Cellular Dynamics international

iCell® Endothelial Cells

User's Guide

Trademarks

iCell is a registered trademark, and Cellular Dynamics and the  logo are trademarks of Cellular Dynamics International, Inc. and may not be used without the express written permission of Cellular Dynamics International, Inc. (CDI).

All other brands, product names, company names, trademarks, and service marks are the properties of their respective owners.

Restrictions and Liabilities

This document is provided "as is." CDI assumes no responsibility for any typographical, technical, or other inaccuracies in this document. CDI reserves the right to periodically change information that is contained in this document; however, CDI makes no commitment to provide any such changes, updates, enhancements, or other additions to this document to you in a timely manner or at all.

OTHER THAN THE LIMITED WARRANTY CONTAINED IN THIS USER'S GUIDE, CDI MAKES NO REPRESENTATIONS, WARRANTIES, CONDITIONS OR COVENANTS, EITHER EXPRESS OR IMPLIED (INCLUDING WITHOUT LIMITATION, ANY EXPRESS OR IMPLIED WARRANTIES OR CONDITIONS OF FITNESS FOR A PARTICULAR PURPOSE, NON-INFRINGEMENT, MERCHANTABILITY, DURABILITY, TITLE, OR RELATED TO THE PERFORMANCE OR NON-PERFORMANCE OF ANY PRODUCT REFERENCED HEREIN OR PERFORMANCE OF ANY SERVICES REFERENCED HEREIN).

This document might contain references to third-party sources of information, hardware or software, products or services and/or third-party web sites (collectively the "Third-Party Information"). CDI does not control, and is not responsible for, any Third-Party Information, including, without limitation the content, accuracy, copyright compliance, compatibility, performance, trustworthiness, legality, decency, links, or any other aspect of Third-Party Information. The inclusion of Third-Party Information in this document does not imply endorsement by CDI of the Third-Party Information or the third party in any way.

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 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.

Copyright Notice

Revision History

Version 1.0, September 2011

Table of Contents

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 Material Safety Data Sheet (MSDS) 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

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 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, 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 five passages when using the specified media.

Components Supplied by Cellular Dynamics

Item	Catalog Number
iCell Endothelial Cells*	(1 unit) ECC-100-010-001
iCell Endothelial Cells Medium Supplement*	(50 ml) ECM-100-031-001
iCell Endothelial Cells User's Guide Also available online at www.cellulardynamics.com/lit/	
Certificate of Analysis Also available online at www.cellulardynamics.com/coa/	
Certificate of Origin	

* Material Safety Data Sheets available online at www.cellulardynamics.com/lit/

Required Equipment and Consumables

Item	Vendor	Catalog Number
Equipment		
Liquid Nitrogen Storage Unit	Multiple Vendors	
Biological Safety Cabinet with UV Lamp	Multiple Vendors	
Tabletop Centrifuge	Multiple Vendors	
37°C Water Bath	Multiple Vendors	
Cell Culture Incubator	Multiple Vendors	
Hemocytometer or Automated Cell Counter	Multiple Vendors	
Optional Equipment		
Cell Culture Incubator with Low O_2 Capacity	Multiple Vendors	
Consumables		
VascuLife VEGF Medium Complete Kit	Lifeline Cell Technologies	LL-0003
PES Filter Unit, 0.2 µm, 500 ml	Multiple Vendors	
Pipettors and Pipettes	Multiple Vendors	
15 ml Centrifuge Tubes	Multiple Vendors	
6-well Cell Culture Plates	Nunc	140675
T75 Flasks	Nunc	156472
96-well Cell Culture Plates	Multiple Vendors	
TrypLE	Invitrogen	12563
Trypan Blue	Gibco	15250
D-PBS	Invitrogen	14190, 14040
Fibronectin	Invitrogen	33016-015
Distilled Water	Multiple Vendors	

