




**CELLular**  
**Dynamics**  
international

**iCell® Neurons**  
**User's Guide**



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CDI does not in any way guarantee or represent that you will obtain satisfactory results from using iCell Neurons as described herein. The only warranties provided to you are included in the Limited Warranty enclosed with this guide. You assume all risk in connection with your use of iCell Neurons.

## Conditions of Use

iCell Neurons 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 Neurons.

## Origin

iCell Neurons are manufactured in the United States of America.

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## Revision History

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# Table of Contents

<b>Before You Begin</b> .....	<b>ii</b>
<b>Chapter 1. Introduction</b> .....	<b>1</b>
Components Supplied by Cellular Dynamics .....	2
Technical Support and Training.....	3
Workflow Diagram .....	4
<b>Chapter 2. Handling and Storage</b> .....	<b>5</b>
Handling iCell Neurons.....	5
Handling iCell Neurons Maintenance Medium and Supplement.....	5
<b>Chapter 3. Preparing Cell Culture Surfaces</b> .....	<b>6</b>
Preparing PLO/Laminin Plates .....	6
Alternate Substrate Options.....	7
<b>Chapter 4. Preparing the Medium</b> .....	<b>8</b>
Preparing Complete iCell Neurons Maintenance Medium .....	8
<b>Chapter 5. Thawing and Plating iCell Neurons</b> .....	<b>9</b>
Thawing iCell Neurons.....	9
Plating iCell Neurons .....	10
Expected Cell Density.....	10
<b>Chapter 6. Maintaining iCell Neurons</b> .....	<b>11</b>
<b>Appendices</b> .....	<b>12</b>
Appendix A. Intellectual Property Rights, Use Restrictions, and Limited License...12	
Appendix B. Limited Warranty .....	13
Appendix C. Limited Liability.....	14

## Before You Begin

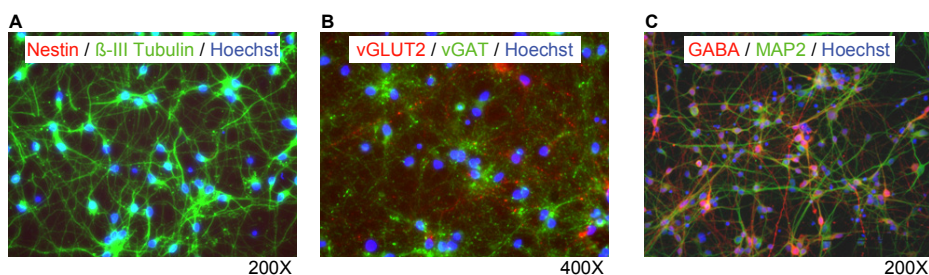
- Immediately transfer the frozen vials to liquid nitrogen storage.
- Read this entire iCell Neurons User's Guide before handling or using iCell Neurons.
- iCell Neurons are for life science research use only. See Appendix A for more information and other restrictions.
- A Material Safety Data Sheet (MSDS) for dimethyl sulfoxide (DMSO), in which iCell Neurons are frozen, is available online at [www.cellulardynamics.com/lit/](http://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 Neurons.

Notes

## Chapter 1. Introduction

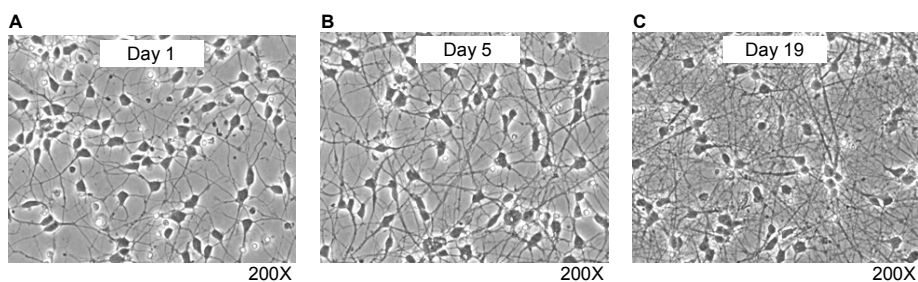
Cellular Dynamics International's (CDI) iCell<sup>®</sup> Neurons are a highly pure population of human neurons derived from induced pluripotent stem (iPS) cells using CDI's proprietary differentiation and purification protocols. iCell Neurons are a mixture of post-mitotic neural subtypes, comprised primarily of GABAergic and glutamatergic neurons (Figure 1), with typical physiological characteristics and responses. These cells provide a reliable source of human neurons suitable for use in targeted drug discovery, toxicity testing, and other life science research.

