FUJIFILM
Cellular Dynamics

iCell® Intestinal Epithelial Cells
User's Guide

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Origin

iCell Intestinal Epithelial Cells are manufactured in the United States of America.

Copyright Notice

Revision History

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Table of Contents

Before You Begin

- Immediately transfer the frozen vials of iCell Intestinal Epithelial Cells to liquid nitrogen storage.
- Read this entire User's Guide before handling or using iCell® Intestinal Epithelial Cells.
- iCell Intestinal Epithelial Cells are FOR RESEARCH USE ONLY and NOT FOR THERAPEUTIC USE. See 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 Intestinal Epithelial Cells are frozen, is available online at 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 Intestinal Epithelial Cells.

Chapter 1. Introduction

iCell Intestinal Epithelial Cells from FUJIFILM Cellular Dynamics, Inc. (FCDI), are a highly purified, yet subtype diverse cells derived from human induced pluripotent stem (iPS) cells using proprietary differentiation protocols developed at FCDI. iCell Intestinal Epithelial Cells (IEC) exhibit hallmarks of small intestine function, including high CYP3A4 and Transepithelial Electrical Resistance (TEER) activity. Thus, these cells provide a reliable source of human intestinal epithelial cells suitable for use in targeted drug and nutritional science discovery, toxicity testing, and other life science research.

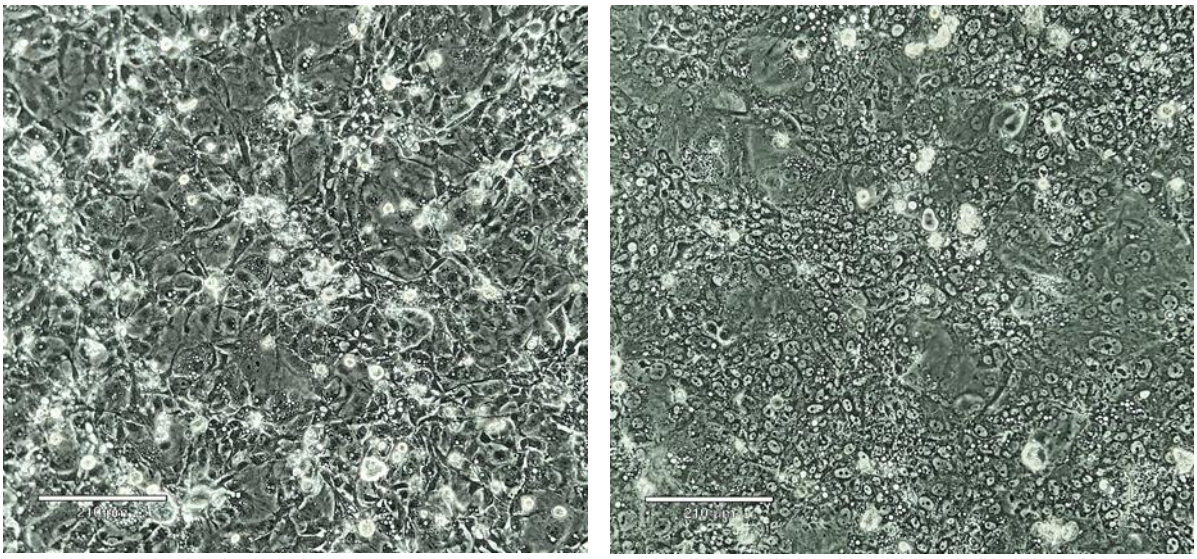


Figure 1: iCell Intestinal Epithelial Cells

Scale bar: 210µm

These example 10X images show iCell Intestinal Epithelial Cells at Day 1 (left) and Day 7 (right) post-thaw on Matrigel-coated 6-well plates.