Notes

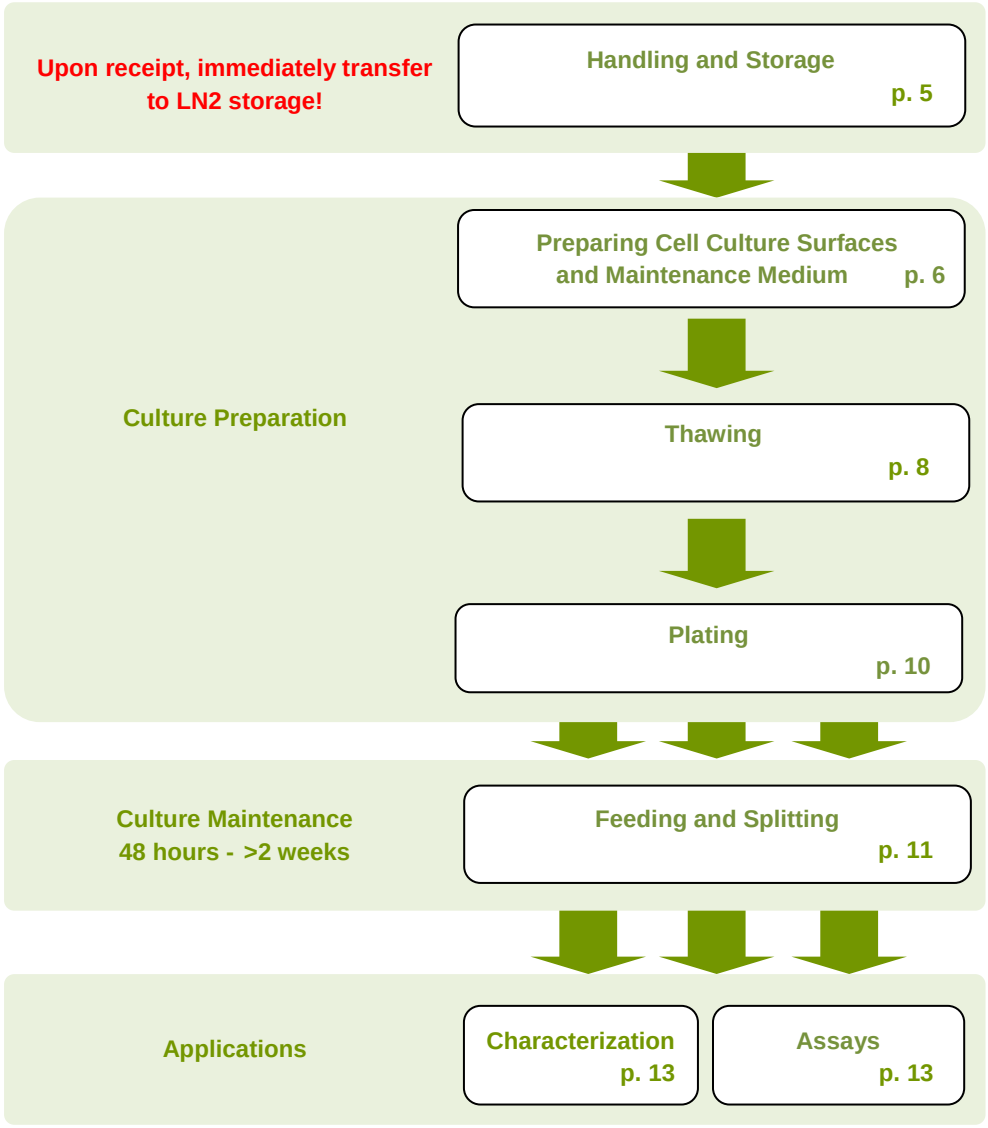
Notes

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) 310-6688 x5 (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

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 liquid nitrogen storage. We strongly recommend transferring the entire cryobox into the storage racks. 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, transfer the bottle to -20°C until ready for use.

Chapter 3. Preparing Cell Culture Surfaces and Maintenance Medium

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

Notes

Preparing Fibronectin Solution

1. **Stock solution:** Resuspend fibronectin at 1 mg/ml in sterile distilled water. You can aliquot and store at -80°C for future use.
2. **Working solution:** If necessary, 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 Cell Culture Vessel

1. Select the cell culture vessel appropriate for your experimental use, for example, a 96-well plate, 6-well plate, or T75 flask.
2. Add fibronectin working solution to the cell culture vessel of choice so that each vessel is coated at 3 µg/cm^2. Example volumes for several types of vessels are as follows:

   - **96-well plate:** Pipette 32 µl of fibronectin working solution into each well.
   - **6-well plate:** Pipette 1 ml of fibronectin working solution into each well.
   - **T75 flask:** Add 7.5 ml of fibronectin working solution into the flask.

