FUJIFILM
Cellular Dynamics

# iCell® Astrocytes 2.0
# User's Guide

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## Conditions of Use

iCell Astrocytes 2.0 are FOR RESEARCH USE ONLY and NOT FOR THERAPEUTIC USE. 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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## Origin

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

## Copyright Notice



## Revision History

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Version 2.0: September 2023

# Table of Contents

# Before You Begin

- Immediately transfer the frozen vials of iCell Astrocytes 2.0 to liquid nitrogen storage.
- Immediately store iCell Astrocytes 2.0 Medium at -20°C until ready for use.
- Read this entire User's Guide before handling or using iCell® Astrocytes 2.0.
- iCell Astrocytes 2.0 are FOR RESEARCH USE ONLY and NOT FOR THERAPEUTIC USE. 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.
- A Safety Data Sheet (SDS) for dimethyl sulfoxide (DMSO), in which iCell Astrocytes 2.0 are frozen, is available online at www.fujifilmcdi.com/product-literature/ or on request from FUJIFILM Cellular Dynamics. Only technically qualified individuals experienced in handling DMSO and human biological materials should access, use, or handle iCell Astrocytes 2.0.

# Chapter 1. Introduction

iCell Astrocytes 2.0 from FUJIFILM Cellular Dynamics, Inc. (FCDI), are a highly pure population of human astrocytes derived from induced pluripotent stem (iPS) cells using FCDI's proprietary differentiation protocols. iCell Astrocytes 2.0 express known astroglia markers (CD44, CD49f, S100β, GFAP) and display functional properties of astrocytes, including glutamate uptake, IL-6 secretion in response to inflammatory stimuli, and enhancement of neural network formation in co-culture with iCell® GlutaNeurons or iCell Induced Excitatory Neurons. These cells provide a reliable source of human astrocytes suitable for use in targeted drug discovery, toxicity testing, and other life science research.

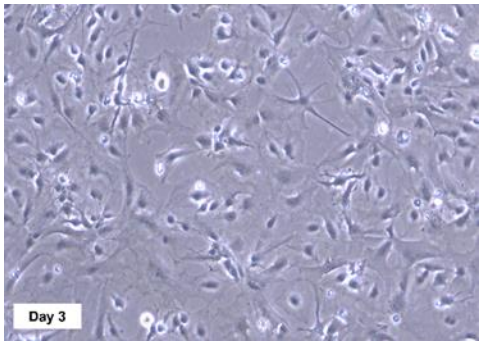



**Figure 1: iCell Astrocytes 2.0 Morphology**

*iCell Astrocytes 2.0 were cultured for 3 days and imaged using brightfield microscopy. iCell Astrocytes 2.0 display characteristic stellate morphology.*

