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iCell® Cardiac Progenitor Cells Prototype User's Guide

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Conditions of Use

iCell Cardiac Progenitor Cells 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 Cardiac Progenitor Cells.

Origin

iCell Cardiac Progenitor Cells are manufactured in the United States of America.

Copyright Notice

Revision History

Version 02: September 2015
Version 0.1: December 2014

Table of Contents

Before You Begin

Notes

- Immediately transfer the frozen vials to liquid nitrogen storage.
- Read this entire iCell® Cardiac Progenitor Cells Prototype User's Guide before handling or using iCell Cardiac Progenitor Cells.
- iCell Cardiac Progenitor Cells 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 Cardiac Progenitor Cells 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 Cardiac Progenitor Cells.

Notes

Chapter 1. Introduction

Cellular Dynamics International's (CDI) iCell Cardiac Progenitor Cells are a highly pure population of human cardiac progenitor cells derived from induced pluripotent stem (iPS) cells using CDI's proprietary differentiation and purification protocols. iCell Cardiac Progenitor Cells exhibit expected physiological characteristics and responses. These cells provide a reliable source of human cardiac progenitor cells suitable for use in targeted drug discovery, toxicity testing, and other life science research.

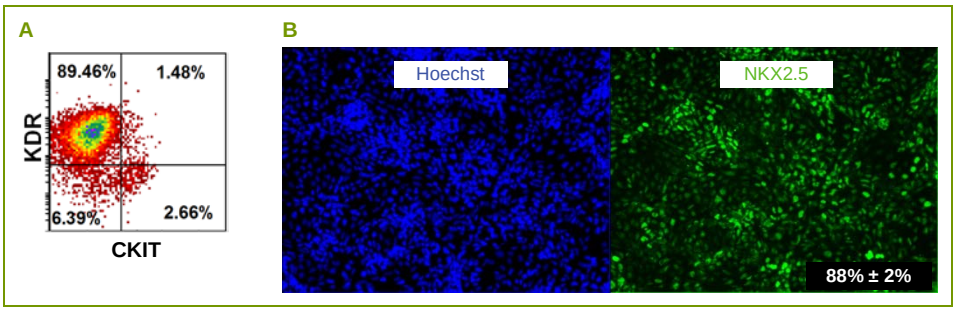

Figure 1: iCell Cardiac Progenitor Cells Represent a Highly Pure Population of Human Cardiac Progenitors

These images show iCell Cardiac Progenitor Cells at day 2 post-plating. iCell Cardiac Progenitor Cells maintain KDR^{+}/CKIT^{-} and NKX2.5^{+} profile as demonstrated by (A) flow cytometry and (B) immunocytochemistry: NKX2.5 (green) and nuclear staining Hoechst (blue).

Components Supplied by Cellular Dynamics

Item	Catalog Number
iCell Cardiac Progenitor Cells Prototype[1]	CPC-301-020-001-PT
iCell Cardiac Progenitor Cells Prototype User's Guide[1]	
Certificate of Testing[2]	
Certificate of Origin If required for shipping purposes	

[1] Safety Data Sheet and User's Guide available online at www.cellulardynamics.com/lit/

[2] Available online at www.cellulardynamics.com/cot/

Required Equipment and Consumables

Note: *As required for the intended use, see the following iCell Cardiac Progenitor Cells Prototype Application Protocols for assay-specific equipment and consumables before thawing cells:*

- *Modeling Cardiac Proliferation: bFGF Induction with High Content Analysis*
- *Modeling Cardiomyocyte Differentiation: Wnt- and Activin/TGFβ-inhibitor Induction with Flow Cytometry Analysis*

These Application Protocols are available online at www.cellulardynamics.com/lit/.

