FUJIFILM
Cellular Dynamics

Quick Guide

iCell® Hepatocytes 2.0

Handling and Storage

Upon receipt, immediately transfer components to the proper storage temperature.

Component	Storage Temperature
iCell Hepatocytes 2.0 Cryovial	Vapor Phase of Liquid Nitrogen
iCell Hepatocytes 2.0 Medium Supplement	-80ºC

Cell Culture Surfaces

For best results, use CELLCOAT® Collagen Type 1 pre-coated plates.

Preparing the Plating Medium

iCell Hepatocytes 2.0 are thawed and maintained in plating medium for 5 days.

1. Thaw iCell Hepatocytes 2.0 Medium Supplement at room temperature.
2. Prepare stock solutions of 10 µg/ml oncostatin M and 5 mM dexamethasone according to the manufacturer's recommendations.
3. Prepare the plating medium (see **Table 2**).
4. Filter the plating medium using a 0.2 µM PES filter unit.
5. Store medium at 4°C for up to 1 week. Do not store at -20ºC.

Thawing the Cells

1. Equilibrate plating medium to room temperature.
2. Equilibrate a 10 ml aliquot of plating medium to 37ºC.
3. Thaw iCell Hepatocytes 2.0 cryovial in a 37ºC water bath for 3 minutes.
4. Transfer the cells to the centrifuge tube containing 37ºC plating medium.
5. Rinse the cryovial with 1 ml of medium and transfer to centrifuge tube.

Avoid repeated pipetting of the cell suspension to maximize cell recovery.

6. Centrifuge the cells at 200 x g for 3 minutes.
7. Discard the supernatant avoiding the cell pellet.
8. Add 2 ml of plating medium to resuspend the cell pellet. Gently pipette to mix.
9. For 10M size vials, add an additional 3 ml of plating medium.
10. Invert twice to mix.

Avoid vigorous shaking or vortexing of the cells.

Plating the Cells

1. Remove a sample of cells to perform a cell count using a hemocytometer (using trypan blue exclusion).
2. Dilute the cell suspension to obtain a desired cell plating density. The recommended plating density is 300,000 viable cells/cm^2.

Culture Vessel	Surface Area	Plating Volume	Cell Number	Cell Density (Viable Cells/ml)
6-well Cell Culture Plate	9.6 cm^2	3 ml	2,880,000	960,000
24-well Cell Culture Plate	1.9 cm^2	600 µl	570,000	950,000
96-well Cell Culture Plate	0.34 cm^2	100 µl	102,000	1,020,000

3. Dispense the cells into the cell culture vessel.
4. Incubate the cells at 37°C, 5% CO_2 for 3 - 4 hours.
5. Equilibrate an aliquot of plating medium to room temperature.
6. Move the 6-well and 24-well plates in the diagonal plane 4 times to dislodge dead cells and debris; this does not apply to 96-well plates.

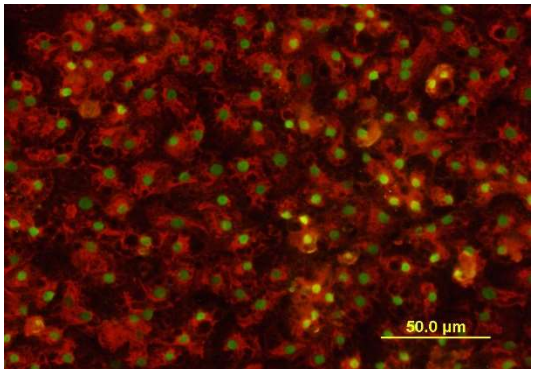

Figure 1: iCell Hepatocytes 2.0, 01279
iCell Hepatocytes 2.0 exhibit expression of Albumin (red) and Hepatocyte Nuclear Factor 4 (green).

Table 1: Required Consumables

Component	Vendor	Catalog #
6-well Cell Culture Multiwell Plate, Collagen Type 1, Clear	Greiner Bio-One	657-950
24-well Cell Culture Multiwell Plate, Collagen Type 1 Clear	Greiner Bio-One	662-950
96-well Cell Culture Microplate, Collagen Type 1, Black	Greiner Bio-One	655-956
Trypan Blue, 0.4% Solution	STEMCELL Technologies	07050

Table 2: Plating Medium Formulation

Component	Vendor Catalog	Volume
RPMI 1640 Medium	ThermoFisher #11875	72 ml
B-27 Supplement (50X)	ThermoFisher #17504	1.5 ml
Recombinant Human Oncostatin M, 10µg/ml[1]	R&D Systems #295-OM	150 µl
Dexamethasone, 5mM[1]	ThermoFisher #ICN19456125	1.5 µl
Gentamicin (50 mg/ml)	ThermoFisher #15750	37.5 µl
iCell Hepatocytes 2.0 Medium Supplement	FUJIFILM Cellular Dynamics #M1024	1.5 ml

[1] Reconstitute according to manufacturer's recommendations.

