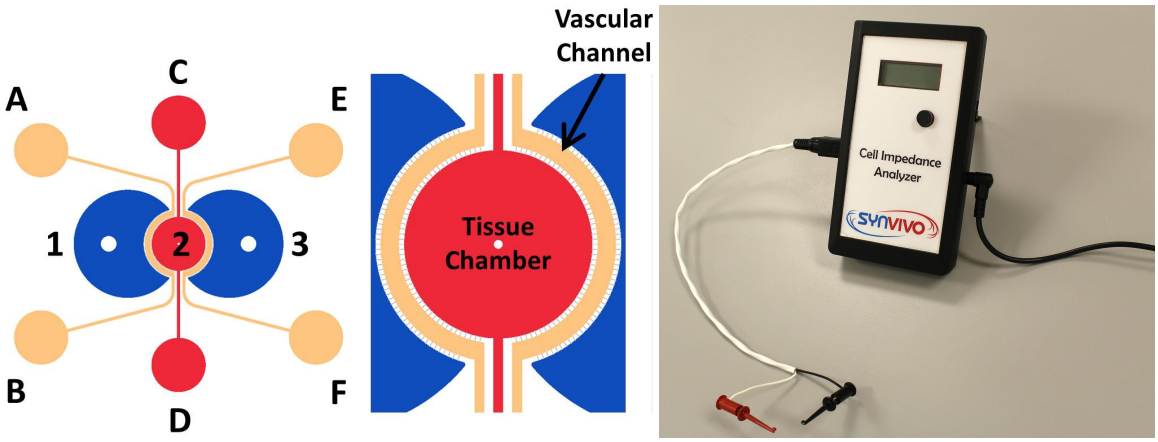
# 3D Blood Brain Impedance Assay Using SynBBB Idealized Network (TEER configuration) Kits and Chips – Technical Manual

Catalog #s 402004, 402003, 102015-SB




*Schematic of the chip used for the SynBBB Model and SynVivo Cell Impedance Analyzer system. Apical chamber (outer channels) are for culture of vascular (endothelial cells) while basolateral chamber (central chamber) are for culture of brain tissue cells (astrocytes, pericytes, neurons). Porous architecture enables communication between the vascular and tissue cells.*

## Overview of SynBBB Model For Impedance Measurement

Delivery of neuroprotective or therapeutic agents to specific regions of the brain presents a major challenge, largely due to the presence of the Blood-Brain Barrier (BBB). Physiologically, the BBB consists of an intricate network of vascular endothelial cells (ECs) that isolate the central nervous system (CNS) from systemic blood circulation. Traditional blood brain barrier (BBB) assays, such as the Transwell® model have significant limitations such as lack of physiological shear stress, real-time visualization capability and large amount of consumables in addition to cumbersome protocols. SynBBB recreates the in vivo microenvironment by mimicking a histological slice of brain tissue cells in communication with endothelial cells across the BBB. **SynBBB is the only *in vitro* BBB model that allows**

- Accurate in vivo hemodynamic shear stress
- Real-time visualization of cellular and barrier functionality
- Significant reduction in cost and time
- Robust and easy to use protocols

This model has been successfully demonstrated for upregulation of tight junction molecules, functional assays (Prabhakarpandian et al., 2013) and validated against in vivo studies showing excellent correlation with permeation of small molecules (Deosarkar et al., 2015). Electrical resistance measurements provide a non-invasive method for real-time monitoring of tight junctions as detailed below. Tight junction formation between cells (e.g., blood brain barrier) can be evaluated by measuring changes in electrical resistance in the intercellular space between cells. The SynVivo Cell Impedance Analyzer, used in conjunction with the SynBBB TEER device, measures electrical impedance (resistance).

## Materials Needed

- SynBBB Impedance (TEER) Chips* (Catalog # 102015-SB): Use ports A, C, and E serve as inlets and ports B, D, and F serve as outlets. Use ports 1, 2, and 3 for silver chloride electrodes.
- SynVivo Impedance Analyzer (Catalog # 304001)
- SynVivo Pneumatic Primer Device (Catalog #205001)
- 1 mL BD plastic syringes or other 1 mL syringes (Catalog # 203004)
- 24 gauge blunt tip needles (Catalog # 204002)
- Tygon microbore tubing, 0.02” ID X 0.06” OD (Catalog # 201005)
- Clamps (Catalog # 202003)
- Forceps
- Syringe Pump capable of flow rates from 10nl/min to 10ul/min
- Fibronectin
- Endothelial Cells (primary or cell line)
- Astrocytes (primary or cell line)

