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iCell[®] Endothelial Cells

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 Endothelial Cells 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 Endothelial Cells.

Conditions of Use

iCell Endothelial Cells 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 Endothelial Cells.

Origin

iCell Endothelial Cells are manufactured in the United States of America.

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Revision History

Version 1.3: August 2015

Version 1.2: August 2014

Version 1.1: November 2013

Version 1.0.1: November 2012

Version 1.0: September 2011

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Before You Begin

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

Notes

Chapter 1. Introduction

Cellular Dynamics International's (CDI) iCell Endothelial Cells are purified human endothelial cells derived from induced pluripotent stem (iPS) cells using CDI's proprietary differentiation protocols. iCell Endothelial Cells exhibit characteristic gene and protein expression (e.g. CD31, CD105, CD144, ZO-1, and von Willebrand Factor) and endothelial cell functions (e.g. tubular network formation, acetylated LDL uptake, barrier function, and wound healing). When thawed, plated, and maintained using the Maintenance Medium as instructed in this User's Guide, iCell Endothelial Cells can be used immediately or maintained over multiple passages. These cells are suitable for use in vascular biology research including angiogenesis, atherosclerosis, inflammation, and many other research areas.

iCell Endothelial Cells Medium Supplement has been specially formulated that when used with VascuLife VEGF Medium (Lifeline Cell Technologies, Cat. No. LL-0003) the resulting Maintenance Medium supports the health, function, and continued growth of endothelial cells while limiting the proliferation of the small percentage of non-endothelial cells that could be present during culture. iCell Endothelial Cells can be maintained in culture for up to 5 passages when using the specified medium.

Components Supplied by Cellular Dynamics

Notes

Item	Catalog Number
iCell Endothelial Cells ¹	ECC-100-010-001 (1 unit)
iCell Endothelial Cells Medium Supplement ¹	ECM-100-030-001 (50 ml)
iCell Endothelial Cells 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	
Optional Equipment		
Cell Culture Incubator with Low O ₂ Capacity	Multiple Vendors	
Consumables		
15 ml Centrifuge Tubes	Multiple Vendors	
6-well Cell Culture Plates	Nunc	140675
96-well Cell Culture Plates	Multiple Vendors	
Dulbecco's Phosphate Buffered Saline without Ca ²⁺ and Mg ²⁺ (D-PBS)	Life Technologies	14190
Sterile Tissue Culture Grade Distilled Water	Multiple Vendors	
Fibronectin	Invitrogen	33016-015
PES Filter Unit, 0.2 µm, 500 ml	Multiple Vendors	
Pipettes	Multiple Vendors	
T75 Flasks	Nunc	156472
Trypan Blue	Gibco	15250
TrypLE	Invitrogen	12563
VascuLife VEGF Medium Complete Kit	Lifeline Cell Technologies	LL-0003

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.

