Cellular Dynamics international

iCell® DopaNeurons
User's Guide

DOCUMENT #DX21839

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CDI does not in any way guarantee or represent that you will obtain satisfactory results from using iCell DopaNeurons 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 DopaNeurons.

Conditions of Use

iCell DopaNeurons 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 DopaNeurons.

Origin

iCell DopaNeurons are manufactured in the United States of America.

Copyright Notice

Revision History

Document #DX21839
Version 1.2: August 2014
Version 1.1: July 2014
Version 1.0: May 2014

Table of Contents

Before You Begin

Notes

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

Notes

Chapter 1. Introduction

Cellular Dynamics International's (CDI) iCell DopaNeurons are a highly pure population of human dopaminergic neurons derived from induced pluripotent stem (iPS) cells using CDI'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.

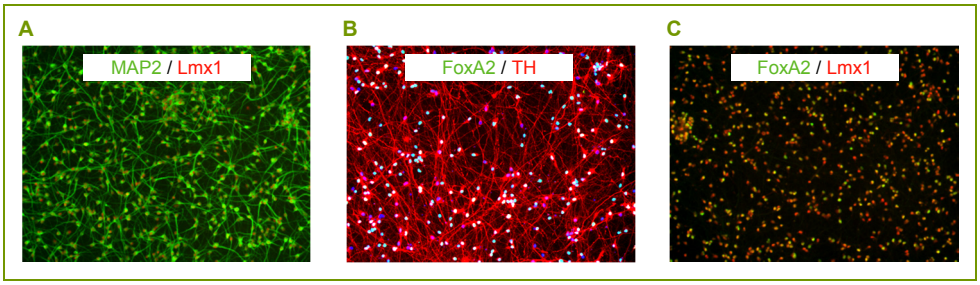

Figure 1: iCell DopaNeurons Represent a Highly Pure Population of Human Neurons
iCell DopaNeurons are comprised primarily of midbrain dopaminergic neurons as demonstrated by immunocytochemistry: (A) microtube-associated protein 2 (MAP2) and LIM homeobox transcription factor 1 (Lmx1), day 7 post-plating;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

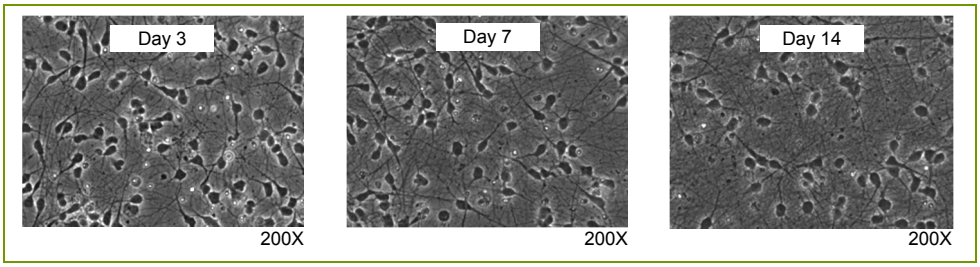

Figure 2: iCell DopaNeurons Exhibit Typical Neuronal Morphology
These images show iCell DopaNeurons at days 3, 7, and 14 post-plating. Re-animated iCell DopaNeurons develop branched networks within 2 - 3 days and remain viable and adherent for an extended period in culture (≥14 days).

