



iCell[®] Cardiomyocytes User's Guide

Document ID: X1000



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Origin

iCell Cardiomyocytes are manufactured in the United States of America.

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Revision History

Document ID: X1000

Version 1.0: February 2019

Version 2.0: September 2021

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Before You Begin

- Immediately transfer the frozen vials to liquid nitrogen storage.
- Read this entire User's Guide before handling or using iCell® Cardiomyocytes.
- iCell Cardiomyocytes are FOR RESEARCH USE ONLY and NOT FOR THERAPEUTIC USE. See www.fujifilmcdi.com/terms-and-conditions/ for USE RESTRICTIONS applicable to the cells and other terms and conditions related to the cells and their use.
- A Safety Data Sheet (SDS) for dimethyl sulfoxide (DMSO), in which iCell Cardiomyocytes are frozen, is available online at www.fujifilmcdi.com/product-literature/ or on request from FUJIFILM Cellular Dynamics. Only technically qualified individuals experienced in handling DMSO and human biological materials should access, use, or handle iCell Cardiomyocytes.

Notes

Chapter 1. Introduction

iCell Cardiomyocytes from FUJIFILM Cellular Dynamics, Inc. (FCDI), are highly purified, human cardiomyocytes derived from induced pluripotent stem (iPS) cells using FCDI'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. When seeded at appropriate densities, iCell Cardiomyocytes also will form electrically connected syncytial layers that beat in synchrony.

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 14 days in the Maintenance Medium without appreciable loss of purity, enabling longer term studies. Thus, the combination of FCDI's purification process and adherence to the procedures described in this User's Guide makes additional use of antibiotics unnecessary.

Components Supplied by FUJIFILM Cellular Dynamics

Notes

Item	Catalog Number
iCell Cardiomyocytes Kit, 01434	R1057 ($\geq 1.0 \times 10^6$ viable cells) or R1007 ($\geq 4.0 \times 10^6$ viable cells) or R1132 ($3 \times \geq 1.0 \times 10^6$ viable cells)
• iCell Cardiomyocytes, 01434 ^a	• C1056 ($\geq 1.0 \times 10^6$ viable cells (350 μ l)) or C1006 ($\geq 4.0 \times 10^6$ viable cells (1 ml))
• iCell Cardiomyocytes Plating Medium ^a	• M1001 (30 ml)
• iCell Cardiomyocytes Maintenance Medium ^a	• M1003 (100 ml)
• iCell Cardiomyocytes User's Guide ^a	• X1000
iCell Cardiomyocytes Kit with iCell Cardiomyocytes Serum-Free Media, 01434 ^c	R1223
iCell Cardiomyocytes Kit with iCell CardioTox Assay Media, 01434 ^c	R1224
iCell Cardiomyocytes Kit, 11713	R1105 ($\geq 1.0 \times 10^6$ viable cells) or R1106 ($\geq 4.0 \times 10^6$ viable cells) or R1117 ($3 \times \geq 1.0 \times 10^6$ viable cells)
• iCell Cardiomyocytes, 11713 ^a	• C1105 ($\geq 1.0 \times 10^6$ viable cells (350 μ l)) or C1106 ($\geq 4.0 \times 10^6$ viable cells (1 ml))
• iCell Cardiomyocytes Plating Medium ^a	• M1001 (30 ml)
• iCell Cardiomyocytes Maintenance Medium ^a	• M1003 (100 ml)
• iCell Cardiomyocytes User's Guide ^a	• X1000
iCell Cardiomyocytes Kit with iCell Cardiomyocytes Serum-Free Media, 11713 ^c	R1225
iCell Cardiomyocytes Kit with iCell CardioTox Assay Media, 11713 ^c	R1226
Certificate of Analysis ^b	

a Safety Data Sheets and User's Guide available online: www.fujifilmcdi.com/product-literature/

b Available online: www.fujifilmcdi.com/coa-lookup/

c These kits enable assay in Serum-Free Medium. Kit components are available in Chapter 8

