FUJIFILM
Cellular Dynamics

iCell® DopaNeurons
User's Guide

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Conditions of Use

iCell DopaNeurons 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 DopaNeurons are manufactured in the United States of America.

Copyright Notice

Revision History

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Before You Begin

Notes

- Upon receipt, immediately transfer the frozen vials to the vapor phase of a liquid nitrogen storage dewar.
- Read this entire User's Guide before handling or using iCell® DopaNeurons.
- iCell DopaNeurons 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 DopaNeurons.

Notes

Chapter 1. Introduction

iCell DopaNeurons from FUJIFILM Cellular Dynamics, Inc. (FCDI), are a highly pure population of human dopaminergic neurons derived from induced pluripotent stem (iPS) cells using FCDI's proprietary differentiation and purification protocols. iCell DopaNeurons are post-mitotic human midbrain dopaminergic neurons with typical physiological characteristics and responses (Figure 1). 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 DopaNeurons 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 (≥14 days), making them amenable to a variety of electrophysiology, neurotoxicity, and neurotransmission assays.

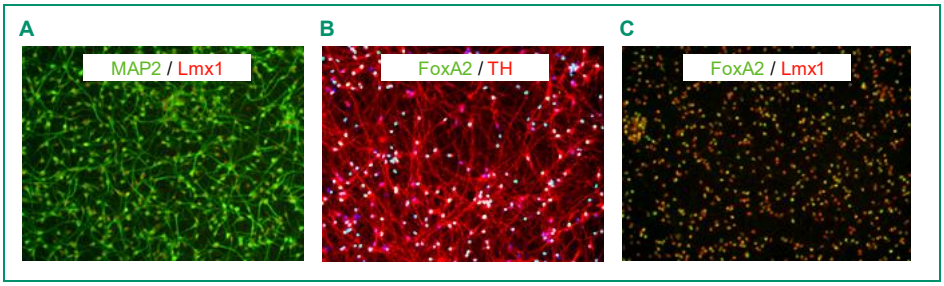


Figure 1: iCell DopaNeurons Represent a Highly Pure Population of Human Neurons

iCell DopaNeurons, 01279 are comprised primarily of midbrain dopaminergic neurons as demonstrated by immunocytochemistry: (A) microtube-associated protein 2 (MAP2) and LIM homeobox transcription factor 1 (Lmx1), day 7 post-plating; (B) forkhead box protein A2 (FoxA2) and tyrosine hydroxylase (TH), day 14 post-plating; and (C) FoxA2 and Lmx1, day 7 post-plating.

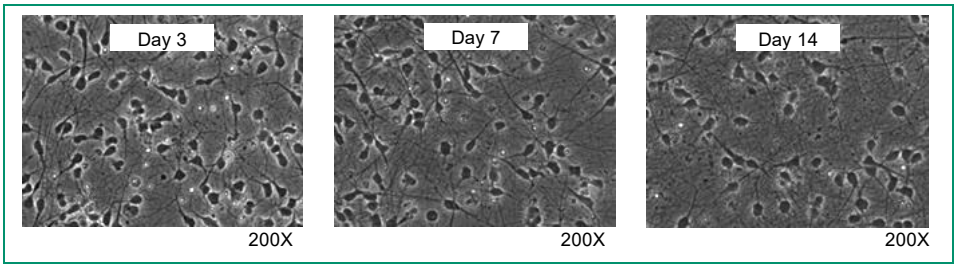


Figure 2: iCell DopaNeurons Exhibit Typical Neuronal Morphology

These images show iCell DopaNeurons, 01279 at days 3, 7, and 14 post-plating. Re-animated iCell DopaNeurons, 01279 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