Table 3: Maintenance Medium Formulation

Component	Vendor Catalog	Volume
RPMI 1640 Medium	ThermoFisher #11875	72 ml
B-27 Supplement (50X)	ThermoFisher #17504	1.5 ml
Dexamethasone, 5mM	ThermoFisher #ICN19456125	1.5 µl
Gentamicin (50mg/ml)	ThermoFisher #15750	37.5 µl
iCell Hepatocytes 2.0 Medium Supplement	FUJIFILM Cellular Dynamics #M1024	1.5 ml

7. Perform 100% medium exchanging with plating medium.
8. Incubate the cells at 37°C, 5% CO_2.

Maintaining the Cells (Day 0 – 5)

1. Equilibrate an aliquot of plating medium to room temperature.
2. Perform 100% medium exchange every day until day 5.
3. Culture the cells at 37°C, 5% CO_2.

Preparing the Maintenance Medium

Maintain iCell Hepatocytes 2.0 in maintenance medium starting at day 5.

1. Thaw iCell Hepatocytes 2.0 Medium Supplement at room temperature.
2. Prepare the maintenance medium (see **Table 3**).
3. Filter maintenance medium using a 0.2µM PES filter unit.
4. Store medium at 4°C for up to 1 week. Do not store at -20ºC.
5. Equilibrate maintenance medium to room temperature before use.

Maintaining the Cells (Day 5+)

Note: *For 3D culture, refer to Application Protocol Modeling 3D Liver Tissue: 3D Hepatocyte Spheroids in Low Attachment Plates available at fujifilmcdi.com/lit/.*

1. Equilibrate an aliquot of maintenance medium to room temperature.
2. After day 5, perform 100% medium exchange every 2 days.
3. Culture the cells at 37°C, 5% CO_2.

For CYP induction assays, do not add dexamethasone to the medium.

Contacting Technical Support

Email: fcdi-support@fujifilm.com

Phone: 1-877-320-6688

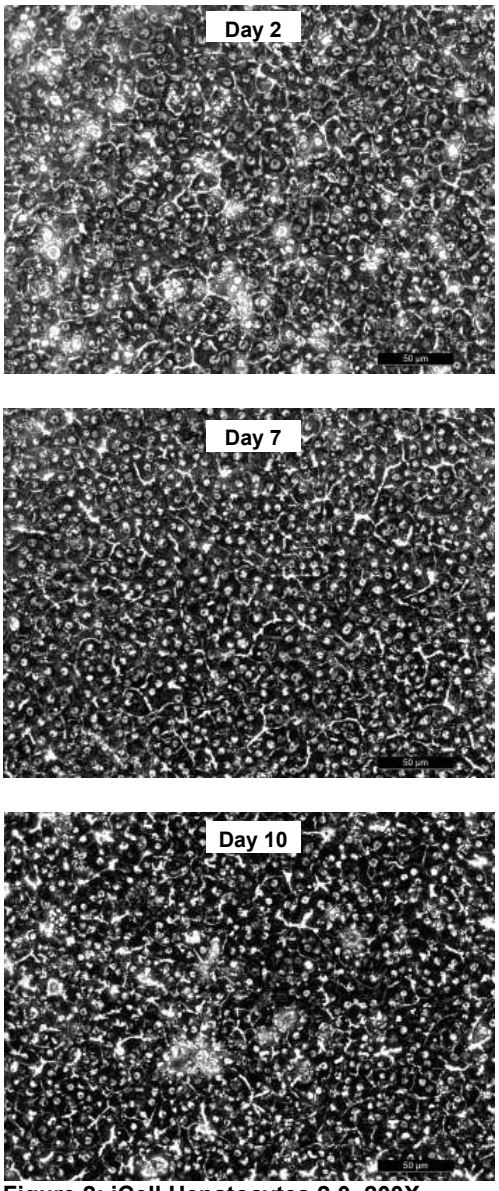

Figure 2: iCell Hepatocytes 2.0, 200X
The iCell Hepatocytes 2.0 at day 2, 7, and 10 post-plating display an adherent monolayer with cobblestone morphology.

Revision History
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