Required Equipment and Consumables

Item	Vendor(s)	Catalog Number(s)
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 ¹	Multiple Vendors	
Liquid Nitrogen Storage Unit	Multiple Vendors	
Phase Contrast Microscope	Multiple Vendors	
Pipettors	Multiple Vendors	
Tabletop Centrifuge	Multiple Vendors	
Consumables		
0.1% Gelatin in Water ²	STEMCELL Technologies	07903
96-well Flat-bottom Microplate, TC-treated, Falcon	Multiple Vendors	
Conical Tubes, 50 ml, Falcon (Centrifuge Tubes)	Multiple Vendors	
Serological Pipettes, 1, 2, 5, 10, 25 ml	Multiple Vendors	
Sterile Distilled Water	Multiple Vendors	
Trypan Blue ²	Gibco	15250

¹ Ensure the automated cell counter is appropriately calibrated before use.

² Similar products are available from multiple vendors.

Technical Support, Knowledge Base, and Training

FCDI'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, in-lab training may be available upon request.

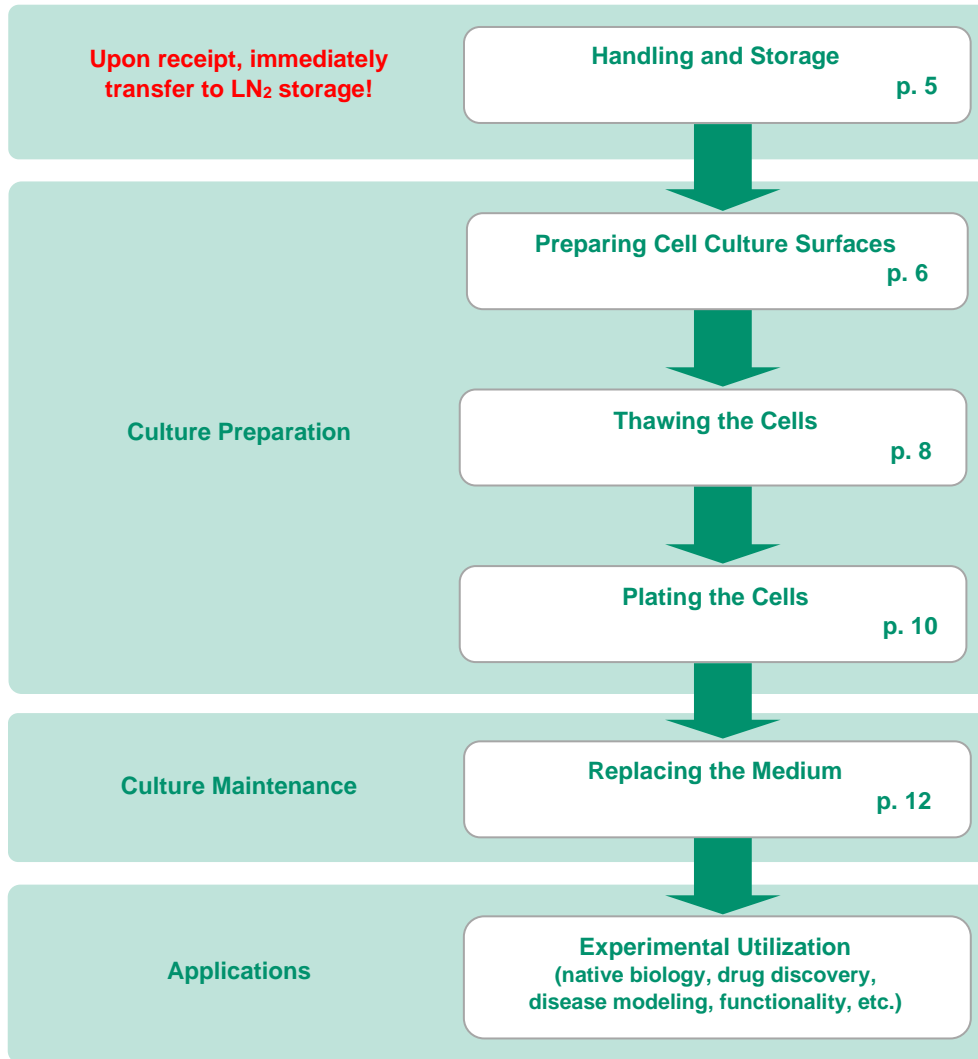
Telephone (877) 320-6688 (US toll-free) / (608) 310-5100 x3
Monday - Friday, 8:30 am - 5:00 pm US Central Time

Email fcdi-support@fujifilm.com

Knowledge Base www.fujifilmcdi.com/knowledge-base/

Workflow Diagram

Notes



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. FCDI 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 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

iCell Cardiomyocytes will plate and function on a variety of substrates including gelatin or 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.

