



# **iCell<sup>®</sup> Endothelial Cells**

## **User's Guide**

Document ID: X1009



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FCDI 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 set forth in [www.fujifilmcdi.com/terms-and-conditions/](http://www.fujifilmcdi.com/terms-and-conditions/). You assume all risk in connection with your use of iCell Endothelial Cells.

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## Origin

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

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## Revision History

Document ID: X1009

Version 1.0: May 2021

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## Before You Begin

- Immediately transfer the frozen vials to liquid nitrogen storage.
- Read this entire User's Guide before handling or using iCell® Endothelial Cells.
- iCell Endothelial Cells are FOR RESEARCH USE ONLY. See [www.fujifilmcdi.com/terms-and-conditions/](http://www.fujifilmcdi.com/terms-and-conditions/) for USE RESTRICTIONS applicable to the cells and other terms and conditions related to the cells and their use.
- A Safety Data Sheet (SDS) for dimethyl sulfoxide (DMSO), in which iCell Endothelial Cells are frozen, is available online at [www.fujifilmcdi.com/product-literature/](http://www.fujifilmcdi.com/product-literature/) or on request from FUJIFILM Cellular Dynamics. Only technically qualified individuals experienced in handling DMSO and human biological materials should access, use, or handle iCell Endothelial Cells.

## Introduction

iCell Endothelial Cells from FUJIFILM Cellular Dynamics, Inc. (FCDI), are purified human endothelial cells derived from induced pluripotent stem (iPS) cells using FCDI'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 Technology, 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 3 passages when using the specified medium.

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## Components Supplied by FUJIFILM Cellular Dynamics

| Item  | Catalog Number  |
|---|---|
| <b>iCell Endothelial Cells Kit, 01434</b> <ul style="list-style-type: none"><li>iCell Endothelial Cells, 01434<sup>1</sup></li><li>iCell Endothelial Cells Medium Supplement<sup>1</sup></li><li>iCell Endothelial Cells User's Guide</li></ul> | <b>R1022</b> <ul style="list-style-type: none"><li>C1021 (<math>\geq 1.0 \times 10^6</math> viable cells)</li><li>M1019 (50 ml)</li><li>X1009</li></ul> |
| <b>iCell Endothelial Cells Kit, 11713</b> <ul style="list-style-type: none"><li>iCell Endothelial Cells, 11713<sup>1</sup></li><li>iCell Endothelial Cells Medium Supplement<sup>1</sup></li><li>iCell Endothelial Cells User's Guide</li></ul> | <b>R1112</b> <ul style="list-style-type: none"><li>C1114 (<math>\geq 1.0 \times 10^6</math> viable cells)</li><li>M1019 (50 ml)</li><li>X1009</li></ul> |
| Certificate of Analysis <sup>2</sup>  |   |

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

2 Available online: [www.fujifilmcdi.com/coa-lookup/](http://www.fujifilmcdi.com/coa-lookup/)

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## Required Equipment and Consumables

| Item  | Vendor(s)                | Catalog Number(s) |
|---|--------------------------|-------------------|
| <b>Equipment</b>  |                          |                   |
| 37°C Water Bath   | Multiple Vendors         |                   |
| Biological Safety Cabinet with UV Lamp  | Multiple Vendors         |                   |
| Cell Culture Incubator  | Multiple Vendors         |                   |
| Hemocytometer or Automated Cell Counter <sup>1</sup>                                      | Multiple Vendors         |                   |
| Liquid Nitrogen Storage Unit  | Multiple Vendors         |                   |
| Pipettors   | Multiple Vendors         |                   |
| Tabletop Centrifuge   | Multiple Vendors         |                   |
| <b>Optional Equipment</b>   |                          |                   |
| Cell Culture Incubator with Low O <sub>2</sub> Capacity                                   | Multiple Vendors         |                   |
| <b>Consumables</b>  |                          |                   |
| 6-well Cell Culture Plates  | Nunc                     | 140675            |
| 96-well Cell Culture Plates   | Nunc                     | 167008            |
| Dulbecco's Phosphate Buffered Saline without Ca <sup>2+</sup> and Mg <sup>2+</sup> (DPBS) | Gibco                    | 14190             |
| Fibronectin   | Gibco                    | 33016-015         |
| PES Filter Unit, 0.2 µm, 500 ml   | Multiple Vendors         |                   |
| Sterile Tissue Culture Grade Distilled Water  | Multiple Vendors         |                   |
| T75 Flasks  | Nunc                     | 156472            |
| Trypan Blue   | STEMCELL Technologies    | 07050             |
| TrypLE  | Thermo Fisher Scientific | 12563             |
| Vasculife VEGF Endothelial Medium Complete Kit  | Lifeline Cell Technology | LL-0003           |

