Cellular Dynamics international

iCell® Astrocytes Prototype User's Guide

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Conditions of Use

iCell Astrocytes 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 Astrocytes.

Origin

iCell Astrocytes are manufactured in the United States of America.

Copyright Notice

Revision History

Version 0.3: October 2014
Version 0.2: August 2014
Version 0.1: March 2013

Table of Contents

Before You Begin

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

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Chapter 1. Introduction

Cellular Dynamics International's (CDI) iCell Astrocytes are a highly pure population of human astrocytes derived from induced pluripotent stem (iPS) cells using CDI's proprietary differentiation and purification protocols. iCell Astrocytes exhibit expected physiological characteristics and responses. These cells provide a reliable source of human astrocytes suitable for use in targeted drug discovery, toxicity testing, and other life science research.

Components Supplied by Cellular Dynamics

Item	Catalog Number
iCell Astrocytes Prototype[1]	ASC-100-020-001-PT
iCell Astrocytes Prototype User's Guide[1]	
Certificate of Testing[2]	
Certificate of Origin If required for shipping purposes	

1 Safety Data Sheet and User's Guide available online at www.cellulardynamics.com/lit/

2 Available by emailing support@cellulardynamics.com or calling (877) 320-6688 (US toll-free) or (608) 310-5100

Required Equipment and Consumables

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	Multiple Vendors	
Liquid Nitrogen Storage Unit	Multiple Vendors	
Pipettors	Multiple Vendors	
Tabletop Centrifuge	Multiple Vendors	
Consumables		
15 ml and 50 ml Centrifuge Tubes	Multiple Vendors	
6-well Cell Culture Plates	Nunc	140675
Astrocyte Culture Medium*	Multiple Vendors	
DMEM	Life Technologies	10569
Growth Factor Reduced Corning Matrigel Matrix (Matrigel)	Corning	354230
Pipettes	Multiple Vendors	

* Recommended astrocyte culture medium: DMEM, High Glucose, GlutaMAX, Pyruvate (Life Technologies, Cat. No. 10569) with 1X N-2 Supplement (Life Technologies, Cat. No. 17502048) and 10% FBS (Hyclone, Cat. No. SH30396.03) or similar commercially available astrocyte culture medium.

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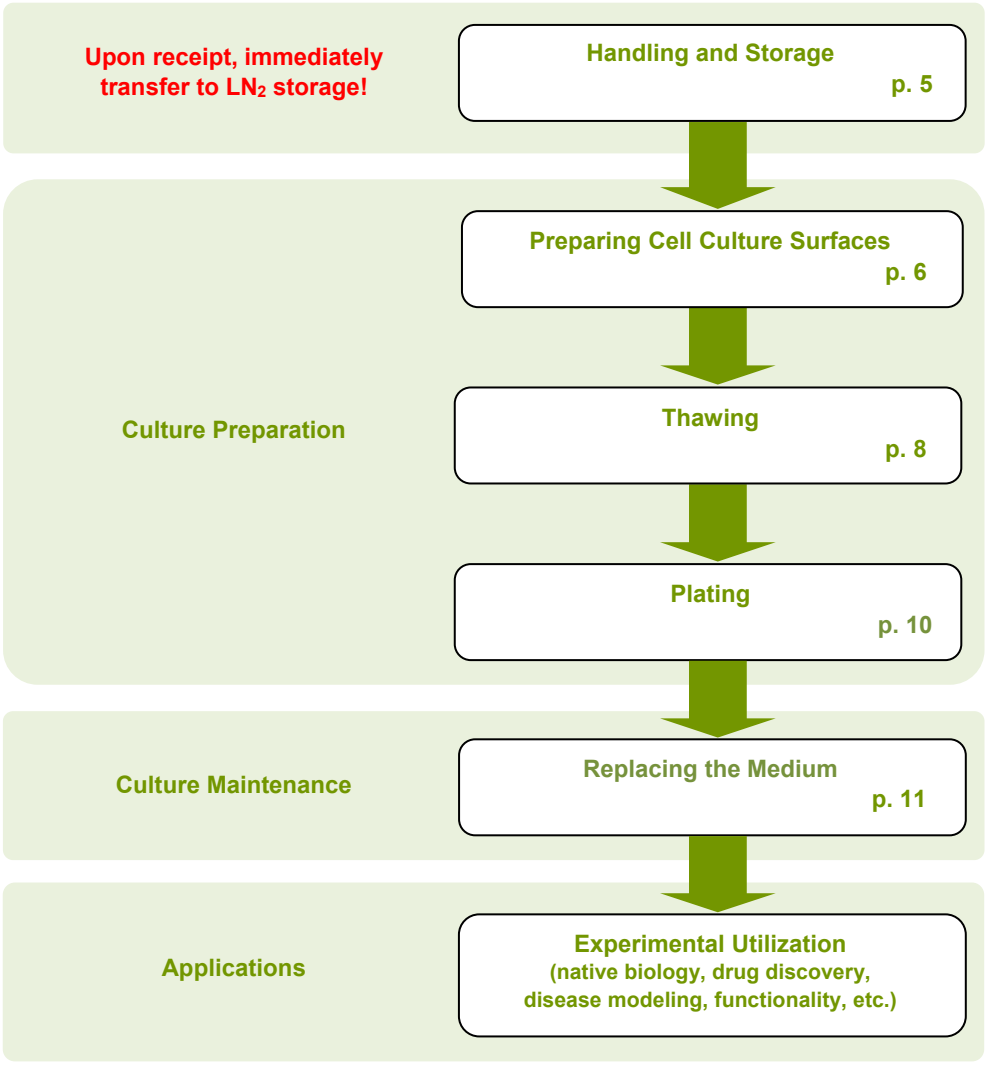
Technical Support and Training

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



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Chapter 2. Handling and Storage

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

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

Chapter 3. Preparing Cell Culture Surfaces

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

1. Dilute Matrigel in ice-cold DMEM to a final concentration of 0.083 mg/ml.
2. Immediately add 1 ml/well of a 6-well cell culture plate.
3. Incubate the plate at room temperature for at least 1 hour before plating iCell Astrocytes.

