FUJIFILM
Cellular Dynamics

iCell® Blood-Brain Barrier Isogenic Kit
User's Guide

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Origin

iCell Blood-Brain Barrier Isogenic Kit components are manufactured in the United States of America.

Copyright Notice

Revision History

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Version 1.0: June 2023

Table of Contents

Before You Begin

- Immediately transfer the frozen vials to liquid nitrogen storage.
- Read this entire User's Guide before handling or using the iCell® Blood-Brain Barrier Isogenic Kit.
- iCell Blood-Brain Barrier Isogenic Kit components 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 Astrocytes, iCell BMEC and iCell Pericytes 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 the iCell Blood-Brain Barrier Isogenic Kit.

Introduction

The blood-brain barrier (BBB) functions to maintain a tightly controlled microenvironment around the brain. For many years, the drug discovery market has needed a robust in vitro BBB model system to evaluate drug permeability 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 assemble such a model system, 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 BBB isogenic kit to model the BBB system in a cell culture insert format. The iCell BBB Isogenic Kit includes iCell Astrocytes, iCell Brain Microvascular Endothelial Cells (iCell BMEC) and iCell Pericytes, (all derived from the same proprietary donor background), and media that enables their long-term survival and superior functional performance assessed by transendothelial electrical resistance (TEER) assays.

The iCell BBB Isogenic 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 function with respect to human health and disease.

Components Supplied by FUJIFILM Cellular Dynamics

Item	Catalog Number
iCell Blood-Brain Barrier Isogenic Kit[1]	**R1241**
• iCell Astrocytes 2.0, 01279[1]	• C1249
• iCell BMEC (Brain Microvascular Endothelial Cells), 01279[1]	• C1239
• iCell Pericytes, 01279[1]	• C1241
• iCell Astrocyte & Pericyte Medium[1]	• M1041 (30 ml)
• iCell BMEC Maintenance Medium[1]	• M1042 (100 ml)
• iCell Plating Supplement 500X[1]	• M1043 (200 µl)
• iCell Blood-Brain Barrier Isogenic Kit User's Guide	• X1045
Certificate of Analysis[2]	
Certificate of Origin – If required for shipping purposes	

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

2 Available online for each cell type: 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	
Hemocytometer or Automated Cell Counter	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
0.1% Gelatin in Water	STEMCELL Technologies	07903
Microcentrifuge Tubes, 0.5 ml	Multiple Vendors	
Penicillin-Streptomycin	Gibco	15140

Recommended vendors are critical to a successful BBB cell culture insert model setup.

Technical Support, and Knowledge Base

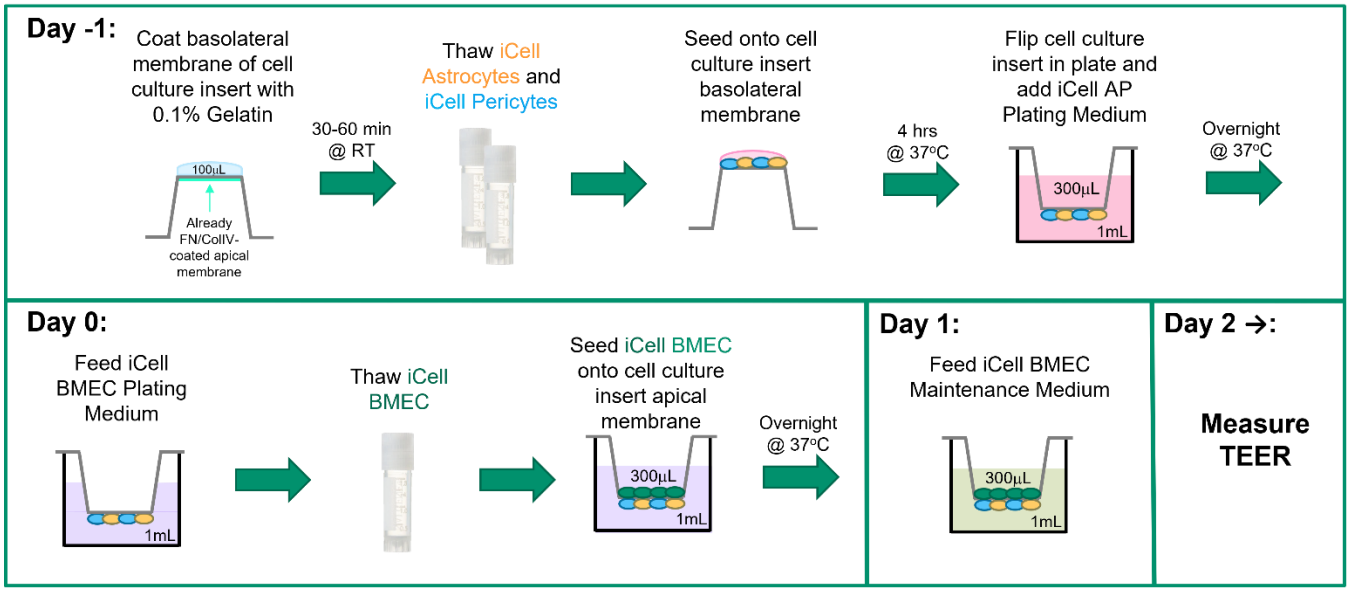
FCDI's Technical Support Scientists have the necessary laboratory and analytical experience to respond to your inquiries. Our web-based Knowledge Base provides solutions for iCell-related questions about plating and media, cell culture, general assay methods, and more. In addition, in-lab training may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

