Cellular Dynamics international

iCell® Hepatocytes 2.0

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 Hepatocytes 2.0 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 Hepatocytes 2.0.

Conditions of Use

iCell Hepatocytes 2.0 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 Hepatocytes 2.0.

Origin

iCell Hepatocytes 2.0 are manufactured in the United States of America.

Copyright Notice

Revision History

Version 1.1: July 2015
Version 1.0: March 2015

Table of Contents

Before You Begin

Notes

- Immediately transfer the frozen vial(s) to liquid nitrogen storage.
- Read this entire iCell® Hepatocytes 2.0 User's Guide before handling or using iCell Hepatocytes 2.0.
- iCell Hepatocytes 2.0 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 Hepatocytes 2.0 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 Hepatocytes 2.0.

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Chapter 1. Introduction

Cellular Dynamics International's (CDI) iCell Hepatocytes 2.0 are a highly pure population of human hepatocytes derived from induced pluripotent stem (iPS) cells using CDI's proprietary differentiation and purification protocols. iCell Hepatocytes 2.0 provide, for the first time, access to commercial quantities of high quality, high purity human liver cells for preclinical drug discovery, hepatotoxicity, and disease research.

When handled and maintained as recommended in this User's Guide, iCell Hepatocytes 2.0 exhibit a mature hepatocyte morphology, characteristic gene and protein expression, and active metabolism. iCell Hepatocytes 2.0 maintain their morphology and function for at least 14 days in culture making them amenable to a variety of assay endpoints.

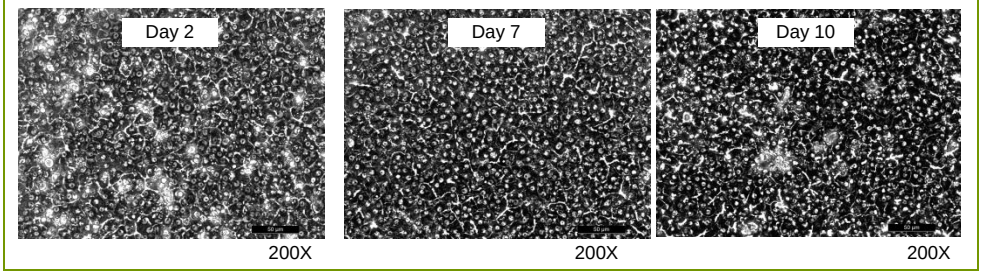

Figure 2: iCell Hepatocytes 2.0 Exhibit Mature Hepatocyte Morphology
These images show iCell Hepatocytes 2.0 at days 2, 7, and 10 post-plating. iCell Hepatocytes 2.0 display an adherent monolayer and cobblestone morphology within 48 hours and remain viable and adherent for an extended period in culture (≥14 days). Some debris in the culture is expected but will decrease over time with medium replacements.

Components Supplied by Cellular Dynamics

Item	Catalog Number
iCell Hepatocytes 2.0[1]	PHC-100-020-001
iCell Hepatocytes 2.0 Medium Supplement[1]	HCS-100-021-001
iCell Hepatocytes 2.0 User's Guide[1]	
Certificate of Analysis[2]	
Certificate of Origin If required for shipping purposes	

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

2 Available online at www.cellulardynamics.com/coa/

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	
Pipettors	Multiple Vendors	
Tabletop Centrifuge	Multiple Vendors	
Consumables		
15 ml Centrifuge Tubes	Multiple Vendors	
B27 Supplement, 50X	Life Technologies	17504
Biocoat Collagen I Multiwell Plates*	Becton Dickinson	354408 (24-well) 354407 (96-well)
Dexamethasone	Fisher Scientific	ICN19456125
Gentamicin	Life Technologies	15750
Oncostatin M	R&D Systems	295-OM
Pipettes	Multiple Vendors	
RPMI	Life Technologies	11875
Trypan Blue	Life Technologies	15250

* CDI recommends using pre-coated cell culture vessels for culturing iCell Hepatocytes 2.0. However, instructions for manually coating cell culture vessels are provided in Chapter 3, Preparing Cell Culture Surfaces.

