

# >> High-throughput assessment of barrier function using human iPSC-derived brain microvascular endothelial cells and retinal pigment epithelial cells

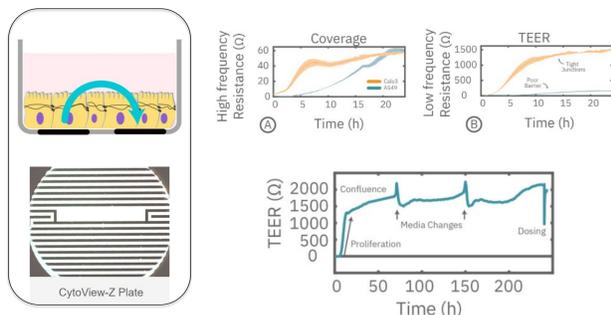
Stacie Chvatal<sup>1</sup>, Emily Cox<sup>1</sup>, Christie Savic<sup>2</sup>, Madelyn Donagan<sup>2</sup>, Rebecca Fiene<sup>2</sup>, Jeanie Liu<sup>2</sup>, Ravi Vaidyanathan<sup>2</sup>, and Coby Carlson<sup>2</sup>

<sup>1</sup>Axion BioSystems, Atlanta, GA, USA; <sup>2</sup>FUJIFILM Cellular Dynamics, Madison, WI, USA

## Maestro Z: Dynamic Cell Tracking Impedance-based TEER Recordings

Epithelial and endothelial cells form barriers in the body. The strength and integrity of these barriers can be assessed via measurements of the electrical resistance across the cell layer, called TEER.

Axion BioSystems' Maestro Z system continuously monitors cell proliferation and TEER to quantify the development of barrier properties over time. Here, we used the Maestro Z to measure coverage and barrier function of iPSC derived endothelial and epithelial cells.



Traditional TEER measurements require a fully confluent cell layer to accurately measure barrier function. In contrast, the Maestro Z tracks coverage and TEER simultaneously and continuously by measuring impedance at multiple frequencies.

### The Maestro Z Product Family

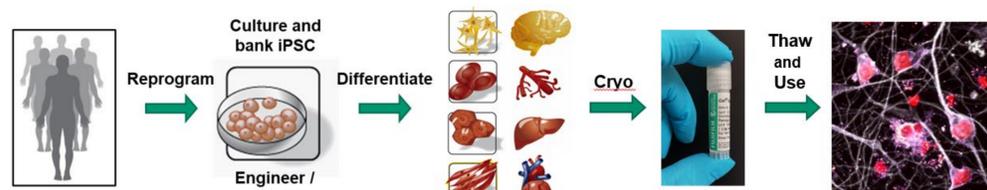


- **Label-free, non-invasive tracking** of cultured cells or spheroids/organoids
- **Integrated environmental control** provides a stable benchtop environment for short- and long-term toxicity studies
- **Automatic and continuous cell monitoring** from 96 or 384 wells simultaneously
- **"One button setup"** automatically docks the plate and adjusts temperature and CO<sub>2</sub> levels
- **Powerful data analysis** to focus on the science, while AxIS Z handles the details with simple setup and automatic experiment tracking
- **See your cells** with the viewing window included in each well of the CytoView-Z 96-well plate
- **State-of-the-art electrode processing chip (BioCore v4)** offers stronger signals, ultra-low frequency content, and enhanced flexibility

Features	Maestro Z	Maestro TrayZ	Maestro ZHT
Throughput:	96-well	Up to 8 x 96-well	384- and 96-well
Environmental Controls:	Built-in	External	Built-in
GxP Compatible:	✓	✓	✓
Barcode Plate Tracking:	✓	✓	✓
Automation API:	✓	No	✓
Dimensions (WxDxH):	280 x 413 x 225 mm	440 x 450 x 60 mm	280 x 452 x 225 mm

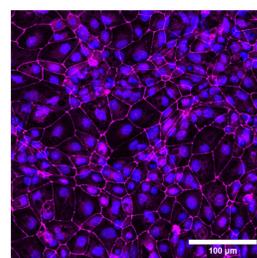


## iPSC Technology: Unlimited Access to Unique Human Cell Types



The inherent power of induced pluripotent stem cell (iPSC) technology offers the ability to generate unlimited quantities of specialized human cell types. By reprogramming somatic cells, such as skin fibroblasts or blood cells, back into a pluripotent state, iPSCs can be differentiated into a wide array of cells, including cardiomyocytes, neurons, and others; thus, overcoming the limitations associated with primary human tissue. Moreover, iPSC-derived cells retain the donor's genetic information, enabling disease modeling and personalized medicine approaches. The renewable and scalable nature of iPSC-derived cells also facilitates high-throughput drug screening and toxicity testing, making this technology a powerful tool in biomedical research and translational applications.

