Cellular Dynamics international
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iCell® Motor Neurons
User's Guide

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Conditions of Use

iCell Motor Neurons are for life science research use only and subject to the use restrictions contained in Appendix A. You are responsible for understanding and performing the protocols described within this guide. 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 Motor Neurons.

Origin

iCell Motor Neurons are manufactured in the United States of America.

Copyright Notice

Revision History

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Table of Contents

Before You Begin

Notes

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

Notes

Chapter 1. Introduction

Cellular Dynamics International's (CDI) iCell Motor Neurons are a highly pure population of motor neurons expressing characteristic motor neuron markers. These cells provide a reliable source of human neurons suitable for elucidating the mechanisms of diseases, such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA), as well as drug development screening.

When handled and maintained as recommended in this User's Guide, iCell Motor Neurons quickly assume a typical neuronal morphology with branching neurites (Figure 1). In addition, these cells display a stable adherent single-cell morphology and remain viable for an extended culture period (≥14 days) making them amenable to a variety of electrophysiology and mechanistic assays.

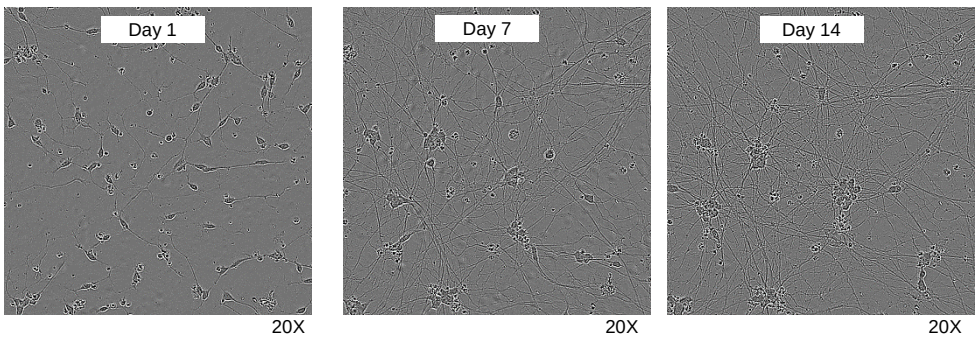

Figure 1: iCell Motor Neurons Exhibit Typical Neuronal Morphology
Brightfield images of iCell Motor Neurons at days 1, 7, and 14 post-plating. Re-animated iCell Motor Neurons develop branched networks within 2 - 3 days and remain viable and adherent for an extended period in culture (≥14 days).

Components Supplied by Cellular Dynamics

Item	Catalog Number
iCell Motor Neurons[1]	MNC-301-030-001-PT
iCell Nervous System Supplement[1]	NSS-301-031-001
iCell Neurons Maintenance Medium[1]	NRM-100-121-001
iCell Neurons Medium Supplement[1]	NRM-100-031-001
iCell Motor Neurons User's Guide[1]	
Certificate of Testing[2]	
Certificate of Origin If required for shipping purposes	

1 Safety Data Sheets 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	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		
0.22 µm Sterile Filter Unit	Multiple Vendors	
15 and 50 ml Centrifuge Tubes	Multiple Vendors	
6-well Cell Culture Plates, Poly-D-Lysine (PDL) Coated	Multiple Vendors	
12-well Cell Culture Plates, Poly-D-Lysine (PDL) Coated	Multiple Vendors	
96-well Cell Culture Plates, Poly-D-Lysine (PDL) Coated	Multiple Vendors	
DAPT, ≥98%, Solid	Sigma-Aldrich	D5942
DMSO, Hybri-Max	Sigma-Aldrich	D2650
Geltrex Matrix	Thermo Fisher Scientific	A15696-01
Pipettes	Multiple Vendors	
Trypan Blue	Thermo Fisher Scientific	15250

* Ensure the automated counter is appropriately calibrated before use.