Notes

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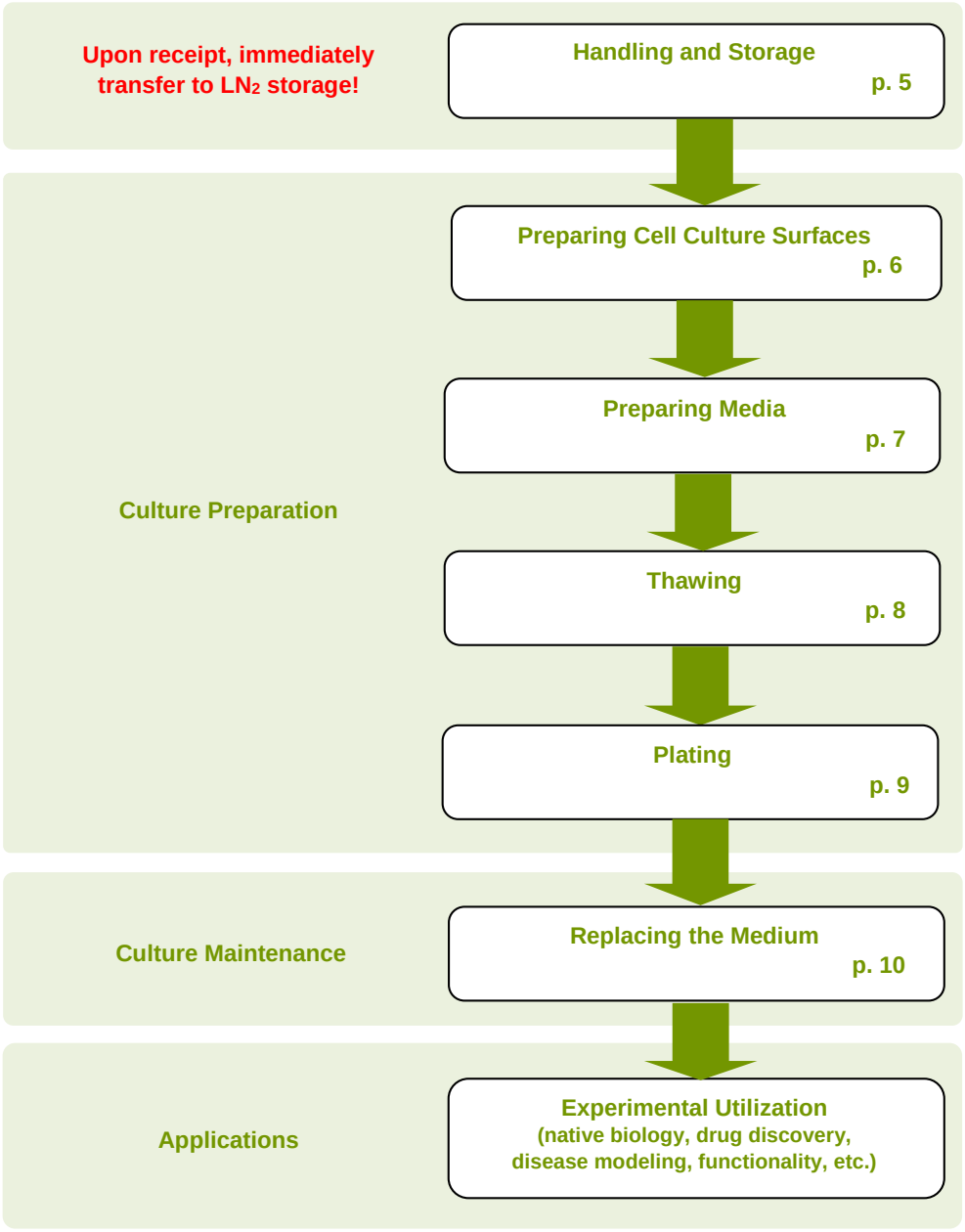
Technical Support and Training

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

Handling iCell Hepatocytes 2.0

iCell Hepatocytes 2.0 are provided as cryopreserved single-cell suspensions in 1.5 ml cryovials. Upon receipt, directly transfer the individual vials containing iCell Hepatocytes 2.0 to the vapor phase of a liquid nitrogen storage dewar.

Note: *Do not store iCell Hepatocytes 2.0 on dry ice or at -80°C as this may impact their performance post-thawing.*

It is critical to maintain cryopreserved iCell Hepatocytes 2.0 at a stable temperature. Minimize exposure of cryopreserved iCell Hepatocytes 2.0 to ambient temperature when transferring vials to liquid nitrogen storage.

Handling iCell Hepatocytes 2.0 Medium Supplement

iCell Hepatocytes 2.0 Medium Supplement is provided in 3 ml tubes and shipped frozen on dry ice. Upon receipt, store iCell Hepatocytes 2.0 Medium Supplement at -80°C until ready for use.

