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iCell[®] Cardiac Progenitor Cells

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 Cardiac Progenitor Cells as described herein. You assume all risk in connection with your use of iCell Cardiac Progenitor Cells.

Conditions of Use

iCell Cardiac Progenitor Cells 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 Cardiac Progenitor Cells.

Origin

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

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Revision History

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Before You Begin

- Immediately transfer the frozen vials to liquid nitrogen storage.
- Read this entire iCell® Cardiac Progenitor Cells 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.

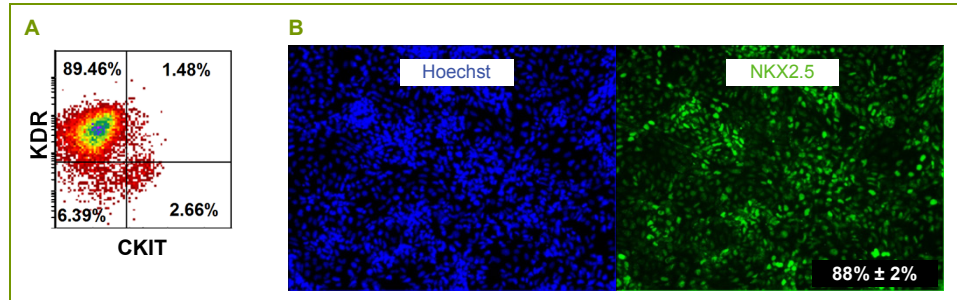


Figure 1: iCell Cardiac Progenitor Cells Represent a Highly Pure Population of Human Cardiac Progenitors

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

Notes

Item	Catalog Number
iCell Cardiac Progenitor Cells Kit, 01279	R1093
• iCell Cardiac Progenitor Cells, 01279 ^{1, 2}	• C1038 (≥5.0 x 10 ⁶ viable cells)
• iCell Cardiac Progenitor Cells User's Guide ²	• X1012

Certificate of Testing³

Certificate of Origin
If required for shipping purposes

1 This product was formerly known by this name and catalog number: iCell Cardiac Progenitor Cells Prototype (Cat. No. CPC-301-020-001-PT).

Note: You may receive product labeled with its former name and catalog number until current stock is depleted. There were no changes in the manufacture of the cells.

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

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

Required Equipment and Consumables

Note: As required for the intended use, see the following iCell Cardiac Progenitor Cells 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: 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 ¹	Multiple Vendors	
Liquid Nitrogen Storage Unit	Multiple Vendors	
Pipettors	Multiple Vendors	
Tabletop Centrifuge	Multiple Vendors	
Consumables		
24-well Flat-bottom Plate, TC-treated, Costar ²	STEMCELL Technologies	38017
6-well Flat-bottom Plate, TC-treated, Costar ²	STEMCELL Technologies	38015
96-well Flat-bottom Microplate, TC-treated, Falcon ²	STEMCELL Technologies	38022
Cocktail B from Hepatocyte Maintenance Supplement Pack (Supplement Pack)	Thermo Fisher Scientific	CM4000
Conical Tubes, 15 ml, Falcon (Centrifuge Tubes) ²	STEMCELL Technologies	38009
Conical Tubes, 50 ml, Falcon (Centrifuge Tubes) ²	STEMCELL Technologies	38010
Dulbecco's Phosphate Buffered Saline without Ca ²⁺ and Mg ²⁺ (D-PBS) ²	STEMCELL Technologies	37350
Fibronectin	Sigma-Aldrich	11051407001 - 1 mg 11080938001 - 5 mg
Gentamicin, 50 mg/ml	Thermo Fisher Scientific	1575060
Serological Pipettes, 5, 10, 25 ml ²	STEMCELL Technologies	38003, 38004, 38005

Notes

Item	Vendor	Catalog Number
Sterile Tissue Culture Grade Distilled Water	Multiple Vendors	
Trypan Blue ²	STEMCELL Technologies	07050
William's E Medium	Thermo Fisher Scientific	A1217601

- 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

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, 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

