
<td>Viable Cell Density</td>
<td>The number of viable cells/ml received from cell counting analysis of the single-cell suspension immediately following thaw.</td>
</tr>
<tr>
<td>Seeded Cell Count</td>
<td>The total number of viable cells added to the cell culture vessel.</td>
</tr>
<tr>
<td>Plated Cell Count</td>
<td>The number of viable cells that have firmly attached to the cell culture vessel at 48 hours post-plating.</td>
</tr>
</tbody>
</table>

Plating Efficiency is calculated as:

\[
\text{Plating Efficiency} = \frac{\text{Plated Cell Count}}{\text{Seeded Cell Count}} \times 100
\]

**Example:** If 600,000 cells were seeded, and 350,000 plated cells were recovered after 48 hours in culture, the Plating Efficiency for that particular lot of cells would be calculated as follows:

\[
58.3\% = \frac{350,000 \text{ Plated Cells}}{600,000 \text{ Seeded Cells}} \times 100
\]

**Note:** 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 online at www.cellulardynamics.com/coa/.

Because iCell Cardiomyocytes are non-proliferative, you may need to 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 (Maintenance Medium) allows highly pure cultures to be maintained for at least 2 weeks.
Determining Viable Cell Density

1. Invert the thawed iCell Cardiomyocytes cell suspension 2 - 3 times to ensure an even cardiomyocyte distribution before performing the cell count.

2. Remove a sample of cells to perform a cell count using a hemocytometer (using trypan blue exclusion to identify viable cells) or an automated cell counter.

3. 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, centrifuge iCell Cardiomyocytes 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 Cardiomyocytes pellet. Note that over-pipetting could reduce cell viability.

Plating iCell Cardiomyocytes

iCell Cardiomyocytes, which recapitulate native human cardiac myocyte physiology and function, are suitable for many cell-based assays. 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 ~63,000 cardiomyocytes/cm² provides a beating syncytium and is the recommended starting density for most cell-based assays. The following table provides the desired cell number and seeding volume for several common cell culture vessels.

Note: This table provides a guide for syncytial formation only. See the application protocols and notes available online at www.cellulardynamics.com/lit/ for recommended densities and seeding volumes for various cell-based assays as well as electrophysiological techniques, such as perforated patch clamp and multielectrode array (MEA).

<table>
<thead>
<tr>
<th>Culture Vessel</th>
<th>Surface Area (cm²)</th>
<th>Seeding Volume (ml)</th>
<th>Cell Number (~6.3 x 10⁴ cells/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-well Cell Culture Plate</td>
<td>9.5</td>
<td>3</td>
<td>600 x 10³</td>
</tr>
<tr>
<td>12-well Cell Culture Plate</td>
<td>3.8</td>
<td>1.2</td>
<td>240 x 10³</td>
</tr>
<tr>
<td>24-well Cell Culture Plate</td>
<td>1.9</td>
<td>0.6</td>
<td>120 x 10³</td>
</tr>
<tr>
<td>96-well Cell Culture Plate</td>
<td>0.32</td>
<td>0.1</td>
<td>20 x 10³</td>
</tr>
<tr>
<td>384-well Cell Culture Plate</td>
<td>0.06</td>
<td>0.025</td>
<td>5 x 10³</td>
</tr>
<tr>
<td>T25 Flask</td>
<td>25</td>
<td>8</td>
<td>1.6 x 10⁶</td>
</tr>
</tbody>
</table>

Table 2: Summary of Recommended Volumes and Measures
All volumes and measures are per well, if applicable.

The number of viable cardiomyocytes 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 online at www.cellulardynamics.com/coa/.

**Plating iCell Cardiomyocytes in 96-well Cell Culture Plates for Cell-based Assays**

The recommended plated density for performing cell-based assays in 96-well cell culture plates using iCell Cardiomyocytes is 20,000 plated cells/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 20,000 cells/well in a 96-well cell culture plate assuming a seeding volume of 100 μl/well, a *Plating Efficiency* of 45%, a post-thaw *Viable Cell Density* of 0.49 x 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 vessel formats by substituting the appropriate recommended cell numbers and seeding volumes.