Chapter 3. Preparing Cell Culture Surfaces

Notes

iCell Hepatocytes 2.0 will plate and function optimally on collagen I-coated cell culture vessels. You can purchase pre-coated vessels (recommended) or coat them manually. Regardless of the coating methodology, have the plating surfaces ready before thawing iCell Hepatocytes 2.0.

Recommended Vessel: Using the Pre-coated Cell Culture Vessel

See Required Equipment and Consumables section in Chapter 1, Introduction, for ordering information for recommended pre-coated cell culture vessels.

Alternative Vessel: Preparing the Collagen I Cell Culture Vessel

1. Prepare 0.02 M acetic acid (Sigma, Cat. No. 320099) in sterile Milli-Q (or equivalent) water.

   Observe safety precautions for handling concentrated acids. Wear eye protection and impervious gloves. Use caution and always add acid slowly to water, never the reverse.

2. Dilute 3 mg/ml collagen I solution (Gibco, Cat. No. A1048301) in 0.02 M acetic acid to a final concentration of 100 µg/ml.

3. Select the cell culture vessel appropriate for your experimental use. Use the volumes specified in the table below in the following coating procedure. Scale volumes appropriately for other vessel formats.

Culture Vessel	Volume of 100 µg/ml Collagen I Solution (ml)	Volume of Water Rinse (ml)
24-well Cell Culture Plate	0.25	0.5
96-well Cell Culture Plate	0.032	0.1

Table 1: Summary of Useful Volumes
*All volumes are **per well**.*

4. Add collagen I solution to each well of the vessel(s).

5. Tap the vessel(s) to distribute liquid evenly.

6. Incubate the vessel(s) at room temperature for at least 1 hour.

7. After incubation, completely aspirate the collagen I solution from each well. Rinse each well 3 times with sterile Milli-Q water and aspirate completely.

   ***Note:** If necessary, air-dry the vessels coated with collagen I and store at 4°C for up to 7 days before use. Equilibrate the vessel(s) at room temperature before plating iCell Hepatocytes 2.0.*

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Chapter 4. Preparing Media

Prepare and store the Plating Medium and Maintenance Medium for iCell Hepatocytes 2.0 as follows:

Plating Medium[1, 2, 3, 4]		
Component	**Amount (ml)**	**Final Concentration**
RPMI	72	96%
B27 Supplement, 50X	1.5	1X
Oncostatin M, 10 µg/ml	0.15	20 ng/ml
Dexamethasone, 5 mM	0.0015	0.1 µM
Gentamicin	0.0375	25 µg/ml
iCell Hepatocytes 2.0 Medium Supplement	1.5	1X

1 Prepare Plating Medium on the day of thaw to ensure optimal viability of cells upon plating.

2 Prepare stock solutions of 10 µg/ml oncostatin M and 5 mM dexamethasone. Follow the manufacturer's instructions for storage and reconstitution.

3 Prepare 10 ml aliquots of Plating Medium in 15 ml centrifuge tubes. Up to 2 vials of iCell Hepatocytes 2.0 can be thawed into 1 tube of 10 ml Plating Medium.

4 Store Plating Medium at 4°C for up to 1 week. Do not store at -20°C.

Maintenance Medium*		
Component	**Amount (ml)**	**Final Concentration**
RPMI	72	96%
B27 Supplement, 50X	1.5	1X
Dexamethasone, 5 mM	0.0015	0.1 µM
Gentamicin	0.0375	25 µg/ml
iCell Hepatocytes 2.0 Medium Supplement	1.5	1X

* Store Maintenance Medium at 4°C for up to 1 week. Do not store at -20°C.

Chapter 5. Thawing iCell Hepatocytes 2.0

Notes

Maintain iCell Hepatocytes 2.0 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 Hepatocytes 2.0 viability and performance.

Note: *Thaw no more than 2 vials of iCell Hepatocytes 2.0 at one time.*

1. Equilibrate a 10 ml aliquot of Plating Medium in a 37°C water bath before thawing iCell Hepatocytes 2.0.
2. Remove the iCell Hepatocytes 2.0 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 **exactly 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 and attachment.
4. Immediately remove the cryovial from the water bath, spray with 70% ethanol, and place in a biological safety cabinet.
5. Gently transfer the iCell Hepatocytes 2.0 cryovial contents into the 15 ml centrifuge tube containing 10 ml of 37°C Plating Medium.