   Note: *Plates coated with Matrigel can be stored at 4°C for up to 1 week. Equilibrate the plates in a 37°C cell culture incubator before use.*

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Chapter 4. Preparing the Medium

CDI recommends thawing, plating, and maintaining iCell Astrocytes in the following medium or in similar commercially available astrocyte culture medium.

Component	Final Concentration
DMEM, High Glucose, GlutaMAX, Pyruvate	89%
FBS	10%
N-2 Supplement (100X)	1X

Table 1: Components of Astrocyte Culture Medium

Chapter 5. Thawing iCell Astrocytes

Notes

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

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

1. Equilibrate the astrocyte culture medium at room temperature for 2 - 4 hours before thawing iCell Astrocytes.
2. Remove the iCell Astrocytes 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) holding the tube stationary (no swirling). Use of a floating microcentrifuge tube rack is recommended.
4. Immediately remove the cryovial from the water bath following the 3 minute incubation, spray with 70% ethanol, and place in a biological safety cabinet.
5. Gently transfer iCell Astrocytes 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 cell viability.*
6. Rinse the empty iCell Astrocyte cryovial with 1 ml of room temperature astrocyte culture medium to recover any residual cells from the cryovial. Transfer the 1 ml astrocyte culture medium rinse from the cryovial drop-wise (~1 drop/second) to the 50 ml centrifuge tube containing the iCell Astrocytes 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 astrocyte culture medium to the cell suspension is critical to minimize osmotic shock and ensure maximum viability and subsequent attachment of the cells to the plating substrate.
7. Slowly add 8 ml of room temperature astrocyte culture medium to the 50 ml centrifuge tube drop-wise (~1 - 2 drops/second). Gently swirl the centrifuge tube while adding the medium.

   

   It is critical to add the 8 ml of astrocytes culture medium slowly to ensure maximum viability and attachment of the cells once plated.

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8. Gently mix the contents of the 50 ml centrifuge tube by swirling or inverting 2 - 3 times. Gentle mixing is critical to ensure maximum viability. Avoid vigorous shaking or vortexing of the cell suspension.

   Note: *iCell Astrocytes can be concentrated at thaw. Transfer the cell suspension to a 15 ml centrifuge tube and centrifuge at 300 x g for 5 minutes. Aspirate the supernatant, leaving 1 ml in the centrifuge tube, and resuspend the cell pellet in astrocyte culture medium.*

   Note: *Thaw no more than 3 vials of iCell Astrocytes at one time. Once thawed, combine the contents of the cryovials before adding the rinse and final volume of astrocyte culture medium. Follow the timing outlined in steps 6 and 7.*

Chapter 6. Plating iCell Astrocytes

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The recommended plating density for iCell Astrocytes is ~4.0 - 5.5 x 10^4 viable cells/cm^2.

1. 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.
2. Dilute the cell suspension using room temperature astrocyte culture medium to obtain a desired cell plating density.
3. Aspirate the Matrigel from pre-coated plates and immediately dispense the cell suspension.
4. Culture iCell Astrocytes in a cell culture incubator at 37°C, 5% CO_2.

Expected Cell Density

~4.0 - 5.5 x 10^4 viable cells/cm^2 is the recommended starting density of iCell Astrocytes for most cell-based assays. However, the optimal density of iCell Astrocytes per unit of surface area can be assay dependent and must be determined empirically based on the intended use. The following table provides the desired cell number and plating volume for several common culture vessels.

Culture Vessel	Surface Area (cm^2)	Plating Volume (ml)	Cell Number (~4.0 - 5.5 x 10^4 cells/cm^2)
6-well Cell Culture Plate	9.6	3	38 - 52 x 10^4
24-well Cell Culture Plate	1.9	0.6	7.6 - 10.4 x 10^4
96-well Cell Culture Plate	0.32	0.2	1.2 - 1.7 x 10^4

Table 2: Summary of Recommended Volumes and Measures
*All volumes and measures are **per well**.*

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Chapter 7. Maintaining iCell Astrocytes

1. Immediately before use, equilibrate the astrocyte culture medium in a 37°C water bath.
2. 24 hours post-plating iCell Astrocytes, carefully aspirate the spent medium and replace (100% exchange) with the appropriate volume of astrocyte culture medium. Recommend volumes are as follows:
   - **6-well cell culture plate:** 2 ml/well
   - **24-well cell culture plate:** 0.6 ml/well
   - **96-well cell culture plate:** 200 µl/well
3. Replace the spent medium every 2 - 3 days.
4. Culture iCell Astrocytes in a cell culture incubator at 37°C, 5% CO_2.

Appendices

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