When handled and maintained as recommended in this User's Guide, iCell Neurons quickly assume a typical neuronal morphology with branching neurites (Figure 2). In addition, these cells display a stable adherent single-cell morphology and remain viable for an extended culture period ( $\geq 14$  days), making them amenable to a variety of electrophysiology, neurotoxicity, and neurotransmission assays.



### Figure 1: iCell Neurons Represent a Highly Pure Population of Human Neurons

*iCell Neurons represent a highly pure population comprised primarily of GABAergic and glutamatergic neurons with low levels of nestin (neuronal progenitor marker) as demonstrated by immunocytochemistry: (A)  $\beta$ -III tubulin (neuronal marker, green) and nestin (red), 7 days post-thaw; (B) punctate staining pattern for the vesicular glutamate transporter 2 (vGLUT2, red) and vesicular GABA transporter (vGAT, green), 14 days post-thaw, indicative of glutamatergic and GABAergic neuronal subtypes, respectively; and (C) gamma-aminobutyric acid (GABA, red) and microtubule-associated protein 2 (MAP2, green), 14 days post-thaw.*



### Figure 2: iCell Neurons Exhibit Typical Neuronal Morphology

*Brightfield images were taken of iCell Neurons at (A) day 1, (B) day 5, and (C) day 19 post-thaw. Re-animated neurons develop branched networks within 24 hours and remain viable and adherent for an extended period in culture ( $\geq 14$  days).*

## Components Supplied by Cellular Dynamics

Notes

Item	Catalog Number
iCell Neurons*	NRC-100-010-001
iCell Neurons Maintenance Medium*	NRM-100-121-001
iCell Neurons Medium Supplement*	NRM-100-031-001
iCell Neurons User's Guide Also available online at <a href="http://www.cellulardynamics.com/lit/">www.cellulardynamics.com/lit/</a>	
Certificate of Analysis Also available online at <a href="http://www.cellulardynamics.com/coa/">www.cellulardynamics.com/coa/</a>	
Certificate of Origin	

\* Material Safety Data Sheets available online at [www.cellulardynamics.com/lit/](http://www.cellulardynamics.com/lit/).

## Required Equipment and Consumables

Item	Vendor	Catalog Number
<b>Equipment</b>		
Liquid Nitrogen Storage Unit	Multiple Vendors	
Biological Safety Cabinet with UV Lamp	Multiple Vendors	
Tabletop Centrifuge	Multiple Vendors	
37°C Water Bath	Multiple Vendors	
Cell Culture Incubator	Multiple Vendors	
Hemocytometer or Automated Cell Counter	Multiple Vendors	
<b>Consumables</b>		
Pipettors and Pipettes	Multiple Vendors	
15 ml and 50 ml Centrifuge Tubes	Multiple Vendors	
6-well Cell Culture Plates	Nunc	140675
96-well Cell Culture Plates	Corning	3603
Poly-L-Ornithine	Sigma	P4957
Laminin	Sigma	L2020
PDL/Laminin Coverslips (electrophysiology applications)	BD Biosciences	354087
TrypLE	Invitrogen	12563

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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.