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

Handling iCell Blood-Brain Barrier Isogenic Kit - Medium Supplements

iCell Astrocyte & Pericyte Medium, iCell BMEC Maintenance Medium and iCell Plating Supplement 500X 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 BBB co-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 -1

Preparing iCell Astrocyte & Pericyte Plating Medium

1. Equilibrate iCell Astrocyte & Pericyte Medium and iCell Plating Supplement to room temperature.
2. Add 60 µl of iCell Plating Supplement and 30 ml of iCell Astrocyte & Pericyte Medium into a 50 ml conical centrifuge tube. Label as iCell Astrocyte & Pericyte (AP) Plating Medium.
3. Add Penicillin-Streptomycin (0.5% final concentration) to the iCell AP Plating Medium prior to use.

   Note: *Only make enough of iCell AP Plating Medium for Day -1 tasks. Do not store beyond 48 hours.*

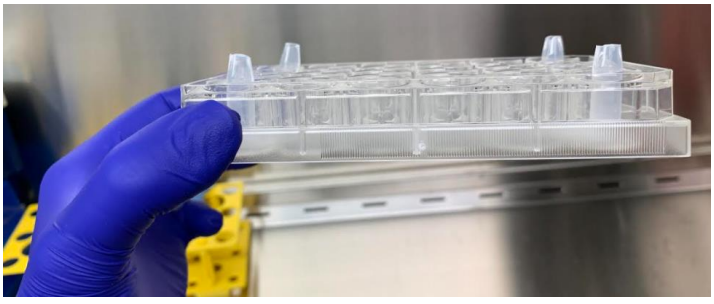
Gelatin Coating of Basolateral Membrane

Immediately prior to thawing iCell Astrocytes and iCell Pericytes, the Fibronectin-Collagen IV-coated cell culture inserts should be warmed to room temperature and coated with 0.1% gelatin solution on the basolateral side.

Note: *Scan the QR to view iCell BBB Tutorial* ***Video 2*** – Gelatin Coating and Plate Prep

1. Inside a Biological Safety Cabinet, format the 24-well plate with the desired number and layout of cell culture inserts.

   Note: *Do not remove the Fibronectin-Collagen IV solution from the apical side of the cell culture inserts.*
2. Gently flip the 24-well plate over, turning the cell culture inserts upside-down.
3. Carefully remove the base of the plate and set aside, leaving the cell culture inserts upside down on the plate lid in the Biological Safety Cabinet.
4. Make four plate spacers by cutting off the bottom tip of 0.5 ml microcentrifuge tubes using a pair of scissors.
5. Place one spacer tube into each of the four corner wells of the 24-well plate base, with the cap of the spacer tube in the bottom of the well. Refer to Figure 1 below.



