

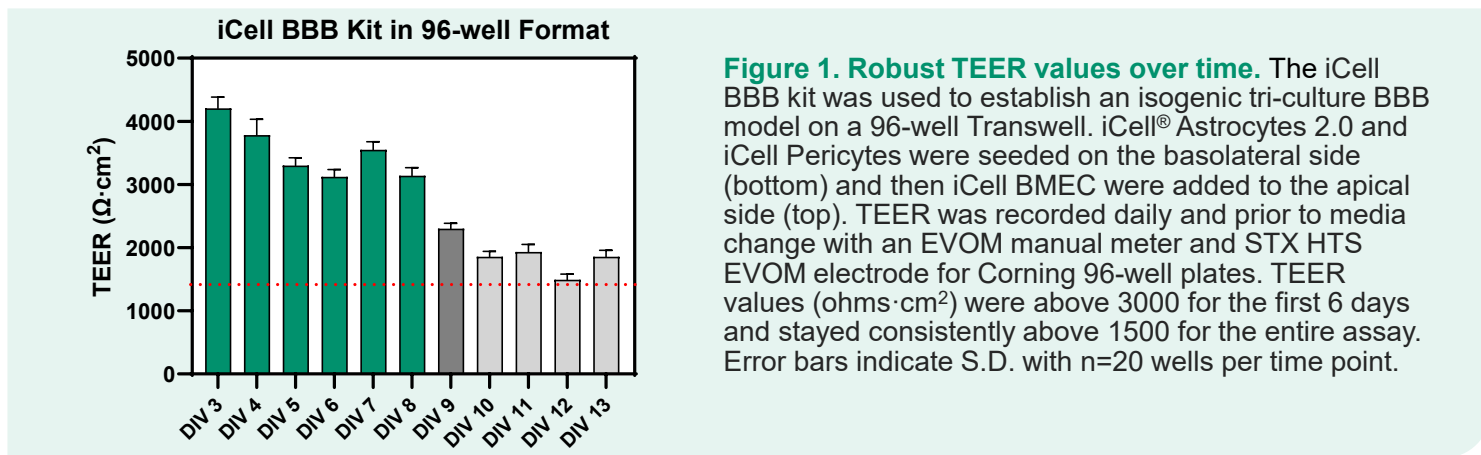
# Adapting the iCell® BBB TEER Assay to 96-well Format

*iCell Lab Note*

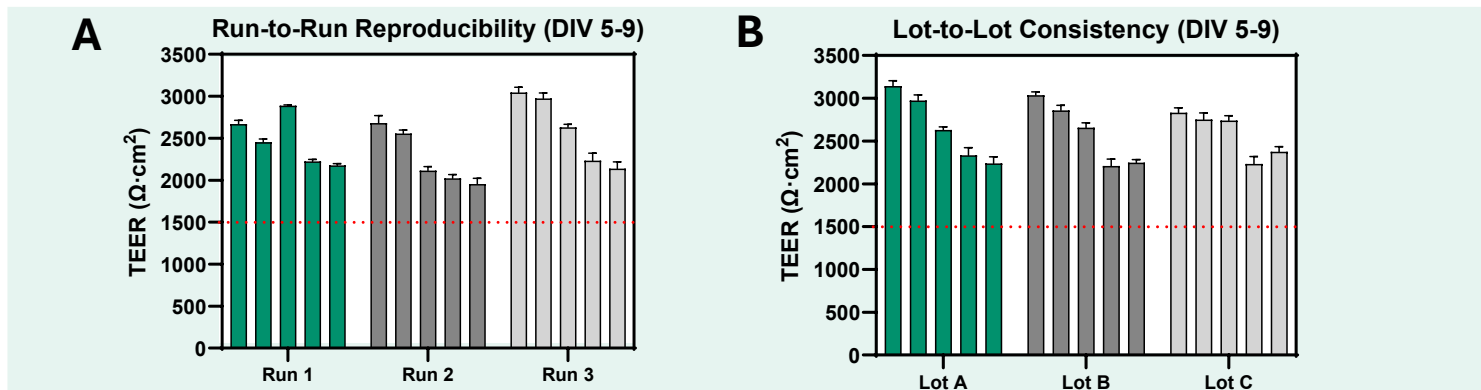
## Introduction.

The blood-brain barrier (BBB) plays a crucial role in maintaining homeostasis within the central nervous system (CNS) by selectively regulating the exchange of molecules between the blood and brain, thereby supporting proper neuronal function. Reliable, high-throughput *in vitro* methods for assessing BBB integrity are essential for advancing research in drug delivery and neurological diseases. Transendothelial Electrical Resistance (TEER) measurement is considered the gold standard for quantifying tightness of the barrier; however, consistent access to the necessary cell types at scale remains limited. To overcome these challenges, FUJIFILM Cellular Dynamics developed the iCell® BBB Kit. This isogenic tri-culture BBB model features human induced pluripotent stem cell (iPSC)-derived brain microvascular endothelial cells (BMECs), astrocytes, and pericytes that are fully compatible with TEER measurements. This iCell Lab Note provides protocol updates for use with an HTS Transwell®-96 permeable support and representative TEER data that can be achieved with this BBB model in a high-throughput assay format.