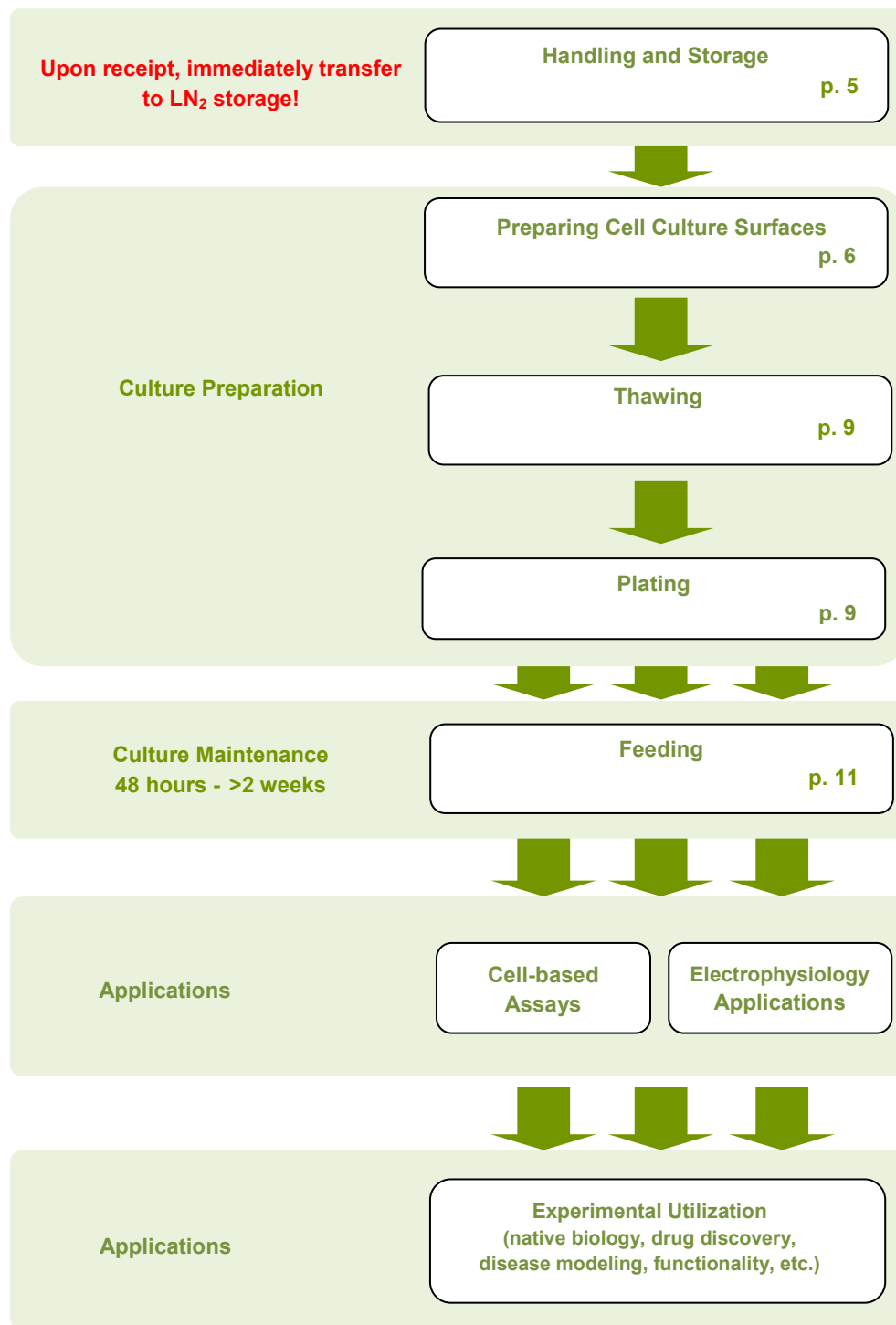
**Telephone** (877) 310-6688 x5 (US toll-free) / (608) 310-5100 x5  
Monday - Friday, 8:30 am - 5:00 pm US Central Time

**Fax** (608) 310-5101

**Email** [support@cellulardynamics.com](mailto:support@cellulardynamics.com)

## Workflow Diagram

Notes



## Chapter 2. Handling and Storage

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### Handling iCell Neurons

iCell Neurons are provided as cryopreserved single-cell suspensions in 1.5 ml cryovials. Upon receipt, directly transfer the cryobox containing iCell Neurons to the vapor phase of liquid nitrogen storage. We strongly recommend transferring the entire cryobox into the storage rack. Avoid transferring individual vials.



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

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### Handling iCell Neurons Maintenance Medium and Supplement

iCell Neurons media are shipped as two components. iCell Neurons Maintenance Medium is shipped at ambient temperature, and iCell Neurons Medium Supplement is shipped frozen on dry ice. Store iCell Neurons Maintenance Medium at 4°C and iCell Neurons Medium Supplement at -20°C before use.

## Chapter 3. Preparing Cell Culture Surfaces

iCell Neurons will plate and function on a variety of substrates. A freshly prepared plate with a base layer of poly-L-ornithine (PLO) and a top coating of laminin is recommended to promote iCell Neurons attachment, viability, and function. Use of commercially available pre-coated poly-D-lysine plates with a top coating of fresh laminin is also described in this chapter. Regardless of the substrate of choice, prepare plating surfaces before thawing iCell Neurons.

