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iCell® DopaNeurons

User's Guide

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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 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 DopaNeurons.

Origin

iCell DopaNeurons are manufactured in the United States of America.

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Before You Begin

- 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.

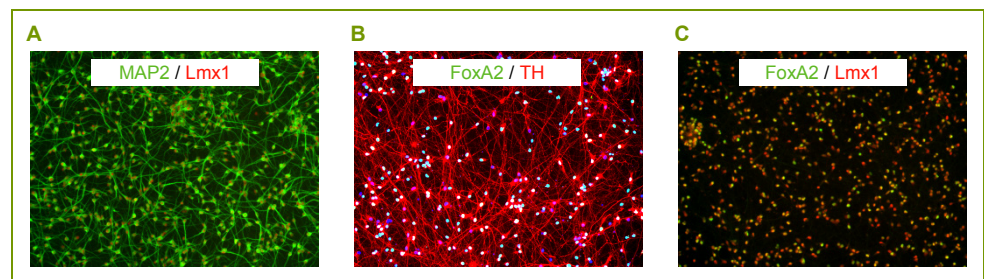


Figure 1: iCell DopaNeurons Represent a Highly Pure Population of Human Neurons
iCell DopaNeurons are comprised primarily of midbrain dopaminergic neurons as demonstrated by immunocytochemistry: (A) microtubule-associated protein 2 (MAP2) and LIM homeobox transcription factor 1 (Lmx1), day 7 post-plating;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

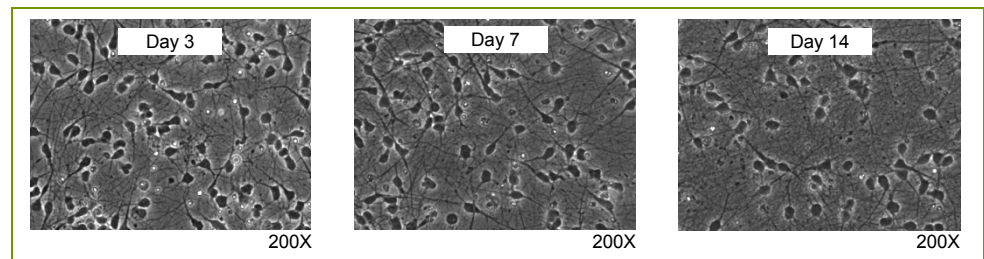


Figure 2: iCell DopaNeurons Exhibit Typical Neuronal Morphology
These images show iCell DopaNeurons at days 3, 7, and 14 post-plating. Re-animated iCell DopaNeurons develop branched networks within 2 - 3 days and remain viable and adherent for an extended period in culture (≥ 14 days).

Components Supplied by Cellular Dynamics

Notes

Item	Catalog Number	
iCell DopaNeurons Kit, 01279	R1032	R1088
• iCell DopaNeurons, 01279 ^{1, 2}	• C1028 ($\geq 5.0 \times 10^6$ viable cells)	• C1087 ($\geq 1.0 \times 10^6$ viable cells)
• iCell Nervous System Supplement ^{1, 2}	• M1031 (1 ml)	• M1031 (1 ml)
• iCell Neural Base Medium 1 ^{1, 2}	• M1010 (100 ml)	• M1010 (100 ml)
• iCell Neural Supplement B ^{1, 2}	• M1029 (2 ml)	• M1029 (2 ml)
• iCell DopaNeurons User's Guide ²	• X1003	• X1003

Certificate of Analysis³

Certificate of Origin

If required for shipping purposes

1 These products were formerly known by these names and/or catalog numbers:

iCell DopaNeurons, 01279 = iCell DopaNeurons (Cat. No. DNC-301-030-001)

iCell Neural Base Medium 1 = iCell DopaNeurons Maintenance Medium (Cat. No. DNM-301-121-001)

iCell Neural Supplement B = iCell DopaNeurons Medium Supplement (Cat. No. DNM-301-031-001)

iCell Nervous System Supplement = iCell Nervous System Supplement (Cat. No. NSS-301-031-001)

Note: You may receive product labeled with the former name and/or catalog number until current stock is depleted. There were no changes in the manufacture of these cells, media, and supplements.