FCDI provides application protocols that recommend assay-specific substrates. See www.fujifilmcdi.com/product-literature/ for a list of available application protocols 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.

Culture Vessel	Surface Area (cm ²)	Volume of 0.1% Gelatin in Water (ml)
6-well Cell Culture Plate	9.6	3
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 <https://www.fujifilmcdi.com/product-literature/>.

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.



Do not allow the gelatin-coated surface to dry.

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 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 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 3 vials of iCell Cardiomyocytes at one time.

1. Equilibrate the Plating Medium at room temperature before thawing iCell Cardiomyocytes.
2. Remove the iCell Cardiomyocytes 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 according to Table 2 below. Avoid submerging the cap and hold the tube stationary (no swirling). Use of a floating microcentrifuge tube rack is recommended.

Vial Size	Volume per Vial (ml)	Thaw Time (min)
iCell Cardiomyocytes $\geq 1.0 \times 10^6$	0.35	2
iCell Cardiomyocytes $\geq 4.0 \times 10^6$	1	4

Table 2: Summary of Vial Size and Thaw Time



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 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.

6. Rinse the empty iCell Cardiomyocytes cryovial with 1 ml of room temperature Plating Medium to recover any residual cells from the vial.
7. 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 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.fujifilmcdi.com/ as a reference for the thawing steps only.

8. Slowly add an additional volume of room temperature Plating Medium to the 50 ml centrifuge tube according to Table 3 below. Gently swirl the centrifuge tube while adding the medium.

Vial Size	Additional Plating Medium (ml)	Total Volume (ml)
iCell Cardiomyocytes $\geq 1.0 \times 10^6$	0.65	2
iCell Cardiomyocytes $\geq 4.0 \times 10^6$	8	10

Table 3: Summary of Vial Size and Additional Plating Medium Volumes



It is critical to add the Plating Medium slowly to ensure maximum viability and attachment of the cells once plated.

9. 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 7 and 8. For example, if pooling 3 vials, add each 1 ml of rinse over 90 seconds (270 seconds total).

Chapter 6. Plating iCell Cardiomyocytes

The recommended seeding density for iCell Cardiomyocytes in standard cell culture plates is 63,000 viable cells/cm². For application-specific plating instructions, see the Application Protocols available online at www.fujifilmcdi.com/product-literature/.

1. Obtain the number of viable cells/vial and viability from the Certificate of Analysis.
2. Invert the thawed iCell Cardiomyocytes 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 total viable cells in the cell suspension.

$$\text{Total Viable Cells (x10}^6\text{)} = \text{Cell Suspension Volume (ml)} \times \text{Cell Concentration (Viable Cells/ml)}$$

5. Calculate the total volume of Plating Medium required for desired cell density.

$$\text{Total Plating Medium Volume (ml):}$$

$$\frac{[\text{Total Viable Cells (x10}^6\text{)} \times \text{Plating Efficiency (as a decimal)}]}{\text{Desired Cell Density (200,000 cells/ml)}}$$

6. Calculate the additional volume of Plating Medium required for desired cell density.

$$\text{Additional Plating Medium (ml)} = \text{Total Plating Medium Volume (ml)} - \text{Cell Suspension Volume (ml)}$$

7. Add the calculated volume of additional Plating Medium to the cell suspension. Invert the cell suspension 2 - 3 times or gently pipette to mix.
8. Aspirate the 0.1% gelatin in water from the pre-coated cell culture vessel(s).
9. Immediately dispense the cell suspension to the cell culture vessel according to Table 4 below. Allow the vessel to remain undisturbed for 15 - 20 minutes in the biosafety cabinet to allow the cells to adhere.