Components Supplied by FUJIFILM Cellular Dynamics

Item	Catalog Number
iCell Intestinal Epithelial Cells Kit, 01279 • iCell Intestinal Epithelial Cells, 01279[1] • iCell Intestinal Epithelial Cell Medium • iCell Plating Supplement B • iCell Intestinal Epithelial Cells User's Guide • iCell Intestinal Epithelial Cells Quick Guide	R1260 (≥ 3.0 x 10^6 viable cells) or R1268 (≥ 1.0 x 10^6 viable cells) • C1267 (≥ 3.0 x 10^6 viable cells) or C1275 (≥ 1.0 x 10^6 viable cells) • M1058 (80 ml) (2 bottles with R1260 or 1 bottle with R1268) • M1049 (100 µl) • X1059 • X1060
iCell Intestinal Epithelial Cells Kit, 21527 • iCell Intestinal Epithelial Cells, 21527[1] • iCell Intestinal Epithelial Cell Medium • iCell Plating Supplement B • iCell Intestinal Epithelial Cells User's Guide • iCell Intestinal Epithelial Cells Quick Guide	R1261 (≥ 3.0 x 10^6 viable cells) or R1269 (≥ 1.0 x 10^6 viable cells) • C1268 (≥ 3.0 x 10^6 viable cells) or C1276 (≥ 1.0 x 10^6 viable cells) • M1058 (80 ml) (2 bottles with R1261 or 1 bottle with R1269) • M1049 (100 µl) • X1059 • X1060
Certificate of Analysis[2]	
Certificate of Origin If required for shipping purposes	

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

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

Required Equipment and Consumables

Item	Vendor(s)	Catalog Number(s)
Equipment		
37°C Water Bath	Multiple Vendors	
Biological Safety Cabinet with UV Lamp	Multiple Vendors	
Cell Culture Incubator	Multiple Vendors	
Liquid Nitrogen Storage Unit	Multiple Vendors	
Phase Contrast Microscope	Multiple Vendors	
Pipettors	Multiple Vendors	
Tabletop Centrifuge	Multiple Vendors	
Hemocytometer or Automated Cell Counter[1]	Multiple Vendors	
Consumables		
DMEM/F-12, HEPES	Thermo Fisher Scientific	11330
Matrigel[2]	Corning	354230
Flat-bottom Plate/Microplate, TC-treated	Multiple Vendors	
HTS Transwell 96-well Permeable Support with 0.4um, PET Membrane[3]	Corning	7369
Costar Transwell 24-well Plate, 0.4um, Polyester Membrane	Corning	3470
Conical Tubes, 50 ml, Falcon (Centrifuge Tubes)	Multiple Vendors	
Serological Pipettes, multiple sizes	Multiple Vendors	
Sterile Reservoirs, 25-100mL	Multiple Vendors	
Penicillin-Streptomycin (10000 U/mL), optional	Multiple Vendors	

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

2 Similar products are available from multiple vendors.

3 Includes: Transwell unit (1 integral tray with 96 wells), reservoir plate (Corning Cat. No. 253391000 for cell growth steps), receiver plate (96 wells for growth or assay steps) and 2 sterile lids; 5 sets per case.

