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Impedance-based TEER Measurements on the Maestro Z with iCell® Brain Microvascular Endothelial Cells

iCell Lab Note

Introduction.

Barrier-forming cells in the human body, such as endothelial cells, play a crucial role in maintaining homeostasis by regulating the selective exchange of substances between compartments, protecting against pathogens, and preserving tissue integrity. Trans-endothelial Electrical Resistance (TEER) is a key functional parameter used to quantify the integrity and permeability of cellular barriers by measuring the resistance to ionic flow across cell layers. Traditionally, TEER has been measured using electrode-based systems with static cell culture inserts; however, alternative approaches such as Maestro Z technology from Axion Biosystems, offer real-time, impedance-based cell analysis. This platform enables monitoring of TEER and barrier function in iCell® Brain Microvascular Endothelial Cells (BMEC) in 96- and 384-well formats. This iCell Lab Note provides the necessary recommendations to get started using these products.

Results.

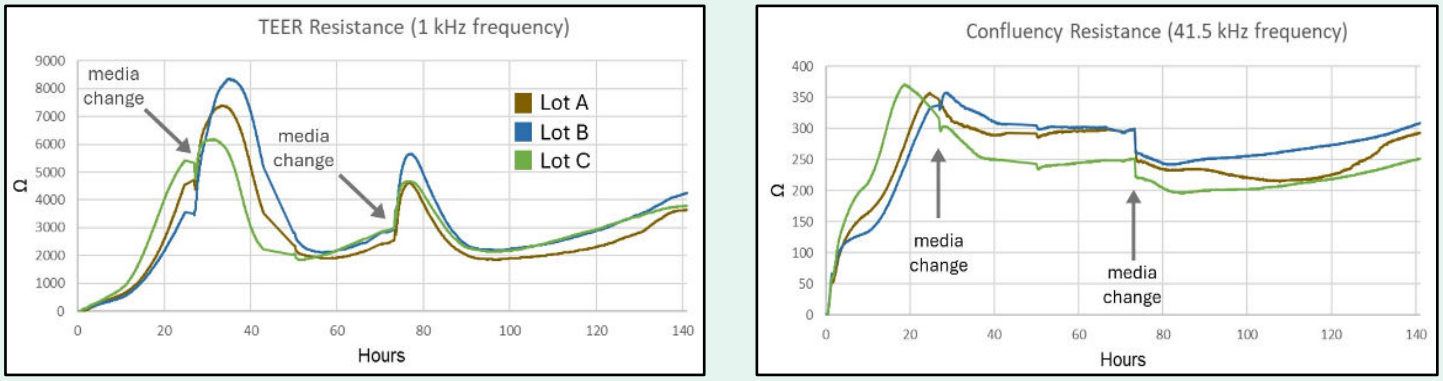

Figure 1. Kinetic TEER measurement profiles. Three different lots of iCell Brain Microvascular Endothelial Cells were cultured in a 384-well CytoView-Z plate for ~6 days (n=18 wells/lot). The 1 kHz frequency data (left) indicate cells yield similar impedance-based TEER measurements over time and finish at approximately the same endpoint value (4000 Ω at 141 hours) for all lots. The 41.5 kHz frequency data (right) demonstrate well confluency is also comparable with closely overlapping traces. Media changes are more impactful on the assay signal at the lower frequency.

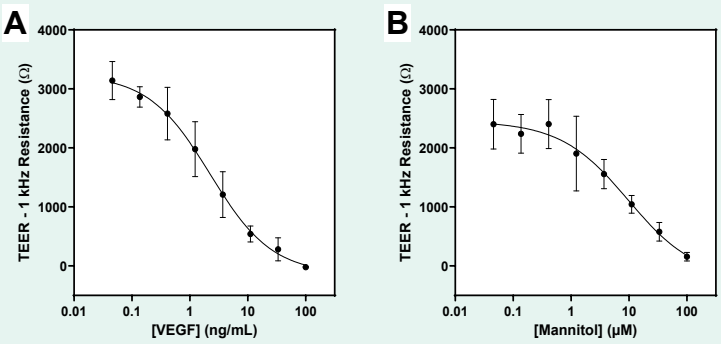

Figure 2. Disruption of barrier integrity. Maestro Z can be used to profile molecules that affect the barrier permeability of iCell Brain Microvascular Endothelial Cells. Here, a titration of (A) VEGF or (B) Mannitol was applied to cells in 384-well format on Day 4, resulting in a decrease in resistance after 24 h (8-pt dose-response, n=4 wells per concentration).

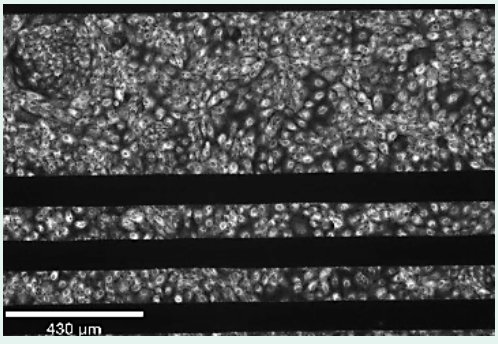

Figure 3. Visualization of the BMEC monolayer. The CytoView-Z 96-well plate has an on-plate viewing window that enables verification of cell attachment, viability, and morphology over time. This additional flexibility supports assay multiplexing. Here is a live-cell image of iCell Brain Microvascular Endothelial Cells stained with MitoTracker Deep Red FM dye.

