FUJIFILM
Cellular Dynamics

iCell® BMEC
User's Guide

Document ID: X1046

Restrictions and Liabilities

This document is provided “as is.” FUJIFILM Cellular Dynamics, Inc. (FCDI), assumes no responsibility for any typographical, technical, or other inaccuracies in this document. FCDI reserves the right to periodically change information that is contained in this document; however, FCDI 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 EXPRESS LIMITED WARRANTY SET FORTH IN WWW.FUJIFILMCDI.COM/TERMS-AND-CONDITIONS/, FCDI MAKES NO, AND DISCLAIMS ALL, REPRESENTATIONS, WARRANTIES, CONDITIONS OR COVENANTS, EXPRESS OR IMPLIED, WITH RESPECT TO ANY PRODUCT REFERENCED HEREIN OR PERFORMANCE OF ANY SERVICES REFERENCED HEREIN, INCLUDING BUT NOT LIMITED TO, 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 websites (collectively the “Third-Party Information”). FCDI 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 FCDI of the Third-Party Information or the third party in any way.

FCDI does not in any way guarantee or represent that you will obtain satisfactory results from using iCell BMEC as described herein. The only warranties provided to you are included in the Limited Warranty set forth in www.fujifilmcdi.com/terms-and-conditions/. You assume all risk in connection with your use of iCell BMEC.

Conditions of Use

iCell BMEC 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.

Trademarks

iCell and MyCell are registered trademarks, and Cellular Dynamics and the Cellular Dynamics logo are trademarks of FUJIFILM Cellular Dynamics, Inc. and may not be used without the express written permission of FUJIFILM Cellular Dynamics, Inc.

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

Origin

iCell BMEC are manufactured in the United States of America.

Copyright Notice

Revision History

Document ID: X1046
Version 2.0: May 2025

Table of Contents

Before You Begin

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

Introduction

The Brain Microvascular Endothelial Cells are a key member of the blood-brain barrier (BBB) that helps maintain a tightly controlled microenvironment around the brain. For many years, the drug discovery market has needed a robust high throughput in vitro Brain Microvascular Endothelial Cell model to evaluate passive and active transport function by measuring drug permeability, drug uptake and barrier function, as well as to study the diseases that affect it.

The inherent power of iPSC technology provides access to the specialized cell types required to generate Brain Microvascular Endothelial Cells, but the field has been plagued with difficulties in reliably manufacturing a consistent supply of these cells at-scale. FUJIFILM Cellular Dynamics Inc., the market leader in iPSC technology and innovation, has developed a new human iPSC-derived iCell Brain Microvascular Endothelial Cells kit. The function of this kit can be assessed in a cell culture insert format. The iCell Brain Microvascular Endothelial Cells Kit includes iCell Brain Microvascular Endothelial Cells (iCell BMEC) and media that enables their long-term survival and superior functional performance assessed by transendothelial electrical resistance (TEER) assays.

The iCell Brain Microvascular Endothelial Cells Kit components have the potential to integrate with emerging organ-on-a-chip technologies and other 3D cell culture systems, thus offering an exciting new capability for the drug discovery community to advance the understanding of BBB or BMEC function with respect to human health and disease.

Components Supplied by FUJIFILM Cellular Dynamics

Item	Catalog Number
iCell BMEC Kit, 01279 • iCell BMEC (Brain Microvascular Endothelial Cells), 01279[1] • iCell BMEC Maintenance Medium[1] • iCell Plating Supplement A[1] • iCell BMEC User's Guide	R1236 • C1239 (≥ 3.0 x 10^6 viable cells) • M1042 (100ml) • M1057 (200 µl) • X1046
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	
Pipettors	Multiple Vendors	
Tabletop Centrifuge	Multiple Vendors	
Consumables		
24-well Plate with BioCoat® Control Cell Culture Inserts, PET Membrane	Corning	354572
Collagen IV	Sigma	C5533
Conical Tubes, 15 ml and 50 ml, Falcon (Centrifuge Tubes)	Multiple Vendors	
Dulbecco's Phosphate Buffered Saline without Ca^{2+} and Mg^{2+} (DPBS), 500mL	Gibco	14190144
Fibronectin, 5mg	Gibco or Sigma	Corning 356008, or Sigma F2006
Microcentrifuge Tubes, 0.5 ml	Multiple Vendors	
Penicillin-Streptomycin	Gibco	15140

