FUJIFILM
Cellular Dynamics

iCell® Induced Excitatory Neurons
User's Guide

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FCDI does not in any way guarantee or represent that you will obtain satisfactory results from using iCell Induced Excitatory Neurons as described herein. The only warranties provided to you are included in the Limited Warranty set forth in www.fujifilmcdi.com/terms-and-conditions/. You assume all risk in connection with your use of iCell Induced Excitatory Neurons.

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iCell Induced Excitatory 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 Induced Excitatory Neurons are manufactured in the United States of America.

Copyright Notice

Revision History

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Table of Contents

Before You Begin

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

Chapter 1. Introduction

iCell Induced Excitatory Neurons from FUJIFILM Cellular Dynamics, Inc. (FCDI), are a highly pure population of human glutamatergic neurons, expressing characteristic receptors (AMPA) and transporters (VGLUT1, VGLUT2). Derived from induced pluripotent stem (iPS) cells engineered to overexpress neurogenin 2 (NGN2), these neurons are fully differentiated at thaw and do not require further exposure to doxycycline or anti-mitotic molecules. Low plating density recommendations extend the value of iCell Induced Excitatory Neurons by enabling plating of up to three 96-well plates per vial of $6x10^6$ cells. Together, iCell Induced Excitatory Neurons offer a convenient and reliable source of human excitatory neuronal cells for use in life science research, early preclinical discovery, and toxicity testing.

When handled and maintained as recommended in this User's Guide, iCell Induced Excitatory Neurons quickly assume a typical neuronal morphology with branching neurites (Figure 1). These cells display a stable adherent single-cell morphology in culture and can be co-cultured with iCell Astrocytes 2.0 to generate advanced *in vitro* neural network models, making these neurons amenable to a variety of neurotoxicity and functional assays.

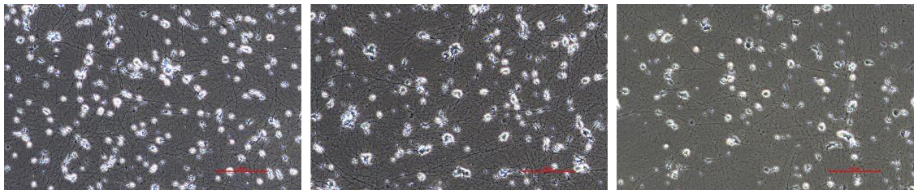


Figure 1: iCell Induced Excitatory Neurons Exhibit Typical Neuronal Morphology

iCell Induced Excitatory Neurons morphology at days 3, 7, and 14 post-plating. Cells were plated at 0.625 x 10^5 cells/cm^2 in a 6-well plate. iCell Induced Excitatory Neurons develop branched networks within 2 - 3 days and can be maintained as mono-cultures over multiple weeks.

Components Supplied by FUJIFILM Cellular Dynamics

Item	Catalog Number
iCell Induced Excitatory Neurons Kit, 01279 • iCell Induced Excitatory Neurons, 01279[1, 2] • iCell Nervous System Supplement[1] • iCell Neural Supplement B[1] • iCell Plating Supplement B[1] • iCell Induced Excitatory Neurons User's Guide	R1245 (≥ 6.0 x 10^6 viable cells) or R1246 (≥ 1.0 x 10^6 viable cells) • C1251 (≥ 6.0 x 10^6 viable cells) or C1252 (≥ 1.0 x 10^6 viable cells) • M1031 (1 ml) • M1029 (2 ml) • M1049 (100 µl) • X1049
iCell Induced Excitatory Neurons GRN R493X HZ KO Kit, 01279[1,2]	R1247 (≥ 6.0 x 10^6 viable cells) R1248 (≥ 1.0 x 10^6 viable cells)
Certificate of Analysis[3]	
Certificate of Origin If required for shipping purposes	

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

[2]These kits contain cells and media to culture disease model induced excitatory neurons. Kit components are available in Chapter 9.