Notes

Item	Catalog Number
iCell DopaNeurons Kit, 01279	R1032 (≥5.0 x 10^6 viable cells) or R1088 (≥1.0 x 10^6 viable cells)
• iCell DopaNeurons, 01279[1]	• C1028 (≥5.0 x 10^6 viable cells) or C1087 (≥1.0 x 10^6 viable cells)
• iCell Nervous System Supplement[1]	• M1031 (1 ml)
• iCell Neural Base Medium 1[1]	• M1010 (100 ml)
• iCell Neural Supplement B[1]	• M1029 (2 ml)
• iCell DopaNeurons User's Guide[1]	• X1003
iCell DopaNeurons PD GBA N370S Kit, 11344[1, 2]	R1229 (≥1.0 x 10^6 viable cells) R1230 (3 vials x ≥1.0 x 10^6 viable cells/vial) R1231 (≥5.0 x 10^6 viable cells)
iCell DopaNeurons PD GBA N370N Mutation-Corrected Isogenic Control Kit, 11344[1, 2]	R1255 (≥1.0 x 10^6 viable cells)
iCell DopaNeurons PD LRRK2 G2019S Kit, 11299[1, 2]	R1232 (≥1.0 x 10^6 viable cells) R1233 (3 vials x ≥1.0 x 10^6 viable cells/vial) R1234 (≥5.0 x 10^6 viable cells)
iCell DopaNeurons PD LRRK2 G2019G Mutation-Corrected Isogenic Control Kit, 11299[1, 2]	R1244 (≥1.0 x 10^6 viable cells) R1243 (≥5.0 x 10^6 viable cells)
iCell DopaNeurons PD SNCA A53T HZ Kit, 01279[1, 2]	R1109 (≥1.0 x 10^6 viable cells) R1110 (3 vials x ≥1.0 x 10^6 viable cells/vial) R1111 (≥5.0 x 10^6 viable cells)
Certificate of Analysis[3]	
Certificate of Origin If required for shipping purposes	

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

2 These kits contain cells and media to culture patient or engineered Parkinson's Disease dopaminergic neurons. Kit components are available in Chapter 8.

3 Available online: www.fujifilmcdi.com/coa-lookup/

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[1]	Multiple Vendors	
Liquid Nitrogen Storage Unit	Multiple Vendors	
Pipettors	Multiple Vendors	
Tabletop Centrifuge	Multiple Vendors	
Consumables		
24-well Flat-bottom Plate, TC-treated, Costar	Multiple Vendors	
6-well Flat-bottom Plate, TC-treated, Costar	Multiple Vendors	
96-well Flat-bottom Microplate, µClear, Poly-D-Lysine, CELLCOAT, or equivalent[2]	Greiner Bio-One	655946
384-well Flat-bottom Microplate, µClear, Poly-D-Lysine, CELLCOAT, or equivalent[2]	Greiner Bio-One	781946

Notes

Conical Tubes, 50 ml, Falcon (Centrifuge Tubes)	Multiple Vendors	
Dulbecco's Phosphate Buffered Saline without Ca^{2+} and Mg^{2+} (D-PBS)	Multiple Vendors	
Laminin	Millipore Sigma	L2020
Poly-L-Ornithine	Millipore Sigma	P4957
Serological Pipettes, 1, 2, 5, 10, 25 ml	Multiple Vendors	
Trypan Blue	Gibco	15250

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

2 Poly-D-Lysine coated microplates are suggested but uncoated 96-well and 384-well plate can be utilized. For all plates, follow Chapter 3.

