iCell® Hepatoblasts Prototype

User's Guide

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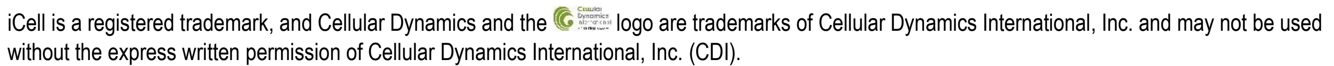

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Conditions of Use

iCell Hepatoblasts 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 Hepatoblasts.

Origin

iCell Hepatoblasts are manufactured in the United States of America.

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Revision History

Version 0.1: April 2016

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Before You Begin

Notes

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

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Notes

Chapter 1. Introduction

iCell Hepatoblasts represent a progenitor population upstream in differentiation from the iCell Hepatocytes 2.0 and iCell Hepatocytes products. This cell type reflects the point in differentiation from induced pluripotent stem (iPS) cells past endoderm but before being a fully specified hepatocyte. Thus, the hepatoblast cells retain the potential to differentiate into both hepatocytes and cholangiocytes. This population of human hepatoblasts is derived from iPS cells using CDI's proprietary differentiation and purification protocols. These cells provide a reliable source of human hepatoblasts that are suitable for use in preclinical drug discovery, toxicity testing, and other life science research.

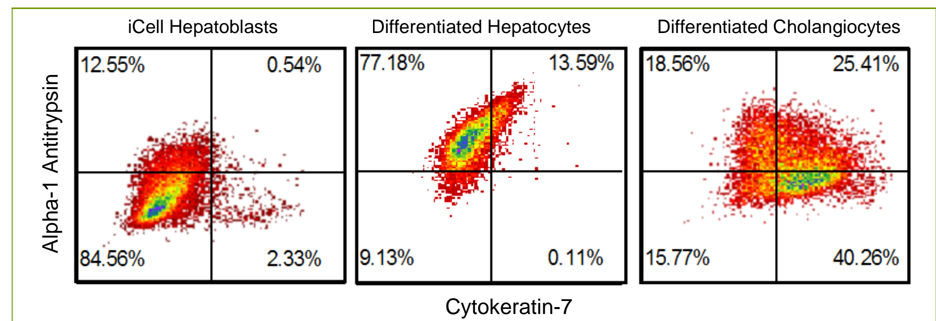


Figure 1: iCell Hepatoblasts Represent a Population of Bipotential Cells
These images show iCell Hepatoblasts at day 0 post-plating and day 7 post-differentiation into hepatocytes or cholangiocytes. iCell Hepatoblasts can be induced to differentiate toward hepatocyte or cholangiocyte lineages, as demonstrated by flow cytometry, resulting in an increase of alpha-1 antitrypsin or cytokeratin-7 expressing cells, respectively.

Components Supplied by Cellular Dynamics

Item	Catalog Number
iCell Hepatoblasts Prototype[1]	HBC-100-020-001-PT
iCell Hepatoblasts Prototype User's Guide[1]	
Certificate of Testing[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/cot/

Required Equipment and Consumables

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

- *Modeling Hepatocyte Differentiation with Flow Cytometry Analysis*
- *Modeling Cholangiocyte Differentiation 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[1]	Multiple Vendors	
Liquid Nitrogen Storage Unit	Multiple Vendors	
Pipettors	Multiple Vendors	
Tabletop Centrifuge	Multiple Vendors	
Consumables		
15 ml Centrifuge Tubes	Multiple Vendors	
Biocoat Collagen I Multiwell Plates[2]	Becton Dickinson	354408 (24-well) 354407 (96-well)
Pipettes	Multiple Vendors	
Trypan Blue	Life Technologies	15250
Plating Medium Components		
B27 Supplement	Life Technologies	17504
Blebbistatin	EMD Scientific	203391
	Sigma	B0560
BMP4	R&D Systems	314-BP
CHIR99021	Biovision	1677-25
	Stemgent	04-0004-10
FGF10	R&D Systems	345-FG
FGF basic (bFGF)	R&D Systems	233-FB
Gentamicin	Life Technologies	15750
HGF	R&D Systems	294-HGN

Notes

Notes

Item	Vendor	Catalog Number
RPMI	Life Technologies	11875

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

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

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.