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## Technical Support, and Knowledge Base

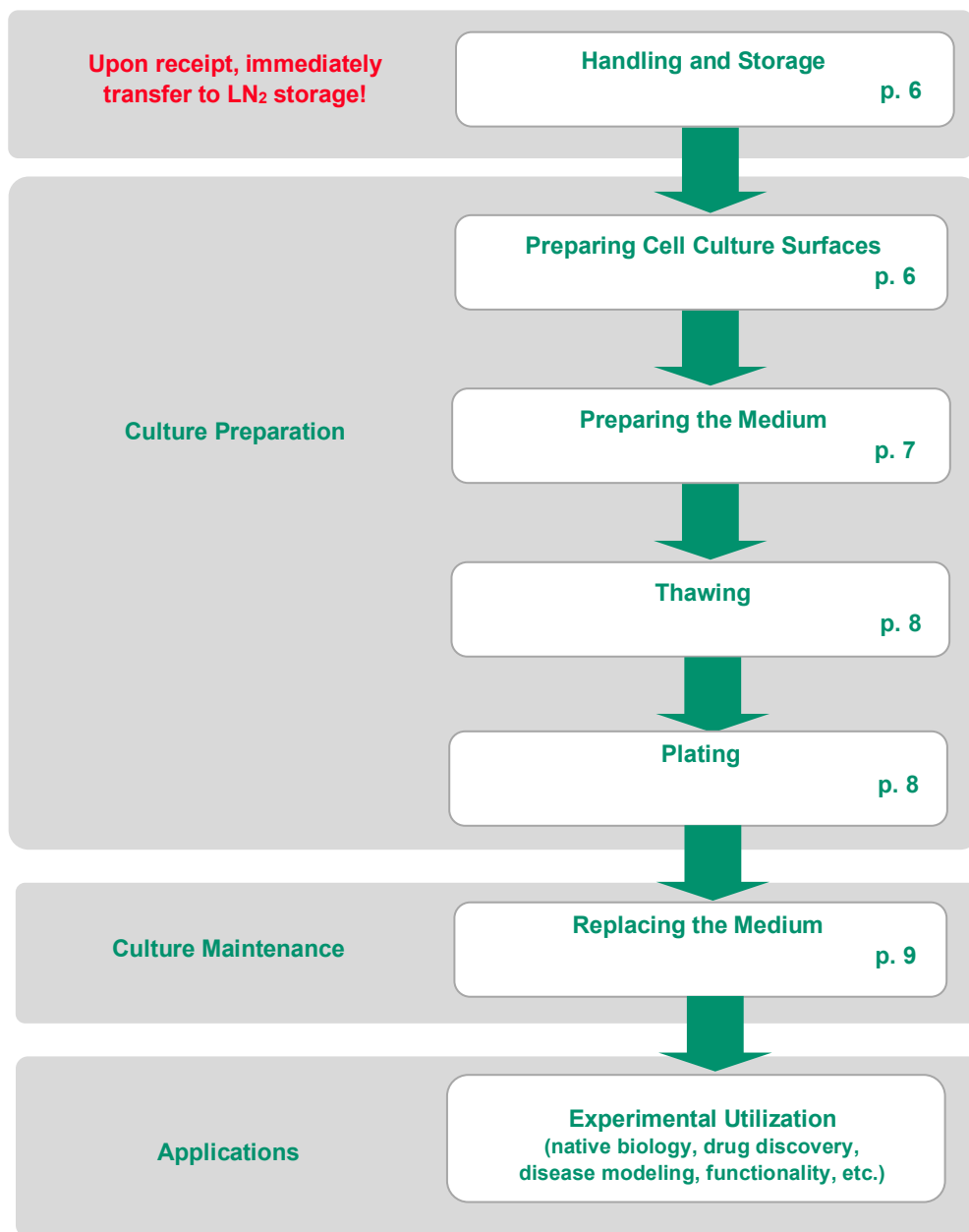
FCDI's Technical Support Scientists have the necessary laboratory and analytical experience to respond to your inquiries. Our web-based Knowledge Base provides solutions for iCell related questions about plating and media, cell culture, general assay methods, and more. In addition, in-lab training may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