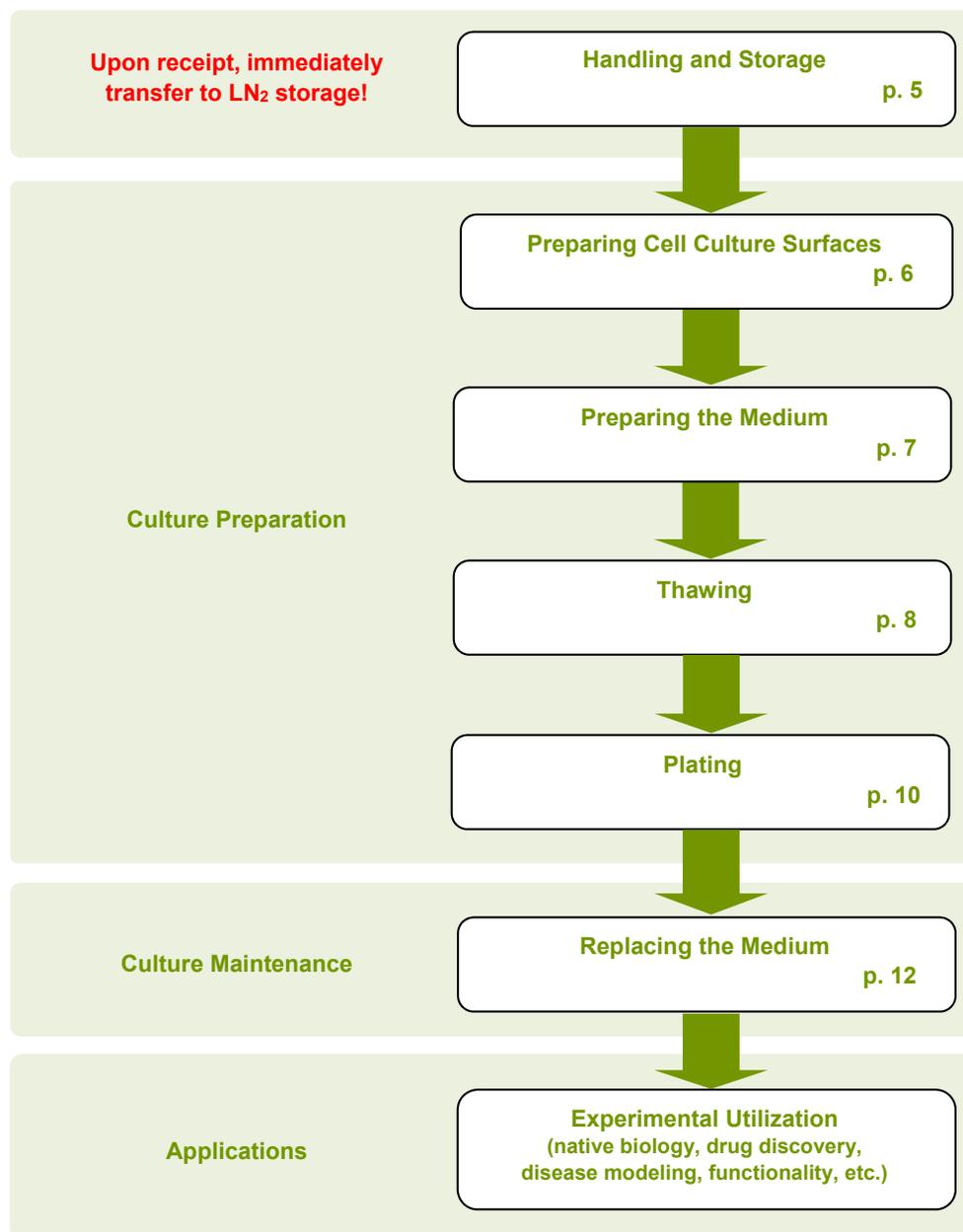
Telephone (877) 320-6688 (US toll-free) / (608) 310-5100 x5
Monday - Friday, 8:30 am - 5:00 pm US Central Time

Fax (608) 310-5101

Email support@cellulardynamics.com

Workflow Diagram

Notes



Chapter 2. Handling and Storage

Handling iCell Endothelial Cells

iCell Endothelial Cells are provided as cryopreserved single-cell suspensions in 1.5 ml cryovials. Upon receipt, directly transfer the cryobox containing iCell Endothelial Cells to the vapor phase of a liquid nitrogen storage dewar. CDI strongly recommends transferring the entire cryobox into the storage rack to avoid transferring individual vials.



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

Handling iCell Endothelial Cells Medium Supplement

iCell Endothelial Cells Medium Supplement is shipped frozen on dry ice. Upon receipt, store the bottle to -20°C until ready for use.

Chapter 3. Preparing Cell Culture Surfaces

Notes

iCell Endothelial Cells behavior is most fully characterized using a fibronectin substrate; however, other substrates can be used.

Preparing the Fibronectin Solution

1. **Stock solution:** Reconstitute fibronectin in sterile water at 1 mg/ml according to the manufacturer's instructions. Aliquot and store at -20°C for future use.
2. **Working solution:** Thaw stock solution at room temperature without agitation. Dilute stock solution in sterile distilled water to a final concentration of 30 µg/ml.