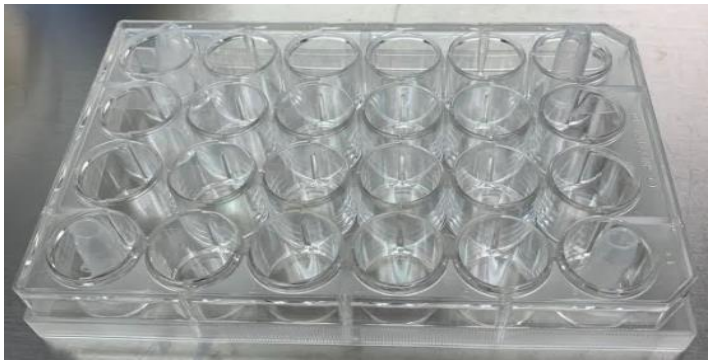


Figure 1: Orientation of Spacer Tubes

6. Add 100 µl of 0.1% gelatin solution to the basolateral 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.
7. Carefully set the 24-well plate base with the spacers, over the coated cell culture inserts, without allowing the inserts to touch the plate base.
8. Gently slide the plate with the gelatin-coated inserts to the back of the Biological Safety Cabinet.
9. Incubate the gelatin solution on the cell culture inserts for a minimum of 30 minutes and proceed to the thawing of the iCell Astrocytes and iCell Pericytes.

Thawing of iCell Astrocytes and iCell Pericytes

Note: *Thaw iCell Astrocytes and iCell Pericytes separately*

1. Warm iCell AP Plating Medium to room temperature prior to thawing cells.
2. Transfer 3 ml of iCell AP Plating Medium to a 15 ml conical centrifuge tube.
3. Immerse the iCell Astrocytes cryovial in a 37°C water bath for 2 minutes (avoid submerging the cap). The use of a floating microcentrifuge tube rack is recommended.
4. Immediately remove the cryovial from the water bath, spray with 70% ethanol, and place into the biological safety cabinet.
5. Gently transfer the iCell Astrocytes cryovial contents to a sterile 15 ml conical centrifuge tube containing 3 ml of iCell AP Plating Medium using a 1 ml pipettor.
6. Rinse the empty iCell Astrocytes cryovial with 1 ml of iCell AP Plating Medium and transfer the plating medium rinse to the 15 ml conical centrifuge tube containing the iCell Astrocytes suspension.
7. Centrifuge the cell suspension at 600 x g for 6 minutes.

> *Centrifugation speed and time are critical for maximum cell recovery.*

8. Aspirate supernatant without disturbing the cell pellet.
9. Resuspend the cell pellet in 500 µl of iCell AP Plating Medium.
10. Repeat steps 2-9 to thaw the iCell Pericytes
11. For iCell Astrocytes and iCell Pericytes, obtain the number of viable cells/vial and the viability from the Certificate of Analysis. Adjust the final cell densities by adding additional iCell AP Plating Medium as needed. See Table 2 below for plating density recommendations.

Cell Type	Cell Number	Plating Volume per Cell Insert (µl)	Cell Density (viable cells/ml)
iCell Astrocytes	$5.5x10^4$	50	$1.1x10^6$
iCell Pericytes	$11x10^4$	50	$2.2x10^6$

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

12. Add 50 µl of the resuspended iCell Astrocytes and 50 µl of the resuspended iCell Pericytes for every cell culture insert to be seeded into a single 15 ml conical centrifuge tube. Gently mix by pipetting the suspension.
13. Gently remove the 24-well plate base with spacers from the gelatin-coated cell culture inserts.

Note: *Scan the QR to view iCell BBB Tutorial* ***Video 3 –*** Gelatin Removal and iCell Astrocytes and iCell Pericytes Seeding

14. Carefully aspirate the gelatin solution from the basolateral side of the cell culture inserts with a P200 pipette, being careful not to puncture the insert membrane.
15. Add 100 µl of the Astrocyte-Pericyte cell suspension to the basolateral side of each cell culture insert.
16. Carefully set the 24-well plate base with the spacers back onto the lid, over the seeded cell culture inserts without allowing the inserts to touch the plate base. Refer to Figure 2 below.