[3]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		
0.22 µm Sterile Filter Unit	Multiple Vendors	
6-well Flat-bottom Plate, TC-treated, Costar	Multiple Vendors	
24-well Flat-bottom Plate, TC-treated, Costar	Multiple Vendors	
96-well Flat-bottom Plate, TC-treated, Falcon	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
BrainPhys Neuronal Medium	STEMCELL Technologies	05790
N-2 Supplement, 100X	Thermo Fisher Scientific	17502-048
Laminin	Millipore Sigma	L2020
Penicillin-Streptomycin, 100X	Thermo Fisher Scientific	15140-122
Poly-L-Ornithine	Millipore Sigma	P4957
Serological Pipettes, multiple sizes	Multiple Vendors	
Trypan Blue	Thermo Fisher Scientific	15250

Technical Support, Knowledge Base, and Training

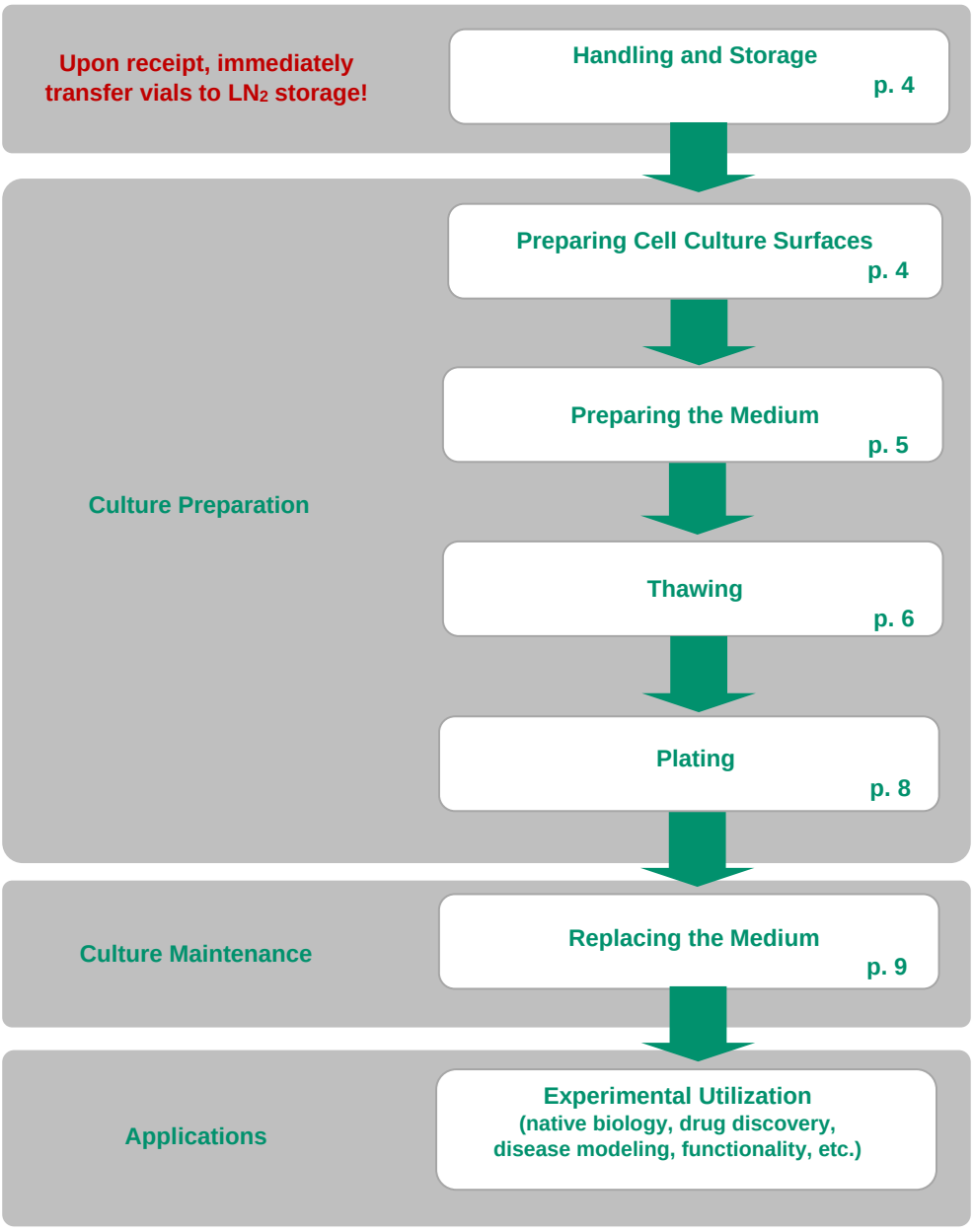
FCDI's Technical Support Scientists have the necessary laboratory and analytical experience to respond to your inquiries. Our web-based Knowledge Base provides solutions for iCell related questions about plating and media, cell culture, general assay methods, and more. In addition, in-lab training may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

iCell Induced Excitatory Neurons are provided as cryopreserved single-cell suspensions in 1.5 ml cryovials. Upon receipt, directly transfer the cryobox containing iCell Induced Excitatory 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 Induced Excitatory Neurons at a stable temperature. Minimize exposure of cryopreserved iCell Induced Excitatory Neurons to ambient temperature when transferring vials to liquid nitrogen

Handling iCell Supplements

iCell Induced Excitatory Neurons are shipped with three (3) additional components: iCell Neural Supplement B, iCell Nervous System Supplement, and iCell Plating Supplement B. These supplements are shipped frozen on dry ice. Upon receipt, store all supplements at -20°C until use.