1. Prepare the thawed cell suspension for plating into a 96-well cell culture plate.
   a. Obtain the *Plating Efficiency* from the Certificate of Analysis. Alternatively, use the lot number to look up your Certificate of Analysis online at www.cellulardynamics.com/coa/ (for purposes of this example, 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, the *Viable Cell Density* is 0.49 x 10^6 cells/ml).
   c. Calculate the *Target Plating Density*:

   \[
   \text{Target Plating Density} = \frac{\text{Desired Cells/Well}}{\text{Seeding Volume/Well (ml)}}
   \]

   For purposes of this example, the desired number of plated cells/well is 20,000, and the suggested seeding volume is 100 μl. Thus, the *Target Plating Density* is

   \[
   0.2 \times 10^6 \text{ cells/ml} = \frac{20,000 \text{ cells/well}}{0.1 \text{ ml}}
   \]

   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

   \[
   10.47 \text{ ml} = \frac{9.5 \text{ ml} \times 0.49 \times 10^6 \text{ cells/ml} \times 0.45}{0.2 \times 10^6 \text{ cells/ml}}
   \]

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

   Notes
   - iCell Cardiomyocytes User's Guide
2. Dispense the **Total Plating Volume** into one or more 96-well cell culture plates.
   
   a. Obtain gelatin-coated 96-well cell culture plate(s). See Chapter 3, Preparing Cell Culture Surfaces, for more information.
   
   b. After aspirating the 0.1 gelatin in water from the wells, gently mix iCell Cardiomyocytes cell suspension and dispense the seeding volume of the cell suspension (i.e. 100 μl) to each well of the 96-well cell culture plate.
   
   c. Culture iCell Cardiomyocytes in a cell culture incubator at 37°C, 7% CO₂.

Media replacement and recommended washing procedures to perform before assaying cardiomyocytes are described in Chapter 6, Maintaining iCell Cardiomyocytes.

**Expected Cell Densities**

The images in Figure 1 show the expected coverage that can be obtained by following the provided plating instructions. iCell Cardiomyocytes were added to a 96-well cell culture plate at the indicated seeding densities to obtain the desired plating densities. The cardiomyocytes 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 demonstrate cell viability, indicate their homogenous distribution, and 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 from that well. Note that the cardiomyocytes have not been maintained long enough in culture to form a syncytium.

![Figure 1: Cell Densities at 48 Hours Post-plating](image)

*These images show the expected density of calcein-positive cardiomyocytes at 48 hours post-plating. The iCell Cardiomyocytes had a Plating Efficiency of ~40%.*
Chapter 6. Maintaining iCell Cardiomyocytes

iCell Cardiomyocytes are shipped cryopreserved, at high purity. The cardiomyocytes preserve a high purity for at least 2 weeks after thawing if plated in iCell Cardiomyocytes Plating Medium (Plating Medium) and maintained in iCell Cardiomyocytes Maintenance Medium (Maintenance Medium) as recommended.

1. 24 hours before use, thaw the Maintenance Medium overnight at 4°C.
2. Immediately before use, equilibrate the Maintenance Medium in a 37°C water bath.
3. 48 hours post-plating iCell Cardiomyocytes, gently wash off the non-adherent cells and debris by pipetting the Plating Medium up and down approximately 5 times while gently washing the surface of the plate.
   
   **Note:** Alternatively, aspirate the Plating Medium to remove the non-adherent cells and perform 2 washes with the appropriate volume of Maintenance Medium, gently washing the surface of the plate with each medium change.

4. Aspirate the Plating Medium and replace with the appropriate volume of Maintenance Medium. Be careful not to touch or disrupt the adhered cardiomyocytes. See Table 2 on page 10 for recommended seeding volumes for several common cell culture vessels.
5. Replace the Maintenance Medium every other day.
6. Culture iCell Cardiomyocytes in a cell culture incubator at 37°C, 7% CO₂.
Appendix A. Intellectual Property Rights, Use Restrictions, and Limited License

A. **OWNERSHIP.** The Products are covered by pending patents and patents: cellulardynamics.com/about-us/patents/. Customer has a limited license to use the Products for internal research purposes for the sole benefit of the Customer, subject to the use restrictions included in subsection B of this Appendix A. Customer acknowledges and agrees that the receipt or purchase of the Products by Customer shall not be construed as a transfer of any title or the grant of any rights in or to the intellectual property embodied in the Products owned or licensed by Cellular Dynamics. In particular, no right or license to make, have made, offer to sell, or sell the Products, to modify or reproduce the Product or any part thereof, or to use the Products in combination with any other product(s), except product(s) provided or expressly licensed to Customer by Cellular Dynamics for such use, is implied or conveyed by the sale or transfer of Products to Customer.