   Avoid repeated pipetting of the thawed iCell Hepatocytes 2.0 cell suspension.
6. Rinse the empty iCell Hepatocytes 2.0 cryovial with 1 ml of 37°C Plating Medium to recover any residual cells from the vial. Transfer the 1 ml of Plating Medium rinse from the cryovial to the 15 ml centrifuge tube containing the iCell Hepatocytes 2.0 cell suspension. Invert the tube slowly to mix the cell suspension.
7. Centrifuge the cell suspension at 200 x g for 3 minutes at room temperature.
8. Carefully aspirate the supernatant, taking care not to disturb the cell pellet.
9. Slowly add 2 ml of 37°C Plating Medium with a wide-bore pipette to resuspend the cell pellet. Avoid vigorous shaking or vortexing of the cell suspension.

   Note: *Avoid repeated pipetting of the thawed iCell Hepatocytes 2.0 to ensure maximum viability and cell attachment.*

   Note: *Thaw no more than 2 vials of iCell Hepatocytes 2.0 at one time. Once thawed, pour the contents of the 2 vials into the same 15 ml centrifuge tube containing 10 ml of 37°C Plating Medium and rinse each vial before inverting the tube.*

Notes

Chapter 6. Plating iCell Hepatocytes 2.0

The recommended plating density of iCell Hepatocytes 2.0 is 3 x 10^5 viable cells/cm^2. Adjust the cell concentration in Plating Medium as needed.

1. Obtain the number of viable cells/vial and viability from the Certificate of Analysis.
2. Remove a sample of cells to confirm the viability of the cells using a hemocytometer (using trypan blue exclusion to identify viable cells) or an automated cell counter.

   Note: *Use a hemocytometer until validation of the automated cell counter can be completed as cell debris may cause altered viability values as calculated on automated counters.*
3. Calculate the final volume of Plating Medium needed to obtain a desired cell plating density (i.e. 1 x 10^6 cells/ml) using the number of viable cells/vial from the Certificate of Analysis.
4. Dispense the cell suspension into the appropriate cell culture vessel(s). Use the volumes specified in the table below for the selected vessel format. For 24-well cell culture plates or larger, gently shake the plates to distribute the cells evenly. Do not shake 96-well cell culture plates.

Culture Vessel	Surface Area (cm^2)	Plating Volume (ml)	Cell Number (3 x 10^5 cells/cm^2)
24-well Cell Culture Plate	1.9	0.57	5.7 x 10^5
96-well Cell Culture Plate	0.32	0.096	0.96 x 10^5

Table 1: Summary of Recommended Volumes and Measures
*All volumes and measures are **per well**.*

5. Culture iCell Hepatocytes 2.0 in a cell culture incubator at 37°C, 5% CO_2 for 3 - 4 hours.
6. During incubation, equilibrate an aliquot Plating Medium at room temperature.
7. Following incubation, shake 24-well cell culture plates in the diagonal planes 4 times to remove dead cells and debris. Do not shake 96-well cell culture plates.
8. Aspirate the Plating Medium from the plate using a pipettor and replace with room temperature Plating Medium.

   Note: *Do not aspirate the medium from more than 12 wells of any vessel format at one time to prevent wells from drying.*
9. Culture iCell Hepatocytes 2.0 in a cell culture incubator at 37°C, 5% CO_2 overnight.

Chapter 7. Maintaining iCell Hepatocytes 2.0

Notes

iCell Hepatocytes 2.0 are shipped cryopreserved at high purity. The hepatocytes preserve a high purity for at least 14 days after thawing if plated and maintained as recommended.

Plating Medium and Maintenance Medium are stable for 1 week when stored at 4°C.

1. Equilibrate Plating Medium at room temperature before use.
2. Approximately 24 hours post-plating iCell Hepatocytes 2.0, aspirate the spent medium and replace (100% exchange) with the appropriate volume of Plating Medium. Recommended volumes are as follows:
   - **24-well cell culture plate:** 0.6 ml/well
   - **96-well cell culture plate:** 100 µl/well
3. Replace 100% of the spent Plating Medium daily until day 5 post-plating.
4. At day 5 post-plating, equilibrate Maintenance Medium at room temperature before use.
5. Aspirate the spent Plating Medium and replace (100% exchange) with the appropriate volume of Maintenance Medium. Recommended volumes are as follows:
   - **24-well cell culture plate:** 0.6 ml/well
   - **96-well cell culture plate:** 100 µl/well
6. Replace 100% of Maintenance Medium every 2 days thereafter.
7. Culture iCell Hepatocytes 2.0 in a cell culture incubator at 37°C, 5% CO_2.

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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

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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.

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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.

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Appendix C. Limited Liability

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