   The fibronectin working solution should remain in the vessel until cells are ready for plating.

   A summary table of important volumes and measures is provided on page 12.
3. Incubate the vessel at room temperature for at least 1 hour before use. Do not use a vessel if the fibronectin working solution has evaporated. Cell culture vessels coated with fibronectin working solution can be stored at 4°C for up to 1 week before use.

Preparing the Maintenance Medium

The Maintenance Medium is comprised of iCell Endothelial Cells Medium Supplement and components of VascuLife VEGF Medium Complete Kit (Lifeline Cell Technologies). iCell Endothelial Cells Medium Supplement has been specifically formulated to complete the VascuLife 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.

Notes

1. Remove 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 VascuLife VEGF Medium Complete Kit in a 37°C water bath. Swirl supplement bottle thoroughly to mix once completely thawed.
3. After the L-glutamine is completely thawed, vortex its vial to dissolve precipitate 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 per 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.

Notes

Thawing iCell Endothelial Cells

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

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

1. Remove the frozen iCell Endothelial Cells cryovial from liquid nitrogen storage.

   Note: *After removal from liquid nitrogen storage, cryovials may be placed on dry ice for up to 10 minutes prior to thawing.*

2. Immerse the vial and gently swirl (avoid submerging the cap) in a 37°C water bath until the cell suspension is fully thawed.
3. When the cell suspension is fully thawed, immediately remove the cryovial from the water bath, spray it with 70% ethanol, and place it in a biological safety cabinet.
4. Gently transfer iCell Endothelial Cells to a sterile 15 ml centrifuge tube using a 1 ml pipette.

   Avoid repeated pipetting of the thawed iCell Endothelial Cell suspension.

5. Using a 1 ml pipette, rinse the iCell Endothelial Cells cryovial with 1 ml of room temperature Maintenance Medium to recover any residual cells. Transfer the 1 ml of Maintenance Medium and any residual cells drop-wise to the 15 ml centrifuge tube containing the iCell Endothelial Cells suspension while gently swirling the tube to completely mix the solution.
6. Using a 10 ml pipette, slowly add 8 ml of 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. Slow addition of the Maintenance Medium to the cell suspension will reduce cellular osmotic shock and increase viability.

7. Gently and slowly pipette the cell suspension once to mix the Maintenance Medium.

Notes

8. Pellet the cells using a tabletop centrifuge at 200 x g for 5 minutes.
9. Aspirate the supernatant, leaving 1 ml in the centrifuge tube.
10. Gently resuspend the pellet in 5 ml of Maintenance Medium. 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.

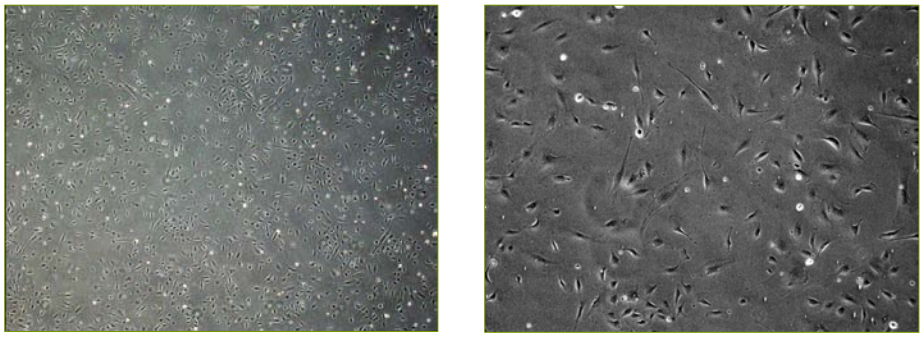
Note: *You can thaw up to three iCell Endothelial Cells vials at one time. However, each vial must be thawed according to the outlined procedure (that is, use 9 ml of Maintenance Medium for each vial: 1 ml for transferring residual cells and 8 ml for dilution). Once thawed and diluted to the desired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

Chapter 5. Plating iCell Endothelial Cells

Notes

The recommended plating density of iCell Endothelial Cells is 10,000 - 15,000 viable cells/cm^2. 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.