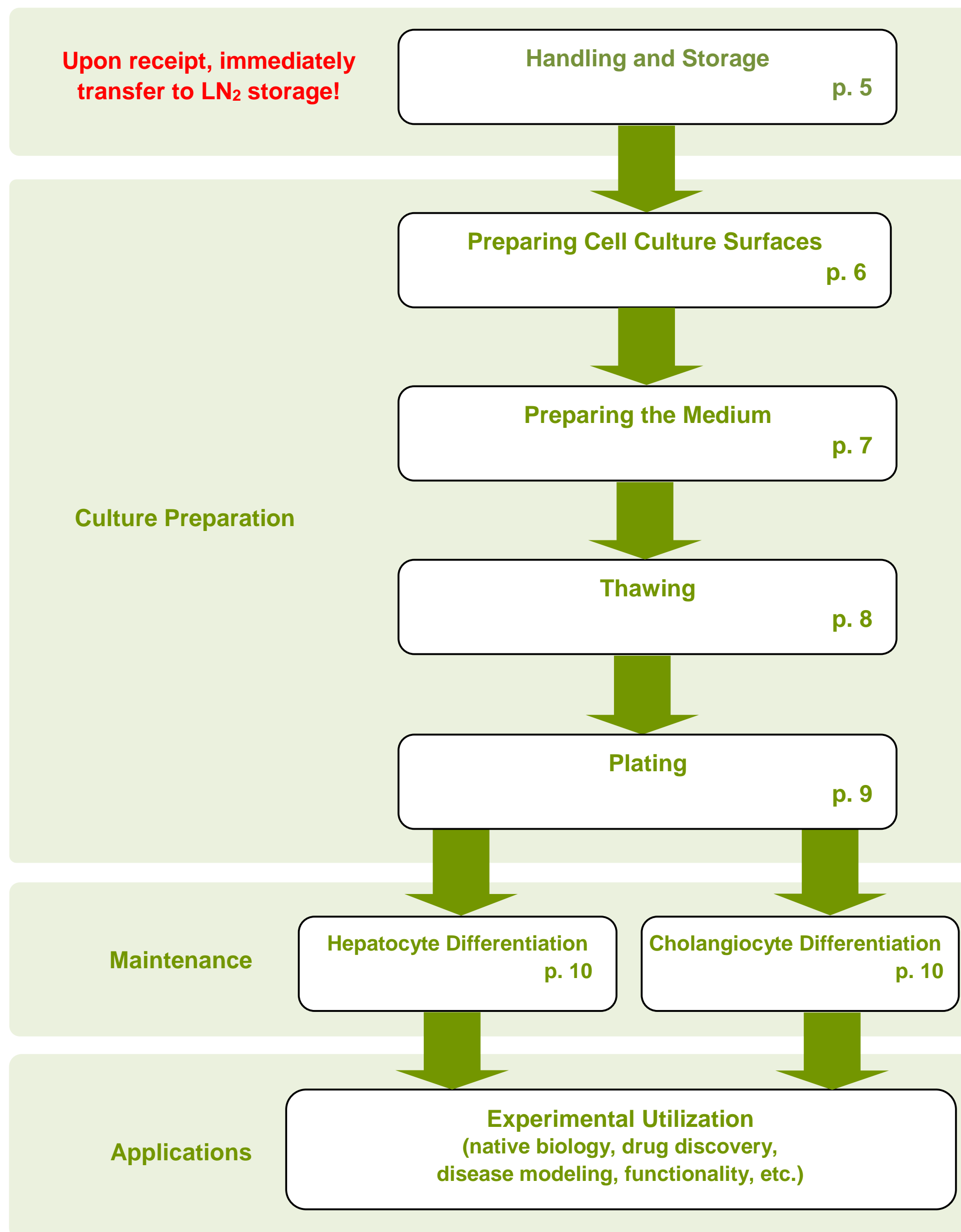
Telephone (877) 320-6688 (US toll-free) / (608) 310-5100 x5
Monday - Friday, 8:30 am - 5:00 pm US Central Time

Fax (608) 310-5101

Email support@cellulardynamics.com

Workflow Diagram

Notes



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Chapter 2. Handling and Storage

Handling iCell Hepatoblasts

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

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

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

Chapter 3. Preparing Cell Culture Surfaces

Notes

iCell Hepatoblasts 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 Hepatoblasts.