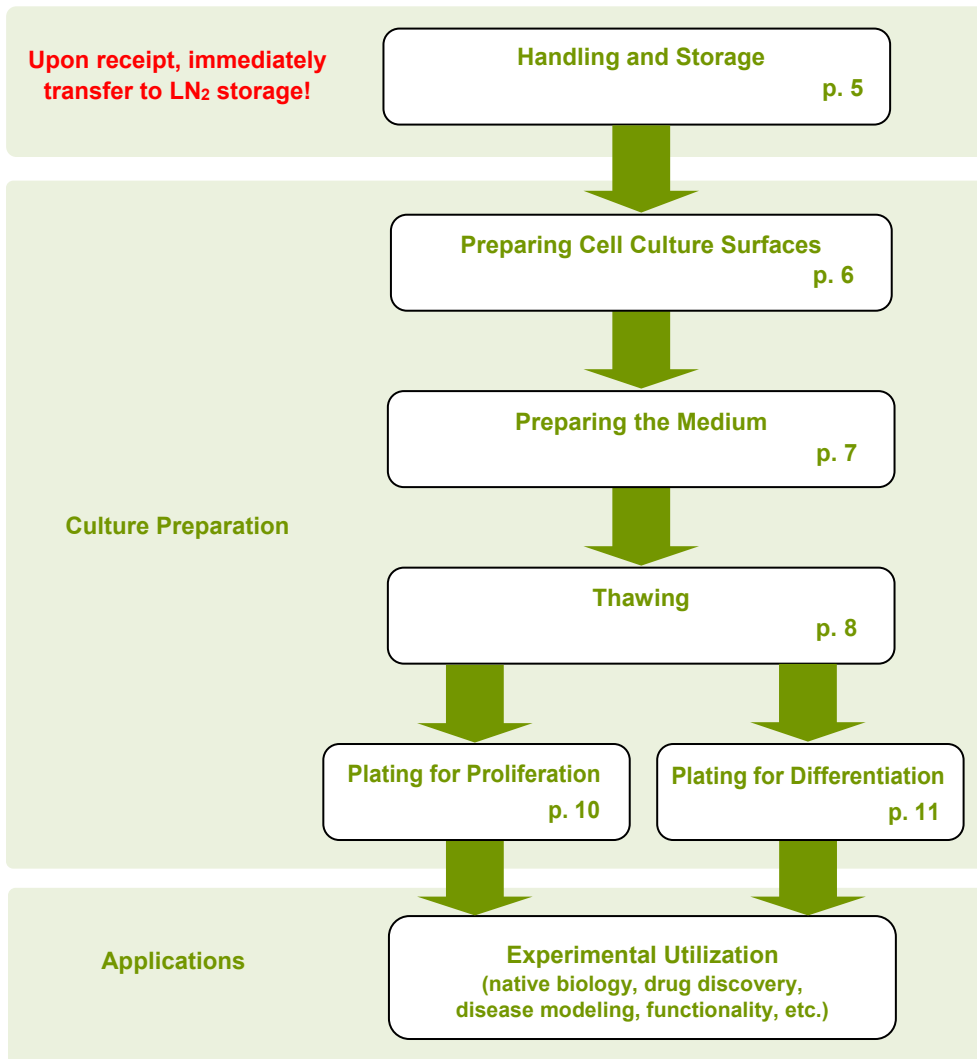
Fax (608) 310-5101

Email support@cellulardynamics.com

Knowledge Base www.cellulardynamics.com/knowledgebase/

Workflow Diagram

Notes



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.

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

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.

Notes

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 Application Protocol: Modeling Cardiac Proliferation: bFGF Induction with High Content Analysis available online: www.cellulardynamics.com/lit/

The following procedure describes how to plate iCell Cardiac Progenitor Cells at 0.78×10^5 viable cells/cm² 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 μ l/well of cell suspension (~25,000 viable cells/well).
6. Immediately proceed to the instructions in the iCell Cardiac Progenitor Cells Application Protocol: Modeling Cardiac Proliferation: bFGF Induction with High Content Analysis for cell culturing with bFGF and labeling for analysis.

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 Application Protocol: Modeling Cardiomyocyte Differentiation: Wnt- and Activin/TGF β -inhibitor Induction with Flow Cytometry Analysis available online: www.cellulardynamics.com/lit/

The following procedure describes how to plate iCell Cardiac Progenitor Cells at 1.56×10^5 viable cells/cm² 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 μ l/well of cell suspension (~50,000 viable cells/well).
6. Immediately proceed to the instructions in the iCell Cardiac Progenitor Cells Application Protocol: Modeling Cardiomyocyte Differentiation: Wnt- and Activin/TGF β -inhibitor Induction with Flow Cytometry Analysis for cell culturing with XAV939 and SB431542, collecting, staining, and labeling for analysis.

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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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C. **DATA.** Customer agrees that if described on Customer's product quotation from Cellular Dynamics it will provide data and information as described therein to Cellular Dynamics regarding Customer's use of the Products.

Appendix B. Product Provided "AS IS"

A. The Products are sold or provided "AS IS."

B. TO THE FULLEST EXTENT PERMITTED BY APPLICABLE LAW, CELLULAR DYNAMICS DISCLAIMS, ALL REPRESENTATIONS, AND WARRANTIES, EXPRESS OR IMPLIED (INCLUDING BUT NOT LIMITED TO, ANY IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE OR NON-INFRINGEMENT), AND ALL LIABILITY, WITH RESPECT TO THE PRODUCTS; AND BY ORDERING AND ACCEPTING THE PRODUCTS CUSTOMER WAIVES ALL RIGHTS AND REMEDIES OTHERWISE AVAILABLE WITH RESPECT TO THE PRODUCTS.

Notes

C. Customer will be solely responsible for (i) Customer's use of the Products for a purpose or in a manner other than that for which they were designed or that is permitted or in breach of the Use Restrictions above; (ii) Customer's failure to follow this User's Guide for the use, storage, and handling of the Products however such failure is caused; (iii) Customer's failure to comply with any of the provisions of Appendix A above; and (iv) any abuse, other misuse or neglect of the Products by Customer or any damage or loss of the Products by events or occurrences beyond a person's (e.g., Cellular Dynamics') control including without limitation, accident, fire, vandalism and natural disasters (acts of God).

D. 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. Because the number of cells in a unit 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.

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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