FUJIFILM
Cellular Dynamics

iCell® Motor Neurons
User's Guide

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Conditions of Use

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

Copyright Notice

Revision History

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Version 2.0: September 2022

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Before You Begin

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

Introduction

iCell Motor Neurons from FUJIFILM Cellular Dynamics, Inc. (FCDI), 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.

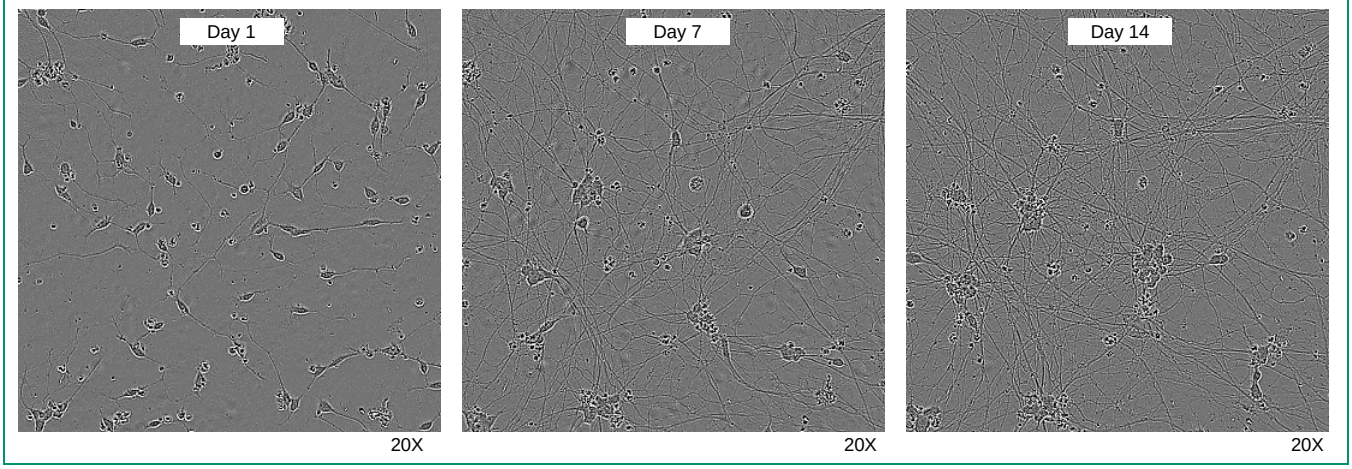


Figure 1: iCell Motor Neurons Exhibit Typical Neuronal Morphology
Representative images of iCell Motor Neurons, 01279 at days 1, 7, and 14 post-plating. The re-animated motor neurons develop branched networks within 2 - 3 days and remain viable and adherent for an extended period in culture (≥14 days).

Components Supplied by FUJIFILM Cellular Dynamics

Item	Catalog Number
iCell Motor Neurons Kit, 01279 • iCell Motor Neurons, 01279[1] • iCell Neural Base Medium 1 • iCell Neural System Supplement • iCell Neural Supplement A • iCell Motor Neurons User's Guide	R1049 (≥ 3 x 10^6 viable cells), or R1051 (≥ 1.0 x 10^6 viable cells • C1048 (≥ 3.0 x 10^6 viable cells), or C1050 (≥ 1.0 x 10^6 viable cells) • M1010 • M1031 • M1032 • X1004
iCell Motor Neurons (TDP43 Q331K) Kit, 01279[3,4]	R1144 (≥ 3 x 10^6 viable cells)
iCell Motor Neurons (TDP43 M337V) Kit, 01279[3,4]	R1145 (≥ 3 x 10^6 viable cells)
Certificate of Analysis[2]	
Certificate of Origin 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/

[3]These kits contain cells and media to culture engineered iCell Motor Neuron disease models. Kit components are available in Page 11.

[4]The iPSCs meet our accepted quality control criteria, including normal karyotype measured by G-band resolution. Additional analysis by high resolution SNP has identified a 1.43Mb amplification on 20q11.21 in our differentiated cells. This amplification does not have any known impact on the phenotype of these cells, or the utility as a disease-model system.

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	
Hemocytometer or Automated Cell Counter[1]	Multiple Vendors	
Liquid Nitrogen Storage Unit	Multiple Vendors	
Pipettors	Multiple Vendors	
Tabletop Centrifuge	Multiple Vendors	
Consumables		
0.2 µm Sterile Filter Unit	Multiple Vendors	
0.4% Trypan Blue Solution	Gibco	15250
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	
Conical Tubes, 15 ml, Falcon (Centrifuge Tubes)	Multiple Vendors	
Conical Tubes, 50 ml, Falcon (Centrifuge Tubes)	Multiple Vendors	
DAPT, ≥98%, Solid	MilliporeSigma	D5942
DMSO, Hybri-Max	MilliporeSigma	D2650
Geltrex Basement Membrane Matrix (Geltrex Matrix)	Thermo Fisher Scientific	A15696-01
Serological Pipettes, 1, 2, 5, 10, 25 ml	Multiple Vendors	