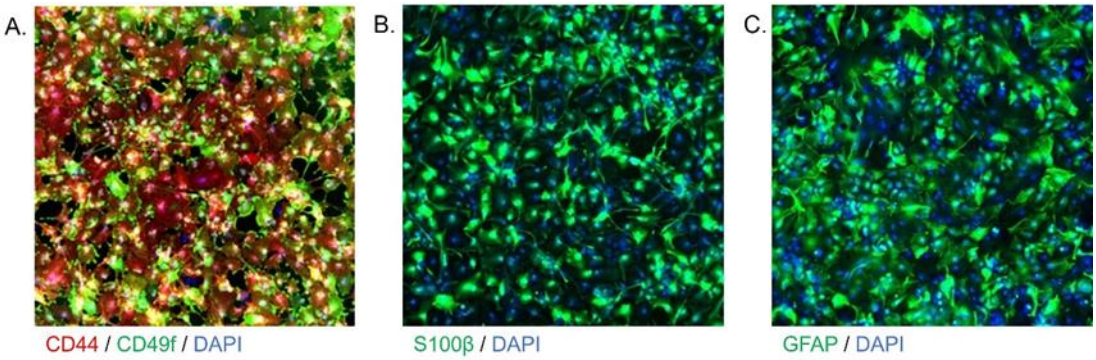



**Figure 2: iCell Astrocytes 2.0 Marker Expression**

*iCell Astrocytes 2.0 were cultured for 7 days prior to fixation and staining for astrocyte specific markers.* ***A)*** *iCell Astrocytes 2.0 co-express high levels of astrocyte markers CD44 (red) and CD49f (green).* ***B)*** *iCell Astrocytes 2.0 express the mature astrocyte marker S100β (green).* ***C)*** *iCell Astrocytes 2.0 express the mature astrocyte marker GFAP (green). Cells were counterstained with DAPI (blue).*

## Components Supplied by FUJIFILM Cellular Dynamics

| Item | Catalog Number |
|---|---|
| iCell Astrocytes 2.0 Kit, 01279<br>• iCell Astrocytes 2.0, 01279[1]<br>• iCell Astrocytes 2.0 Medium<br>• iCell Astrocytes 2.0 User's Guide | R1240<br>• C1249 (≥ 1.0 x $10^6$ viable cells)<br>• M1048<br>• X1048 |
| Certificate of Analysis[2] | |
| Certificate of Origin<br>If required for shipping purposes | |

[1]Safety Data Sheet and User's Guide available online: www.fujifilmcdi.com/product-literature/

[2]Available online: www.fujifilmcdi.com/coa-lookup/

## Required Equipment and Consumables

| Item | Vendor(s) | Catalog Number(s) |
|---|---|---|
| **Equipment** | | |
| 37°C Water Bath | Multiple Vendors | |
| Biological Safety Cabinet with UV Lamp | Multiple Vendors | |
| Cell Culture Incubator | Multiple Vendors | |
| Liquid Nitrogen Storage Unit | Multiple Vendors | |
| Pipettors | Multiple Vendors | |
| Tabletop Centrifuge | Multiple Vendors | |
| **Consumables** | | |
| 0.2 µm Sterile Filter Unit | Multiple Vendors | |
| 6-well Cell Culture Plates | Multiple Vendors | |
| 96-well Cell Culture Plates | Multiple Vendors | |
| Conical Tubes, 50 ml, Falcon (Centrifuge Tubes) | Multiple Vendors | |
| Dulbecco's Phosphate Buffered Saline without $Ca^{2+}$ and $Mg^{2+}$ (D-PBS) | Thermo Fisher Scientific | 14190 |
| DMEM/F-12, HEPES | Thermo Fisher Scientific | 11330 |
| Matrigel Growth Factor Reduced (GFR) Basement Membrane Matrix | Corning | 354230 |
| Serological Pipettes, multiple sizes | Multiple Vendors | |