Chapter 3. Preparing Cell Culture Surfaces

iCell Induced Excitatory Neurons will plate and function on a freshly prepared plate with a base layer of poly-L-ornithine (PLO) and a top coating of Matrigel. This matrix combination is recommended to promote iCell Induced Excitatory Neurons attachment, viability, and function.

Prepare plating surfaces before thawing iCell Induced Excitatory 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 0.01% PLO Solution (ml)	Volume of D-PBS Rinse (ml)	Volume of 0.083 mg/ml Matrigel Solution (ml)
6-well Cell Culture Plate	1	2	1
24-well Cell Culture Plate	0.6	1.2	0.6
96-well Cell Culture Plate	0.1	0.2	0.1

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

2. Add 0.01% PLO solution to each well of the vessel(s).
3. Store the vessel(s) at 4°C overnight.
4. Thaw Matrigel according to manufacturer's instructions. Keep on ice.
5. Dilute 0.9 mg of Matrigel in 10.8 ml of ice-cold DMEM/F-12 or BrainPhys Neuronal Medium for a final Matrigel concentration of 0.083 mg/ml.
6. Completely aspirate the 0.01% PLO solution from each well of the treated cell culture vessel(s). Rinse each well 3 times with D-PBS (without calcium and magnesium) and aspirate completely.

   ***Note:** Rinsing each well thoroughly is critical to avoid PLO-induced cell toxicity.*

7. Add appropriate volume of 0.083 mg/ml Matrigel solution to each well and incubate the vessel(s) in a 37°C cell culture incubator for 1 hour.

   Note: *Alternatively, add the Matrigel solution to each well, wrap the vessel(s) in plastic paraffin film, and store overnight at 4°C. Equilibrate the vessel(s) in a 37°C cell culture incubator before use.*

8. Aspirate the Matrigel solution immediately before the addition of the cell suspension.

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

Chapter 4. Preparing the Medium

iCell Induced Excitatory Neurons are cultured in Complete BrainPhys Medium, comprised of BrainPhys Neuronal Medium, iCell Neural Supplement B, iCell Nervous System Supplement, N-2 supplement, Laminin and Penicillin-Streptomycin. Complete BrainPhys Medium is serum-free and has been specially formulated to maintain the health, function, and purity of iCell Induced Excitatory Neurons. iCell Induced Excitatory Neurons can be maintained in culture for 2 weeks in this medium without appreciable loss of viability or purity.

Complete BrainPhys Medium	Volume (ml)
BrainPhys Neuronal Medium	95
iCell Neural Supplement B	2
iCell Nervous System Supplement	1
N-2 Supplement	1
Laminin (~1 mg/ml)*	~0.1
Penicillin-Streptomycin (optional)	1

Table 2: Volumes for Complete BrainPhys Medium

*It is recommended to calculate volumes of laminin that is needed from the Protein Content (mg/ml) found on the manufacturer's CoA.

Induced Excitatory Neurons Plating Medium	Volume (ml)
Complete BrainPhys Medium	25
iCell Plating Supplement B (1000X)	0.025

Table 3: Volumes for Induced Excitatory Neurons Plating Medium

Preparing Complete BrainPhys Medium

1. Thaw iCell Neural Supplement B, iCell Nervous System Supplement, and N-2 supplement at room temperature on the day of medium preparation.

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

2. Spray all medium components with 70% ethanol and place in a biological safety cabinet.
3. Using sterile technique, add the entire contents of the iCell Neural Supplement B (~2 ml), iCell Nervous System Supplement vial (~1 ml), N-2 supplement (~1 ml) and Penicillin-Streptomycin (~1 ml) into BrainPhys Neuronal Medium (95 ml) to make the Complete BrainPhys Medium. Filter the Complete BrainPhys Medium through a 0.22 µm sterile filter unit.

4. Store the Complete BrainPhys Medium at 4°C, protected from light, for up to 2 weeks.

   Note: *Freeze remaining N-2 supplement in 1 ml aliquots. Do not refreeze the other individual medium components or Complete BrainPhys Medium.*

Preparing Induced Excitatory Neuron Plating Medium

1. Thaw iCell Plating Supplement B at room temperature on the day of medium preparation.

   Note: *iCell Plating Supplement B precipitant can be warmed to 37°C and mixed to dissolve precipitant back into solution.*

2. Using sterile technique, transfer 25 ml of Complete BrainPhys Medium into a separate 50 ml conical vial.
3. Add 25 µl of iCell Plating Supplement B (1000X) into the 25 ml of Complete BrainPhys Medium to make Induced Excitatory Neuron Plating Medium.