Preparing the Fibronectin Cell Culture Vessel

1. Select the cell culture vessel appropriate for your experimental use. Add fibronectin working solution to coat to 3 µg/cm², using the example volumes specified below. Scale volumes appropriately for other vessel formats.
 - **6-well cell culture plate:** 1 ml/well
 - **96-well cell culture plate:** 32 µl/well
 - **T75 flask:** 7.5 ml

Note: The fibronectin working solution should remain in the vessel until cells are ready to plate.

A summary table of recommended volumes and measures is provided on page 10.

2. Incubate the vessel(s) at room temperature for at least 1 hour.



Do not use a vessel if the fibronectin working solution has evaporated.

Note: If necessary, store vessels coated with fibronectin at 4°C for up to 1 week before use.

Chapter 4. Preparing the Medium

The Maintenance Medium is comprised of iCell Endothelial Cells Medium Supplement and components of the Vasculife VEGF Medium Complete Kit. iCell Endothelial Cells Medium Supplement has been specifically formulated to complete the Vasculife VEGF Medium resulting in a Maintenance Medium that maintains the health and function of iCell Endothelial Cells. The Maintenance Medium is stored at 4°C and can be used for up to 1 month.

1. Remove the Vasculife VEGF Medium Complete Kit from a -20°C freezer and discard the FBS LifeFactor bottle.
2. Thaw iCell Endothelial Cells Medium Supplement and remaining vials from the Vasculife VEGF Medium Complete Kit in a 37°C water bath. Swirl supplement bottle to mix thoroughly once thawed completely.
3. After the L-glutamine completely thaws, quickly vortex the bottle before use.
4. Spray all bottles and vials with 70% ethanol and place in a biological safety cabinet.
5. Using sterile technique, add the following components at the volumes specified to the 500 ml Vasculife Basal Medium bottle to make the Maintenance Medium:

Component	Volume/500 ml
rh VEGF LifeFactor	0.5 ml
rh EGF LifeFactor	0.5 ml
rh FGF basic LifeFactor	0.5 ml
rh IGF-1 LifeFactor	0.5 ml
Ascorbic Acid LifeFactor	0.5 ml
Hydrocortisone Hemisuccinate LifeFactor	0.5 ml
Heparin Sulfate LifeFactor	0.5 ml
L-glutamine LifeFactor*	10 ml
iCell Endothelial Cells Medium Supplement	50 ml

* You will have approximately 15 ml extra L-glutamine LifeFactor that will not be added to the medium.

6. Filter the Maintenance Medium using a 500 ml, 0.2 µm PES filter unit.
7. Store the Maintenance Medium at 4°C, protected from light, for up to 1 month.

Chapter 5. Thawing iCell Endothelial Cells

Maintain iCell Endothelial Cells in liquid nitrogen until immediately before thawing to ensure maximal performance of the cells. Complete the following steps of the thawing protocol in a time-efficient manner to facilitate optimal iCell Endothelial Cells viability and performance.

Note: Thaw no more than 3 vials of iCell Endothelial Cells at one time.

1. Equilibrate the Maintenance Medium at room temperature before thawing iCell Endothelial Cells.
2. Remove the iCell Endothelial Cells 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 and gently swirl (avoid submerging the cap) until the cell suspension is fully thawed.
4. When the cell suspension is fully thawed, immediately remove the cryovial from the water bath, spray with 70% ethanol, and place in a biological safety cabinet.
5. Gently transfer iCell Endothelial Cells cryovial contents to a sterile 15 ml centrifuge tube using a 1 ml pipettor.



Avoid repeated pipetting of the thawed iCell Endothelial Cells suspension.