## Technical Support, Knowledge Base, and Training

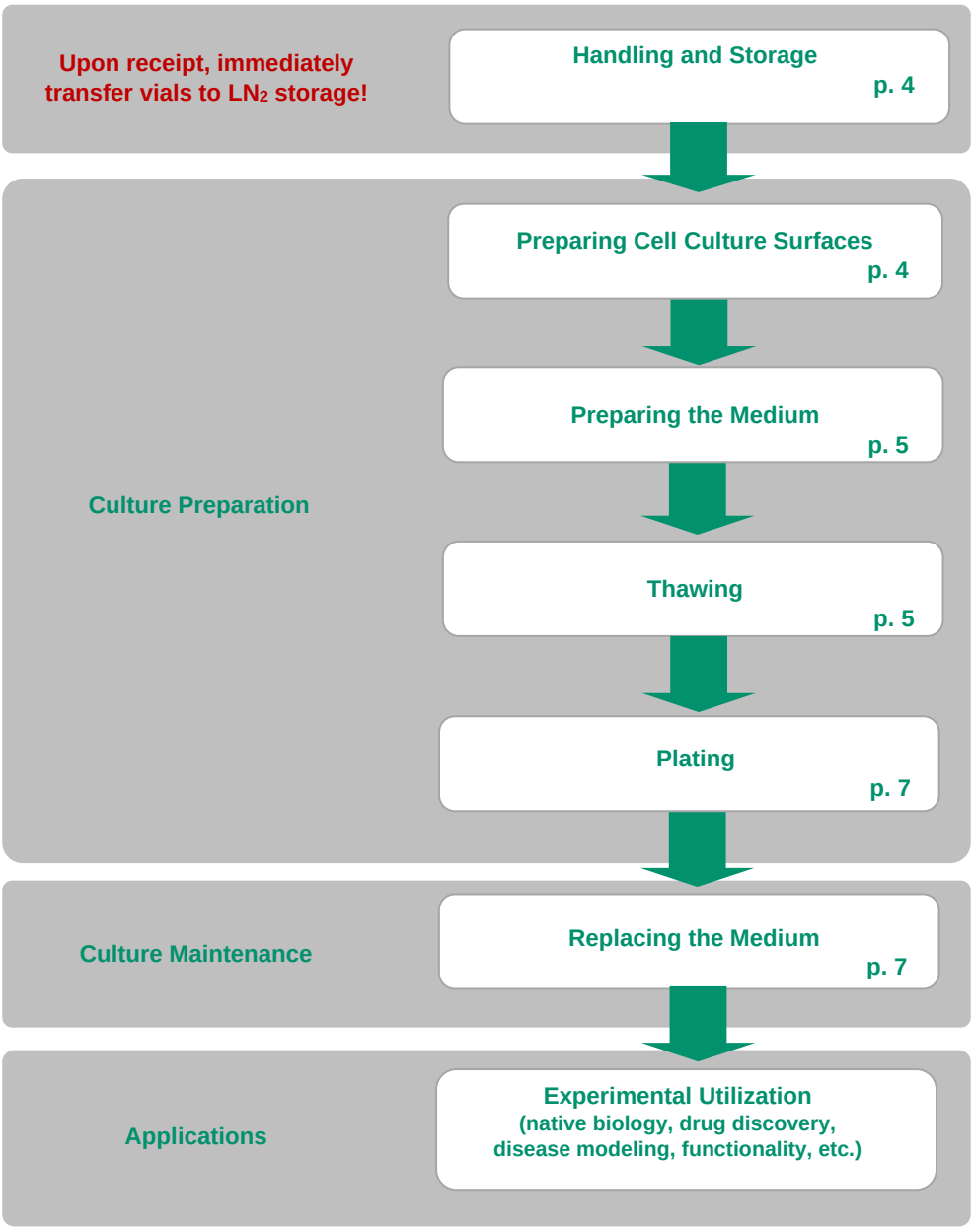
FCDI's Technical Support Scientists have the necessary laboratory and analytical experience to respond to your inquiries. Our web-based Knowledge Base provides solutions for iCell related questions about plating and media, cell culture, general assay methods, and more. In addition, in-lab training may be available upon request.

**Telephone** (877) 320-6688 (US toll-free) / (608) 310-5100 x3
Monday - Friday, 8:30 am - 5:00 pm US Central Time

**Email** fcdi-support@fujifilm.com

**Knowledge Base** www.fujifilmcdi.com/knowledge-base/

## Workflow Diagram



# Chapter 2. Handling and Storage

## Handling iCell Astrocytes 2.0

iCell Astrocytes 2.0 are provided as cryopreserved single-cell suspensions in 1.5 ml cryovials. Upon receipt, directly transfer the cryobox containing iCell Astrocytes 2.0 to the vapor phase of a liquid nitrogen storage dewar. FCDI strongly recommends transferring the entire cryobox into the storage rack to avoid transferring individual vials.

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

## Handling iCell Astrocytes 2.0 Medium

iCell Astrocytes 2.0 Medium is shipped frozen on dry ice. Upon receipt, store iCell Astrocytes 2.0 Medium at -20°C until ready for use and at 4°C for up to 2 weeks post-thawing. If media will be used for longer than 2 weeks, aliquot and freeze again after the initial thaw. Do not subject media to more than a single refreeze and thaw cycle.