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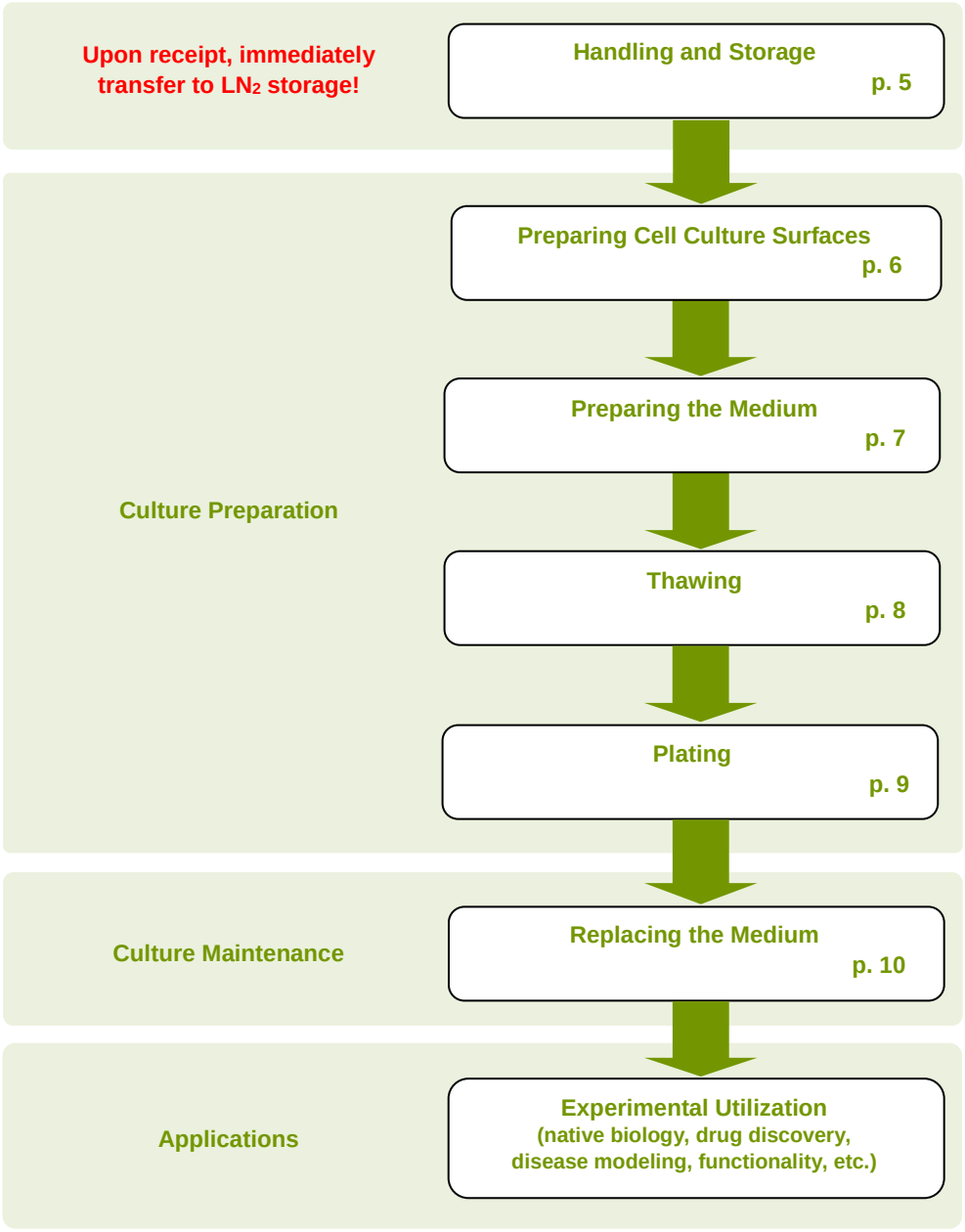
Technical Support and Training

CDI's Technical Support Scientists have the necessary laboratory and analytical experience to respond to your inquiries. 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 x5 Monday - Friday, 8:30 am - 5:00 pm US Central Time
Fax	(608) 310-5101
Email	support@cellulardynamics.com
Knowledge Base	www.cellulardynamics.com/knowledgebase/

Workflow Diagram



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Chapter 2. Handling and Storage

Handling iCell Motor Neurons

iCell Motor Neurons are provided as cryopreserved single-cell suspensions in 1.5 ml cryovials. Upon receipt, directly transfer iCell Motor Neurons to the vapor phase of a liquid nitrogen storage dewar.