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	
Pipettors	Multiple Vendors	
Tabletop Centrifuge	Multiple Vendors	
Consumables		
15 ml and 50 ml Centrifuge Tubes	Multiple Vendors	
96-well Cell Culture Plates	Multiple Vendors	
Cocktail B from Hepatocyte Maintenance Supplement Pack (Supplement Pack)	Life Technologies	CM4000
Dulbecco's Phosphate Buffered Saline without Ca^{2+} and Mg^{2+} (D-PBS)	Multiple Vendors	
Fibronectin	Roche Applied Sciences	11051407001 (1 mg) 11080938001 (5 mg)
Gentamicin, 50 mg/ml	Life Technologies	15750
Pipettes	Multiple Vendors	
Sterile Tissue Culture Grade Distilled Water	Multiple Vendors	
William's E Medium	Life Technologies	A12176-01

* Ensure automated cell counter is appropriately calibrated before use.

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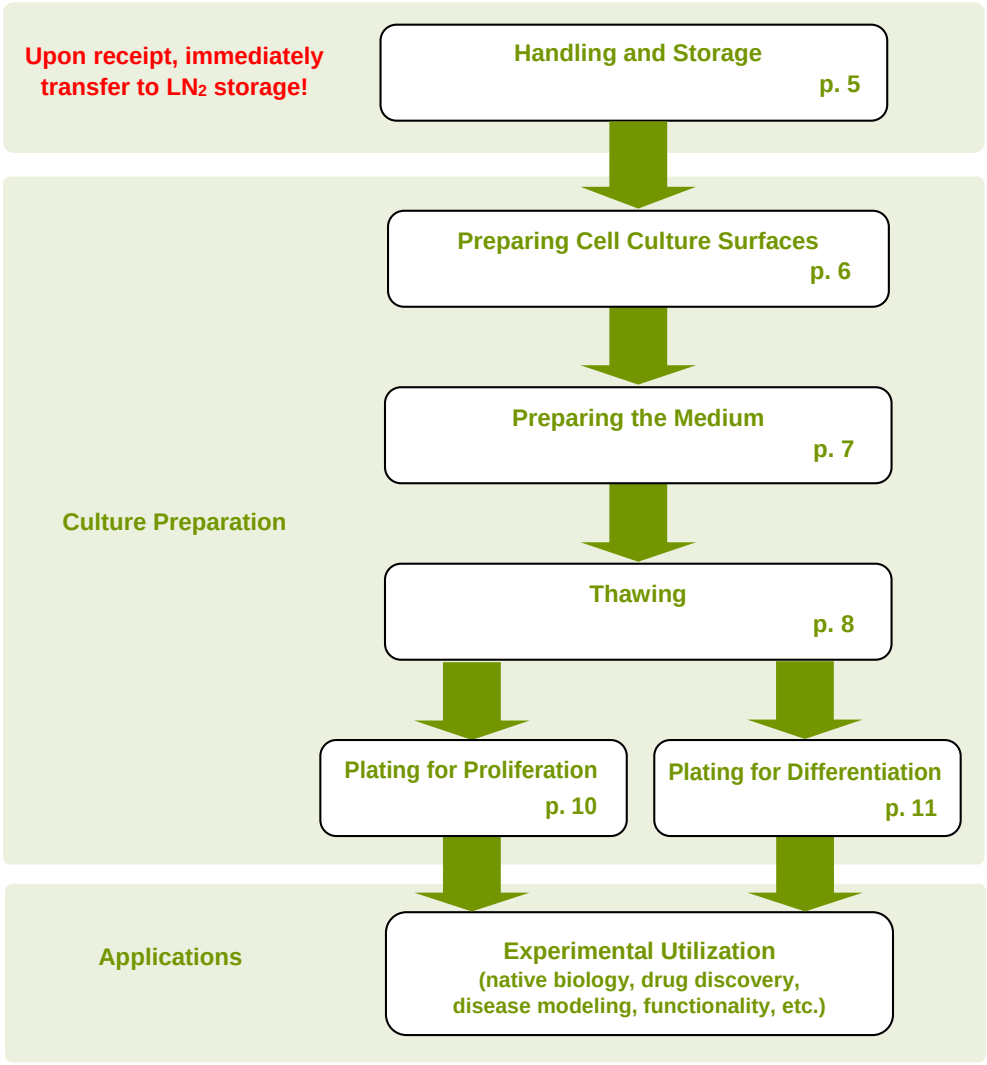
Technical Support and Training

