SYNBBB 3D TEER CONFIGURATION BLOOD BRAIN BARRIER MODEL TECHNICAL MANUAL

For Kits and Chips: Catalog #s 402004, 402003, 102015-SB3

601 Genome Way NW, Suite 2023E • Huntsville, AL 35806 USA • T: 256-726-4838
www.synvivobio.com

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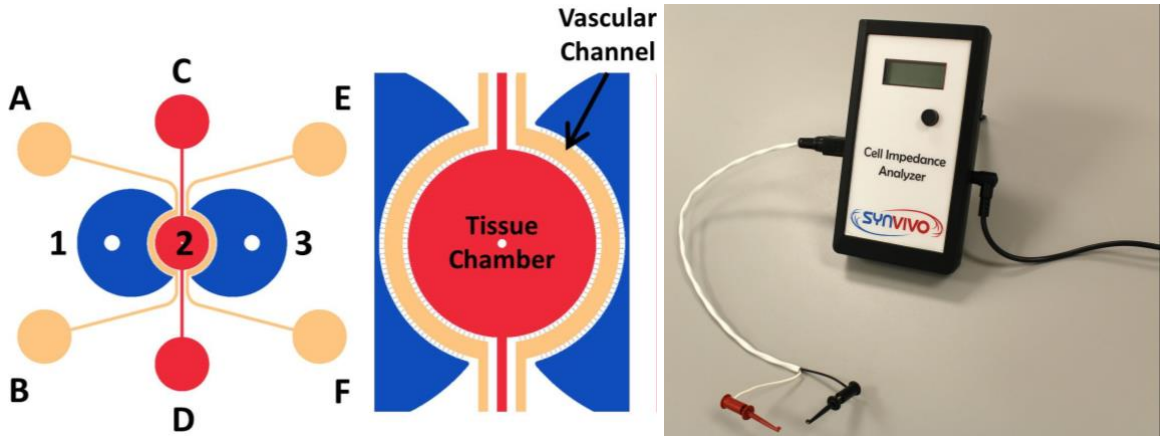
INTRODUCTION



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

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 that isolate the central nervous system from systemic blood circulation. Traditional BBB assays, such as the 2D cell culture insert model have significant limitations such as lack of physiological shear stress, real-time visualization capability and large number of consumables. SynVivo™ 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:**

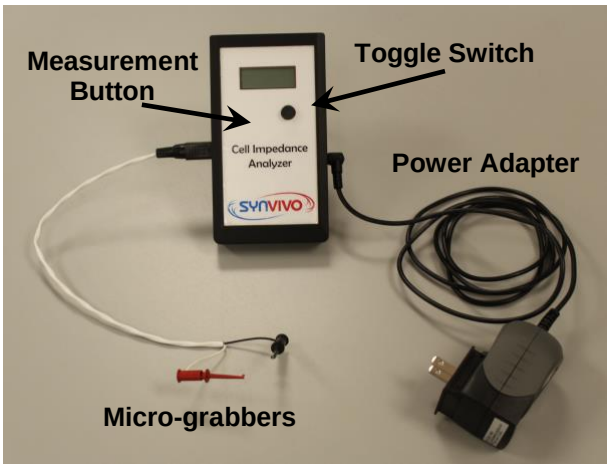
- Accurate in vivo hemodynamic shear stress
- Real-time visualization of cellular and barrier functionality
- Significant reduction in cost and time
- Robust and easy to use protocols

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 chip, measures electrical impedance (resistance, denoted by “Z”).

SYNVIVO CELL IMPEDANCE ANALYZER

Cell Impedance Analyzer Specifications

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



The SynVivo® Cell Impedance Analyzer is designed to measure changes in impedance-base resistance (Z) across cells cultured in SynVivo SynBBB chips. 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™ SynBBB chip by attaching micrograbber clips to AgCl electrodes inserted into the electrode ports of the chip. The measurement button is pressed once to display the operational frequency, and once again to acquire the resistance Z and phase measurement.