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

Handling iCell Neuronal Medium and Supplements

iCell Motor Neurons medium is shipped as three components: iCell Neurons Maintenance Medium, iCell Neurons Medium Supplement, and iCell Nervous System Supplement. iCell Neurons Maintenance Medium is shipped at ambient temperature while iCell Neurons Medium Supplement and iCell Nervous System Supplement are shipped frozen on dry ice. Upon receipt, store iCell Neurons Maintenance Medium at 4°C and iCell Neurons Medium Supplement and iCell Nervous System Supplement at -20°C until ready for use.

Chapter 3. Preparing Cell Culture Surfaces

Notes

iCell Motor Neurons will plate and function on poly-D-lysine (PDL) coated plates supplemented with a top coating of Geltrex Matrix, which is recommended to promote iCell Motor Neurons attachment, long term viability, and function.

Prepare plating surfaces before thawing iCell Motor Neurons.

1. Select the cell culture vessel appropriate for your experimental use. Use the volumes specified in the table below in the following coating procedure. Scale volumes appropriately for other vessel formats.

Culture Vessel	Volume of Geltrex Matrix (ml)
6-well Cell Culture Plate	1
12-well Cell Culture Plate	0.8
96-well Cell Culture Plate	0.1

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

2. Add an appropriate volume of the Geltrex Matrix, as specified above, to each well of the vessel(s).
3. Incubate the vessel(s) at room temperature for at least 1 hour.
4. Aspirate the Geltrex Matrix immediately before the addition of the cell suspension.

Do not allow the Geltrex-coated surface to dry. Drying of the culture surface can lead to cell clumping and migration.

Notes

Chapter 4. Preparing the Medium

iCell Motor Neurons medium (Complete Maintenance Medium) is comprised of three components: iCell Neurons Maintenance Medium, iCell Neurons Medium Supplement, and iCell Nervous System Supplement. DAPT should be added to the Complete Maintenance Medium for the first week of culture to prevent outgrowth of proliferative cells. iCell Motor Neurons can be maintained in culture for at least 2 weeks in this medium without appreciable loss of viability or purity.

Complete Maintenance Medium Components	Volume	Final Concentration
iCell Neurons Maintenance Medium	100 ml	Not Applicable
iCell Neurons Medium Supplement	2 ml	Not Applicable
iCell Nervous System Supplement	1 ml	Not Applicable

Table 2: Volumes for Complete Maintenance Medium Preparation

1. Thaw iCell Neurons Supplement and iCell Nervous System Supplement at room temperature on the day of medium preparation.

   Do not thaw the supplements in a 37°C water bath.

2. Spray all medium components with 70% ethanol and place in a biological safety cabinet.
3. Dissolve DAPT in DMSO to achieve a concentration of 20 mM (8.6 mg/ml).
4. Using sterile technique, add iCell Neurons Medium Supplement (~2 ml) and iCell Nervous System Supplement (~1 ml) to iCell Neurons Maintenance Medium (~100 ml) to make the Complete Maintenance Medium.
5. Add the Complete Maintenance Medium (50 ml) to two 50 ml centrifuge tubes.
6. Store one tube at 4°C, protected from light, for use during the second week of culture.

   To the other tube, add 12.5 µl of DAPT to achieve a 5 µM final concentration. Filter the Complete Maintenance Medium + DAPT through a 0.22 µm sterile filter unit. Store this medium at 4°C, protected from light, for use during thawing, plating, and the first week of culture.

   Note: *Do not refreeze the individual components of the Complete Maintenance Medium. Complete Maintenance Medium is stable for 2 weeks when stored at 4°C.*

Chapter 5. Thawing iCell Motor Neurons

Notes

Maintain iCell Motor 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 Motor Neurons viability and performance.

Note: *Thaw no more than 1 vial of iCell Motor Neurons at one time.*

1. Equilibrate the Complete Maintenance Medium + DAPT at room temperature before thawing iCell Motor Neurons.
2. Remove the iCell Motor Neurons cryovial from the liquid nitrogen storage tank.