Technical Support, Knowledge Base, and Training

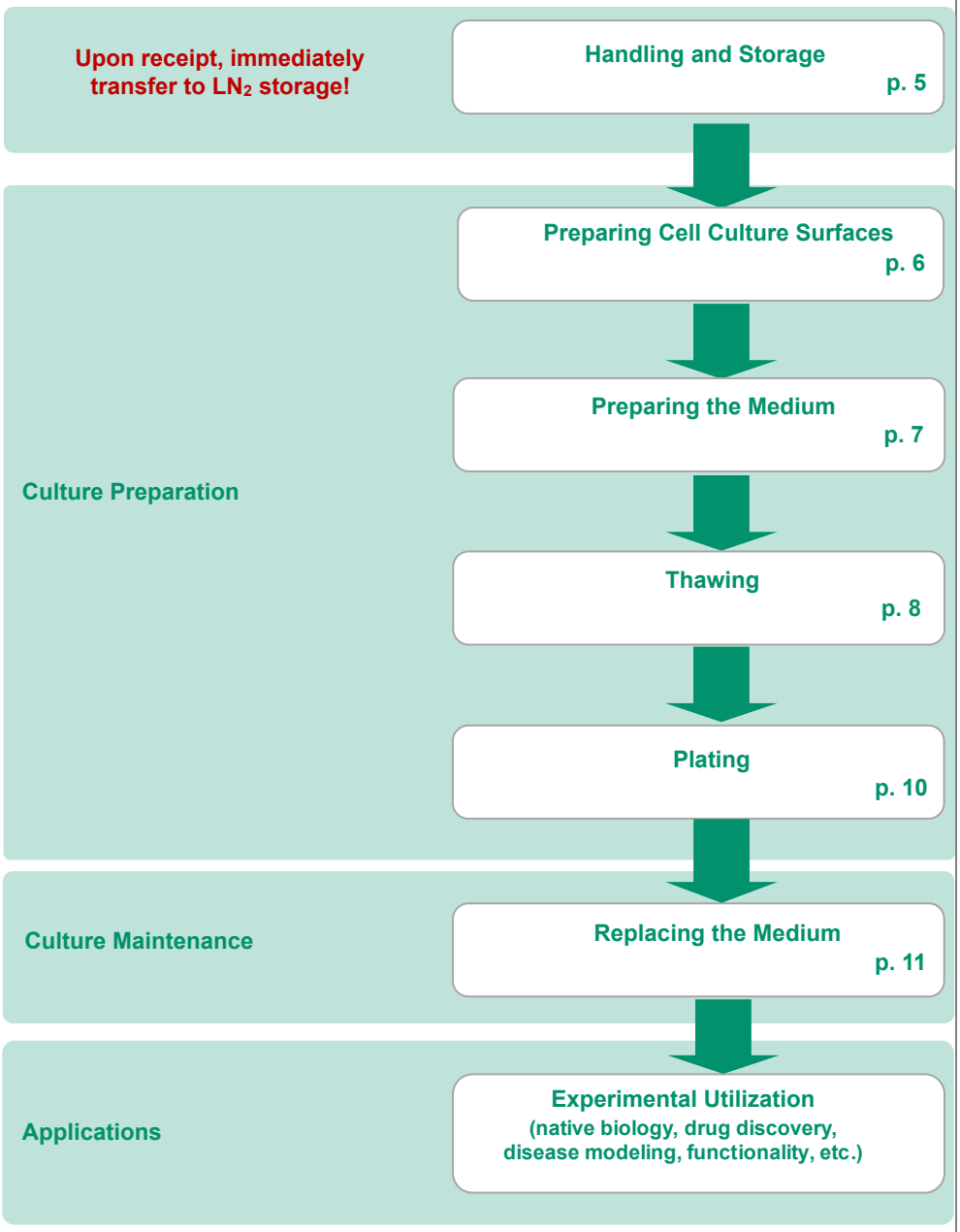
FCDI's Technical Support Scientists have the necessary laboratory and analytical experience to respond to your inquiries. Our web-based Knowledge Base provides solutions for iCell related questions about plating and media, cell culture, general assay methods, and more. In addition, in-lab training may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



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Chapter 2. Handling and Storage

Handling iCell DopaNeurons

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

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

Handling iCell Medium and Supplements

iCell DopaNeurons are shipped with three additional components: iCell Neural Base Medium 1, iCell Neural Supplement B, and iCell Nervous System Supplement. iCell Neural Base Medium 1 is shipped at ambient temperature while iCell Neural Supplement B and iCell Nervous System Supplement are shipped frozen on dry ice. Upon receipt, store iCell Neural Base Medium 1 at 4°C and iCell Neural Supplement B and iCell Nervous System Supplement at -20°C until ready for use.

Chapter 3. Preparing Cell Culture Surfaces

Notes

iCell DopaNeurons will plate and function on a freshly prepared plate with a base layer of poly-L-ornithine (PLO) and a top coating of laminin, which are recommended to promote iCell DopaNeurons attachment, viability, and function.

Prepare plating surfaces before thawing iCell DopaNeurons.

1. Thaw fresh stock laminin solution at room temperature or overnight at 4°C. Do not thaw the laminin solution in a 37°C water bath. Do not vortex the laminin solution.
2. 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 10 µg/ml Laminin 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**.*

3. Add 0.01% PLO solution to each well of the vessel(s).
4. Incubate the vessel(s) at room temperature for at least 1 hour.
5. Dilute fresh stock laminin solution in D-PBS (without calcium and magnesium) to a final concentration of 10 µg/ml immediately before use. Do not vortex the laminin solution.
6. After incubation, completely aspirate the PLO solution from each well. Rinse each well 3 times with D-PBS and aspirate completely.

   ***Note:** Rinsing each well thoroughly is critical to avoid PLO-induced cell toxicity.*
7. Add 10 µg/ml laminin solution to each well and incubate the vessel(s) in a 37°C cell culture incubator for at least 1 hour.

   ***Note:** Alternatively, add the laminin solution to each well, wrap the vessel(s) in parafilm, and store overnight at 4°C. Equilibrate the vessel(s) in a 37°C cell culture incubator before use.*
8. Aspirate the laminin solution immediately before the addition of the cell suspension.

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

Notes

Chapter 4. Preparing the Medium

The Complete Maintenance Medium for iCell DopaNeurons is comprised of three components: iCell Base Medium 1, iCell Neural Supplement B, and iCell Nervous System Supplement. The Complete Maintenance Medium is serum- and antibiotic-free and has been specially formulated to maintain the health and function of iCell DopaNeurons while limiting the proliferation of progenitor or non-neuronal cells. iCell DopaNeurons can be maintained in culture for at least 2 weeks in this medium without appreciable loss of viability or purity.