*** Store at Room Temperature. Shelf life is 6 months at room temperature**

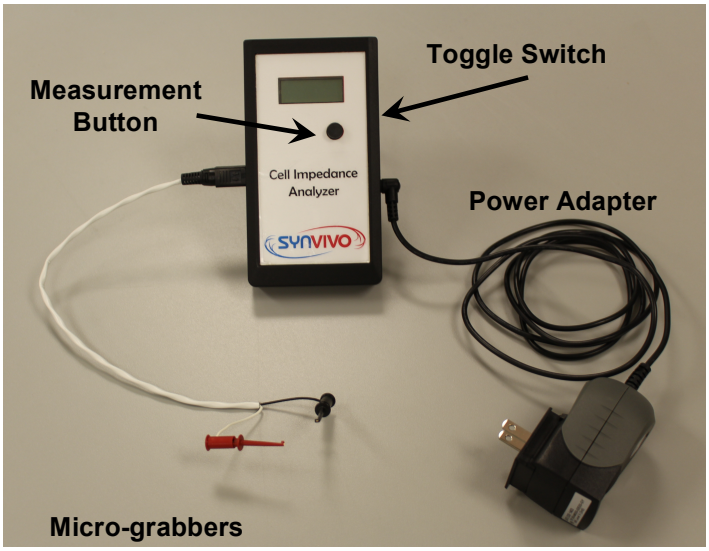
## Specifications of Impedance Analyzer

| | |
|---|---|
| **Impedance Measurement Range** | |
| *Minimum* | 20 kOhm |
| *Maximum* | 300 kOhm |
| **Operating Frequencies** | |
| *500, 1,000, and 10,000 Hz* | |
| **Connections** | |
| *Electrode* | Micro-grabber Connectors |
| *Power* | AC Adapter |

**Important Note: The SynVivo® Cell Impedance Analyzer is compatible only with SynBBB TEER chips.**

## Operation Basics

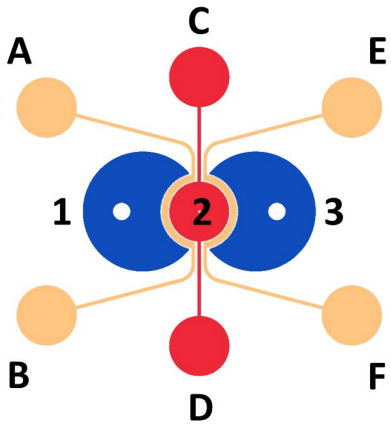
The SynVivo® Cell Impedance Analyzer is designed to measure changes in impedance (resistance) across cells cultured in SynVivo IMN2-Z devices.




A toggle switch on the right side of the Analyzer changes the operational frequency between 500, 1,000, and 10,000 Hz. The Analyzer is interfaced to the SynVivo IMN2-Z device by attaching micrograbber clips to the electrodes in the device.

The measurement button is pressed once to display the operational frequency, and once again to acquire the impedance (Z) and phase measurement.

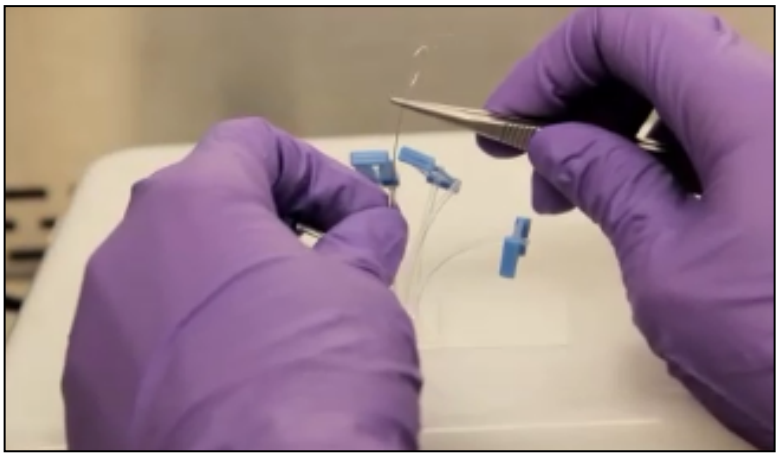
Follow the steps outlined in the next section to operate the SynVivo® Cell Impedance Analyzer.