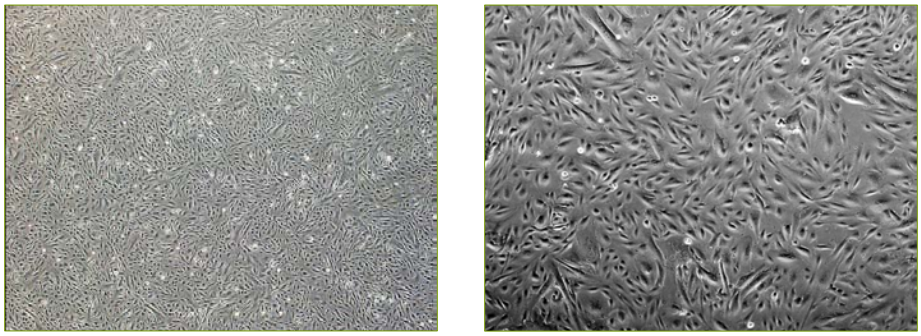
Figure 1 and Figure 2 illustrate the expected coverage that can be obtained by following the provided plating instructions. iCell Endothelial Cells were added to a 6-well plate at 10,000 cells/cm^2 to achieve a confluent monolayer about 3 - 4 days later.



4X 10X

Figure 1: Plating Density at 24 Hours

These images illustrate the expected density of iCell Endothelial Cells after 24 hours of incubation. These cells are somewhat sparse but healthy.



4X 10X

Figure 2: Plating Density at 96 Hours

These images illustrate the expected density of iCell Endothelial Cells after 96 hours of incubation. These cells have grown to form a confluent monolayer.

Notes

Chapter 6. Maintaining iCell Endothelial Cells

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

iCell Endothelial Cells are best cultured in a low oxygen incubator (37°C, 5% CO_2, 5% O_2) but can also be cultured in a standard cell culture incubator (37°C, 5% CO_2).

1. Immediately before use, warm the Maintenance Medium in a 37°C water bath.
2. 24 hours after thawing and plating iCell Endothelial Cells, aspirate the spent medium and replace with the appropriate volume of fresh Maintenance Medium. Recommended volumes are as follows:
   - **96-well plate:** 200 µl/well
   - **6-well plate:** 2 ml/well
   - **T75 flask:** 15 ml
3. Exchange the Maintenance Medium every other day.
4. Passage iCell Endothelial Cells every 3 - 4 days. The following instructions apply to one well of a 6-well plate, but volumes can be scaled according to vessel size:
   a. Use a plate that was coated with fibronectin working solution up to 1 week in advance.
   b. Aspirate spent medium from the confluent cell culture.
   c. Rinse iCell Endothelial Cells with 2 ml of D-PBS and aspirate D-PBS.
   d. Add 1 ml of TrypLE or 0.25% trypsin/EDTA. Incubate at room temperature until cells are loosened from the plate (approximately 5 minutes). Tap vessel gently as necessary to dislodge cells from plate. Pipette up and down once to ensure complete removal of cells. Add cells to a 15 ml conical tube. Check the plate microscopically to confirm that cells have been 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.

   e. Rinse the well with 2 ml of Maintenance Medium and then add this medium to the 15 ml conical tube to quench the TrypLE or trypsin/EDTA.
   f. Resuspend cells by pipetting gently. Remove a sample of cells to perform a cell count using a hemocytometer or an automated cell counter.
   g. Spin cells in a tabletop centrifuge at 200 x g for 5 minutes to pellet the cells and aspirate the supernatant to the last 1 ml.

h. Resuspend cells in appropriate amount of Maintenance Medium to plate at a density of 10,000 - 15,000 iCell Endothelial Cells per cm^2. For a 6-well plate, there should be 96,000 - 144,000 cells plated per well in 2 ml of Maintenance Medium. Suggested cell numbers for additional types of vessels are provided in Table 1.

i. Aspirate the fibronectin working solution from the vessel to be plated.

j. Add iCell Endothelial Cells into the vessel.