Technical Support, Knowledge Base, and Training

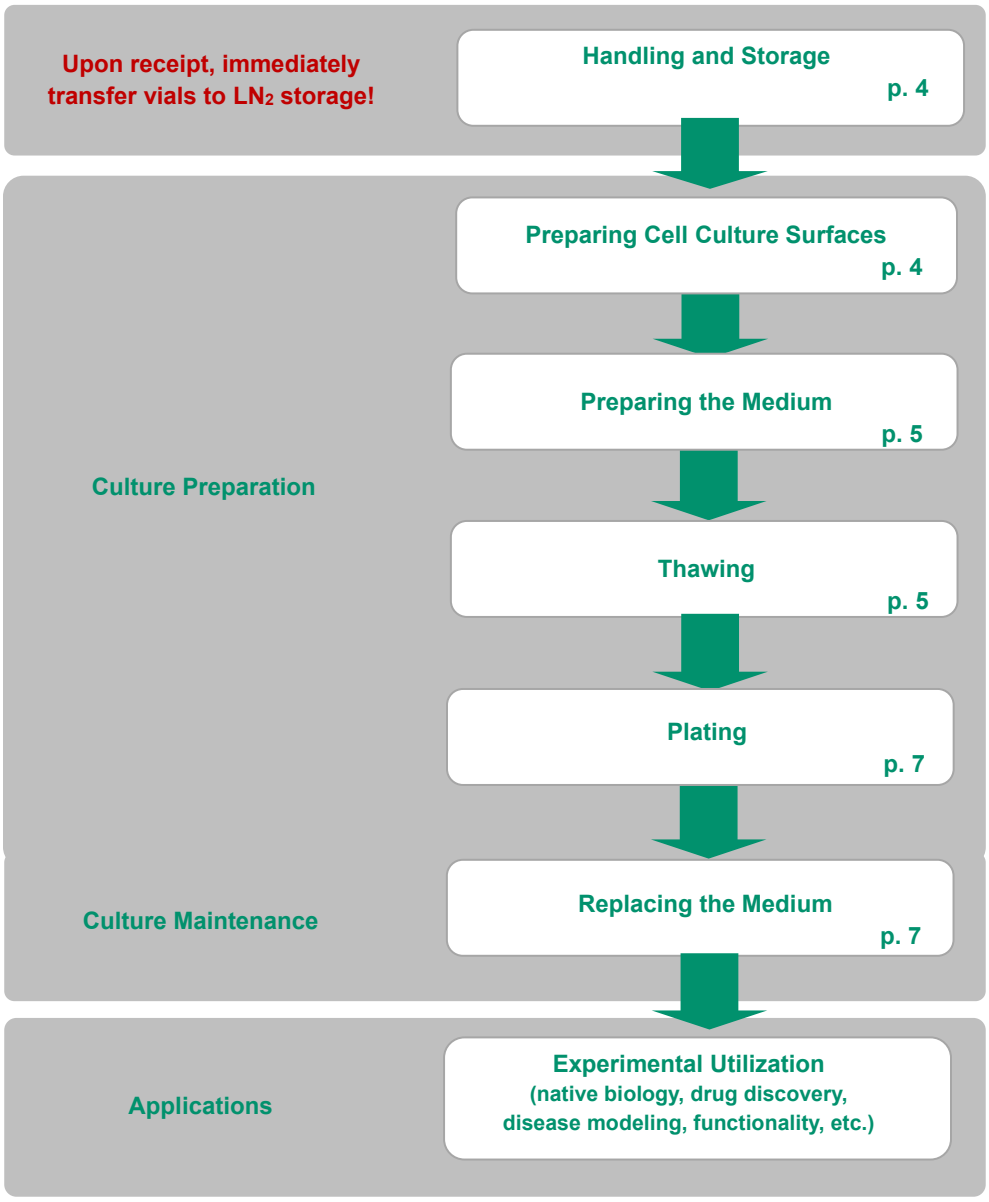
FCDI's Technical Support Scientists have the necessary laboratory and analytical experience to respond to your inquiries. Our web-based Knowledge Base provides solutions for iCell related questions about plating and media, cell culture, general assay methods, and more. In addition, in-lab training may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

Knowledge Base www.fujifilmcdi.com/knowledge-base/

Workflow Diagram



Chapter 2. Handling and Storage

Handling iCell Intestinal Epithelial Cells

iCell Intestinal Epithelial Cells are provided as cryopreserved single-cell suspensions in 1.5 ml cryovials. Upon receipt, directly transfer the cryobox containing iCell Intestinal Epithelial 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 Intestinal Epithelial Cells at a stable temperature. Minimize exposure of cryopreserved iCell Intestinal Epithelial Cells to ambient temperature when transferring vials to liquid nitrogen

Handling iCell Intestinal Epithelial Cell Medium and Supplement

iCell Intestinal Epithelial Cells are shipped with two (2) additional components: iCell Intestinal Epithelial Cell Medium and iCell Plating Supplement B (1000X). These medium components are shipped frozen on dry ice. Upon receipt, store each component at -20°C until use.