2 Safety Data Sheets and User's Guide available online: www.cellulardynamics.com/lit/

3 Available online: 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 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 ²	STEMCELL Technologies	38017
6-well Flat-bottom Plate, TC-treated, Costar ²	STEMCELL Technologies	38015
96-well Flat-bottom Microplate, TC-treated, Falcon ²	STEMCELL Technologies	38022
Conical Tubes, 50 ml, Falcon (Centrifuge Tubes) ²	STEMCELL Technologies	38010
Dulbecco's Phosphate Buffered Saline without Ca ²⁺ and Mg ²⁺ (D-PBS) ²	STEMCELL Technologies	37350
Laminin	Sigma-Aldrich	L2020
Poly-L-Ornithine	Sigma-Aldrich	P4957
Serological Pipettes, 1, 2, 5, 10, 25 ml ²	STEMCELL Technologies	38001, 38002, 38003, 38004, 38005
Trypan Blue ²	STEMCELL Technologies	07050

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

2 Similar products are available from multiple vendors.

Technical Support, Knowledge Base, 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

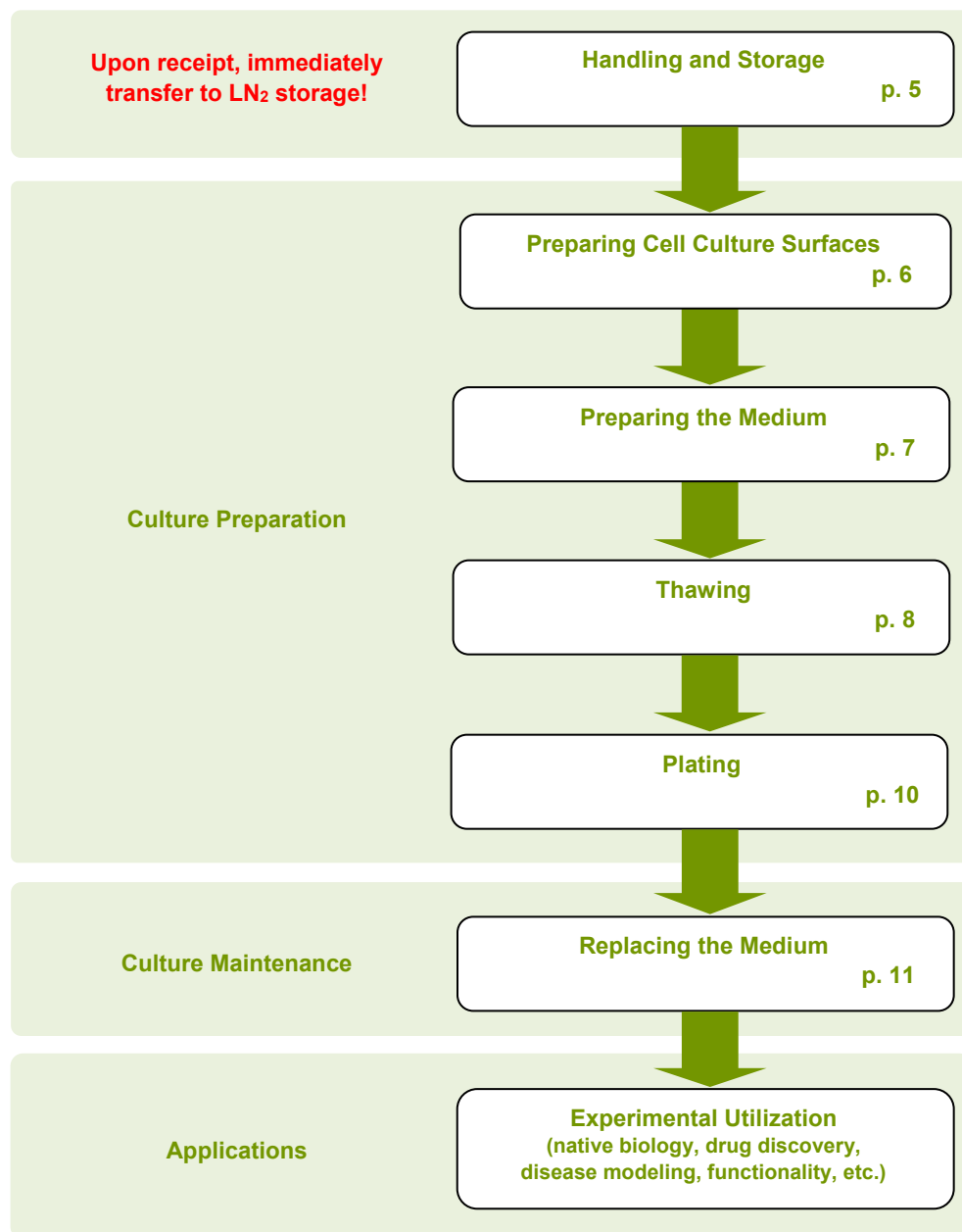
Fax (608) 310-5101

Email support@cellulardynamics.com

Knowledge Base www.cellulardynamics.com/knowledgebase/

Workflow Diagram

Notes



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 (formerly known as iCell DopaNeurons Maintenance Medium), iCell Neural Supplement B (formerly known as iCell DopaNeurons Medium Supplement), 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

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 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 fresh stock laminin solution 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.

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: *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

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.

Notes

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 $\geq 1.6 \times 10^5$ viable cells/cm². 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% CO₂.

Expected Cell Density