Components Supplied by Cellular Dynamics

Item	Catalog Number
iCell DopaNeurons[1]	DNC-301-030-001
iCell DopaNeurons Maintenance Medium[1]	DNM-301-121-001
iCell DopaNeurons Medium Supplement[1]	DNM-301-031-001
iCell Nervous System Supplement[1]	NSS-301-031-001
iCell DopaNeurons User's Guide[1]	
Certificate of Analysis[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 online at www.cellulardynamics.com/coa/

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	Multiple Vendors	
Liquid Nitrogen Storage Unit	Multiple Vendors	
Pipettors	Multiple Vendors	
Tabletop Centrifuge	Multiple Vendors	
Consumables		
15 ml and 50 ml Centrifuge Tubes	Multiple Vendors	
24-well Cell Culture Plates	Nunc	142475
6-well Cell Culture Plates	Nunc	140675
96-well Cell Culture Plates	Corning	3603
Dulbecco's Phosphate Buffered Saline without Ca^{2+} and Mg^{2+} (D-PBS)	Life Technologies	14190
Laminin	Sigma	L2020
Pipettes	Multiple Vendors	
Poly-L-Ornithine	Sigma	P4957
Trypan Blue	Gibco	15250

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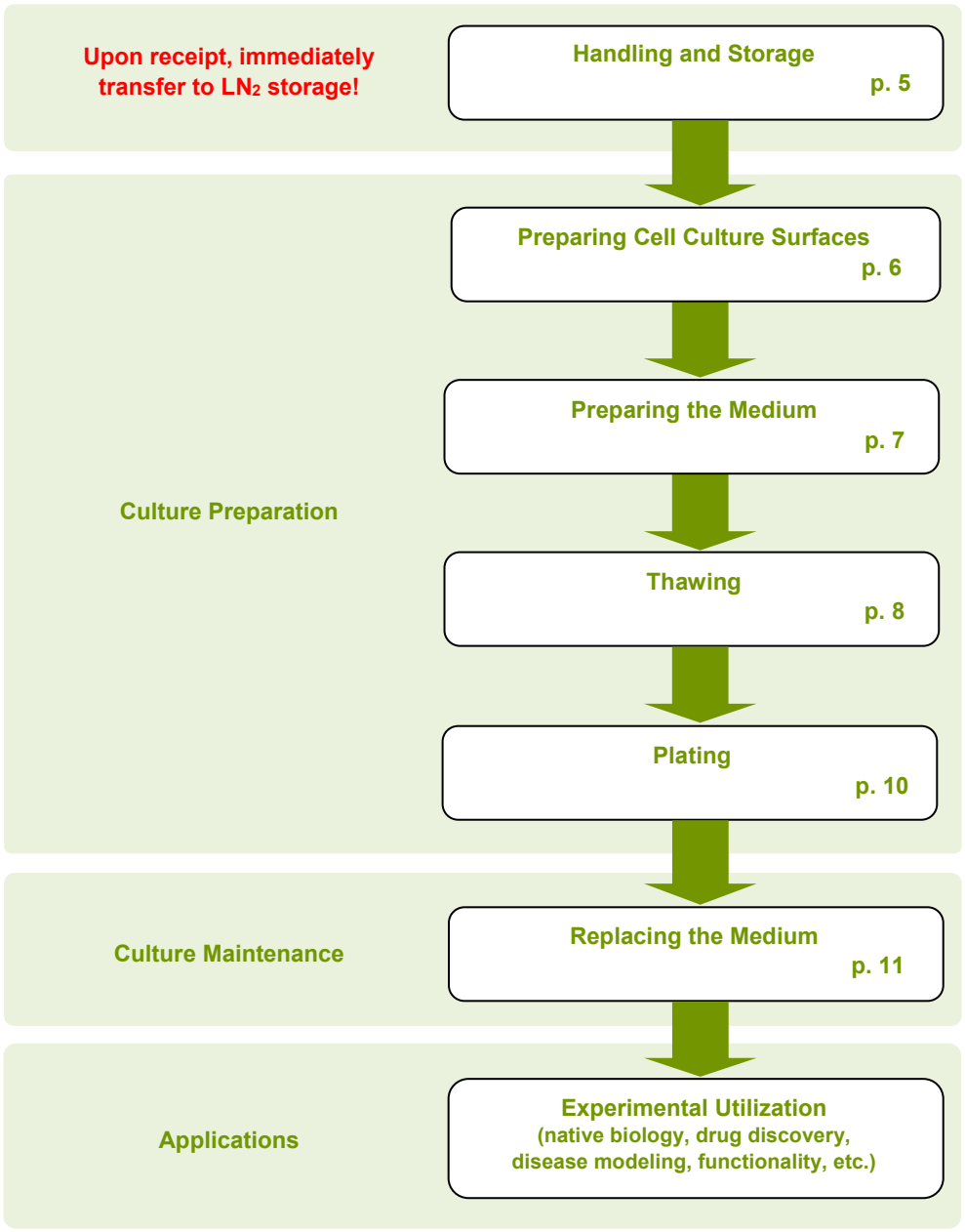
Technical Support and Training