6. Rinse the empty iCell Endothelial Cells cryovial with 1 ml of room temperature Maintenance Medium to recover any residual cells from the vial. Transfer the 1 ml of Maintenance Medium rinse and any residual cells drop-wise (~1 drop/sec) to the 15 ml centrifuge tube containing the iCell Endothelial Cells 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 Maintenance Medium to the cell suspension is critical to minimize osmotic shock and ensure maximum viability and subsequent attachment of the cells to the plating substrate.

7. Slowly add 8 ml of room temperature Maintenance Medium to the 15 ml centrifuge tube. Gently flick and swirl the centrifuge tube while adding the Maintenance Medium.



It is critical to add the 8 ml of Maintenance Medium slowly to ensure maximum viability and attachment of the cells once plated.

8. Gently and slowly pipette the cell suspension once to mix the Maintenance Medium.
9. Centrifuge the cell suspension at 200 x g for 5 minutes at room temperature.

Notes

10. Aspirate the supernatant, leaving 1 ml in the centrifuge tube.

11. Gently resuspend the cell pellet in 5 ml of Maintenance Medium.

Note: Thaw up to 3 vials of iCell Endothelial Cells at one time. However, each vial must be thawed according to the outlined procedure (i.e. use 9 ml of Maintenance Medium for each vial: 1 ml for transferring residual cells and 8 ml for dilution). Once thawed and diluted to the desired density, you can pool the cell suspensions for plating.

Chapter 6. Plating iCell Endothelial Cells

The recommended plating density of iCell Endothelial Cells is 10,000 - 15,000 viable cells/cm². Adjust the cell concentration in the Maintenance Medium as needed. Just before adding cells, aspirate the fibronectin working solution from the prepared cell culture vessel.

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 Maintenance Medium to obtain a desired cell plating density.
3. Aspirate the fibronectin solution from the pre-coated cell culture vessel(s).
4. Immediately dispense the cell suspension into the pre-coated cell culture vessel(s).
5. Culture iCell Endothelial Cells in a cell culture incubator at 37°C, 5% CO₂, 5% O₂.

Note: *iCell Endothelial Cells are best cultured in a low oxygen incubator (37°C, 5% CO₂, 5% O₂) but can also be cultured in a standard cell culture incubator (37°C, 5% CO₂).*

Expected Cell Density

Figure 1 shows the expected coverage that can be obtained by following the provided plating instructions. iCell Endothelial Cells were added to a 6-well cell culture plate at 10,000 cells/cm² to achieve a confluent monolayer about 3 - 4 days later. The following table provides the desired cell number and plating volume for several common cell culture vessels.

Culture Vessel	Surface Area (cm ²)	Plating Medium (ml)	Cell Number (1 x 10 ⁴ cells/cm ²)	Cell Number (1.5 x 10 ⁴ cells/cm ²)
6-well Cell Culture Plate	9.6	2	96 x 10 ³	144 x 10 ³
96-well Cell Culture Plate	0.32	0.2	3.2 x 10 ³	4.8 x 10 ³
T75 Flask	75	15	750 x 10 ³	1,125 x 10 ³

Table 1: Summary of Recommended Volumes and Measures

All volumes and measures are per well, if applicable.

Notes

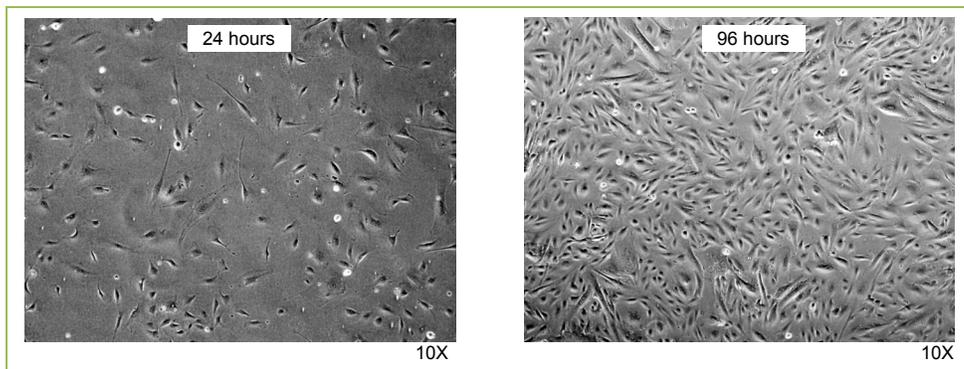


Figure 1: iCell Endothelial Cells at 24 and 96 Hours Post-plating

At 24 hours, the cells are somewhat sparse but healthy while at 96 hours they have grown to form a confluent monolayer and are ready to passage.