5. iCell Endothelial Cells can be maintained by routine splitting for up to five 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.

Culture Vessel	Surface Area	Fibronectin Working Solution	Maintenance Medium	Cell Number (to plate 10K/cm^2)	Cell Number (to plate 15K/cm^2)
96-well plate	0.32 cm^2	32 µl	200 µl	3,200	4,800
6-well plate	9.6 cm^2	1 ml	2 ml	96,000	144,000
T75 flask	75 cm^2	7.5 ml	15 ml	750,000	1,125,000

Table 1: Summary of Useful Volumes and Measures
*All volumes and measures are **per well**, if applicable.*

Notes

Notes

Chapter 7. iCell Endothelial Cells Characterization and Assays

iCell Endothelial Cells perform well in many assays, such as cell viability, cytokine stimulation, and impedance/barrier function. The optimal plated density and timing after plating is dependent on the assay and must be determined empirically based on the intended use of the endothelial cells.

Following are results of some of the characterization studies and assays performed to demonstrate the marker expression and functionality of iCell Endothelial Cells.

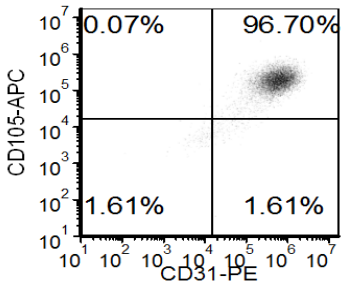
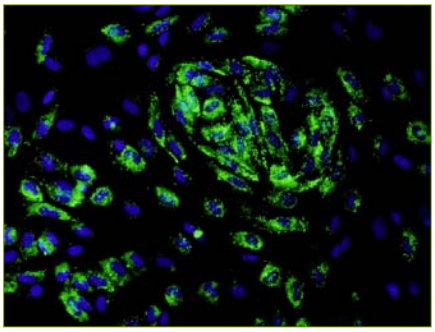


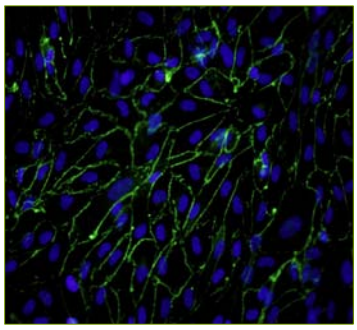
Figure 3: Cell Surface Marker Expression
iCell Endothelial Cells were thawed, plated, and assayed using FACS the following day for expression of endoglin (CD105) and PECAM-1 (CD31) on the cell surface.

40X (brightfield)



40X (fluorescence)

Figure 4: von Willibrand Expression
The image on the right shows the expression of von Willebrand Factor (vWF, green). Nuclei are counterstained with Hoechst (blue). The image on the left is the same image taken in brightfield. Cells were thawed, grown for two passages, and harvested for plating. The cells were plated at a density of 10,000 cells/cm^2, grown for 1 week to be over-confluent, and stained to detect vWF.



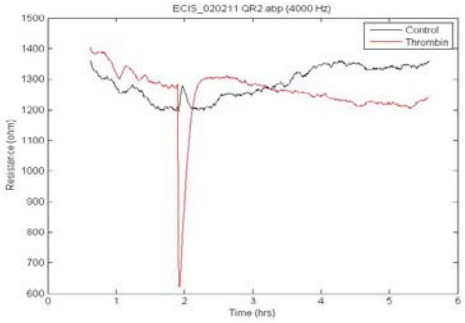
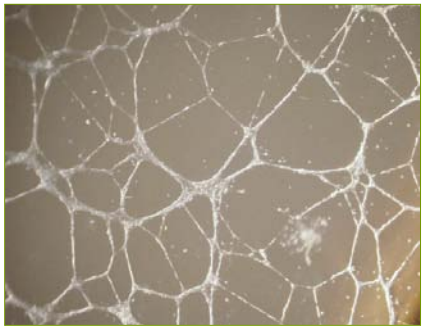
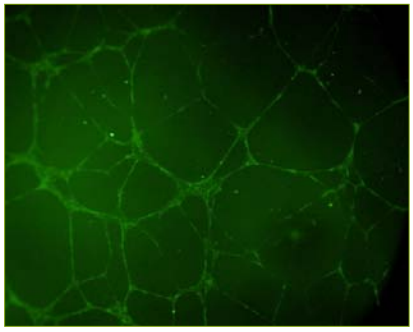