Culture Vessel	Surface Area (cm ²)	Plating Volume (ml)	Cell Number	Cell Density (cells/ml)
6-well Plate	9.6	3	600,000	200,000
96-well Plate	0.32	0.1	20,000	200,000

Table 4: 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.

10. Culture iCell Cardiomyocytes in a cell culture incubator at 37°C, 5% CO₂ for 48 hours.

Expected Cell Density

iCell Cardiomyocytes can be used at varying plating densities, ranging from 10,000 - 100,000 viable cells/cm². The optimal density of iCell Cardiomyocytes can be assay dependent and must be determined empirically based on the intended use. A density of 63,000 viable cells/cm² is the recommended starting density for most assays as it provides a beating syncytium.

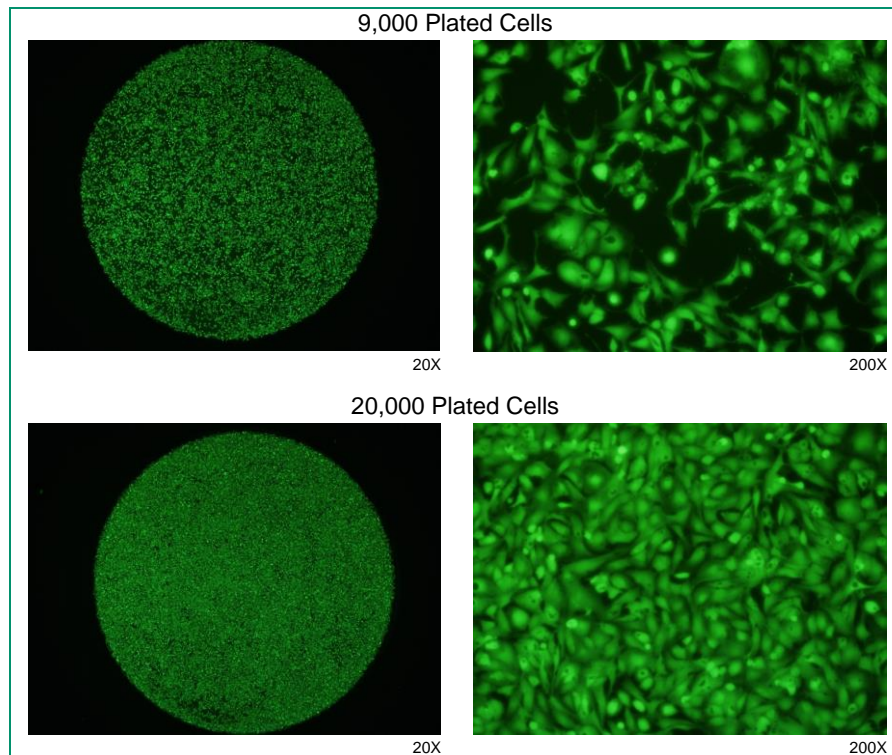


Figure 1: Cell Densities at 48 Hours Post-Plating

iCell Cardiomyocytes, 01434 at 48 hours labeled with calcein-AM demonstrates cell viability, homogenous distribution and expected morphology in a 96-well plate.

Chapter 7. Maintaining iCell Cardiomyocytes

Notes

1. Immediately before use, equilibrate iCell Cardiomyocytes Maintenance Medium (Maintenance Medium) in a 37°C water bath.
2. 48 hours post-plating iCell Cardiomyocytes, aspirate the Plating Medium using a pipettor and replace with the appropriate volume of Maintenance Medium according to Table 5 below. Be careful not to touch or disrupt the adhered cardiomyocytes.

Culture Vessel	Surface Area (cm ²)	Media Volume (ml)
6-well Cell Culture Plate	9.6	3
96-well Cell Culture Plate	0.32	0.1