1. Thaw the iCell Neural Supplement B and iCell Nervous System 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 vial (~2 ml) and the iCell Nervous System Supplement vial (~1 ml) to the iCell Neural Medium 1 bottle (~100 ml) to make the Complete Maintenance Medium.
4. Store the Complete Maintenance Medium at 4°C, protected from light, for up to 4 weeks.

   Note: *FCDI recommends using room temperature Complete Maintenance Medium to thaw iCell DopaNeurons.*

   Note: *Do not refreeze individual medium components or Complete Maintenance Medium.*

Chapter 5. Thawing iCell DopaNeurons

Notes

Maintain iCell DopaNeurons 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 DopaNeurons viability and performance.

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

1. Equilibrate the Complete Maintenance Medium at room temperature before thawing iCell DopaNeurons.
2. Remove the iCell DopaNeurons 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, spray with 70% ethanol and place in a biological safety cabinet.
5. Gently transfer the iCell DopaNeurons 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 DopaNeurons cell suspension.

6. Rinse the empty iCell DopaNeurons 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 DopaNeurons 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 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 to the 50 ml centrifuge tube drop-wise (~2 - 3 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.

Notes

8. Gently mix the contents of the 50 ml centrifuge tube by swirling 3 - 4 times. Gentle mixing is critical to ensure maximum viability. Avoid vigorous shaking or vortexing of the cell suspension.
9. Transfer the cell suspension to a 15 ml centrifuge tube.
10. Centrifuge the cell suspension at 400 x g for 5 minutes at room temperature.
11. Carefully aspirate the supernatant, leaving 1 ml in the centrifuge tube.

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

12. Gently resuspend the cell pellet in an appropriate volume (e.g., 3 ml) of Complete Maintenance Medium by pipetting up and down 2 - 3 times.

Chapter 6. Plating iCell DopaNeurons

Notes

The recommended plating density for iCell DopaNeurons is ≥1.6 x 10^5 viable cells/cm^2. See Figure 3 for images showing cells plated at alternative plating densities.

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 Complete Maintenance Medium to obtain a desired cell plating density.
3. Aspirate the laminin solution from the pre-coated cell culture plates and immediately dispense the cell suspension.
4. Culture iCell DopaNeurons in a cell culture incubator at 37°C, 5% CO_2.

Expected Cell Density

iCell DopaNeurons can be plated at various densities to accommodate different applications (Figure 3). However, 1.6 - 2.3 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 when plating at a moderate density of 2.0 x 10^5 cells/cm^2.

Culture Vessel	Surface Area (cm^2)	Plating Volume (ml)	Cell Number (2.0 x 10^5 cells/cm^2)	Cell Density (cells/ml)
6-well Cell Culture Plate	9.6	3	1,920,000	640,000
24-well Cell Culture Plate	1.9	0.6	380,000	630,000
96-well Cell Culture Plate	0.32	0.2	64,000	320,000
384-well Cell Culture Plate*	0.1	0.05	20,000	400,000

Table 2: Summary of Recommended Volumes and Measures

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

*Surface area of 384-well plate may vary by vendor.

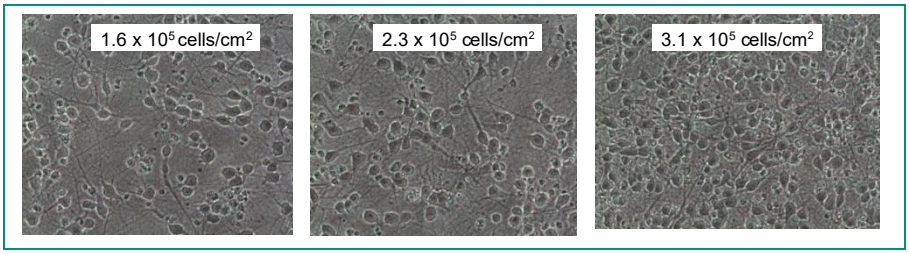

Figure 3: iCell DopaNeurons Plated at Various Densities

These images show iCell DopaNeurons, 01279 at day 14 post-plating when plated at 1.6 x 10^5, 2.3 x 10^5, and 3.1 x 10^5 viable cells/cm^2 into a PLO/laminin-coated 96-well cell culture plate.

Notes

Chapter 7. Maintaining iCell DopaNeurons

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

Prior to each media change, Complete Maintenance Medium should be equilibrated to room temperature.