Figure 5: Cell Barrier Function

For the image on the left, iCell Endothelial Cells were thawed, grown for one passage, and harvested. They were subsequently plated at a density of 15,000 cells/cm^2 and grown for 1 week to become over-confluent in order to maximize tight junction formation. Cells were stained with ZO-1, which is specific to tight junctions between the cells (green).The nuclei were counterstained with Hoechst (blue). For the image on the right, to quantify barrier function, iCell Endothelial Cells were thawed, grown for one passage, and plated on an ECIS chip at 50,000 cells/well. Resistance was measured 48 hours later using ECIS (Applied Biophysics). The control well is shown in black, and thrombin was added at 5 U/ml to the well shown in red. Thrombin disrupted the barrier function, as shown by decreased resistance, and the barrier function recovered after a short period of time.



4X (brightfield)



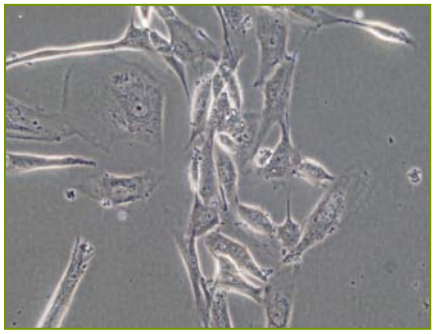
4X (Ulex)

Figure 6: Tube Formation

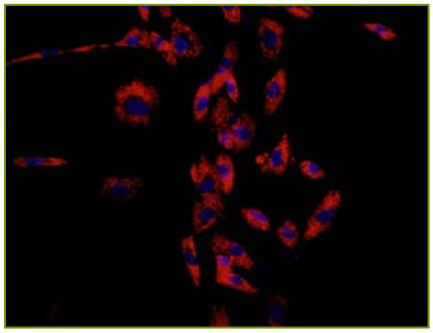
The above images show the capacity for iCell Endothelial Cells to form tubes in a thick layer of Matrigel. The image on the left is taken in brightfield, while the image on the right is the same field of cells but stained with Ulex (green). 100,000 iCell Endothelial Cells were plated at passage 4 into one well of a 24-well plate pre-coated with 200 µl of Matrigel, incubated overnight, and stained the following day. Tube formation and binding of Ulex are characteristics typical of endothelial cells.

Notes

Notes



40X Magnification (brightfield)



40X Magnification (Ulex)

Figure 7: Acetylated LDL Uptake

The image (above right) shows the uptake of acetylated LDL (red). Nuclei are counterstained with Hoechst (blue). The image on the left is the same image taken in brightfield. Cells were thawed, grown for two passages, and plated at 10,000 cells/cm^2. The experiment was performed 48 hours later.

Optional Assay Reagents

Reagent	Vendor	Catalog Number
von Willebrand Factor	Dako	A0082
Alex Fluor 488 goat anti-rabbit IgG (von Willebrand Factor Secondary Antibody)	Molecular Probes	A11034
ZO-1, Alexa Fluor 488 Conjugate	Molecular Probes	339188
Thrombin	Sigma	T8885
Ulex europaeus, UEA-I	Sigma	L9006
Acetylated LDL, Alexa Fluor 594 Conjugate	Molecular Probes	L35353

Notes

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

A. **OWNERSHIP**. Customer acknowledges that iCell Endothelial Cells (the "Products") sold to Customer embody intellectual property deemed to be of significant value to Cellular Dynamics and its licensors, and that such intellectual property may be protected by the law of patents, copyrights, trade secrets, and other laws. Customer acknowledges and agrees that neither this Appendix nor the purchase of the Products by Customer shall be construed as a transfer of any title or the grant of any rights in and to the intellectual property embodied in the Products owned or licensed by Cellular Dynamics. The Products are covered by one or more pending patents. Customer has a limited license to use the Products under the patents, subject to the use restrictions contained in this User's Guide and subsection B of this Appendix A, below. Customer hereby grants to Cellular Dynamics a perpetual, royalty-free, fully paid up, non-exclusive, worldwide, unlimited license to use for any purpose any and all improvements made by Customer to the intellectual property embodied in the Products. Such rights include, without limitation, improvements related to reanimation or thawing, cell functionality, cell differentiation, cell plating, cell improvement assays; or protocols relating to the same. Customer is not granting any rights to and retains all rights to any and all findings and data relating to the performance of the Customer's products, compounds or molecules on the Products.