Technical Support, and Knowledge Base

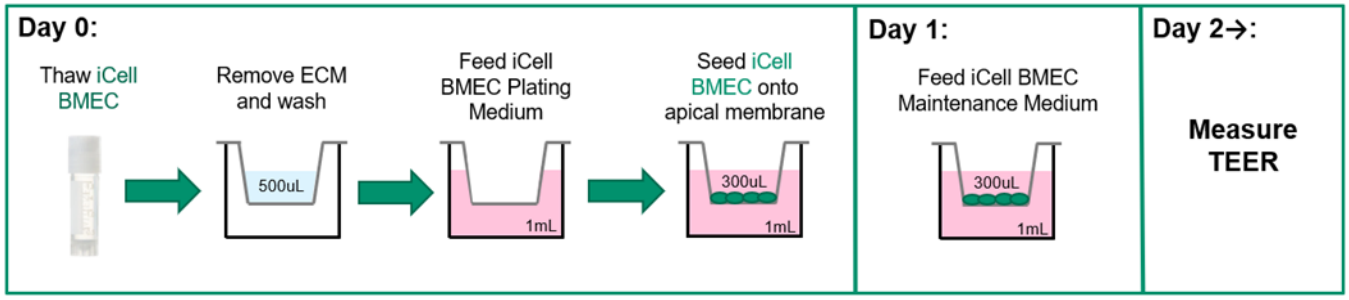
FCDI's Technical Support Scientists have the necessary laboratory and analytical experience to respond to your inquiries. Our web-based Knowledge Base provides solutions for iCell-related questions about plating and media, cell culture, general assay methods, and more. In addition, in-lab training may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

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

Workflow Diagram



Handling and Storage

Handling iCell BMEC - Cells

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

Handling iCell BMEC - Medium and Supplement

iCell BMEC Maintenance Medium and iCell Plating Supplement A are shipped frozen on dry ice. Upon receipt, store the bottles at -20°C until ready for use.

Preparing Cell Culture Surfaces

Preparing the Fibronectin-Collagen IV Coating of Apical Membrane

Cell culture inserts for BMEC mono-culture are coated on the apical side with a Fibronectin-Collagen IV matrix. Coated cell culture inserts should be used within 1 week of coating.

Note: *Scan the QR to view iCell BBB Tutorial* ***Video 1*** – Fibronectin-Collagen IV Coating of Cell Culture Inserts

1. Reconstitute Fibronectin and Collagen IV in DPBS to 1 mg/ml concentration individually.
2. Calculate the volume of Fibronectin, Collagen IV, and DPBS needed to coat the desired number of cell culture inserts using the information in Table 1.

Culture Vessel	Fibronectin Volume (µl)	Collagen IV Volume (µl)	DPBS Volume (µl)	Total Volume (µl)
1 insert of 24-well Cell Culture Insert	10	40	50	100
12 inserts of 24-well Cell Culture Insert	120	480	600	1200

Table 1: Summary of Recommended Volumes
This table assumes an ECM concentration of 1mg/ml.

3. Add the calculated volumes of Fibronectin, Collagen IV, and DPBS together in a sterile tube and mix gently.
4. Add 100 µl of the Fibronectin-Collagen IV solution to the apical side of each 24-well plate cell culture insert, being careful not to puncture the insert membrane. Ensure that there is an even coating of the solution.

5. Store coated plates with the Fibronectin-Collagen IV solution for a minimum of 12-24 hours at 4°C before use. Allow to warm to room temperature prior to seeding cells.

   Note: *The inserts may also be wrapped in parafilm, covered in aluminum foil, and stored at 4°C for up to 1 week. Allow to warm to room temperature prior to seeding cells.*

Day 0

Preparing iCell BMEC Plating and Maintenance Medium

1. Warm iCell BMEC Maintenance Medium to room temperature prior to use.
2. Add 2 µl iCell Plating Supplement A per 1 ml iCell BMEC Maintenance Medium and label as iCell BMEC Plating Medium.