# Chapter 3. Preparing Cell Culture Surfaces

iCell Astrocytes 2.0 will plate and function on cell culture plates pre-coated with Matrigel. The following procedure details coating 6-well and 96-well cell culture plates. Scale volumes appropriately for other well formats.

1. Thaw Matrigel stock solution overnight on ice in a 2 - 8°C refrigerator.
2. Dilute Matrigel stock to 0.062 mg/ml using cold DMEM/F12. Do not vortex.
3. Dispense the Matrigel solution into the cell culture vessel(s) according to Table 1.

| Culture Vessel | Volume of 0.062 mg/ml Matrigel Solution (ml) |
|---|---|
| 6-well Cell Culture Plate | 1 |
| 96-well Cell Culture Plate | 0.1 |

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

4. Incubate the vessel(s) in a 37°C cell culture incubator for at least 1 hour.

   ***Note:** Matrigel should be aspirated from the cell culture plate only immediately before the addition of the cells.*

# Chapter 4. Preparing the Medium

iCell Astrocytes 2.0 Medium (Medium) has been specially formulated to maximize the cell viability and recovery at thaw, and to maintain the health and function of iCell Astrocytes 2.0 in culture over time, respectively. Thaw and store the media as follows:

1. 24 hours before use, thaw the Medium overnight at 4°C.
2. Prepare aliquots of media and store at 4°C for up to 2 weeks.

   ***Note:*** *The medium aliquots can be stored at -20°C. Do not thaw and refreeze the medium aliquots multiple times.*

# Chapter 5. Thawing iCell Astrocytes 2.0

Maintain iCell Astrocytes 2.0 in liquid nitrogen until immediately before thawing to ensure maximal performance of the cells. Completing the following steps of the thawing procedure in a time-efficient manner facilitates optimal iCell Astrocytes 2.0 viability and performance.

1. Equilibrate the Medium at room temperature before thawing iCell Astrocytes 2.0.
2. Remove the iCell Astrocytes 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 2 minutes or until thawed (avoid submerging the cap). Use of a floating microcentrifuge tube rack is recommended.
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 Astrocytes 2.0 cryovial contents to a sterile 50 ml centrifuge tube using a 1 ml pipettor.

   ***Note:*** *Use of a 50 ml centrifuge tube facilitates suitable mixing to minimize osmotic shock and increase astrocyte viability.*

   *Avoid repeated pipetting of the thawed iCell Astrocytes 2.0 cell suspension.*
6. Rinse the empty iCell Astrocytes 2.0 cryovial with 1 ml of room temperature Medium to recover any residual cells from the vial.
7. Transfer the 1 ml Medium rinse from the cryovial drop-wise (~1 drop/sec) to the 50 ml centrifuge tube containing the iCell Astrocytes 2.0 cell suspension. Gently swirl the tube while adding the medium to mix the solution completely and minimize the osmotic shock on the thawed cells.

   *Drop-wise addition of the Medium to the cell suspension is critical to minimize osmotic shock and ensure maximum viability and attachment.*
8. Slowly add 8 ml of room temperature Medium to the 50 ml centrifuge tube. Add the first 1 ml drop-wise. Add the remaining 7 ml over the next minute. Gently swirl the centrifuge tube while adding the medium.

*It is critical to add the 8 ml of Medium slowly to ensure maximum viability of the cells.*

9. Gently mix the contents of the 50 ml centrifuge tube by swirling 3 - 4 times. Gentle mixing is critical to ensure maximum viability. Avoid vigorous shaking or vortexing of the cell suspension.
10. Transfer the cell suspension to a 15 ml centrifuge tube.
11. Centrifuge the cell suspension at 400 x g for 5 minutes at room temperature.
12. Carefully aspirate the supernatant without disturbing the cell pellet.
13. Gently resuspend the cell pellet in 2 ml of Medium by pipetting up and down 2 - 3 times.