Chapter 5. Thawing iCell Induced Excitatory Neurons

Store iCell Induced Excitatory Neurons in liquid nitrogen (vapor phase) until immediately before thawing to ensure maximal recovery and performance of the cells. Complete the following steps of the thawing procedure in a time-efficient manner to facilitate optimal iCell Induced Excitatory Neurons viability and performance.

Note: *Thaw no more than 2 vials of iCell Induced Excitatory Neurons at one time.*

1. Equilibrate the Induced Excitatory Neuron Plating Medium in a 37°C water bath before thawing iCell Induced Excitatory Neurons.
2. Remove the iCell Induced Excitatory Neurons 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 exactly 3 minutes (avoid submerging the cap), holding the tube stationary and without swirling. Use of a floating microcentrifuge tube rack is recommended.

Precise timing is critical to maximize 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 Induced Excitatory 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 Induced Excitatory Neurons cell suspension.

6. Rinse the empty iCell Induced Excitatory Neurons cryovial with 1 ml of Induced Excitatory Neuron Plating Medium to recover any residual cells from the vial.
7. Transfer the 1 ml of the Induced Excitatory Neuron Plating Medium rinse from the cryovial drop-wise (~1 drop/sec) to the 50 ml centrifuge tube containing the iCell Induced Excitatory 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 Induced Excitatory Neuron Plating Medium to the cell suspension is critical to minimize osmotic shock and ensure maximum viability and attachment.

8. Let cell suspension sit undisturbed at room temperature for 5 minutes.
9. Slowly add an additional 1 ml of Induced Excitatory Neuron Plating Medium to the 50 ml centrifuge tube drop-wise (~1 drops/sec) while gently swirling.

It is critical to add the 1 ml of Induced Excitatory Neuron Plating Medium slowly to ensure maximum viability and attachment of the cells once plated. Avoid vigorous shaking or vortexing of the cell suspension.

10. Let cell suspension sit at room temperature for 5 minutes.
11. Slowly add 7 ml of Induced Excitatory Neuron Plating Medium to the 50 ml centrifuge tube drop-wise (~1 drops/sec) while gently swirling.

It is critical to add 7 ml of Complete BrainPhys Medium slowly to ensure maximum viability and attachment of the cells once plated. Avoid vigorous shaking or vortexing of the cell suspension.

12. Centrifuge the cell suspension at 300 x g for 3 minutes at room temperature.
13. Carefully aspirate the supernatant, leaving 1 ml in the centrifuge tube.
14. Gently resuspend the cell pellet in an appropriate volume (e.g., 3 ml) of Induced Excitatory Neuron Plating Medium by pipetting up and down 2 - 3 times.

Chapter 6. Plating iCell Induced Excitatory Neurons

The recommended plating density for iCell Induced Excitatory Neurons is 0.625 x 10^5 viable cells/cm^2 (or 20,000 cells/well for a 96-well plate). Three 96-well plates can be seeded from one vial of iCell Induced Excitatory Neurons (6x10^6 cells/vial). Table 4 provides the desired cell number and plating volume for several common cell culture plates when plating at a density of 0.625 x 10^5 viable cells/cm^2.

1. Obtain the number of viable cells/vial and viability from the Certificate of Analysis.
2. Calculate the final volume of Induced Excitatory Neuron Plating Medium needed to obtain the desired cell plating density using the number of viable cells/vial from the Certificate of Analysis. See Table 4 below for examples.
3. Dilute the cell suspension with room temperature Induced Excitatory Neuron Plating Medium to obtain a desired cell plating density.
4. 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.*

5. Dispense the cell suspension into the appropriate cell culture vessel(s).
6. Culture iCell Induced Excitatory Neurons in a cell culture incubator at 37°C, 5% CO_2 for 24 hours.

