FUJIFILM
Cellular Dynamics

Quick Guide

iCell® Astrocytes

Handling and Storage

Upon receipt, immediately transfer the cryovial to liquid nitrogen storage.

Preparing Cell Culture Surfaces

1. Thaw laminin stock solution at room temperature.
2. Dilute laminin stock in DPBS to 10 µg/ml. Do not vortex.
3. Dispense the 10 µg/ml laminin solution into the cell culture vessel(s) according to the table below.

Culture Vessel	Volume of 10 µg/ml Laminin Solution
6-well Cell Culture Plate	1 ml
96-well Cell Culture Plate	100 µl

4. Incubate at 37°C for at least 1 hour.

Preparing the Maintenance Medium

5. Prepare maintenance medium (see **Table 2**).
6. Filter maintenance medium using a 0.2 µm PES filter unit.
7. Store maintenance medium at 4°C for up to 2 weeks.
8. Equilibrate maintenance medium to room temperature before use.

Thawing the Cells

1. Thaw iCell® Astrocytes cryovial in a 37°C water bath for 3 minutes. Clean with 70% ethanol.
2. Transfer the cells to a 50 ml centrifuge tube.
3. Rinse the cryovial with 1 ml of maintenance medium and add it to the centrifuge tube dropwise while swirling.
4. Slowly add 8 ml of maintenance medium to the tube dropwise while gently swirling the tube.
5. Gently mix by inverting the centrifuge tube or slowly pipetting.
6. Centrifuge at 300 x g for 5 minutes.
7. Carefully aspirate the supernatant.
8. Resuspend the cell pellet in 3 ml of maintenance medium.

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 with maintenance medium to obtain a desired cell plating density. The recommended cell density for most cell-based assays is 40,000 – 55,000 viable cells/cm^2, but it can be assay dependent.

Culture Vessel	Surface Area (cm^2)	Plating Volume	Cell Number
6-well cell culture plate	9.6	3 ml	384,000 - 528,000
96-well cell culture plate	0.32	200 µl	12,800 - 17,600

3. Aspirate the laminin solution from the pre-coated plates.
4. Dispense the cells into the cell culture vessel.
5. Culture the cells at 37°C, 5% CO_2.

Maintaining the Cells

1. Replace 50-75% of the medium every 2-3 days.
2. Culture the cells at 37°C, 5% CO_2.

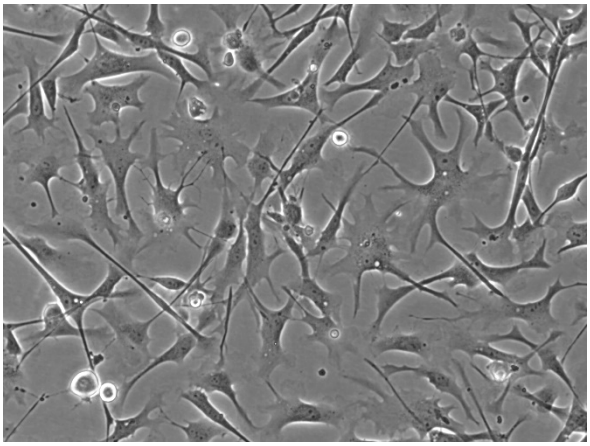

Figure 1: iCell Astrocytes, 100X
iCell Astrocytes, 01434 at 24 hours post-plating (46,000 viable cells/cm^2).

Table 1: Required Consumables

Component	Vendor	Catalog #
Dulbecco's Phosphate Buffered Saline, No Ca^{2+} or Mg^{2+} (DPBS)	ThermoFisher	14190
Laminin	MilliporeSigma	L2020

Table 2: Maintenance Medium Formulation

Component	Vendor Catalog #	Volume (ml)
DMEM/F-12, HEPES	ThermoFisher # 11330	97
Fetal Bovine Serum	GE Healthcare Life Sciences #SH30071.03	2
N-2 Supplement, 100X	ThermoFisher #17502048	1

Contacting Technical Support

Email: fcdi-support@fujifilm.com

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Conditions of Use

The cells are for RESEARCH USE ONLY. See https://fujifilmcdi.com/assets/tnc/standard.pdf for USE RESTRICTIONS applicable to the cells and other terms and conditions related to the cells and their use.

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