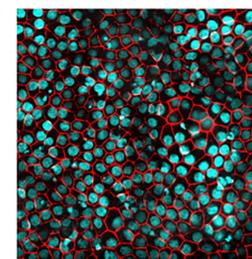
### iCell® Brain Microvascular Endothelial Cells (BMEC), 01279



iCell BMEC are the barrier-forming cells of the blood-brain barrier (BBB). BMEC are unlike other endothelial cells lining peripheral blood vessels in that they display distinctive morphological and structural features as tightly-packed cells with uniform size and clear cell boundaries. They express markers such as Claudin-5 and ZO-1, GLUT1, BCRP, MRP1 and TFRC. Their functional performance is typically measured by TEER in a Transwell assay format.

### iCell Retinal Pigment Epithelial Cells (RPE), 01279

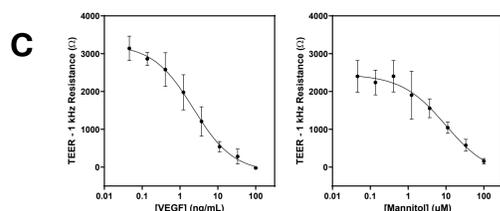
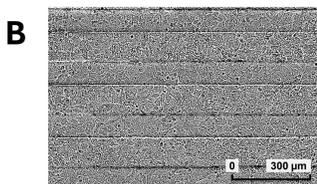
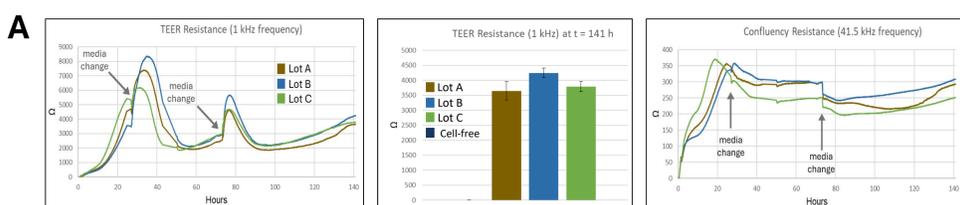
iCell RPE express mature cell type-specific markers, including BEST1 and CRALBP, within 28 days post-thaw and exhibit key functions such as phagocytosis and cytokine release. Importantly, these cells also form organized and polarized monolayers with well-defined tight junctions, making this model suitable for transport, barrier function, and permeability studies.



The human iPSC-derived cell types used in this study were iCell® products from FUJIFILM Cellular Dynamics.

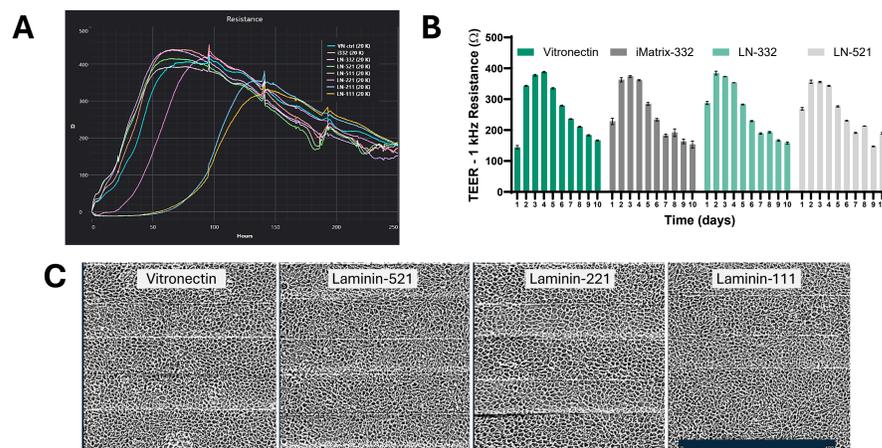
## Impedance-based TEER to Characterize Cellular Function and Performance

### Lot-to-lot consistency and Barrier Disruption



TEER assay development on the Maestro Z platform. (A) Real-time impedance assays were monitored for 3 different lots of iCell® BMEC cultured in a 384-well CytoView-Z plate for 6 days. The data indicate similar traces of impedance (1 kHz) over time and essentially the same endpoint TEER value (3600-4200 Ω at 141 h) across all 3 lots. (B) Visualization of the BMEC monolayer on a CytoView-Z plate enables verification of cell attachment, morphology, and viability. Assay multi-plexing is also possible, if desired. (C) Importantly, this application can be used to profile molecules that disrupt barrier formation, including VEGF and Mannitol (8-pt dose response, n=4 wells at each concentration).

### Screening for ECM Influences on Cell Behavior



Maestro Z TEER assay to optimize cell culture ECM conditions. The recommended ECM for RPE is Vitronectin; however, investigating full-length laminin isoforms that are now available and are known to provide structural support and to modulate the mechanical status and function of RPE cells is critical. In this study, we compared the impedance signal from iCell RPE cultured on various recombinant laminins mainly from BioLamina. (A) Real-time measurements of 1 kHz TEER resistance highlight the kinetic differences observed. (B) Data can also be plotted at specific 24 h intervals over 10 days, comparing ECM control with two sources of Laminin-332, as well as the popular LN-521. (C) Cells on 96-well CytoView-Z plates can be observed through the on-plate viewing window. RPE morphology on Day 10 appears to be very similar despite the cells having experienced dynamic impedance changes.