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

Complete Maintenance Medium is stable for 4 weeks when stored at 4°C. Repeated warming of Complete Maintenance Medium may decrease stability.

1. The first medium change post-plating should be performed after 48 hours. Replace approximately 50% of the medium.
2. Replace approximately 50% of the medium every 2 - 3 days.

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

3. Culture iCell DopaNeurons in a cell culture incubator at 37°C, 5% CO_2.

Chapter 8. Parkinson's Disease Modeling Kits and Citation Guidance

Notes

iCell DopaNeurons Parkinson's Disease Kit Components Supplied by FUJIFILM Cellular Dynamics

Item	Catalog #	Catalog #	Catalog #
iCell DopaNeurons PD GBA N370S Kit, 11344	R1229	R1230	R1231
• iCell DopaNeurons PD GBA N370S, 11344	• C1147 - 1 vial (≥1.0 x 10^6 viable cells)	• C1147 - 3 vials (≥1.0 x 10^6 viable cells)	• C1148 - 1 vial (≥5.0 x 10^6 viable cells)
• iCell Nervous System Supplement	• M1031 (1 ml)		
• iCell Neural Base Medium	• M1010 (100 ml)		
• iCell Neural Supplement B	• M1029 (2 ml)		
• iCell DopaNeurons User's Guide	• X1003		
iCell DopaNeurons PD GBA N370N Mutation-Corrected Isogenic Control Kit, 11344	R1255		
• iCell DopaNeurons PD GBA N370N Mutation-Corrected Isogenic Control, 11344	• C1258 - 1 vial (≥1.0 x 10^6 viable cells)		
• iCell Nervous System Supplement	• M1031 (1 ml)		
• iCell Neural Base Medium	• M1010 (100 ml)		
• iCell Neural Supplement B	• M1029 (2 ml)		
• iCell DopaNeurons User's Guide	• X1003		
iCell DopaNeurons PD LRRK2 G2019S Kit, 11299	R1232	R1233	R1234
• iCell DopaNeurons PD LRRK2 G2019S, 11299	• C1149 - 1 vial (≥1.0 x 10^6 viable cells)	• C1149 - 3 vials (≥1.0 x 10^6 viable cells)	• C1150 - 1 vial (≥5.0 x 10^6 viable cells)
• iCell Nervous System Supplement	• M1031 (1 ml)		
• iCell Neural Base Medium	• M1010 (100 ml)		
• iCell Neural Supplement B	• M1029 (2 ml)		
• iCell DopaNeurons User's Guide	• X1003		
iCell DopaNeurons PD LRRK2 G2019G Mutation-Corrected Isogenic Control Kit, 11299	R1244		R1243
• iCell DopaNeurons PD LRRK2 G2019G Mutation-Corrected Isogenic Control, 11299	• C1256 - 1 vial (≥1.0 x 10^6 viable cells)		• C1255 - 1 vial (≥5.0 x 10^6 viable cells)
• iCell Nervous System Supplement	• M1031 (1 ml)		
• iCell Neural Base Medium	• M1010 (100 ml)		
• iCell Neural Supplement B	• M1029 (2 ml)		
• iCell DopaNeurons User's Guide	• X1003		
iCell DopaNeurons PD SNCA A53T HZ Kit, 01279	R1109	R1110	R1111
• iCell DopaNeurons PD SNCA A53T HZ, 01279	• C1112 - 1 vial (≥1.0 x 10^6 viable cells)	• C1112 - 3 vials (≥1.0 x 10^6 viable cells)	• C1113 - 1 vial (≥5.0 x 10^6 viable cells)
• iCell Nervous System Supplement	• M1031 (1 ml)		
• iCell Neural Base Medium	• M1010 (100 ml)		

• iCell Neural Supplement B • iCell DopaNeurons User's Guide	• M1029 (2 ml) • X1003

Notes

Guide for Citing of GBA and LRRK2 iCell DopaNeurons in Publications

Publications and presentations featuring GBA and LRRK2 iCell DopaNeurons should reference the Parkinson's Progression Markers Initiative (PPMI) and the Golub Capital iPSC PPMI Sub-study (http://www.ppmi-info.org) as the original iPS cell line(s) source for the differentiated cells used in these experiments. Publications should acknowledge that the Parkinson's Progression Initiative (PPMI) is a public-private partnership funded by The Michael J. Fox Foundation for Parkinson's Research and other funding partners. Publications using these cells should be made available without charge to the research community through the PPMI website, when not prohibited by publication copyright terms and conditions. For assistance in uploading to the PPMI website, please contact: https:/www.ppmi-info.org/contact-us/.