Chapter 3. Preparing Cell Culture Surfaces

Matrigel is the preferred substrate for the cell culture surface to support attachment, viability, and long-term activity of iCell Intestinal Epithelial Cells. The Matrigel coating procedure is described below.

1. Thaw an unopened ampule of Matrigel overnight at 4°C and split into aliquots. The unused aliquots can be stored at -20°C. If using previously aliquoted Matrigel, it may be thawed overnight at 4°C or on ice for at least 1h, depending on aliquot size. Do not allow Matrigel stock to come to room temperature.
2. Dilute thawed Matrigel stock 30-fold by volume in DMEM/F12 to obtain a 1:30 dilution.
3. Add the volume of diluted Matrigel specified in the table below to each well of the vessel(s). Scale volumes appropriately for other vessel formats.

Culture Vessel	Surface Area (cm^2)	Volume of Diluted Matrigel (ml)
6-well Cell Culture Plate	9.6	1
12-well Cell Culture Plate	3.5	0.5
24-well Cell Culture Plate	1.9	0.25
96-well Cell Culture Plate	0.32	0.1
24-well Transwell Insert	0.33	0.1 (apical)
96-well Transwell Insert	0.143	0.05 (apical)

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

4. Incubate the plate(s) at room temperature for at least 1 hour (preferred method) or in a 37°C cell culture incubator for at least 30 minutes.

   ***Note:** Coated plate(s) plates can be stored at 4°C, wrapped in parafilm, for up to 1 week. Prior to use, place coated plate(s) at room temperature for at least 1 hour or in a 37°C cell culture incubator for at least 30 minutes.*

5. Aspirate the Matrigel solution from the plate immediately before the addition of the cell suspension.

Do not allow the coated surface to dry prior to the addition of cells.

Chapter 4. Preparing the Medium

1. Thaw iCell Intestinal Epithelial Cell Medium at 4°C overnight.
2. Thaw iCell Plating Supplement B at room temperature for 30 minutes on the day of thawing cells.
3. For thawing, add iCell Plating Supplement B to iCell Intestinal Epithelial Cell Medium at 1:1000 ratio on the day of thawing cells. Determine specific volume of Intestinal Epithelial Cell Thaw Medium to prepare based on desired culture vessel type. Refer to the table below and Chapters 5 - 6.

   Note: *Add 1% Penicillin/Streptomycin if desired*

Component	Volume (ml)	Final Concentration
iCell Intestinal Epithelial Cell Medium (± Pen-Strep)	45	100%
iCell Plating Supplement B	0.045	1X

Table 2: Intestinal Epithelial Cell Thaw Medium

All volumes and measures accommodate thawing and plating of 96-well transwell vessel.

iCell Intestinal Epithelial Cell Medium can be stored at 4°C for up to 2 weeks.

4. For maintenance, provide cultures with a full feed of iCell Intestinal Epithelial Cell Medium.

   Note: *Add 1% Penicillin/Streptomycin if desired*

Chapter 5. Thawing iCell Intestinal Epithelial Cells

Store iCell Intestinal Epithelial Cells in liquid nitrogen (vapor phase) until immediately before thawing to ensure maximal recovery and performance of the cells. Complete the following steps of the thawing procedure in a time-efficient manner to facilitate optimal iCell Intestinal Epithelial Cells viability and performance.

Note: *Thaw no more than 2 vials of iCell Intestinal Epithelial Cells at one time.*

1. Equilibrate the appropriate amount of Intestinal Epithelial Cell Thaw Medium and Matrigel-coated vessels to room temperature before thawing iCell Intestinal Epithelial Cells.
2. Remove the iCell Intestinal Epithelial Cells cryovial from the liquid nitrogen storage tank. Keep vials on dry ice until ready to thaw.