## Results.

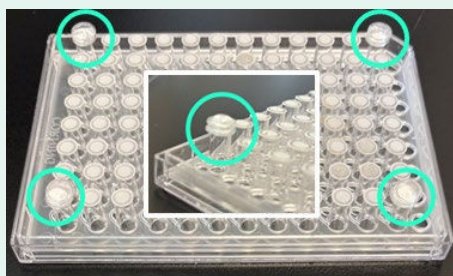


**Figure 1. Robust TEER values over time.** The iCell BBB kit was used to establish an isogenic tri-culture BBB model on a 96-well Transwell. iCell® Astrocytes 2.0 and iCell Pericytes were seeded on the basolateral side (bottom) and then iCell BMEC were added to the apical side (top). TEER was recorded daily and prior to media change with an EVOM manual meter and STX HTS EVOM electrode for Corning 96-well plates. TEER values (ohms·cm<sup>2</sup>) were above 3000 for the first 6 days and stayed consistently above 1500 for the entire assay. Error bars indicate S.D. with n=20 wells per time point.



**Figure 2. Reproducible and Consistent Assay Performance.** TEER assay was performed with the iCell BBB Kit on a 96-well Transwell and focused on recordings from day in vitro (DIV) 5-9. **(A)** Run-to-run reproducibility was demonstrated across three independent experimental runs (Run 1- Run 3) on three different days. **(B)** Lot-to-lot consistency under the same assay conditions was performed with three different production lots of iCell BMEC (Lot A, B, and C) tested all on the same 96-well Transwell (the same lots of iCell Astrocytes 2.0 and iCell Pericytes were used). In both examples, TEER values exceeded 2000 ohms·cm<sup>2</sup> and were sustained over the 5 days of measurement. The red dotted line at 1500 ohms·cm<sup>2</sup> indicates the expected threshold for *in vitro* BBB models. Error bars indicate SEM with n=12 wells per data point.

## Technical Tips and Tricks.



**Figure 3. Bumpers.** Generation of an *in vitro* BBB model with human iPSC-derived cell types on both sides (top and bottom) of the membrane of a 96-well Transwell required unconventional steps to enable cell culture. Cabinet door bumpers (from Amazon) were adhered to the 4 corners during cell seeding to ensure adequate spacing of the plates.

**Table 1. Materials Needed**

Product	Vendor	Cat. #
iCell Blood-Brain Barrier Isogenic Kit	FCDI	R1241
iCell BMEC, 01279 ( $\geq 3M$ cells/vial)	(incl. in kit)	C1239
iCell Pericytes, 01279 ( $\geq 1.5M$ cells/vial)	(incl. in kit)	C1241
iCell Astrocytes 2.0, 01279 ( $\geq 1M$ cells/vial)	(incl. in kit)	C1249
iCell BMEC Maintenance Medium	(incl. in kit)	M1042
iCell Plating Supplement, 500X	(incl. in kit)	M1043
iCell Astrocyte & Pericyte Medium	(incl. in kit)	M1041
Human Fibronectin	Sigma	F2006
Human Collagen Type IV	Sigma	C5533
Gelatin, 0.1% in water	SCT	07903
HTS Transwell-96 Permeable Support †	Corning	7369
EVOM Manual Meter for TEER ‡	WPI	EVM-MT-03-02
STX HTS EVOM Electrode (Corning 96) ‡	WPI	EVM-EL-03-03-05

† Transwell product includes untreated receiver and reservoir plates.  
‡ All TEER data was measuring using equipment from World Precision Instruments (WPI).

## Methods.

The original iCell Blood-Brain Barrier Isogenic Kit [User's Guide](#) provides detailed protocol information to create a tri-culture BBB model on a 24-well cell culture insert. It is required to review the User's Guide thoroughly prior to executing the protocol in this iCell Lab Note. Table 2 below highlights key differences for scaling to 96-well format, including a reduced density for each cell type and a lower concentration of ECM components.

**Table 2. Key Differences between Transwell Formats**

Feature	24-well	96-well
Insert Surface Area	0.33 cm <sup>2</sup>	0.143 cm <sup>2</sup>
Insert / Well Volumes	300 $\mu$ l / 1 ml	75 $\mu$ l / 235 $\mu$ l
Apical ECM conc. (in $\mu$ g/ml)	100 $\mu$ l of FN (100) and Col-1 (400)	50 $\mu$ l of FN (10) and Col-1 (40)
Basolateral ECM volume	100 $\mu$ l of Gelatin	30 $\mu$ l of Gelatin
# of Astrocytes/Pericytes (basolateral)	55k ASC and 110k PERI in 100 $\mu$ l	10k ASC and 15k PERI in 30 $\mu$ l
# of BMEC (apical)	215k BMEC in 300 $\mu$ l	31k BMEC in 75 $\mu$ l
# of wells per iCell BBB Kit	$\geq 12$ wells	$\geq 96$ wells

## Summary.

There was a strong demand for adapting the TEER assay using the iCell BBB Kit to a high-throughput 96-well format. In response, FUJIFILM Cellular Dynamics published this iCell Lab Note to detail the protocol modifications involved in scaling from a 24-well to a 96-well Transwell. Robust TEER measurements from the tri-culture BBB model using EVOM equipment from World Precision Instruments (WPI), with values exceeding 1500 ohms·cm<sup>2</sup> sustained for up to 2 weeks. The assay demonstrated reliable and consistent performance across multiple runs and cell lots. This model closely replicates the physiological architecture of the BBB, and high TEER values confirm the formation of a tight cellular barrier. Overall, this system enables reproducible, high-throughput assessment of BBB integrity over extended culture periods, supporting future studies with permeability/Papp, efflux transporter, and transport assays for both small and large molecules.

## Highlights.

Use of cryopreserved cells, optimized culture media, and validated protocols make this an assay-ready BBB model.

A robust 96-well TEER assay to assess BBB integrity can be generated from one iCell BBB kit that includes all 3 cell types.

EVOM technology and 96-well Transwell inserts bring the consistency necessary for reliable TEER recordings.



Scan here to download the [iCell BBB Kit User's Guide](#)

Contact **Technical Support** (FCDI-Support@fujifilm.com) for more protocol details and supportive data.