iCell DopaNeurons can be plated at various densities to accommodate different applications (Figure 3). However, $1.6 - 2.3 \times 10^5$ viable cells/cm² 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 2.0×10^5 cells/cm².

Culture Vessel	Surface Area (cm ²)	Plating Volume (ml)	Cell Number (2.0×10^5 cells/cm ²)
6-well Cell Culture Plate	9.6	2	1.9×10^6
24-well Cell Culture Plate	1.9	0.6	3.8×10^5
96-well Cell Culture Plate	0.32	0.2	6.4×10^4

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

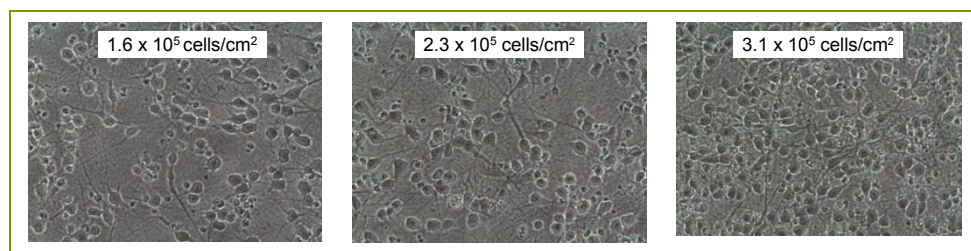


Figure 3: iCell DopaNeurons Plated at Various Densities

These images show iCell DopaNeurons at day 14 post-plating when plated at 1.6×10^5 , 2.3×10^5 , and 3.1×10^5 viable cells/cm² into a PLO/laminin-coated 96-well cell culture plate.

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 post-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% CO₂.

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

A. **OWNERSHIP.** The Products are covered by pending patents and patents: cellulardynamics.com/about-us/patents/. Customer has a limited license to use the Products for internal research purposes for the sole benefit of the Customer, subject to the use restrictions and third party licenses included in subsections B and C of this Appendix A. Customer acknowledges and agrees that the receipt or purchase of the Products by Customer shall not be construed as a transfer of any title or the grant of any rights in or to the intellectual property embodied in the Products owned or licensed by Cellular Dynamics. In particular, no right or license to make, have made, offer to sell, or sell the Products, to modify or reproduce the Product or any part thereof, or to use the Products in combination with any other product(s), except product(s) provided or expressly licensed to Customer by Cellular Dynamics for such use, is implied or conveyed by the sale or transfer of Products to Customer.