Cryovials can be kept on dry ice for up to 60 minutes before thawing.

3. Immerse the cryovial in a 37°C water bath for 2 minutes (avoid submerging the cap), holding the tube stationary and without swirling. Use of a floating microcentrifuge tube rack is recommended.

Precise timing is critical to maximize 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 Intestinal Epithelial Cells cryovial contents drop-wise to the sterile 50 ml conical tube.

Avoid repeated pipetting of the thawed iCell Intestinal Epithelial Cells cell suspension.

6. Rinse the empty iCell Intestinal Epithelial Cells cryovial with 1 ml of the Intestinal Epithelial Cell Thaw Medium to recover any residual cells from the vial. Add this rinse to the 50 ml conical tube containing the cell suspension.
7. Slowly add 8 ml of Intestinal Epithelial Cell Thaw Medium dropwise to the 50 ml conical tube containing the cell suspension.

   Note: *Drop-wise addition of the medium to the cell suspension is critical to minimize osmotic shock and ensure maximum viability and recovery.*
8. Gently swirl or invert the tube to mix the cell suspension.
9. Centrifuge the cell suspension at 400 x g for 5 minutes at room temperature.
10. Carefully aspirate the supernatant, gently resuspend the cell pellet in Intestinal Epithelial Cell Thaw Medium by pipetting up and down 3 - 4 times slowly.

For 3M vial size: resuspend in 3 mL medium (per thawed vial)

For 1M vial size: resuspend in 1 mL medium (per thawed vial)

   Note: *Once thawed and suspended in Intestinal Epithelial Cell Thaw Medium as described in Step 10, the contents of the conical tubes can be pooled before proceeding to count the cells.*
11. Optional: Obtain a cell count using an automated cell counter or a hemocytometer.

    Note: *Seeding density of iCell Intestinal Epithelial Cells should be based on the Certificate of Testing (CoT) or Certificate of Analysis (CoA).*

Chapter 6. Plating iCell Intestinal Epithelial Cells

The recommended seeding density for iCell Intestinal Epithelial Cells depends on the desired application. For most applications, a seeding density of 300,000 cells/cm^2 is recommended. For assays involving transepithelial electrical resistance (TEER) output, a seeding density of 200,000 cells/cm^2 is recommended.

1. Obtain the number of viable cells/vial and viability from the Certificate of Testing or Certificate of Analysis.
2. Calculate the final volume of Intestinal Epithelial Cell Thaw Medium needed to obtain the desired cell suspension density using the number of viable cells/vial from the Certificate of Testing or Certificate of Analysis. See **Table 3** below for examples.
3. Dilute the cell suspension with room temperature Intestinal Epithelial Cell Thaw Medium to obtain a desired cell plating density.
4. Gently swirl the cell suspension 2 – 3 times and immediately dispense the cell suspension into the cell culture vessel.

   Note: *For seeding transwell inserts, plate cells in the apical (top) compartment.*
5. Culture iCell Intestinal Epithelial Cells in a cell culture incubator at 37°C, 5% CO_2.

Culture Vessel	Surface Area (cm^2)	Plating Volume (ml)	Cell Number for 300k/cm^2 Density	Cell Number for 200k/cm^2 Density
24-well Cell Culture Plate	1.9	0.5	5.7 x 10^5	N/A
48-well Cell Culture Plate	0.95	0.3	2.85 x 10^5	N/A
96-well Cell Culture Plate	0.32	0.2	9.6 x 10^4	N/A
24-well Transwell Insert	0.33	0.15 (apical) 0.6 (basolateral)	N/A	6.6 x 10^4
96-well Transwell Insert	0.143	0.1 (apical) 0.235 (basolateral)	N/A	2.86 x 10^4

Table 3: Summary of Recommended Volumes and Measures

*All volumes and measures are **per well**.*

Chapter 7. Maintaining iCell Intestinal Epithelial Cells

iCell Intestinal Epithelial Cells require a short maturation period post-thaw (~ 7 days) for optimal functionality.