Chapter 7. Maintaining iCell Endothelial Cells

iCell Endothelial Cells are shipped cryopreserved at high purity. The cells preserve a high purity if maintained in prepared Maintenance Medium and cultured as recommended.

1. Immediately before use, equilibrate the Maintenance Medium in a 37°C water bath. Do not equilibrate the Maintenance Medium in 37°C water bath multiple times. Aliquot the medium into small working volumes during cell maintenance.
2. 24 hours post-plating iCell Endothelial Cells, aspirate the spent medium and replace with the appropriate volume of Maintenance Medium. Recommended volumes are as follows:
 - **6-well cell culture plate:** 2 ml/well
 - **96-well cell culture plate:** 200 µl/well
 - **T75 flask:** 15 ml
3. Replace the Maintenance Medium every other day.
4. Culture iCell Endothelial Cells in a cell culture incubator at 37°C, 5% CO₂, 5% O₂.

Note: *iCell Endothelial Cells are best cultured in a low oxygen incubator (37°C, 5% CO₂, 5% O₂) but can also be cultured in a standard cell culture incubator (37°C, 5% CO₂).*

5. Passage iCell Endothelial Cells every 3 - 4 days. The following instructions apply to one well of a 6-well cell culture plate. Scale volumes appropriately for other vessel formats.
 - a. Use a plate coated with fibronectin up to 1 week in advance.
 - b. Aspirate the spent medium from the confluent cell culture.
 - c. Rinse iCell Endothelial Cells with 2 ml of D-PBS and aspirate the D-PBS.
 - d. Add 1 ml of TrypLE or 0.25% trypsin/EDTA.
 - e. Incubate at room temperature until cells are loosened from the plate (approximately 5 minutes).
 - f. Gently tap the vessel as necessary to dislodge cells from the plate. Pipette up and down once to ensure the complete detachment of cells. Transfer the cell suspension to a 15 ml centrifuge tube. Microscopically check the plate to confirm that cells were removed.



Do not remove cells from the plate by scraping. If cells are not harvested according to these instructions, there may be a loss of viability, poor recovery, or loss of cell surface markers.

- g. Rinse the well with 2 ml of Maintenance Medium. Transfer the 2 ml of Maintenance Medium rinse from the well to the 15 ml centrifuge tube containing the iCell Endothelial Cells suspension to quench the TrypLE or trypsin/EDTA.
- h. Gently mix the cell suspension by pipetting. Remove a sample of cells to perform a cell count using a hemocytometer or an automated cell counter.

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- i. Centrifuge the cell suspension at 200 x g for 5 minutes.
- j. Aspirate the supernatant, leaving 1 ml in the centrifuge tube.
- k. Gently resuspend the cell pellet in the appropriate amount of Maintenance Medium to plate iCell Endothelial Cells at a density of 10,000 - 15,000 cells/cm².
- l. Aspirate the fibronectin working solution from the coated vessel.
- m. For 6-well cell culture plates, dispense 96,000 - 144,000 cells/well in 2 ml of Maintenance Medium. Suggested cell numbers for other vessel formats are provided in Table 1 on page 10.

Note: *iCell Endothelial Cells can be maintained by routine splitting for up to 5 passages. To avoid a poor quality cell culture, do not allow cells to become over-confluent at any time. It is recommended to passage cells at or just before attaining confluence.*

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

A. OWNERSHIP. The Products are covered by pending patents and patents: www.cellulardynamics.com/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 included in subsection B 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.

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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 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

Notes

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.

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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