   Note: *Only make enough of iCell BMEC Plating Medium for Day 0 tasks. Do not store beyond 48 hours.*
3. Add Penicillin-Streptomycin (1% final concentration) to the iCell BMEC Plating and Maintenance Medium prior to use.
4. Store the remaining iCell BMEC Maintenance Medium at 4°C for up to 2 weeks.

Thawing iCell BMEC

1. Warm iCell BMEC Plating Medium and Fibronectin-Collagen IV coated cell culture inserts to room temperature prior to use.
2. Thaw iCell BMEC cryovial in a 37°C water bath for 2 minutes and 30 seconds. Clean with 70% ethanol.
3. Gently transfer the iCell BMEC cryovial contents to a sterile 15 ml conical centrifuge tube using a 1 ml pipettor.
4. Rinse the empty iCell BMEC cryovial with 1 ml of iCell BMEC Plating Medium and slowly add the plating medium rinse to the 15 ml conical centrifuge tube containing the iCell BMEC suspension. Mix by gently pipetting.
5. Obtain the number of viable cells/vial and the viability from the Certificate of Analysis. Adjust the final cell density by adding additional iCell BMEC Plating Medium as needed. See Table 3 below for plating density recommendations.

Do not centrifuge the cells.

Cell Type	Viable Cell Number	Plating Volume per Cell Insert (µl)	Cell Density (viable cells/ml)
iCell BMEC	214,500	300	0.715×10^6

Table 3: Summary of Recommended Volumes and Measures
All volumes and measures are per 24-well plate cell culture insert (0.33cm^2 surface area)

6. Gently aspirate the Fibronectin-Collagen IV solution from the apical compartment of the coated cell culture inserts made on Day -1.
7. Add 0.5 ml of DPBS to the apical compartment of the coated cell culture inserts to be seeded.
8. Gently aspirate the DPBS from the apical compartment of the coated cell culture inserts to be seeded.

   Note: *Refer to Video 5 – Medium Removal and iCell BMEC Seeding*

   Note: *Scan the QR to view iCell BBB Tutorial* **Video 5** – Medium Removal and iCell BMEC Seeding

9. Add 1 ml of iCell BMEC Plating Medium to the basolateral compartment of each seeded cell culture insert.
10. Add 300 µl of iCell BMEC cell suspension to the apical compartment of each cell culture insert.
11. Place the plate to a 37°C, 5% CO_2 incubator and incubate overnight.

Day 1

Changing from Plating to Maintenance Medium

1. Warm iCell BMEC Maintenance Medium to room temperature prior to use.
2. Gently aspirate the iCell BMEC Plating Medium from the basolateral and then the apical compartments of the cell culture inserts.
3. Add iCell BMEC Maintenance Medium to the apical (300 µl) and then the basolateral (1 ml) compartments of each cell culture insert.
4. Return the plate to a 37°C, 5% CO_2 incubator.

Co-cultures should be given a complete feed of iCell BMEC Maintenance Medium to the apical (300 µl) and basolateral (1 ml) compartments of each cell culture insert every other day until assay.

Representative TEER Data and Images

Transendothelial electrical resistance (TEER) of iCell BMEC mono-cultures were measured using an EVOM2 Epithelial Ohm Meter with Endohm-6G chamber attachment (World Precision Instruments). Measurements were taken on days 2 through 6 of assay set-up.