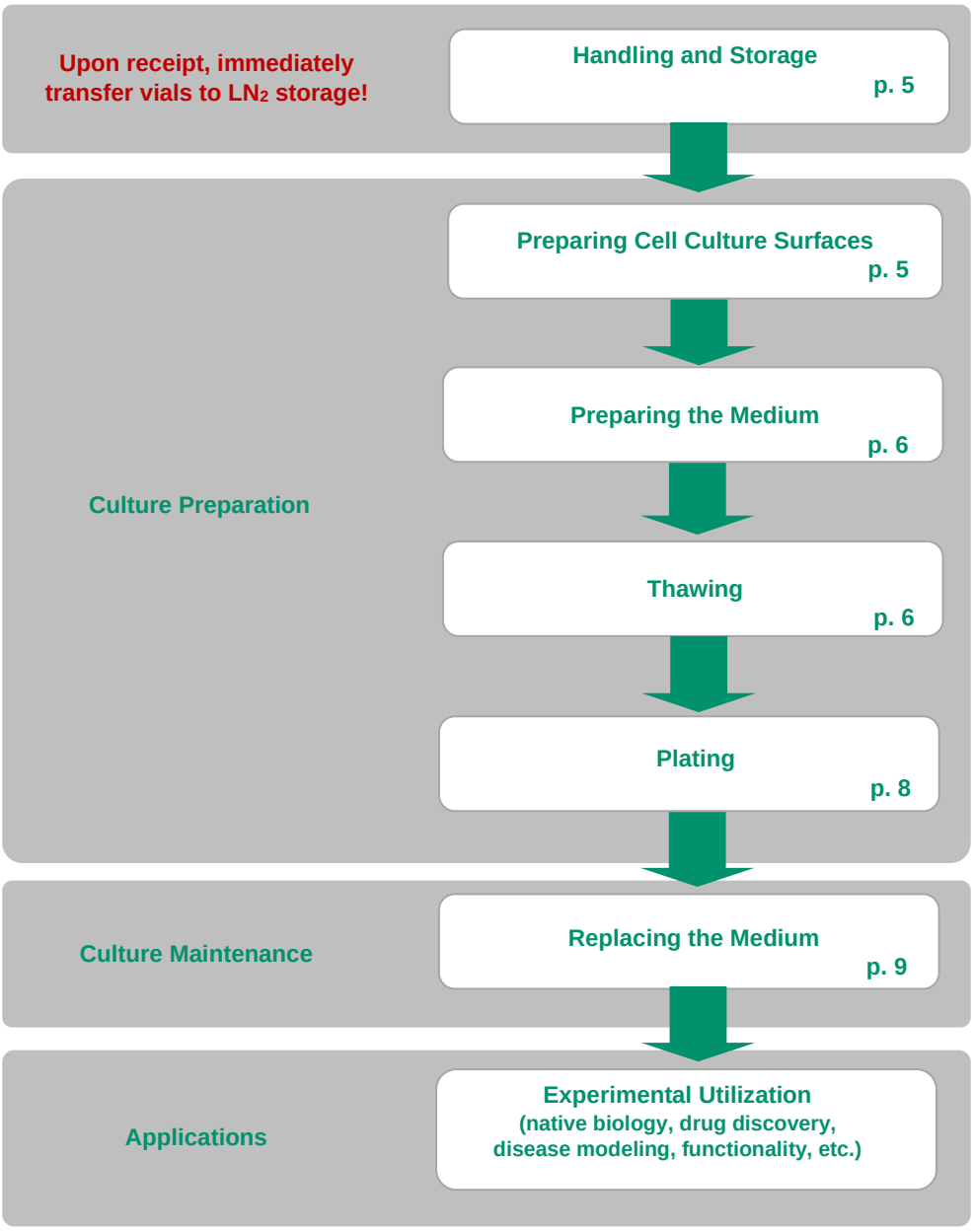
[1] Ensure the automated cell counter is appropriately calibrated before use.

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



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 the cryobox containing iCell Motor Neurons 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 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 are shipped with three additional components: iCell Neural Base Medium 1, iCell Neural Supplement A, and iCell Nervous System Supplement. iCell Neural Base Medium 1 is shipped at ambient temperature while iCell Neural Supplement A and iCell Nervous System Supplement are shipped frozen on dry ice. Upon receipt, store iCell Base Medium 1 at 4°C and iCell Neural Supplement A and iCell Nervous System Supplement at -20°C until ready for use. Do not subject media to more than a single refreeze and thaw cycle.

Preparing Cell Culture Surfaces

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

1. Select the PDL-coated cell culture vessel appropriate for your experimental use. Add the appropriate volume of Geltrex solution to each well according to the table below. Scale volumes appropriately for other vessel formats.

Culture Vessel	Geltrex Volume (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 and Measures
*All volumes and measures are **per well**.*

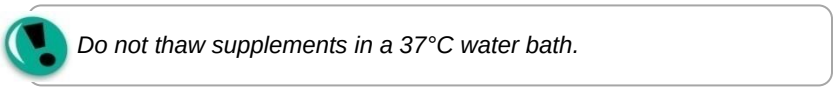
2. Incubate the vessel(s) in a 37°C cell culture incubator for at least 1 hour.
3. Aspirate the Geltrex matrix immediately before the addition of the cell suspension.