Recommended Vessel: Using the Pre-coated Cell Culture Vessel

See Required Equipment and Consumables section in Chapter 1, Introduction, for ordering information for the 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 tissue culture grade distilled 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 sterile tissue culture grade distilled 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 Hepatoblasts.*

Notes

Chapter 4. Preparing the Medium

Prepare and store the Plating Medium for iCell Hepatoblasts as follows:

Component[1, 2, 3, 4, 5]	Amount (ml)	Final Concentration
RPMI	98	98%
B27 Supplement, 50X	2	1X
Blebbistatin, 25 mM	0.01	2.5 µM
BMP4, 25 µg/ml	0.2	50 ng/ml
CHIR99021, 10mM	0.03	3 µM
FGF10, 100 µg/ml	0.06	60 ng/ml
bFGF, 100 µg/ml	0.01	10 ng/ml
Gentamicin, 50 mg/ml	0.05	25 µg/ml
HGF, 25 µg/ml	0.08	20 ng/ml

1 Prepare Plating Medium on the day of thaw to ensure optimal viability of cells upon plating.
2 Follow the manufacturer's instructions for storage and reconstitution of component stock solutions.
3 Filter the medium using a 150 ml, 0.2 µm PES filter unit.
4 Prepare 10 ml aliquots of Plating Medium in 15 ml centrifuge tubes.
5 Store Plating Medium at 4°C for up to 1 week. Do not store at -20°C.

Chapter 5. Thawing iCell Hepatoblasts

Notes

Maintain iCell Hepatoblasts 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 Hepatoblasts viability and performance.

Note: *Thaw no more than 3 vials of iCell Hepatoblasts at one time.*

1. Equilibrate a 10 ml aliquot of Plating Medium in a 37°C water bath before thawing iCell Hepatoblasts.
2. Remove the iCell Hepatoblasts 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 3 minutes (avoid submerging the cap) while gently swirling.
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 Hepatoblasts cryovial contents into the 15 ml centrifuge tube containing 10 ml of 37°C Plating Medium.

Avoid repeated pipetting of the thawed iCell Hepatoblasts cell suspension.

6. Rinse the empty iCell Hepatoblasts 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 Hepatoblasts 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.

Avoid multiple centrifugations as this may impact viability and attachment of the cells.

8. Carefully aspirate the supernatant, taking care not to disturb the cell pellet.
9. Gently resuspend cell pellet in 5 ml of room temperature Plating Medium with a wide-bore pipette. Avoid vigorous shaking or vortexing of the cell suspension.

Avoid repeated pipetting of the thawed iCell Hepatoblasts cell suspension.

Note: *Thaw no more than 3 vials of iCell Hepatoblasts at one time. Once thawed, pour the contents of the 2 or 3 vials into the same 50 ml centrifuge tube containing 20 or 30 ml of Plating Medium, respectively. Rinse each vial before inverting the final suspension of thawed cells.*

Notes

Chapter 6. Plating iCell Hepatoblasts

The following procedure describes how to plate iCell Hepatoblasts at 3 x 10^5 viable cells/cm^2. Scale volumes appropriately for other cell culture vessel formats.

1. Invert the thawed iCell Hepatoblasts cell 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. Calculate the final volume of Plating Medium needed to obtain a desired cell plating density (i.e. 1 x 10^6 viable cells/ml).
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 2: Summary of Recommended Volumes and Measures
*All volumes and measures are **per well**.*

5. Culture iCell Hepatoblasts in a cell culture incubator at 37°C, 5% CO_2 overnight.

Chapter 7. Differentiating iCell Hepatoblasts

Notes

iCell Hepatoblasts represent a population of bipotential cells that can differentiate into hepatocytes or cholangiocytes.

Inducing Hepatocytes Differentiation

iCell Hepatoblasts will differentiate into a highly pure hepatocyte population within 7 days when cultured in Hepatocyte Differentiation Medium for 1 day, then in Hepatocyte Maintenance Medium for 6 days. The differentiation can be determined and quantified as number of alpha-1 antitrypsin expressing cells.

For assay instructions, see the iCell Hepatoblasts Prototype Application Protocol: Modeling Hepatocyte Differentiation with Flow Cytometry Analysis available online at www.cellulardynamics.com/lit/.

Inducing Cholangiocytes Differentiation

iCell Hepatoblasts will differentiate into cholangiocytes within 7 days when cultured in Cholangiocyte Differentiation Medium containing activin A for 1 day, then in Cholangiocyte Maintenance Medium for 6 days. The differentiation can be determined and quantified as the number of cytokeratin-7 expressing cells.

For assay instructions, see the iCell Hepatoblasts Prototype Application Protocol: Modeling Cholangiocyte Differentiation with Flow Cytometry Analysis available online at www.cellulardynamics.com/lit/.

Notes

Appendices

Appendix A. Intellectual Property Rights, Use Restrictions, and Limited License

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