Expected Cell Density

iCell Induced Excitatory Neurons can be plated at various densities to accommodate different applications (Figure 2). However, 0.625 x 10^5 viable cells/cm^2 is the recommended density range for most applications. The following table 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 (0.625 x 10^5 cells/cm^2)
6-well Cell Culture Plate	9.6	2	6.0 x 10^5
24-well Cell Culture Plate	1.9	0.6	1.2 x 10^5
96-well Cell Culture Plate	0.32	0.1	2.0 x 10^4

Table 4: Summary of Recommended Volumes and Measures

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

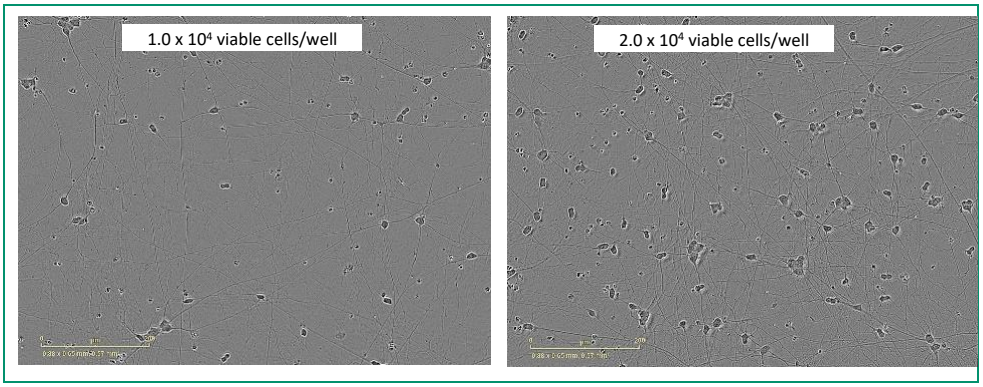


Figure 2: iCell Induced Excitatory Neurons Plated at Two Densities

iCell Induced Excitatory Neurons were seeded at 1.0 x 10^4 and 2.0 x 10^4 viable cells/well into a PLO/Matrigel-coated 96-well cell culture plate and cultured for 7 days.

Chapter 7. Maintaining iCell Induced Excitatory Neurons

iCell Induced Excitatory Neurons are shipped cryopreserved at high purity. The cells maintain a high purity for at least 2 weeks post-plating if maintained in Complete BrainPhys Medium and cultured as recommended in a standard cell culture incubator (37°C, 5% CO_2).

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

1. Immediately before use, aliquot the amount of Complete BrainPhys Medium needed for the media change into a 50 ml conical tube and equilibrate to room temperature for at least 30 minutes.

   Do not equilibrate the Complete BrainPhys Medium to 37°C. Repeated warming of the Complete BrainPhys medium may decrease stability.

2. 24 hours post-plating iCell Induced Excitatory Neurons, perform the first medium change as follows:
   - **96-well cell culture plate:** Add 100 µl of the Complete BrainPhys Medium to each well (200 µl total volume).
   - **Larger than 96-well cell culture plate formats:** Replace 100% of the medium

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

3. Culture iCell Induced Excitatory Neurons in a cell culture incubator at 37°C, 5% CO_2.
4. Replace medium every 2 - 3 days:
   - **96-well cell culture plate:** Replace 50% of the medium
   - **Larger than 96-well cell culture plate formats:** Replace 100% of the medium
5. Culture iCell Induced Excitatory Neurons in a cell culture incubator at 37°C, 5% CO_2.

Chapter 8. Co-Culture of iCell Induced Excitatory Neurons with iCell Astrocytes 2.0

iCell Induced Excitatory Neurons can be cultured with iCell Astrocytes 2.0 to generate neural co-cultures for use in multiple applications including microelectrode array (MEA) recordings. Follow the protocol and plating densities recommended in “Measuring Neural Network Activity on MEA: Co-culture of iCell Induced Excitatory Neurons with iCell Astrocytes 2.0” to set up co-cultures for use with 48-well MEA plates on the Maestro Pro MEA system (Axion). For the complete application protocol, visit:

[Insert QR Code here]

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

This protocol is designed to be used with iCell Astrocytes 2.0. Contact Technical Support for guidance on using iCell Astrocytes, 01434.

Chapter 9. Induced Excitatory Neurons Disease Modeling Kits

iCell Induced Excitatory Neurons Disease Modeling Kit Components Supplied by FUJIFILM Cellular Dynamics

Item	Catalog #	Catalog #
iCell Induced Excitatory Neurons GRN R493X HZ KO Kit, 01279	R1247	R1248
• iCell Induced Excitatory Neurons GRN R493X HZ KO, 01279	• C1253 - 1 vial (≥ 6.0 x 10^6 viable cells)	• C1254 - 1 vial (≥ 1.0 x 10^6 viable cells)
• iCell Nervous System Supplement • iCell Neural Supplement B • iCell Plating Supplement B • iCell Induced Excitatory Neurons User’s Guide	• M1031 (1 ml) • M1029 (2 ml) • M1049 (100 µl) • X1049	