# Chapter 6. Plating iCell Astrocytes 2.0

The recommended plating density for iCell Astrocytes 2.0 is 20,000 – 90,000 viable cells/cm$^2$ dependent on plate and application.

1. Obtain the number of viable cells/vial and viability from the Certificate of Analysis.
2. Calculate the final volume of Medium needed to obtain the desired cell plating density using the number of viable cells/vial from the Certificate of Analysis. See Table 2 below for examples.
3. Dilute the cell suspension with room temperature Medium to obtain a desired cell plating density.
4. Mix the cell suspension by swirling 2 – 3 times or by gently pipetting up and down.
5. Aspirate the Matrigel solution from the pre-coated cell culture plates.

> *Do not allow the coated surface to dry prior to the addition of cells.*

6. Dispense the cell suspension into the appropriate cell culture vessel(s).
7. Culture iCell Astrocytes 2.0 in a cell culture incubator at 37°C, 5% $CO_2$.

### Expected Cell Density

iCell Astrocytes 2.0 can be plated at various densities to accommodate different applications, ranging from 20,000 – 90,000 viable cells/cm$^2$. The optimal density of iCell Astrocytes 2.0 per unit of surface area will be assay dependent and must be determined empirically based on the intended use. The following table (Table 2) provides the desired cell number and plating volume for several common cell culture vessels.

| Culture Vessel | Surface Area (cm$^2$) | Plating Volume (ml) | Cell Number | Cell Density (cells/cm$^2$) |
|---|---|---|---|---|
| 6-well Cell Culture Plate | 9.6 | 2 | 2.88 x $10^5$ | 3.0 x $10^4$ |
| 96-well Cell Culture Plate | 0.32 | 0.1 | 1.28 x $10^4$ | 4.0 x $10^4$ |

**Table 2: Summary of Recommended Volumes and Measures**

*All volumes and measures are **per well**.*

# Chapter 7. Maintaining iCell Astrocytes 2.0

iCell Astrocytes 2.0 are shipped cryopreserved at high purity. The cells maintain a high purity if maintained in iCell Astrocytes 2.0 Medium and cultured as recommended in a standard cell culture incubator (37°C, 5% $CO_2$).

1. Immediately before use, equilibrate the Medium to room temperature.
2. Replace 50 - 75% of the Medium every 2 - 3 days.
3. Culture iCell Astrocytes 2.0 in a cell culture incubator at 37°C, 5% $CO_2$.

# Chapter 8. Co-Culture of iCell Astrocytes 2.0 with iCell Neurons on MEA

iCell Astrocytes 2.0 can be cultured with iCell GlutaNeurons (Figure 3) or iCell Induced Excitatory Neurons (Figure 4) in microelectrode array (MEA) plates to generate electrically active neural networks. These co-cultures can be easily maintained for extended periods of time and benefit MEA assay performance by establishing a more consistent and longer-lasting model of neural network activity. FCDI provides specific Application Protocols for culturing iCell GlutaNeurons with iCell Astrocytes as well as iCell Induced Excitatory Neurons with iCell Astrocytes. These are done in a 48-well MEA plate format to establish robust baseline network activity on the Maestro Pro MEA system (Axion Biosystems). For the complete application protocol, visit:

SCAN ME

Measuring Neural Network Activity on MEA: Co-culture of iCell GlutaNeurons with iCell Astrocytes

[Insert QR Code here]

Neural Network Activity on MEA: Co-culture of iCell Induced Excitatory Neurons with iCell Astrocytes 2.0