### Preparing PLO/Laminin Plates

1. Select the culture dish appropriate for your experimental use. Use the volumes specified in the following table for the selected culture format in the following coating procedure. Scale volumes appropriately for other culture formats.

Plate Format	Volume of PLO Solution (0.01%) per Well	Volume of Water (washes) per Well	Volume of Laminin Solution (3.3 µg/ml) per Well
6-well plate	1 ml	2 ml	3 ml
96-well plate	100 µl	200 µl	100 µl
384-well plate	25 µl	50 µl	25 µl

**Note:** For plating cells onto coverslips, 12 mm BD BioCoat Poly-D-Lysine/Laminin Coverslips are recommended.

2. Add the volume of 0.01% PLO solution specified in the table in step 1 to each well of the plate.
3. Incubate plate(s) at room temperature for at least 1 hour.
4. Prepare a 3.3 µg/ml laminin solution by diluting the stock laminin (1 mg/ml) 1:300 into sterile-filtered Milli-Q (or equivalent) water.

**Note:** Thaw stock laminin solution at room temperature or overnight at 4°C. Do not thaw stock laminin in a 37°C water bath. Do not vortex laminin solution.

5. After incubation, aspirate the PLO solution from each well. Rinse each well twice with the volume of sterile Milli-Q water recommended in the table in step 1 and aspirate completely.

**Note:** Aliquot remaining stock laminin solution into small working volumes and store at -20°C.

6. Add the volume of 3.3 µg/ml laminin solution specified in the table in step 1 to each well and incubate the plates in a 37°C cell culture incubator for at least 1 hour.

**Note:** Alternatively, laminin can be added to each well and the plates stored overnight at 4°C. Warm plates in 37°C cell culture incubator before use.

7. Aspirate laminin solution immediately before addition of cell suspension.



Do not allow the laminin-coated surface to dry.

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## Alternate Substrate Options

iCell Neurons can be plated onto the following substrate:

BD BioCoat Poly-D-Lysine Plates (BD Biosciences, Cat. No. 354413 (6WP), Cat. No. 354640 (96WP), or Cat. No. 356663 (384WP)) coated with fresh laminin as described in steps 4, 6, and 7 in the previous section.

Additionally, iCell Neurons have been plated onto other substrates. Contact CDI's Technical Support for more information.

## Chapter 4. Preparing the Medium

Notes

### Preparing Complete iCell Neurons Maintenance Medium

Complete iCell Neurons Maintenance Medium (hereafter referred to as Complete Maintenance Medium) is comprised of (1) iCell Neurons Maintenance Medium and (2) iCell Neurons Medium Supplement. Complete Maintenance Medium is serum- and antibiotic-free and has been specially formulated to maintain the health and function of iCell Neurons while limiting the proliferation of progenitor or non-neuronal cells. iCell Neurons therefore can be maintained in culture for at least 2 weeks in Complete Maintenance Medium without appreciable loss of viability or purity.

1. Thaw iCell Neurons Medium Supplement overnight at 4°C (or in a 37°C water bath just until thawed).
2. Spray iCell Neurons Maintenance Medium bottle and iCell Neurons Medium Supplement vial with 70% ethanol and place in a biological safety cabinet.
3. Using sterile technique, add the entire contents of the iCell Neurons Medium Supplement (~2 ml) to the iCell Neurons Maintenance Medium (~100 ml) to make Complete Maintenance Medium.
4. Store Complete Maintenance Medium at 4°C, protected from light, for up to 3 weeks.

**Note:** CDI recommends using room temperature Complete Maintenance Medium to thaw iCell Neurons.

**Note:** Do not re-freeze iCell Neurons Medium Supplement or freeze Complete Maintenance Medium.

## Chapter 5. Thawing and Plating iCell Neurons

### Thawing iCell Neurons

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

**Note:** Thaw no more than 1 vial of iCell Neurons at a time. Cell suspensions from multiple vials may be pooled after each is taken through step 7 if desired.