B. **USE RESTRICTIONS.** The Products are licensed for internal research purposes only, and may not be used for any other purpose. The Products must be used in accordance with this User's Guide to which Customer, by ordering and accepting the Products, agrees. Customer shall not make, have made, offer to sell, or sell the Products. Unless expressly permitted in a contract executed between Customer and Cellular Dynamics, Customer shall not use the Products (or any modifications Customer makes to the Products or any cells derived, developed or expanded from the Products) in (i) the manufacture of any products, or (ii) any services for a third party. Customer may not transfer the Products (or any modifications Customer makes to the Products or any cells derived, developed or expanded from the Products) to any third party without Cellular Dynamics' prior written consent. Customer shall not reverse engineer the Products. Customer shall not use the Products, components or modifications thereof, or any cells derived, developed or expanded therefrom, in humans, in clinical trials, for diagnostic purposes involving human subjects, or for any investigational or other therapeutic use. Customer shall not use the Products directly or indirectly to derive or make any human gamete or gamete precursor cell. Customer shall use the Products in accordance with all applicable laws and regulations and any applicable institutional review board approved protocol and/or privacy office approval. Customer is not entitled to receive any data or information from Cellular Dynamics that directly identifies the donor of the biological materials from which the Products indirectly are derived or were made. Customer shall not attempt in any way to determine the identity of the donor of the biological materials from which the Products indirectly are derived or were made.

C. **THIRD PARTY LICENSES.** Customer must also execute the Letter of Acknowledgement with Memorial Sloan-Kettering Cancer Center, attached to or accompanying 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), in connection with such purchase or transfer.

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,

Notes

this warranty automatically shall be void, and any claims under it invalid, (i) if Customer's 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.

C. TO THE FULLEST EXTENT PERMITTED BY APPLICABLE LAW, CELLULAR DYNAMICS DISCLAIMS ALL REPRESENTATIONS, AND WARRANTIES, EXPRESS OR IMPLIED, WITH RESPECT TO THE PRODUCTS, INCLUDING BUT NOT LIMITED TO, ANY IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE OR NON-INFRINGEMENT, AND CUSTOMER WAIVES ALL RIGHTS AND REMEDIES, WITH RESPECT TO ANY DEFECTIVE PRODUCTS OTHER THAN THE EXPRESS WARRANTY AND REMEDY THEREFOR STATED ABOVE IN THIS APPENDIX B.

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.

F. Cellular Dynamics makes no warranty of any kind or nature, neither express nor implied, for any product sold together with, or as a part of, the Products (e.g., an accessory accompanying a Product or a discrete component part of a Product that is a kit) that is not manufactured by Cellular Dynamics. Any such accessory to or part of the Products shall have the warranty, if any, that is offered and granted (and, for clarity, extended by its terms to Customer) by the manufacturer of such other accessory or component product accessories.

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

TO THE FULLEST EXTENT PERMITTED UNDER APPLICABLE LAW, CELLULAR DYNAMICS SHALL NOT HAVE ANY LIABILITY FOR INCIDENTAL, COMPENSATORY, PUNITIVE, CONSEQUENTIAL, INDIRECT, SPECIAL OR OTHER SIMILAR DAMAGES, HOWEVER CAUSED AND REGARDLESS OF FORM OF ACTION WHETHER IN CONTRACT, TORT (INCLUDING NEGLIGENCE), STRICT PRODUCT LIABILITY OR OTHERWISE, EVEN IF CELLULAR DYNAMICS HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. NOTWITHSTANDING ANY OTHER TERM OR IMPLICATION TO THE CONTRARY, UNDER NO CIRCUMSTANCES SHALL CELLULAR DYNAMICS' LIABILITY TO CUSTOMER EXCEED THE AMOUNT PAID BY CUSTOMER FOR THE PRODUCTS TO CELLULAR DYNAMICS.

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Notes