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) 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

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 DopaNeurons Maintenance Medium and Supplements

iCell DopaNeurons medium is shipped as three components: iCell DopaNeurons Maintenance Medium, iCell DopaNeurons Medium Supplement, and iCell Nervous System Supplement. iCell DopaNeurons Maintenance Medium is shipped at ambient temperature while iCell DopaNeurons Medium Supplement and iCell Nervous System Supplement are shipped frozen on dry ice. Upon receipt, store iCell DopaNeurons Maintenance Medium at 4°C and iCell DopaNeurons Medium Supplement 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 1 mg/ml 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 3.3 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 1 mg/ml laminin solution 1:300 in D-PBS (without calcium and magnesium) to a final concentration of 3.3 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 twice with D-PBS and aspirate completely.

   ***Note:** Rinsing each well thoroughly is critical to avoid PLO-induced cell toxicity.*
7. Add 3.3 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

Complete iCell DopaNeurons Maintenance Medium (Complete Maintenance Medium) is comprised of three components: iCell DopaNeurons Maintenance Medium, iCell DopaNeurons Medium Supplement, and iCell Nervous System Supplement. 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 DopaNeurons Medium Supplement and iCell Nervous System Supplement at room temperature on the day of medium preparation.

   

   Do not thaw iCell DopaNeurons medium 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 DopaNeurons Medium Supplement (~2 ml) and the iCell Nervous System Supplement (~1 ml) to the iCell DopaNeurons Maintenance Medium (~100 ml) to make Complete Maintenance Medium.
4. Store the Complete Maintenance Medium at 4°C, protected from light, for up to 4 weeks.

   Note: *CDI 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 **exactly 3 minutes** (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 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.

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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.

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)
6-well Cell Culture Plate	9.6	2	1.9 x 10^6
24-well Cell Culture Plate	1.9	0.6	3.8 x 10^5
96-well Cell Culture Plate	0.32	0.2	6.4 x 10^4

Table 2: Summary of Recommended Volumes and Measures
*All volumes and measures are **per well**.*

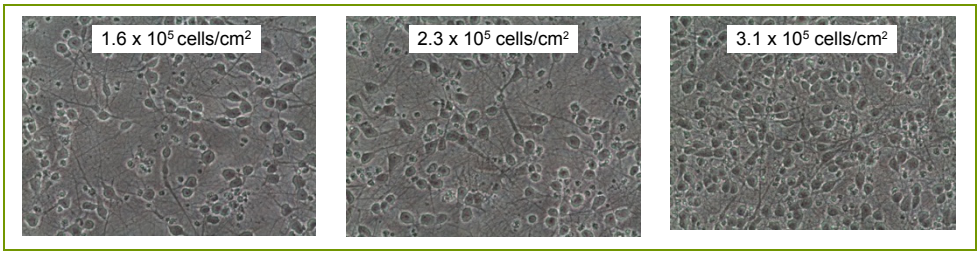

Figure 3: iCell DopaNeurons Plated at Various Densities
These images show iCell DopaNeurons at day 14 post-plating when plated at 1.6 x 10^5, 2.3 x 10^5, and 3.1 x 10^5 viable cells/cm^2 into a PLO/laminin-coated 96-well cell culture plate.