## Representative Data

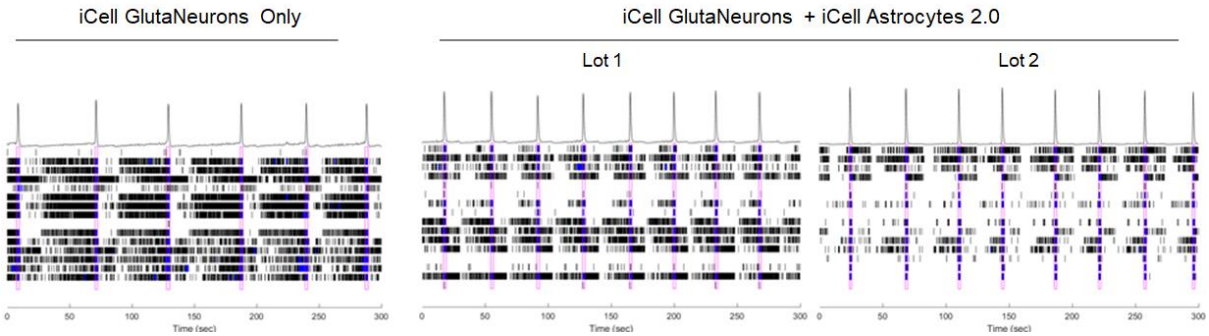



**Figure 3: iCell Astrocytes 2.0 Promote Network Formation in Co-culture with iCell GlutaNeurons**

*iCell Astrocytes 2.0 (two lots) were plated with iCell GlutaNeurons in a 1:6 ratio and cultured in a 48-well BioCircuit MEA plate (Axion Biosystems) using Complete BrainPhys Medium and following the FCDI MEA co-culture application protocol. Representative MEA raster plots from Neural Metric Tool, recorded at 21 days, indicate improved neural network synchrony in co-culture with iCell Astrocytes 2.0 compared to iCell GlutaNeurons alone.*

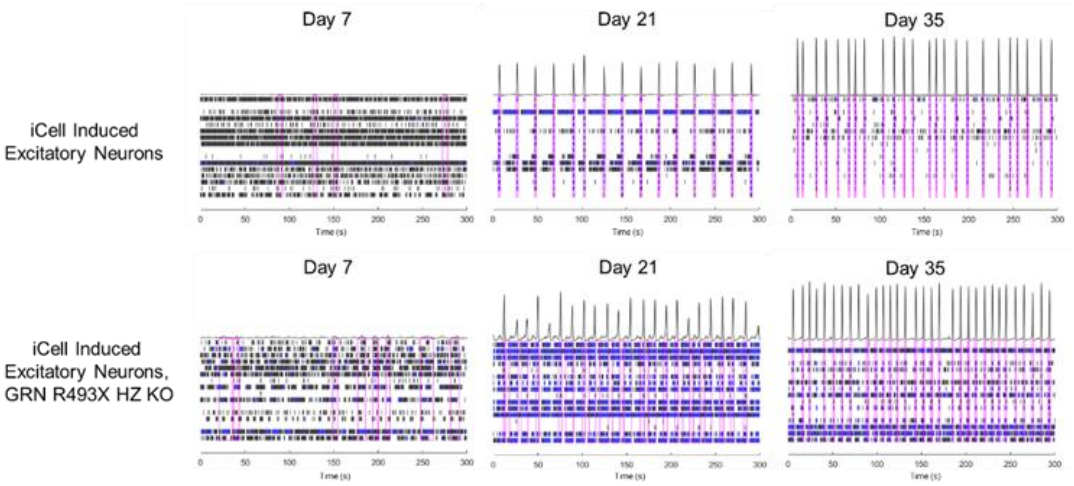



**Figure 4: Development of MEA Activity in iCell Astrocytes 2.0 and iCell Induced Excitatory Neurons Co-Cultures.**
*iCell Induced Excitatory Neurons or iCell Induced Excitatory Neurons GRN R493X HZ KO, an engineered model for frontotemporal dementia (FTD), were co-cultured with iCell Astrocytes 2.0 for 4 weeks in a 48-well BioCircuit MEA plate (Axion Biosystems). Representative raster plots from Neural Metric Tool, recorded on Day 7, 21, and 35, visually demonstrate differences in neural activity development between control and GRN R493X HZ KO iCell Induced Excitatory Neurons.*