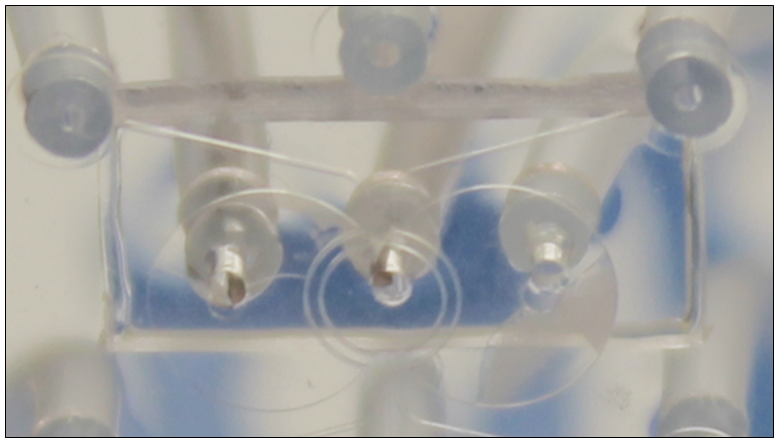

**Steps for Measuring Electrical Resistance**

*The following protocol should be carried out within a laminar flow hood to maintain sterility.*

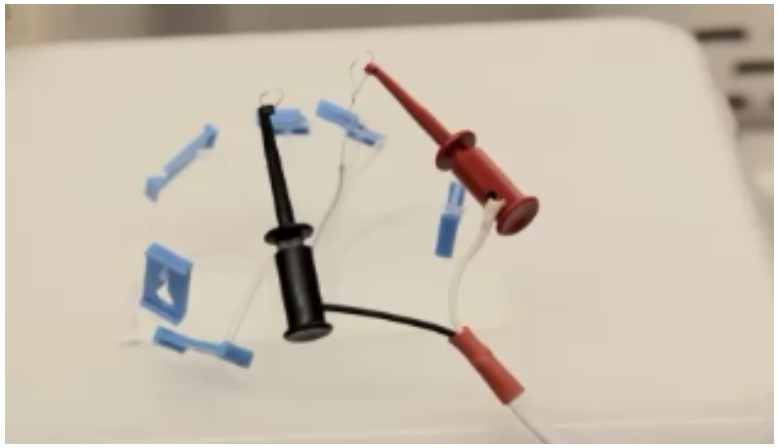
1. Place the fully perfused SynBBB chip on a hotplate set to 37 °C. If a hotplate is not available, allow the chip to come to room temperature for ~10 minutes before proceeding.
2. Using tweezers, thread an electrode through electrode port 2 on the chip, pushing the electrode tip down in contact with the glass slide.



3. Thread the 2nd electrode into electrode port 1. Ensure that both electrode tips are touching the glass slide.



4. Turn on the SynVivo Cell Impedance Analyzer by plugging in the power cord. The Analyzer will calibrate automatically.
5. Attach a micro-grabber clip to each electrode, being sure not to pull the electrodes out of place.



6. Select the desired operating frequency using the toggle located on the right side of the Analyzer (see Choosing the Operating Frequency below). Press the measurement button to display the impedance (Z) and phase values on the screen.


7. If another frequency is desired, switch the toggle and press the measurement button, once to display the current operating frequency, and once again to display the impedance and phase values.
8. Repeat steps 3 through 7 on electrode port 3.

## Choosing the Operating Frequency

The table below gives optimized operating frequencies for certain cell types.

| Cell Type | Operating Frequency |
|---|---|
| Human Umbilical Vein Endothelial Cell (HUVEC) | 10 kHz |
| Rat Brain Microvascular Endothelial Cells (RBEC) | 1 kHz |

Use the following protocol to determine the optimal operating frequency for a specific cell type:

1. Measure the baseline impedance and phase values of a coated and fully perfused chip at operating frequencies of 500, 1,000, and 10,000 Hz.
2. Coat the device with the desired cells, and grow under desired conditions.
3. Acquire impedance and phase values at operating frequencies of 500, 1,000, and 10,000 Hz.
4. Choose the operating frequency with a phase value closest to 0, ideally between 0 and ±15.