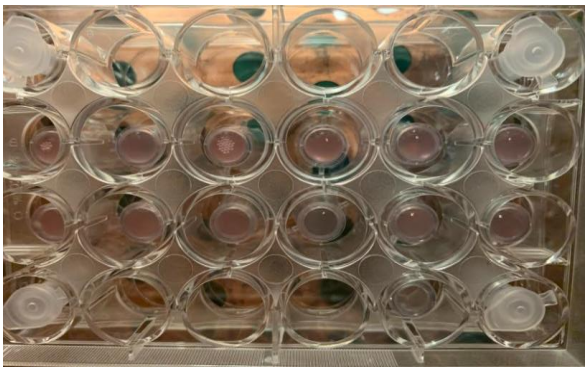


Figure 2

17. Carefully transfer the upside-down 24-well plate with the Astrocyte-Pericyte seeded inserts to a 37°C, 5% CO_2 incubator and incubate for 4 hours.
18. After 4 hours, transfer the upside-down 24-well plate with the Astrocyte-Pericyte seeded inserts into a Biological Safety Cabinet.
19. Gently lift the plate base with the spacers off the seeded cell culture inserts and remove the spacers.
20. Set the plate base back onto the lid, over the seeded cell culture inserts.
21. Slowly flip the entire plate so that the inserts are in the correct orientation.

Note: *Refer to Video 4 – Final Plate Flip and 4 Hour Feeding*

Note: *Scan the QR to view iCell BBB Tutorial* **Video 4** – Final Plate Flip and 4 Hour Feeding

22. Add 1 ml of iCell AP Plating Medium to the basolateral compartment of each seeded cell culture insert.

23. Carefully remove the Fibronectin-Collagen IV solution from the apical side of each seeded cell culture insert.
24. Add 300 µl of the iCell AP Plating Medium to the apical compartment of each seeded cell culture insert.
25. Place the plate to a 37°C, 5% CO_2 incubator and incubate overnight.

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 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 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	$21.45x10^4$	300	$0.715x10^6$

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 iCell AP Plating Medium from the apical and basolateral compartments of the Astrocyte-Pericyte seeded cell culture inserts made on Day -1.

   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

7. Add 1 ml of iCell BMEC Plating Medium to the basolateral compartment of each seeded cell culture insert.
8. Add 300 µl of iCell BMEC cell suspension to the apical compartment of each seeded cell culture insert.
9. 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 apical and basolateral compartments of the tri-culture cell culture inserts.
3. Add iCell BMEC Maintenance Medium to the apical (300 µl) and 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

Transendothelial electrical resistance (TEER) of iCell BBB tri-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.

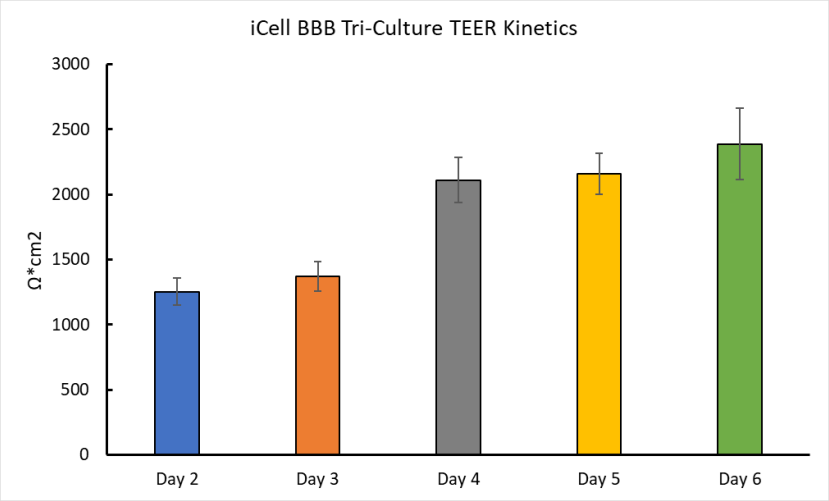


Figure 3