CDI's Technical Support Scientists have the necessary laboratory and analytical experience to respond to your inquiries. In addition, 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

iCell Cardiac Progenitor Cells are provided as cryopreserved single-cell suspensions in 1.5 ml cryovials. Upon receipt, directly transfer the cryobox containing iCell Cardiac Progenitor Cells 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 Cardiac Progenitor Cells at a stable temperature. Minimize exposure of cryopreserved iCell Cardiac Progenitor Cells to ambient temperature when transferring vials to liquid nitrogen storage.

Chapter 3. Preparing Cell Culture Surfaces

Notes

iCell Cardiac Progenitor Cells will function on cell culture vessels pre-coated with fibronectin. The following procedure details coating 96-well cell culture plates. Scale volumes appropriately for other vessel formats.

1. Dilute 1 mg/ml fibronectin solution in sterile D-PBS to a final concentration of 5 µg/ml immediately before use.

   Note: *Reconstitute fibronectin in sterile water at 1 mg/ml according to the manufacturer's instructions. Aliquot and store at -20°C.*

2. Add 100 µl/well of the 5 µg/ml fibronectin solution to the 96-well cell culture plate(s).
3. Incubate the cell culture plate(s) at 37°C for at least 1 hour before plating iCell Cardiac Progenitor Cells.

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Chapter 4. Preparing the Medium

The Maintenance Medium for iCell Cardiac Progenitor Cells is comprised of William's E Medium, Cocktail B, and gentamicin. The Maintenance Medium is serum-free.

1. Prepare the Maintenance Medium by diluting Cocktail B in William's E Medium to 1X immediately before use.

   Note: *Cocktail B is provided in the Supplement Pack and supplies the cardiac progenitor cells with a source of energy in the serum-free William's E Medium. Also provided in the Supplement Pack is dexamethasone, which is not used for preparing the Maintenance Medium.*

   Note: *Stored separately, William's E Medium and Cocktail B are stable at 4°C for 1 year according to the manufacturer*.

2. Dilute gentamicin in Maintenance Medium at a final concentration of 25 µg/ml.
3. Invert to mix. Do not filter.
4. Store the Maintenance Medium at 4°C, protected from light, for up to 2 weeks.

Chapter 5. Thawing iCell Cardiac Progenitor Cells

Notes

Maintain iCell Cardiac Progenitor Cells 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 Cardiac Progenitor Cells viability and performance. See Chapters 6 and 7 for plating instructions for inducing proliferation or differentiation, respectively.

Note: *Thaw no more than 3 vials of iCell Cardiac Progenitor Cells at one time.*

1. Equilibrate the Maintenance Medium at room temperature for 2 - 4 hours before thawing iCell Cardiac Progenitor Cells.
2. Remove the iCell Cardiac Progenitor Cells cryovial from the liquid nitrogen storage tank.

   Note: *If necessary, place cryovials on dry ice for up to 10 minutes before thawing.*
3. 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.
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 Cardiac Progenitor Cells cryovial contents to a 50 ml centrifuge tube using a 1 ml pipettor.

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

   

   Avoid repeated pipetting of the thawed iCell Cardiac Progenitor Cells suspension.
6. Rinse the empty iCell Cardiac Progenitor Cells cryovial with 1 ml of room temperature Maintenance Medium to recover residual cells from the cryovial. Transfer the 1 ml of Maintenance Medium rinse from the cryovial drop-wise (~1 drop every 4 - 5 seconds) to the 50 ml centrifuge tube containing the iCell Cardiac Progenitor Cells 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 Maintenance Medium to the cell suspension is critical to minimize osmotic shock and ensure maximum viability and subsequent attachment of the cells to the plating substrate.

