

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	-80°C
iCell Plating Supplement B	-20°C

Cell Culture Surfaces

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

Preparing the Plating Medium

iCell Hepatocytes 2.0 are thawed in thawing medium (described in the Thawing the Cells section) and cultured 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 plating medium (see **Table 2**).
4. Filter 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 -20°C.

Thawing the Cells

1. Equilibrate plating medium to room temperature.
2. Thaw iCell Plating Supplement B at room temperature.
3. Prepare thawing medium by adding iCell Plating Supplement B (1000X) to an aliquot of plating medium.
4. **Note:** Only make enough thawing medium for Day 0 tasks. Do not store beyond 48 hours.
5. Equilibrate a 10 ml aliquot of thawing medium in a 50 ml centrifuge tube to 37°C.
6. Thaw iCell Hepatocytes 2.0 cryovial in a 37°C water bath for 3 minutes.
7. Transfer the cells to the centrifuge tube containing 37°C thawing medium.
8. Rinse the cryovial with 1 ml of thawing medium and transfer to centrifuge tube.

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

9. Centrifuge the cells at 200 x g for 3 minutes.
10. Aspirate the supernatant, avoiding the cell pellet.
11. Add 2 ml of thawing medium to resuspend the cell pellet. Gently pipette to mix. For 10M size vials, add an additional 3 ml of thawing medium. Invert centrifuge tube twice to mix.

Avoid vigorous shaking or vortexing of the cells.

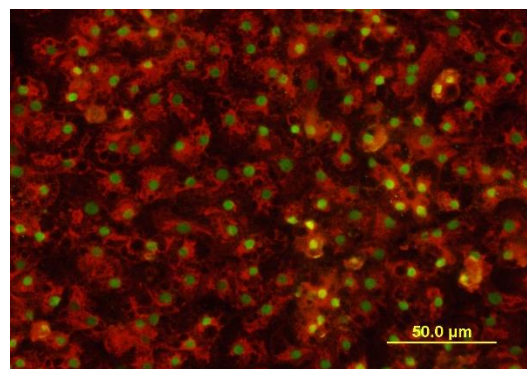


Figure 1: iCell Hepatocytes 2.0, 01279
iCell Hepatocytes 2.0 exhibit expression of Albumin (red) and Hepatocyte Nuclear Factor 4 (green).

Table 1: Required Consumables

Component	Vendor	Catalog #
6-well Cell Culture Multiwell Plate, Collagen Type 1, Clear	Greiner Bio-One	657950
24-well Cell Culture Multiwell Plate, Collagen Type 1 Clear	Greiner Bio-One	662950
96-well Cell Culture Microplate, Collagen Type 1, Black	Greiner Bio-One	655956
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 #CN19456125	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 #CN19456125	1.5 µl
Gentamicin (50mg/ml)	ThermoFisher #15750	37.5 µl
iCell Hepatocytes 2.0 Medium Supplement	FUJIFILM Cellular Dynamics #M1024	1.5 ml

Plating the Cells

1. Remove a sample of cells to perform a cell count using a hemocytometer using trypan blue exclusion.
2. Using thawing medium, dilute the cell suspension to obtain a desired cell plating density. The recommended plating density is 300,000 viable cells/cm².

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% CO₂ for 16-24 hours.

Maintaining the Cells (Day 1 – 4)

1. Equilibrate an aliquot of plating medium to room temperature.
2. On day 1, move the 6-well and 24-well plates diagonally 4 times to dislodge dead cells and debris; *this does not apply to 96-well plates*.
3. Perform 100% medium exchange every day until day 4.
4. Culture the cells at 37°C, 5% CO₂.

Preparing the Maintenance Medium


Culture iCell Hepatocytes 2.0 in maintenance medium starting on day 5.

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

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 www.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% CO₂.

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

Contacting Technical Support

Email: fcidi-support@fujifilm.com

Phone: 1-877-320-6688

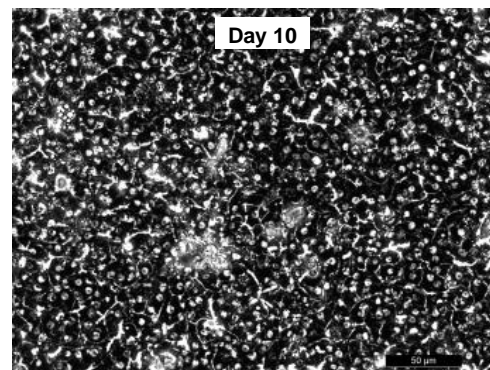
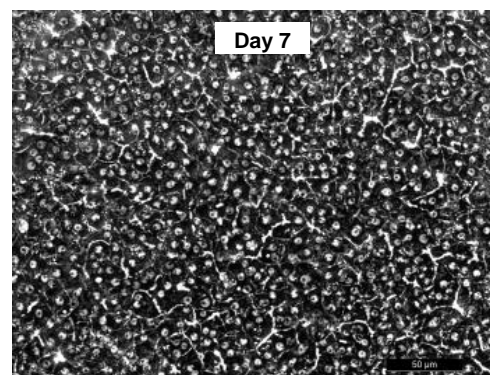
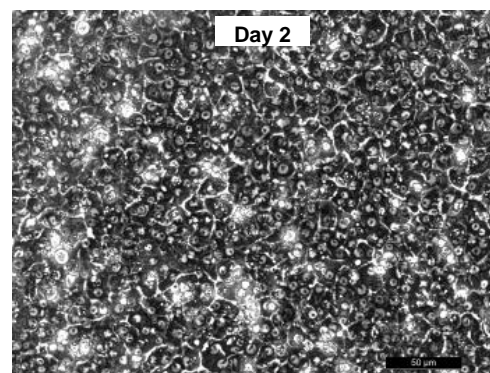



Figure 2: iCell Hepatocytes 2.0, 200X
The iCell Hepatocytes 2.0 at day 2, 7, and 10 post-plating display an adherent monolayer with cobblestone morphology.

Conditions of Use

The cells are for RESEARCH USE ONLY. See www.fujifilmcdi.com/terms-and-conditions/ for USE RESTRICTIONS applicable to the cells and other terms and conditions related to the cells and their use.

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