1. Remove the frozen iCell Neurons cryovial from the liquid nitrogen storage tank.

**Note:** If necessary, cryovials can be placed on dry ice for up to 10 min before thawing.

2. Place the vial and gently swirl (avoid submerging the cap) in a 37°C water bath for **exactly 3 min**.
3. Immediately remove the cryovial from the water bath following the 3 min incubation, spray with 70% ethanol, wipe dry, and place in a biological safety cabinet.
4. Gently transfer the iCell Neurons cryovial contents to a 50 ml centrifuge tube using a 1 ml pipettor.



Avoid repeated pipetting of the thawed iCell Neurons cell suspension.

5. Rinse the cryovial with 1 ml of room temperature Complete Maintenance Medium to recover any residual cells from the vial. Transfer the 1 ml of Complete Maintenance Medium rinse from the cryovial drop-wise (~1 drop/sec) to the 50 ml centrifuge tube containing the iCell Neurons cell suspension. Gently swirl the tube while adding the medium to completely mix the solution and to minimize the osmotic shock on the thawed cells.



Drop-wise addition of the Complete Maintenance 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.

6. Slowly add 8 ml of room temperature Complete Maintenance Medium to the 50 ml centrifuge tube (~1 - 2 drops/sec). Gently swirl the centrifuge tube while adding the medium.



It is **critical** to add the 8 ml of Complete Maintenance Medium slowly to ensure maximum viability and attachment of the cells once plated.

7. 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.

## Plating iCell Neurons

1. Aspirate the laminin solution from pre-coated plates.
2. Immediately dispense 3 ml of cell suspension to each of 3 wells of a pre-coated 6-well plate (scale appropriately for other plate formats or coverslips).

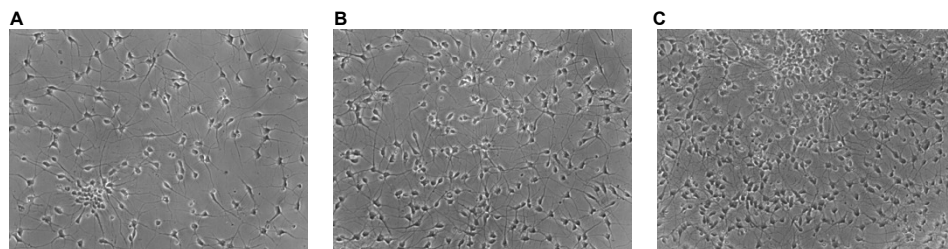
**Note:** To obtain a desired cell seeding density, determine viability and total viable cell number using a hemocytometer (using trypan blue exclusion to identify viable cells) or an automated cell counter and dilute the cell suspension using room temperature Complete Maintenance Medium.

**Note:** Seeding iCell Neurons at less than 67,500 cells/cm<sup>2</sup> could result in reduced viability over time.

3. Place cultures in a cell culture incubator set to 37°C and 5% CO<sub>2</sub>.

## Expected Cell Density

iCell Neurons can be plated at various densities to accommodate different application needs. The following images show iCell Neurons on day 3 post-thaw when seeded at (A) 37,500, (B) 75,000, and (C) 150,000 viable cells per cm<sup>2</sup> on a PLO/laminin-coated plate.



**Figure 3: iCell Neurons Plated at Various Densities**

These images show iCell Neurons on day 3 post-thaw when seeded at (A) 37,500, (B) 75,000, and (C) 150,000 viable cells per cm<sup>2</sup>.

## Chapter 6. Maintaining iCell Neurons

iCell Neurons are shipped cryopreserved at high purity. The cells maintain a high purity for at least 2 weeks after thawing if plated and maintained in Complete Maintenance Medium as instructed.



*Complete Maintenance Medium is stable for 3 weeks when stored at 4°C.*

1. Immediately before use, warm the Complete Maintenance Medium in a 37°C water bath.
2. 24 hours after plating the iCell Neurons, aspirate the spent medium in the well and replace (100% exchange) with the appropriate volume of Complete Maintenance Medium. Recommended volumes are as follows:
  - 2 ml/well for 6-well plates
  - 1 ml/well for 12-well plates
  - 200 µl/well for 96-well plates
  - 25 µl/well for 384-well plates
3. Exchange 50 - 75% of media every 3 - 5 days after initial feeding. iCell Neurons should be cultured in a cell culture incubator at 37°C and 5% CO<sub>2</sub>.

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