Assay Technology.

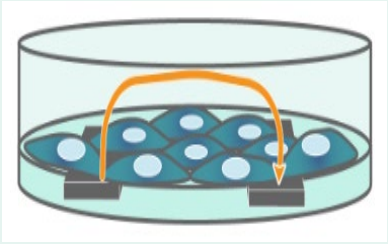

Figure 4. TEER Assay Schematic. Each well in a CytoView-Z plate contains one inter-digitated electrode that spans the entire bottom surface, with a recording area of 20 mm^2 or 4.9 mm^2 for 96- vs. 384-well format, respectively. An impedance-based system applies a small AC current and measures the electrical resistance across the cell layer to quantify barrier integrity and monitor changes in real-time. A high resistance TEER value corresponds to a tighter and continuous barrier, while a low value signifies a leaky barrier.

Methods.

Refer to the User's Guide for preparation of the Plating Medium and additional information on thawing and handling iCell Brain Microvascular Endothelial Cells.

- ~20 ml of Plating Medium will be needed on Day 0.
- Dilute Fibronectin (FN) to 100 µg/ml in DPBS and coat plate with 50 µl per well at 37ºC for 1-2 hours.
  - **FN alone is sufficient for iCell BMEC performance in the CytoView-Z plates.** †
- Rinse wells 1X with PBS and replace with 50 ml of Plating Medium. Centrifuge the plate at 200 x g for 1 min.
- Dock the plate and "Measure Baseline" with media only in every well of the CytoView-Z plate.
- Follow the User's Guide to properly thaw iCell BMEC.
  - Two vials will be required to fill an entire 96-well plate.¶
- Do not spin the cell suspension. Instead, dilute to a cell density of at least 400,000 cells/ml and add 150 µl/well of a 96-well CytoView-Z plate.
  - Recommended seeding density is 60-80K BMEC per well.
  - Include some cell-free controls (e.g., the 4 corner wells).
- Allow the plated cells to sit at room temp for 1 h in the hood to reduce edge effects. Also, fill the on-plate reservoirs with sterile H_2O for better humidity control.
- Record impedance on an Axion instrument that supports Maestro Z technology.
- Analyze data with AxIS Z software.

Table 1. Materials Needed

Product	Vendor	Cat. #
iCell® BMEC Kit, 01279 ¶	FCDI	R1236
• iCell BMEC (≥3M cells/vial)	(incl. in kit)	C1239
• iCell BMEC Maintenance Medium	(incl. in kit)	M1042
• iCell Plating Supplement A, 500X	(incl. in kit)	M1057
Fibronectin	Sigma	F2006
96-well CytoView-Z plate ◊	Axion	Z96-IMP-96B
Maestro Z platform ➤	Axion	Various
Human VEGF-165, PeproTech®	Thermo Fisher	100-20-10UG
D-Mannitol	Sigma Aldrich	63560

¶ Three (3) vials of cells will be required to fill an entire 384-well CytoView-Z plate. Cell number and volumes per well should be scaled accordingly (~25% of 96-well format).
† Recommended ECM for TEER assay on cell culture inserts is a combination of FN and Collagen-IV (FN/Col-IV). Numerous other ECM types were shown to support BMEC attachment and robust impedance values, incl. FN/Col-IV, iMatrix Laminin-511, FN alone, and BioLamina LN521.
◊ The catalog # for 384-well CytoView-Z plates is Z384-IMP-384B.
➤ All Maestro instruments support impedance-based TEER assays in 96-well format; however, only the Maestro Pro and the Maestro ZHT can record from a 384-well CytoView-Z plate.

Summary.

iCell® Brain Microvascular Endothelial Cells provide a highly reproducible human iPSC-derived model for evaluating barrier integrity and tight junction function. When paired with the Maestro Z platform from Axion, researchers can obtain continuous, impedance-based TEER measurements in 96- or 384-well formats without manual intervention. This system enables high-throughput, real-time assessment of barrier formation, stability, and compound-induced disruption. Compared to traditional "chopstick-electrode" devices, the Maestro Z offers a simplified workflow that enhances assay consistency and throughput, making it a reliable solution for investigating endothelial barrier function, screening modulators of permeability, and supporting the development of advanced in vitro BBB models.

Highlights.

Cryopreserved iCell Brain Microvacular Endothelial Cells are supplied with an optimized culture medium, so they are ready-to-use in this assay.

TEER and barrier function can be monitored in 96- or 384-well format, enabling high-throughput screening assays.

Impedance-based TEER offers a hands-free alternative to traditional measurements of barrier formation/function.

Scan here to download the iCell BMEC User's Guide

Contact **Technical Support** for more protocol details and supportive data.
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