1. Immediately before feeding iCell Intestinal Epithelial Cells, equilibrate an appropriate amount of iCell Intestinal Epithelial Cell Medium to room temperature.

Do not equilibrate the Maintenance Medium in 37°C water bath multiple times. Aliquot the medium into small working volumes during cell maintenance.

2. Approximately 24 hours post-plating, remove the plates from the incubator, gently aspirate spent medium, and perform a full medium exchange with iCell Intestinal Epithelial Cell Medium. Recommended volumes are given in the table below:

Culture Vessel	Volume (ml/well)
24-well Cell Culture Plate	0.5
48-well Cell Culture Plate	0.3
96-well Cell Culture Plate	0.2
24-well Transwell Insert	0.15 (apical) 0.6 (basolateral)
96-well Transwell Insert	0.075 (apical) 0.235 (basolateral)

Table 4: Summary of Recommended Volumes and Measures

*All volumes and measures are **per well**.*

3. Replace iCell Intestinal Epithelial Cell Medium every other day.
4. Culture iCell Intestinal Epithelial Cells in a cell culture incubator at 37°C, 5% CO_2.

It is recommended to change the medium a few wells at a time to minimize the time without medium for the cells.

Chapter 8. Representative TEER Data

Transepithelial electrical resistance (TEER) of iCell Intestinal Epithelial Cells were measured using an EVOM2 Epithelial Ohm Meter with Endohm-6G attachment or STX HTS EVOM Electrode for Corning 96 attachment (World Precision Instruments). Measurements were taken on days 4 through 9 post-thaw.

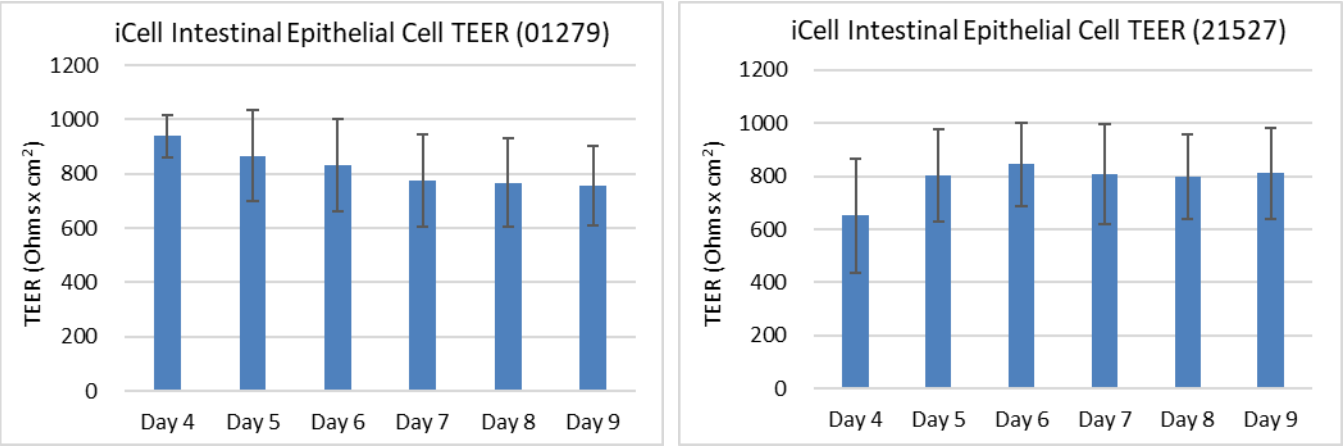


Figure 2: Representative post-thaw TEER data of iCell Intestinal Epithelial Cells utilizing a Costar Transwell 24-well plate (0.4µm, Polyester Membrane)

Average TEER normalized by transwell membrane surface area of three iCell Intestinal Epithelial Cell lots per donor on days 4 through 9 post-thaw.