B. **USE RESTRICTIONS.** The Products and/or components of 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 the iCell Endothelial Cell User's Guide. No other right, express or implied, is conveyed by the sale of the Products. In particular, no right to make, have made, offer to sell, or sell the Products is implied by the sale or purchase of the Products. The license granted herein does not imply or convey the right to use the Products in combination with any other product(s) whose manufacture, sale, or use is covered by any patent. Customer may not transfer the Products to any third party without Cellular Dynamics's prior written consent. Customer shall not reverse engineer, modify or otherwise alter the Products in any way. Customer shall not use the Products or any components thereof in humans, in clinical trials or for diagnostic purposes involving human subjects, for any therapeutic use or investigational use, nor for any purpose in contravention of any applicable law, regulation, ordinance, institutional review board approved protocol, or privacy office approval.

Appendix B. Limited Warranty

A. 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's sole and exclusive liability) under this limited warranty shall be replacement of the defective Products by Cellular Dynamics.

B. Cellular Dynamics reserves the right to make changes in design, production, manufacture, or characteristics of the Products or to improve on the Products at any time and in any way, without incurring any obligations to replace or modify any Products previously sold.

C. Under no circumstances shall Cellular Dynamics's 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. This warranty does not apply to any defect or nonconformance caused by (i) Customer's use

Notes

of the Products for a purpose or in a manner other than that for which they were designed or that is permitted or in breach of this User's Guide, (ii) the failure by Customer to follow Cellular Dynamics' User's Guide for use, storage, and handling of the Products; or (iii) as a result of any other abuse, misuse or neglect of the Products by Customer. This warranty applies only to Customer and not to third parties. This warranty is not assignable.

D. TO THE FULLEST EXTENT PERMITTED BY APPLICABLE LAW, CELLULAR DYNAMICS DISCLAIMS ALL OTHER 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. CUSTOMER'S SOLE REMEDY FOR BREACH OF WARRANTY IS STATED ABOVE.

E. 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 label, Customer must notify Cellular Dynamics in writing of any nonconformity of the Product, describing the nonconformity in detail; otherwise all Products shall be conclusively deemed accepted without qualification. Customer's failure to notify Cellular Dynamics in such time period voids the limited warranty described above. 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 replacement Product based on a breach of the above limited warranty. Any action by Customer for Cellular Dynamics' breach of this limited warranty must be commenced within 18 months following the date of such breach.

F. Customer acknowledges that the Products are subject to U.S. export control laws and regulations. Customer represents and warrants that it is the ultimate end-user of the Products, and further represents and warrants that it will not knowingly sell, export, re-export, transfer, divert, or otherwise dispose of the Products (including other materials or goods derived from or based on the Products) to any other destination, entity, or person without the prior authorization of any relevant U.S. federal government agency and Cellular Dynamics. Customer represents and warrants that it will not use the Products for any purpose prohibited by the laws or regulations of the United States and/or other government authorities to which Customer is subject without the prior authorization from any government entity whose laws and regulations may apply to the use of the Products.

G. Cellular Dynamics makes no warranty of any kind or nature, neither express nor implied, for any Products or part of the Products that is not manufactured by Cellular Dynamics. Any Products, or other such part or accessories to the Products shall have the warranty, if any, that is offered and granted by the manufacturer of such other products and accessories.

H. 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 warranty 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. CUSTOMER UNDERSTANDS THAT ANY RISKS OF LOSS HEREUNDER ARE REFLECTED IN THE PRICE OF THE PRODUCTS AND THAT THESE TERMS WOULD HAVE BEEN DIFFERENT IF THERE HAD BEEN A DIFFERENT ALLOCATION OF RISK.

Notes