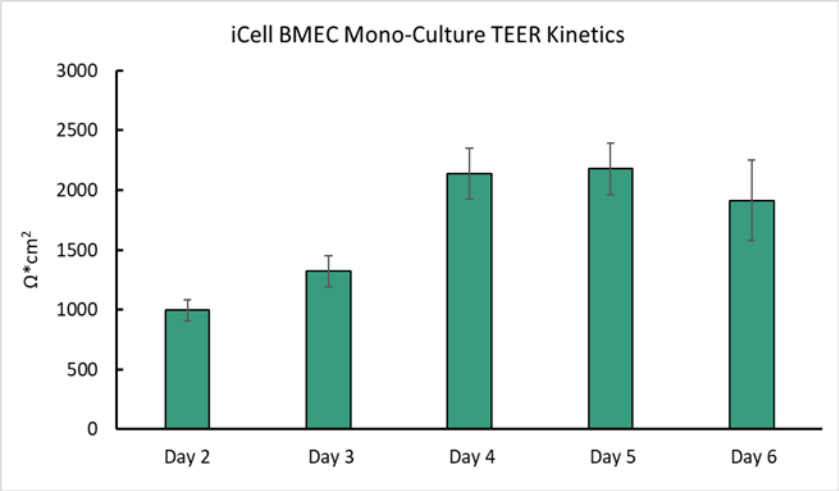


Figure 3

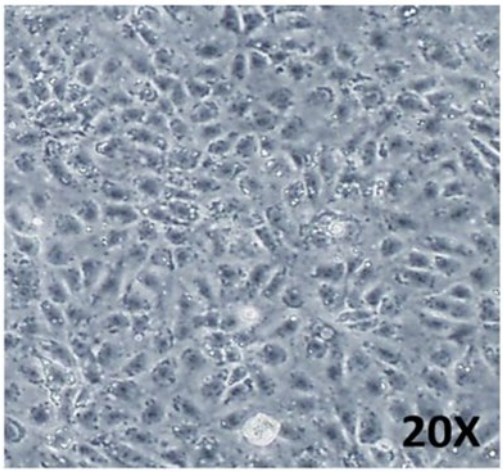


Figure 4: Bright field image of iCell BMEC using 20x objective on Day 5 post-thaw.

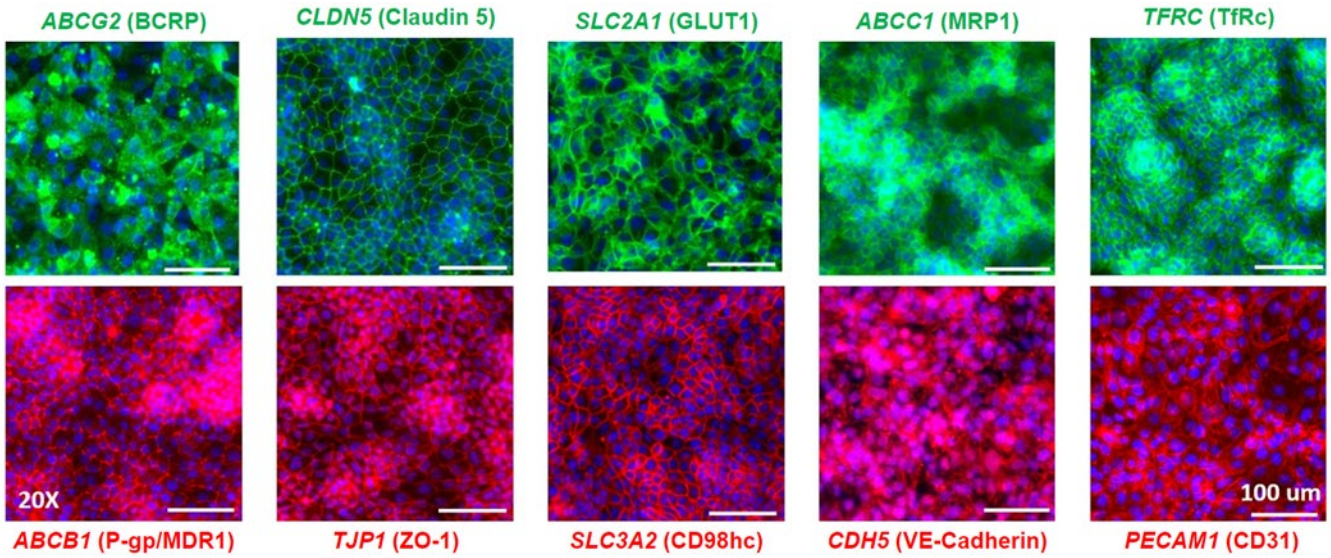


Figure 5: Immunocytochemistry images of iCell BMEC using 20x objective on Day 5 post-thaw.