Notes

Chapter 7. Maintaining iCell DopaNeurons

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

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

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

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

   Repeated warming of Complete Maintenance Medium may decrease stability.

2. 2 - 3 days posting-plating iCell DopaNeurons, aspirate the spent medium and replace (75 - 100% exchange) with the appropriate volume of Complete Maintenance Medium. Recommended volumes are as follows:

   - **6-well cell culture plate:** 2 ml/well
   - **24-well cell culture plate:** 0.6 ml/well
   - **96-well cell culture plate:** 200 µl/well

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

3. Replace 75 - 100% of the medium every 2 - 3 days.
4. Culture iCell DopaNeurons in a cell culture incubator at 37°C, 5% CO_2.

Appendices

Notes

Appendix A. Intellectual Property Rights, Use Restrictions, and Limited License

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Appendix B. Limited Warranty

A. During the Warranty Period (as defined below) and subject to subsection F of this Appendix B. Cellular Dynamics warrants that its Products conform to the specifications contained in the Certificate of Analysis for the Product shipped to Customer. Customer's sole and exclusive remedy (and Cellular Dynamics' sole and exclusive liability) with respect to any defective Products shall be replacement of the defective Products by Cellular Dynamics pursuant to this Appendix B.

B. Under no circumstances shall Cellular Dynamics' liability to Customer exceed the amount paid by Customer for the Products to Cellular Dynamics. Cellular Dynamics will bear all reasonable shipping costs if the Products are replaced pursuant to this warranty. For clarity, this warranty automatically shall be void, and any claims under it invalid, (i) if Customer's

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use of the Products is other than solely in accordance with this User's Guide and Cellular Dynamics' Terms and Conditions (or such other written agreement between Cellular Dynamics and Customer under which the Products are sold or transferred to Customer) or for a purpose or in a manner other than that for which the Products were designed; or (ii) if Customer fails to follow this User's Guide for the use, storage, and handling of the Products however such failure is caused; or (iii) if Customer fails to comply with any of the provisions of Appendix A in this User's Guide; or (iv) if there is any abuse, other misuse or neglect of the Products by Customer or to the extent of any damage or loss of the Products by events or occurrences beyond a person's (e.g., Cellular Dynamics') control including without limitation, accident, fire, vandalism and natural disasters (acts of God). This warranty applies only to Customer and not to third parties. This warranty is not assignable.

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D. Within five (5) business days of thawing the Product but prior to the expiration date of the Product as listed on the Certificate of Analysis and/or Product's label (the "Warranty Period"), Customer must notify Cellular Dynamics in writing of any nonconformity of the Products, describing the nonconformity in detail. Customer's failure to properly notify Cellular Dynamics in the Warranty Period voids the limited warranty set forth above in this Appendix B.

E. Customers who believe they have a warranty claim should call Cellular Dynamics' Technical Support line at (608) 310-5100 ext. 5 or email at support@cellulardynamics.com to request a replacement Product based on a breach of the limited warranty set forth above in this Appendix B. Any action by Customer for Cellular Dynamics' breach of this limited warranty, for which Customer has given timely and proper notice of such breach during the Warranty Period and otherwise in accordance with this Appendix B, must be commenced by Customer within 18 months following the date of such breach.

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G. Customer acknowledges and agrees that Cellular Dynamics may fill Customer's order with any number of units of Products. Such units may be more units than Customer ordered. Customer will not be charged extra for any adjustments made by Cellular Dynamics. The number of cells in a unit is determined by the Product's Certificate of Analysis. The number of cells that are contained in a unit accounts for both viability and plating efficiency percentages. Because this may vary from lot to lot, Cellular Dynamics reserves the right to fill the order with that number of units which is sufficient to fill Customer's order and such adjustments shall not constitute a breach of the limited warranty set forth herein.

Appendix C. Limited Liability

Notes

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