   Note: *If necessary, place the cryovial on dry ice for up to 10 minutes before thawing.*
3. Immerse the cryovial in a 37°C water bath for 2 minutes and 30 seconds (avoid submerging the cap), holding the tube stationary (no swirling). Use of a floating microcentrifuge tube rack is recommended.

   Precise timing is critical to maximizing viable cell recovery.
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 Motor Neurons 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 neuron viability.*

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

   Drop-wise addition of the Complete Maintenance Medium + DAPT to the cell suspension is critical to minimize osmotic shock and ensure maximum viability and attachment.
7. Slowly add 8 ml of room temperature Complete Maintenance Medium + DAPT to the 50 ml centrifuge tube drop-wise (~2 - 3 drops/sec) while gently swirling.

   It is critical to add the 8 ml of Complete Maintenance Medium + DAPT slowly to ensure maximum viability and attachment of the cells once plated. Avoid vigorous shaking or vortexing of the cell suspension.

Notes

Chapter 6. Plating iCell Motor Neurons

The recommended plating density for iCell Motor Neurons is 1 x 10^5 viable cells/cm^2 (3.2 x 10^4 cells/well for a 96-well cell culture plate).

1. Transfer the ~10 ml iCell Motor Neurons cell suspension to a 15 ml centrifuge tube.
2. Centrifuge the cell suspension at 400 x g at room temperature for 5 minutes.
3. Carefully aspirate the supernatant, leaving ≥0.5 ml in the centrifuge tube and determine the remaining volume by pipetting.

   Leaving <0.5 ml of medium risks aspirating a portion of the cell pellet.

4. Gently resuspend the cell pellet in 5 ml of room temperature Complete Maintenance Medium + DAPT by flicking the tube and then pipetting up and down 2 - 3 times.

   Avoid excessive pipetting of the cell suspension.

5. Remove a sample of cells to perform a cell count using a hemocytometer (using trypan blue exclusion to identify cells).
6. Dilute with Complete Maintenance Medium + DAPT to obtain a desired cell plating density. Table 3 provides the cell number and plating volume for several common cell culture vessels when plating at a density of 1 x 10^5 viable cells/cm^2.
7. Aspirate the Geltrex Matrix from the pre-coated cell culture plates and immediately dispense a volume of the cell suspension as indicated in Table 3.
8. Culture iCell Motor Neurons in a cell culture incubator at 37°C, 5% CO_2.

Culture Vessel	Surface Area (cm^2)	Plating Volume (ml)	Cell Number (1 x 10^5 cells/cm^2)
6-well Cell Culture Plate	9.5	2	9.5 x 10^5
12-well Cell Culture Plate	3.8	1	3.8 x 10^5
96-well Cell Culture Plate	0.32	0.2	3.2 x 10^4

Table 3: Summary of Recommended Volumes and Measures
*All volumes and measures are **per well**.*

Chapter 7. Maintaining iCell Motor Neurons

Notes

When plated and maintained in Complete Maintenance Medium, iCell Motor Neurons remain viable for an extended culture period (≥14 days) while retaining a high level of purity.

Complete Maintenance Medium is stable for 2 weeks when stored at 4°C.

1. Immediately before use, equilibrate the Complete Maintenance Medium to room temperature for at least 30 minutes.

   Note: *During the first week in culture, you exchange spent medium with Complete Maintenance Medium + DAPT. After the first week, you exchange spent medium with only Complete Maintenance Medium.*

   Note: *Do not equilibrate the medium to* 37°C.

   Repeated warming of the Complete Maintenance Medium may decrease stability.

2. Perform a 75% medium exchange on day 2 post-plating with Complete Maintenance Medium + DAPT and then every 2 - 3 days in this manner.

   It is critical to gently dispense the Complete Maintenance Medium to the side of the well to avoid cell detachment.

3. After 1 week in culture, perform a 50% medium exchange with only Complete Maintenance Medium and then every 2 - 3 days in this manner.
4. Culture iCell Motor Neurons in a cell culture incubator at 37°C, 5% CO_2.

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