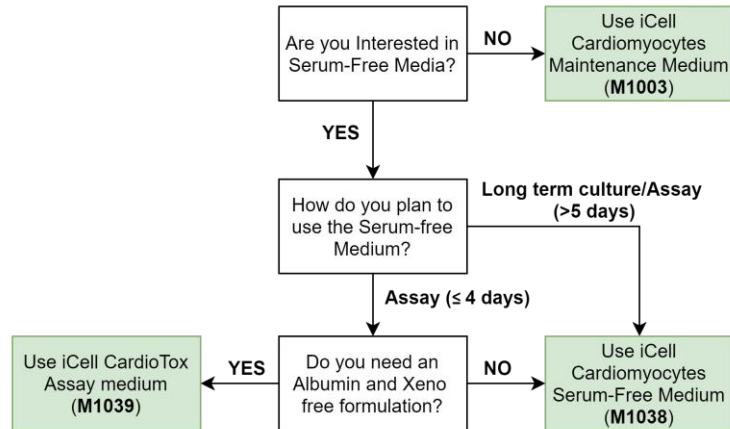
Table 5: Summary of Recommended Volumes and Measures

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

3. Replace the Maintenance Medium every other day.
4. Culture iCell Cardiomyocytes in a cell culture incubator at 37°C, 5% CO₂.

Chapter 8. Assaying iCell Cardiomyocytes

iCell Cardiomyocytes can be assayed in Serum-containing (iCell Cardiomyocytes Maintenance Medium), Serum-free (iCell Cardiomyocytes Serum-Free Medium) or Serum-free **and** Albumin-free (iCell CardioTox Assay Medium) medium. See flow chart below for reference:



Note: The iCell Cardiomyocytes are initially thawed and cultured as described in this User's Guide for 14 days. Once the maintenance media is replaced with iCell CardioTox Assay Medium, the cells can be assayed for up to 4 days. When the maintenance medium is replaced with iCell Cardiomyocytes Serum-Free Medium, the cells can be cultured for up to 14 days.



Refer to the iCell Cardiomyocytes Serum-Free Medium Quick Guide or iCell CardioTox Assay Medium Quick Guide for an assay workflow.

Serum-Free Medium Kit Components Supplied by FUJIFILM Cellular Dynamics

Notes

Item	Catalog Number
iCell Cardiomyocytes Kit w/ iCell Cardiomyocytes Serum-Free Medium, 01434	R1223
<ul style="list-style-type: none"> •iCell Cardiomyocytes, 01434 •iCell Cardiomyocytes Plating Medium •iCell Cardiomyocytes Maintenance Medium •iCell Cardiomyocytes Serum-Free Medium •iCell Cardiomyocytes User's Guide 	<ul style="list-style-type: none"> •C1006 ($\geq 4.0 \times 10^6$ viable cells) •M1001 (30 ml) •M1003 (100 ml) •M1038 (100 ml) •X1000
iCell Cardiomyocytes Kit w/ iCell CardioTox Assay Medium, 01434	R1224
<ul style="list-style-type: none"> •iCell Cardiomyocytes, 01434 •iCell Cardiomyocytes Plating Medium •iCell Cardiomyocytes Maintenance Medium •iCell CardioTox Assay Medium •iCell Cardiomyocytes User's Guide 	<ul style="list-style-type: none"> •C1006 ($\geq 4.0 \times 10^6$ viable cells) •M1001 (30 ml) •M1003 (100 ml) •M1039 (100 ml) •X1000
iCell Cardiomyocytes Kit, w/ iCell Cardiomyocytes Serum-Free Medium, 11713	R1225
<ul style="list-style-type: none"> •iCell Cardiomyocytes, 11713 •iCell Cardiomyocytes Plating Medium •iCell Cardiomyocytes Maintenance Medium •iCell Cardiomyocytes Serum-Free Medium •iCell Cardiomyocytes User's Guide 	<ul style="list-style-type: none"> •C1106 ($\geq 4.0 \times 10^6$ viable cells) •M1001 (30 ml) •M1003 (100 ml) •M1038 (100 ml) •X1000
iCell Cardiomyocytes Kit w/ iCell CardioTox Assay Medium, 11713	R1226
<ul style="list-style-type: none"> •iCell Cardiomyocytes, 11713 •iCell Cardiomyocytes Plating Medium •iCell Cardiomyocytes Maintenance Medium •iCell CardioTox Assay Medium •iCell Cardiomyocytes User's Guide 	<ul style="list-style-type: none"> •C1106 ($\geq 4.0 \times 10^6$ viable cells) •M1001 (30 ml) •M1003 (100 ml) •M1039 (100 ml) •X1000

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