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

Preparing the Medium

iCell Motor Neurons medium (Complete Maintenance Medium) is comprised of three components: iCell Neural Base Medium 1, iCell Neural Supplement A, and iCell Nervous System Supplement. DAPT is 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.

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



2. Spray all medium components with 70% ethanol and place in a biological safety cabinet.
3. Reconstitute DAPT to achieve a concentration of 20 mM according to the manufacturer's recommendations.
4. Using sterile technique, add the entire contents of the iCell Neural Supplement A vial (~2ml) and iCell Nervous System Supplement vial (~1ml) to the iCell Neural Base Medium 1 (~100ml) to make the Complete Maintenance Medium.
5. Dispense 50 ml of Complete Maintenance Medium to a 50 ml centrifuge tube.
6. Add 12.5 µl of 20 mM DAPT to the 50 ml of Complete Maintenance Medium.
7. Filter the Complete Maintenance Medium + DAPT through a 0.2 µm sterile filter unit.
8. Store Complete Maintenance Medium + DAPT at 4°C, protected from light, for use during thawing, plating, and first week of culture.
9. Store remaining Complete Maintenance Medium at 4°C, protected from light, for use during the second week of culture.
10. Equilibrate medium to room temperature before use.

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

Thawing iCell Motor Neurons

Maintain iCell Motor Neurons in liquid nitrogen until immediately before thawing to ensure maximal performance of the cells. Completing the following steps 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 cryovials on dry ice for up to 10 minutes before thawing.*

3. Thaw the cryovial in a 37°C water bath for 2 minutes and 30 seconds (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 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.
7. 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 the 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.

8. Slowly add 8 ml of room temperature Complete Maintenance Medium + DAPT to the 50 ml centrifuge tube. Add the first 1 ml drop-wise over 30-60 seconds. Then add the remaining volume over the next ~30 seconds. Gently swirl the centrifuge tube while adding the medium.

   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.

Plating iCell Motor Neurons

The recommended seeding density for iCell Motor Neurons in standard cell culture plates is 100,000 viable cells/cm^2 or 32,000 cells/well for a 96-well cell culture plate.

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

Leaving less than 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 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 viable cells) or an automated cell counter.
6. Dilute the cell suspension with room temperature Complete Maintenance Medium + DAPT to obtain a desired cell plating density.
7. Aspirate the Geltrex matrix from the pre-coated cell culture plates and immediately dispense the cell suspension.
8. Culture iCell Motor Neurons in a cell culture incubator at 37°C, 5% CO_2.

Expected Cell Density

iCell Motor Neurons can be plated at various densities to accommodate different applications. However, 1.0 x 10^5 viable cells/cm^2 is the recommended density for most applications. The following table provides the desired cell number and plating volume for several common cell culture vessels when plating at a density of 1.0 x 10^5 cells/cm^2.

Culture Vessel	Surface Area (cm^2)	Plating Volume (ml)	Cell Number (1.0 x 10^5 cells/cm^2)	Cell Density (cells/ml)
6-well Cell Culture Plate	9.6	2	9.6 x 10^5	4.8 x 10^5
12-well Cell Culture Plate	3.8	1	3.8 x 10^5	3.8 x 10^5
96-well Cell Culture Plate	0.32	0.1	3.2 x 10^4	3.2 x 10^5

Table 2: Summary of Recommended Volumes and Measures

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

Maintaining iCell Motor Neurons

When plated and maintained in Complete Maintenance Medium, iCell Motor Neurons can persist in culture while retaining a high level of purity for at least 2 weeks post-plating.

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

1. Equilibrate the Complete Maintenance Medium to room temperature for at least 30 minutes.

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

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

   Repeated warming of 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 <u>critical</u> 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.

Disease Modeling Kit Components Supplied by FUJIFILM Cellular Dynamics

Item	Catalog Number
iCell Motor Neurons (TDP43 Q331K) Kit, 01279	R1144 (≥ 3 x 10^6 viable cells)
• iCell Motor Neurons (TDP43 Q331K), 01279[a]	• C1161 (≥ 3.0 x 10^6 viable cells)
• iCell Neural Base Medium 1	• M1010
• iCell Neural System Supplement	• M1031
• iCell Neural Supplement A	• M1032
• iCell Motor Neurons User's Guide	• X1004
iCell Motor Neurons (TDP43 M337V) Kit, 01279	R1145 (≥ 3 x 10^6 viable cells)
• iCell Motor Neurons (TDP43 M337V), 01279[a]	• C1162 (≥ 3.0 x 10^6 viable cells)
• iCell Neural Base Medium 1	• M1010
• iCell Neural System Supplement	• M1031
• iCell Neural Supplement A	• M1032
• iCell Motor Neurons User's Guide	• X1004

[a]The starting iPSCs meet our accepted quality control criteria, including normal karyotype measured by G-band resolution. Additional analysis by high resolution SNP has identified a 1.43Mb amplification on 20q11.21 in our differentiated cells. This amplification does not have any known impact on the phenotype of these cells, or the utility as a disease-model system.