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Appendix B. Limited Warranty

A. During the Warranty Period (as defined below) and subject to subsection F of this Appendix B. Cellular Dynamics warrants that its Products conform to the specifications contained in the Certificate of Analysis for the Product shipped to Customer. Customer's sole and exclusive remedy (and Cellular Dynamics' sole and exclusive liability) with respect to any defective Products shall be replacement of the defective Products by Cellular Dynamics pursuant to this Appendix B.  

B. Under no circumstances shall Cellular Dynamics' liability to Customer exceed the amount paid by Customer for the Products to Cellular Dynamics. Cellular Dynamics will bear all reasonable shipping costs if the Products are replaced pursuant to this warranty. For clarity, this warranty automatically shall be void, and any claims under it invalid, (i) if Customer's use of the Products is other than solely in accordance with this User's Guide and Cellular Dynamics' Terms and Conditions (or such other written agreement between Cellular Dynamics and Customer under which the Products are sold or transferred to Customer) or for a purpose or in a manner other than that for which the Products were designed; or (ii) if Customer fails to follow this User's Guide for the use, storage, and handling of the Products however such failure is caused; or (iii) if Customer fails to comply with any of the provisions of Appendix A in this User's Guide; or (iv) if there is any abuse, other misuse or neglect of the Products by Customer or to the extent of any damage or loss of the Products by events or occurrences beyond a person's (e.g., Cellular Dynamics') control including without
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D. Within five (5) business days of thawing the Product but prior to the expiration date of the Product as listed on the Certificate of Analysis and/or Product’s label (the “Warranty Period”), Customer must notify Cellular Dynamics in writing of any nonconformity of the Products, describing the nonconformity in detail. Customer’s failure to properly notify Cellular Dynamics in the Warranty Period voids the limited warranty set forth above in this Appendix B.

E. Customers who believe they have a warranty claim should call Cellular Dynamics’ Technical Support line at (608) 310-5100 ext. 5 or email at support@cellulardynamics.com to request a replacement Product based on a breach of the limited warranty set forth above in this Appendix B. Any action by Customer for Cellular Dynamics’ breach of this limited warranty, for which Customer has given timely and proper notice of such breach during the Warranty Period and otherwise in accordance with this Appendix B, must be commenced by Customer within 18 months following the date of such breach.

F. Cellular Dynamics makes no warranty of any kind or nature, neither express nor implied, for any product sold together with, or as a part of, the Products (e.g., an accessory accompanying a Product or a discrete component part of a Product that is a kit) that is not manufactured by Cellular Dynamics. Any such accessory to or part of the Products shall have the warranty, if any, that is offered and granted (and, for clarity, extended by its terms to Customer) by the manufacturer of such other accessory or component product accessories.

G. Customer acknowledges and agrees that Cellular Dynamics may fill Customer’s order with any number of units of Products. Such units may be more units than Customer ordered. Customer will not be charged extra for any adjustments made by Cellular Dynamics. The number of cells in a unit is determined by the Product’s Certificate of Analysis. The number of cells that are contained in a unit accounts for both viability and plating efficiency percentages. Because this may vary from lot to lot, Cellular Dynamics reserves the right to fill the order with that number of units which is sufficient to fill Customer’s order and such adjustments shall not constitute a breach of the limited warranty set forth herein.

Appendix C. Limited Liability

TO THE FULLEST EXTENT PERMITTED UNDER APPLICABLE LAW, CELLULAR DYNAMICS SHALL NOT HAVE ANY LIABILITY FOR INCIDENTAL, COMPENSATORY, PUNITIVE, CONSEQUENTIAL, INDIRECT, SPECIAL OR OTHER SIMILAR DAMAGES, HOWEVER CAUSED AND REGARDLESS OF FORM OF ACTION WHETHER IN CONTRACT, TORT (INCLUDING NEGLIGENCE), STRICT PRODUCT LIABILITY OR OTHERWISE, EVEN IF CELLULAR DYNAMICS HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. NOTWITHSTANDING ANY OTHER TERM OR IMPLICATION TO THE CONTRARY, UNDER NO CIRCUMSTANCES SHALL CELLULAR DYNAMICS’ LIABILITY TO CUSTOMER EXCEED THE AMOUNT PAID BY CUSTOMER FOR THE PRODUCTS TO CELLULAR DYNAMICS.

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