## Mono-Culture of Endothelial Cells for Impedance Measurement

Shear-induced endothelial cell tight junctions, which cannot be achieved in the Transwell® model, are easily achieved in the SynVivo model using fluid perfusion. Formation of tight junction changes can be measured using biochemical or electrical analysis (assessing changes in electrical resistance) with the SynVivo Cell Impedance Analyzer (Catalog # 304001).

*Note: Most primary endothelial cells are usable only until passage 8. For endothelial cell lines, refer to vendor specifications for passage information. Most endothelial cell lines do not sustain shear stress, which is required for formation of tight junctions.*

A. **Coating The Device with Endothelial Cell Culture Matrix (e.g. fibronectin).** This process is performed using Pneumatic Primer (SynVivo Cat# 205001).

1. Using fine-point forceps, insert approximately 1 inch segments of Tygon tubing into the outlet ports (B, and F) for the vascular channel and the electrode ports (1, 2, and 3).
2. Thaw and dilute fibronectin to 100-200 µg/mL in chilled serum-free media or 1X PBS.
3. Using a 1ml syringe, draw up the fibronectin and attach to a syringe pump. Attach a piece of tubing with an appropriate length to reach the inlet port (A) of the vascular channel.
4. Allow the fibronectin to flow into the vascular channel at 3 µL/min until 2 droplets form on the outlet tubing. Stop the flow, clamp both port tubing and cut the tubing connected to the syringe just above the clamp.
5. Repeat this process for the other vascular channel, using inlet port E.
6. Place tubing into the tissue chamber ports (C and D) and clamp all tubing.
7. Place the chip at 37°C in an incubator for 1 hour. The chip can go as long as overnight, if needed.
8. Connect the chip to the Pneumatic Primer by locking the needle into the LuerLock connector on the box. *Note: Multiple devices can be primed simultaneously using the multiple port manifold, available from SynVivo (cat # 207001)*
9. Turn the knob on the controller box and adjust the pressure to ~5-7 psi. Apply the pressure for ~5-20 minutes. Devices will take at least 15 minutes to completely fill.
10. Turn off the pressure and cut the Tygon tubing connected to the Pneumatic Primer.
11. Allow the device to incubate at 37° for a minimum of 1 hour before use
12. Flush fresh media into device just before seeding endothelial cells.

## B. Baseline Impedance Measurement

1. Acquire baseline impedance measurements across electrode ports 1 and 2 using the SynVivo Cell Impedance Analyzer (see Analyzer protocol above for details).
2. Repeat baseline impedance measurement across ports 2 and 3.
3. Subtract the baseline impedance measurement from subsequent impedance measurements with endothelial cells.

## C. Culture of Endothelial Cells

1. Endothelial cells should be dissociated, centrifuged and concentrated to approximately 2-5x$10^7$ cells/mL. Note: Do not expose primary cells to trypsin longer than necessary. Most primary cells will dissociate in about 3 minutes.
2. Place a drop of water at the base of the inlet port (A) tubing to be removed.
3. Gently remove the tubing - the water should cover the port once the tubing is removed, and should remain there until new Tygon tubing is inserted into the port. This will prevent air from entering the device.
4. Remove the clamp on the outlet port (B).
5. Prepare a syringe and tubing with the cell suspension and mount onto a syringe pump.
6. Ensure that the tubing is free of air bubbles and allow a convex meniscus to form at the end of the tubing.
7. Insert the tubing into the port (A)—the meniscus and the drop of water will form a bridge to prevent air from entering the device as the tubing is inserted.
8. Clean the fluid from the surface of the device.
9. Begin the injection at 5 µL/min.
10. Observe the device as the cells are flowing. Once the vascular channel is filled with cells, clamp the outlet tubing (port B) and stop the flow.
11. Carefully clamp the inlet tubing and cut the tubing just above the clamp to separate the device from the syringe.
12. Repeat this process for the other vascular channels using port E and F. Cells should be uniformly dispersed at this moment
13. Allow the cells to attach for at least 4 hours before changing media.

    a. To set up a media change program using a syringe pump, use the following steps:
        i. Program the pump to flush out the vascular channel without cells every 3 hours at 2µl/min for 3 minutes to refresh the media.
            1. Program summary: Media Change
                a. Step 1: Constant Rate

i. Mode: Infuse
ii. Set rate: 2 µl/min
iii. Time: 0:03:00 (3 minutes).

b. Step 2: Pause
i. Mode: Pause
ii. Target time: 3:00:00 (3 hours)

c. Step 3: Repeat from Step 1

14. Flow can be started on primary endothelial cells after 24 hours, though some cells may need up to 72 hours (with daily media changes) before they can withstand flow.

a. For complete confluent monolayers, introduce flow using programmable syringe pump with the flow rate climbing from 0.01µl/min to 1µl/min over a 24-hour period (Table 1).
i. The program example uses the "Step" feature of the pump, which infuses media at a user-defined flow rate over a specific period of time.