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7. Add 3 ml of room temperature Maintenance Medium to the 50 ml centrifuge tube. Add the first 1 ml drop-wise over 30 - 60 seconds. Then add the remaining 2 ml over the next ~30 seconds. Gently swirl the centrifuge tube while adding the medium.

It is critical to add the 3 ml of Maintenance Medium slowly to ensure maximum viability and attachment of the cells once plated.

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 Cardiac Progenitor Cells at one time. Once thawed, combine the contents of the cryovials before adding the rinse and final volume of Maintenance Medium. Follow the timing outlined in steps 6 and 7. For example, if pooling 3 cryovials, add each 1 ml of rinse over 90 seconds (270 seconds total).*

Chapter 6. Plating iCell Cardiac Progenitor Cells for Cardiac Proliferation

Notes

iCell Cardiac Progenitor Cells will proliferate when cultured in fibroblast growth factor (FGF)-containing Maintenance Medium for 2 days. The proliferation rate can be determined and quantified as number of $NKX2.5^+$ cells.

For assay instructions, see the iCell Cardiac Progenitor Cells Prototype Application Protocol: Modeling Cardiac Proliferation: bFGF Induction with High Content Analysis available online at www.cellulardynamics.com/lit/.

The following procedure describes how to plate iCell Cardiac Progenitor Cells at 0.78×10^5 viable cells/cm^2 into a 96-well cell culture plate. Scale volumes appropriately for other cell culture vessel formats.

1. Invert the thawed iCell Cardiac Progenitor Cells suspension 2 - 3 times to ensure an even cell 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. Dilute the cell suspension in room temperature Maintenance Medium to 2.8×10^5 viable cells/ml.
4. Aspirate the fibronectin solution from a pre-coated 96-well cell culture plate.
5. Invert the cell suspension 6 times. Immediately dispense 90 µl/well of cell suspension (~25,000 viable cells/well).
6. Immediately proceed to the instructions in the iCell Cardiac Progenitor Cells Prototype Application Protocol: Modeling Cardiac Proliferation: bFGF Induction with High Content Analysis for cell culturing with bFGF and labeling for analysis.

Notes

Chapter 7. Plating iCell Cardiac Progenitor Cells for Cardiomyocyte Differentiation

iCell Cardiac Progenitor Cells will differentiate into cardiomyocytes within 6 - 8 days when cultured in XAV939/SB431542 containing Maintenance Medium for 2 days, then in XAV939/SB431542-free Maintenance Medium for 4 - 6 days. The differentiation can be determined and quantified as number of cardiac troponin T $(cTNT)^+$ cells.

For assay instructions, see the iCell Cardiac Progenitor Cells Prototype Application Protocol: Modeling Cardiomyocyte Differentiation: Wnt- and Activin/TGFβ-inhibitor Induction with Flow Cytometry Analysis available online at www.cellulardynamics.com/lit/.

The following procedure describes how to plate iCell Cardiac Progenitor Cells at 1.56×10^5 viable cells/cm^2 into a 96-well cell culture plate. Scale volumes appropriately for other cell culture vessel formats.

1. Invert the thawed iCell Cardiac Progenitor Cells suspension 2 - 3 times to ensure an even cell 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. Dilute the cell suspension in room temperature Maintenance Medium to 5.6×10^5 viable cells/ml.
4. Aspirate the fibronectin solution from a pre-coated 96-well cell culture plate.
5. Invert the cell suspension 6 times. Immediately dispense 90 µl/well of cell suspension (~50,000 viable cells/well).
6. Immediately proceed to the instructions in the iCell Cardiac Progenitor Cells Prototype Application Protocol: Modeling Cardiomyocyte Differentiation: Wnt- and Activin/TGFβ-inhibitor Induction with Flow Cytometry Analysis for cell culturing with XAV939 and SB431542, collecting, staining, and labeling for analysis.

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