**Telephone** (877) 320-6688 (US toll-free) / (608) 310-5100 x3  
Monday - Friday, 8:30 am - 5:00 pm US Central Time

**Email** [fcdi-support@fujifilm.com](mailto:fcdi-support@fujifilm.com)

**Knowledge Base** [www.fujifilmcdi.com/knowledge-base/](http://www.fujifilmcdi.com/knowledge-base/)

## Workflow Diagram



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## 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. FCDI 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 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 at -20°C until ready for use.

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## Preparing Cell Culture Surfaces

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

Regardless of the substrate of choice, prepare plating surfaces before thawing iCell Endothelial Cells.

### Preparing the Fibronectin Solution

- Stock solution:** Reconstitute fibronectin in sterile distilled water at 1 mg/ml according to the manufacturer's instructions.
- Working solution:** Dilute stock solution in sterile distilled water to a final concentration of 30 µg/ml.

### Preparing the Fibronectin Cell Culture Vessel

- Reconstitute fibronectin to 1 mg/ml according to the manufacturer's recommendations.
- Dilute 1 mg/ml fibronectin using sterile water to 30 µg/ml.
- Add 30 µg/ml fibronectin solution to coat to 3 µg/cm<sup>2</sup>, using the volumes specified below. Scale volumes appropriately for other vessel formats.

| Cell Culture Vessel       | Surface Area         | Fibronectin Solution Volume |
|---------------------------|----------------------|-----------------------------|
| T-75 Flask                | 75 cm <sup>2</sup>   | 7.5 ml                      |
| 6-well Cell Culture Plate | 9.6 cm <sup>2</sup>  | 1 ml                        |
| 96-well                   | 0.32 cm <sup>2</sup> | 32 µl                       |

- Incubate the vessel at least 1 hour at room temperature.
- Aspirate the fibronectin solution before adding cells.



6. Store fibronectin-coated vessels at 4°C for up to 1 week.



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

## Preparing the Medium

The Maintenance Medium is comprised of iCell Endothelial Cells Medium Supplement and components of the Vasculife VEGF Endothelial 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. Obtain the Vasculife VEGF Endothelial Medium Complete Kit and discard the FBS LifeFactor bottle.
2. Thaw iCell Endothelial Cells Medium Supplement and remaining vials from the Vasculife VEGF Endothelial 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 Vasculife Basal Medium bottle to make the Maintenance Medium:

| Component                                   | Volume/bottle |
|---|---------------|
| Vasculife Basal Medium <sup>1</sup>         | 475 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 <sup>2</sup>         | 10 ml         |
| iCell Endothelial Cells Medium Supplement   | 50 ml         |
| <i>Antimicrobial Supplement<sup>3</sup></i> | 0.5 ml        |

1 The bottle of Vasculife Basal Medium contains 475 ml of medium. After adding all medium components, the final volume will equal 538 ml. It is not necessary to remove basal medium from the bottle before adding the supplements.

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

3 This component is optional.

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

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## 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.

1. Equilibrate the Maintenance Medium at room temperature.
2. Remove the iCell Endothelial Cells cryovial from the liquid nitrogen storage tank.  
**Note:** *If necessary, transport cryovials on dry ice.*
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.
6. Rinse the empty iCell Endothelial Cells cryovial with 1 ml of Maintenance Medium to recover any residual cells from the vial. Transfer the 1 ml of rinse drop-wise to the 15 ml centrifuge tube containing the iCell Endothelial Cells suspension. Gently swirl the tube while adding the medium.



*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 Maintenance Medium to the 15 ml centrifuge tube while gently swirling.
8. Gently pipette the cell suspension once to mix.
9. Centrifuge the cell suspension at 200 x g for 5 minutes at room temperature.
10. Aspirate the supernatant.
11. Gently resuspend the cell pellet in 5 ml of Maintenance Medium.