***Table 1: Step programming to increase the flow rate from 0.01 to 1 µL/min over 24 hours***

| SEQ | Flow Rate (µl/min) | Time (h:m:s) | Direction |
|---|---|---|---|
| 1 | 0.01 | 2:00:00 | INFUSE |
| 2 | 0.025 | 2:00:00 | INFUSE |
| 3 | 0.05 | 2:00:00 | INFUSE |
| 4 | 0.075 | 2:00:00 | INFUSE |
| 5 | 0.1 | 2:00:00 | INFUSE |
| 6 | 0.2 | 2:00:00 | INFUSE |
| 7 | 0.3 | 2:00:00 | INFUSE |
| 8 | 0.4 | 2:00:00 | INFUSE |
| 9 | 0.5 | 2:00:00 | INFUSE |
| 10 | 0.6 | 2:00:00 | INFUSE |
| 11 | 0.8 | 2:00:00 | INFUSE |
| 12 | 1 | 2:00:00 | INFUSE |

15. Use the SynVivo Cell Impedance Analyzer to acquire measurements at the desired time points.

## Co-Culture Impedance Assay

*The following protocol is an example of co-culture using brain endothelial cells and astrocytes/astrocyte conditioned media. This protocol may be adapted for any vascular-tissue model. All steps, when possible, should be carried out within a laminar flow hood to maintain sterility.*

### A. Coating the Tissue Chamber

1. Using fine-point forceps, insert approximately 1 inch segments of Tygon tubing into the outlet ports (B, D, and F) for the vascular channel and the electrode ports (1, 2, and 3).
2. Place all materials on ice to cool: devices, forceps, syringe with needle and tubing. Keep devices on ice through the entire process.
3. Dilute Matrigel 1:5 with chilled serum-free media or 1X PBS.
4. Draw Matrigel into a small segment tubing on syringe – it is recommended to only draw up what is necessary for one device (about 30 ul). This is to prevent excess Matrigel from polymerizing in the syringe and excess tubing.
5. Quickly insert tubing into port (C) and infuse by hand until one drop of pink Matrigel mixture drops from electrode port 2 tubing. Clamp electrode port 2, and infuse until outlet tubing (D) is infused.
6. Detach syringe from tubing and bury entire device in ice for 1 hour.
7. After 1 hour, wash with serum-free media (don't be too gentle – this step will help remove clumps). Clamp ports (C) and (D).

**Follow steps above for seeding and culture of endothelial cells before going forward to the next step.**

### B. Astrocyte Seeding Protocol

1. All tubing should be clamped before beginning this procedure.
2. Astrocytes should be dissociated centrifuged and concentrated to approximately 1-3x$10^7$ cells/mL.
3. Place a drop of water at the base of the inlet port (C) tubing to be removed.
4. Gently remove the tubing - the water should cover the port once the tubing is removed, and should remain there until new Tygon tubing is inserted into the port. This will prevent air from entering the device.
5. Remove the clamp on the outlet port (D).
6. Prepare a syringe and tubing with the cell suspension and mount onto a syringe pump.
7. Ensure that the tubing is free of air bubbles and allow a convex meniscus to form at the end of the tubing.
8. Insert the tubing into the port (C)—the meniscus and the drop of water will form a bridge to prevent air from entering the device as the tubing is inserted.
9. Clean the fluid from the surface of the device.

10. Begin the injection at 5 µL/min.
11. Observe the device as the cells are flowing. Once the tissue chamber is filled with cells, clamp the outlet tubing (port D) and stop the flow.
12. Cut the inlet tubing approximately 1 inch above the device, and then clamp. This will help disperse the cells more evenly throughout the vascular chamber.
13. Allow astrocytes to attach for at least 4 hours before changing media.
14. Acquired impedance measurements at desired time points using the Cell Impedance Analyzer.
15. For conditioned media from astrocytes, repeat above process without cells.