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## Plating iCell Endothelial Cells

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

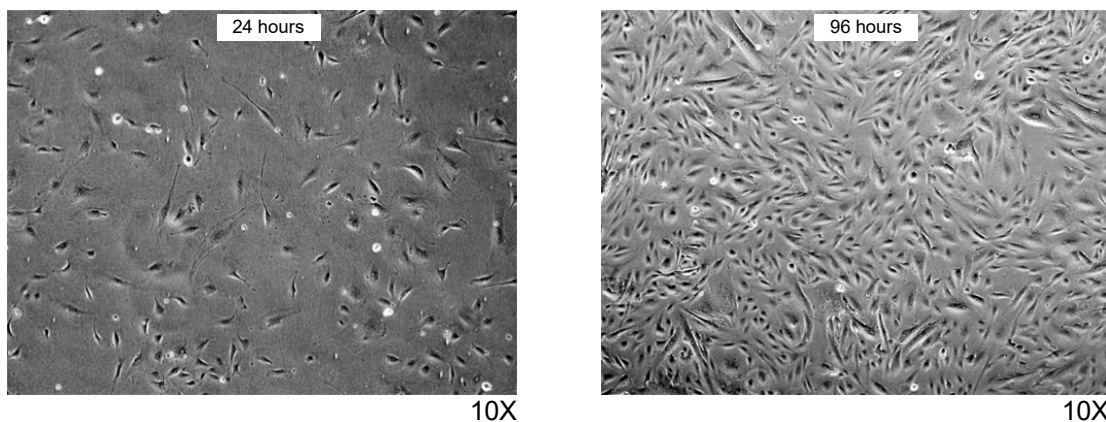
1. Remove a sample of cells to perform a cell count using a hemocytometer (using trypan blue exclusion to identify viable cells).
2. Dilute the cell suspension using Maintenance Medium to obtain a desired cell plating density.
3. Aspirate the fibronectin solution from the pre-coated cell culture vessel(s).
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% CO<sub>2</sub>, 5% O<sub>2</sub>.



*iCell Endothelial Cells are best cultured in a hypoxic incubator (37°C, 5% CO<sub>2</sub>, 5% O<sub>2</sub>), but can be cultured in a standard cell culture incubator (37°C, 5% CO<sub>2</sub>).*

## Expected Cell Density

iCell Endothelial Cells were added to a 6-well cell culture plate at 10,000 cells/cm<sup>2</sup> to achieve a confluent monolayer about 3-4 days later.



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

*At 24 hours, iCell Endothelial Cells, 01434 are somewhat sparse but healthy while at 96 hours they have grown to form a confluent monolayer and are ready to passage.*

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## 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. Equilibrate an aliquot of Maintenance Medium in a 37°C water bath. Do not equilibrate the Maintenance Medium in 37°C water bath multiple times.
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.

## Passaging the iCell Endothelial Cells

Passage iCell Endothelial Cells every 3-4 days. The following applies to one well of a 6-well cell culture plate. Scale volumes appropriately for other vessel formats.

1. Aspirate the spent medium from the confluent cell culture.
2. Add 2 ml of DPBS to the well to rinse the culture.
3. Aspirate the DPBS and add 1 ml of TrypLE or 0.25% Trypsin/EDTA.
4. Incubate at room temperature until cells begin to detach from the plate (approximately 5 minutes).

5. Gently tap the vessel to dislodge the cells from the plate. Pipette up and down once to ensure complete cell detachment.



*Do not remove cells from the plate by scraping, as this may cause a loss of viability, poor recovery, or loss of cell surface markers.*

6. Transfer the cell suspension to a 15 ml centrifuge tube.
7. Rinse the well with 2 ml of Maintenance Medium. Transfer to the 15 ml centrifuge tube.
8. Gently mix the cell suspension by pipetting.
9. Remove a sample of cells to perform a cell count using a hemocytometer and trypan blue exclusion dye.
10. Centrifuge the cell suspension at 200 x g for 5 minutes.
11. Aspirate the supernatant.
12. Dilute the cell pellet in the appropriate volume of Maintenance Medium to the desired cell density.
13. Aspirate the fibronectin solution from the pre-coated vessel.
14. Dispense cells into the vessel.
15. Incubate the cells in a cell culture incubator.

**Note:** *iCell Endothelial Cells can be maintained by routine splitting for up to 3 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.*

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