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

Choosing an 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
Human Brain Microvascular Endothelial Cells	1 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 chip 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 degrees, ideally between 0 and ±15 degrees.

SYNBBB TEER MODEL PROTOCOLS

Workflow

The following workflow chart shows the basic steps of setting up a SynBBB TEER model:

Establishing the Basement Membrane

Principle:

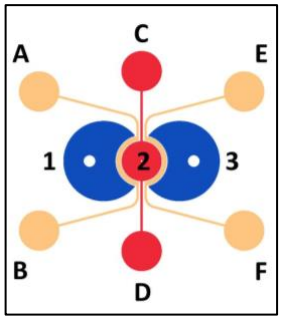
The extracellular matrix (ECM), or basement membrane, is a structural framework of proteins that provides a scaffold for cells comprising a tissue. Fibronectin is a major component of vascular endothelial networks, where it serves as a scaffold protein and plays a role in vascular morphogenesis. This protocol will use human fibronectin as the ECM, but the protocol can be adapted for other types of ECM, such as collagen or Matrigel (see "SynBBB 3D Blood Brain Barrier Model Technical Manual" or contact Synvivo technical support for more details).

Equipment:

- SynVivo SynBBB Impedance (TEER) Chips (SynVivo 102015-SB3)
- Tygon tubing (0.02 inch ID x 0.06 inch OD, or 0.05 cm ID x 0.15 cm OD; SynVivo 201005)
- Blue slide clamps (SynVivo 202001)
- 1 mL syringe with Luer-Lok tip (25 pack; SynVivo 203005)
- 24-gauge blunt-tipped needles (0.5 inches or 1.27 cm long; SynVivo 204003)
- Pneumatic Primer (SynVivo 205001)
- Forceps
- Scissors

Reagents:

- 1X PBS without calcium or magnesium
  or
- Serum-free endothelial cell basal medium
- Human fibronectin



Protocol for Monoculture:

1. Using forceps, insert 1-inch segments of Tygon tubing into the outlet Ports (B, D, and F) 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. Attach a 24-gauge blunt-tipped needle to a 1ml syringe. Cut and attach an approximately 5-inch segment of Tygon tubing to the end of the needle.
4. Draw up the diluted fibronectin only into the Tygon tubing (not into the needle or syringe). Attach the free end of the tubing to inlet **Port A** of the vascular channel.

5. Gently push fibronectin into the vascular channel until 2 droplets form on the outlet tubing in **Port B**. Cut the tubing connected to the syringe approximately 1 inch from the chip.
6. Repeat this process for the other vascular channel, using inlet **Port E** and outlet **Port F**.
7. Place the chip at 37°C in an incubator for 1 hour. The chip can go as long as overnight, if needed.
8. The chip is ready to be primed with the Pneumatic Primer.

Protocol for Co-culture:

1. Using forceps, insert 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). Clamp the tubing attached to electrode **Port 2**.
2. Thaw and dilute fibronectin to 100-200 µg/mL in chilled serum-free media or 1X PBS.
3. Attach a 24-gauge blunt-tipped needle to a 1ml syringe. Cut and attach an approximately 5-inch segment of Tygon tubing to the end of the needle.
4. Draw up the diluted fibronectin only into the Tygon tubing (not into the needle or syringe). Attach the free end of the tubing to inlet **Port A** of the vascular channel.
5. Gently push fibronectin into the vascular channel until 2 droplets form on the outlet tubing in **Port B**. Cut the tubing connected to the syringe approximately 1 inch from the chip.
6. Repeat this process for the other vascular channel (inlet **Port E** and outlet **Port F**) and the tissue channel (inlet **Port C** and outlet **Port D**).
7. Place the chip at 37°C in an incubator for 1 hour. The chip can go as long as overnight, if needed.
8. The chip is ready to be primed with the Pneumatic Primer.

Priming SynBBB TEER Chips

Principle:

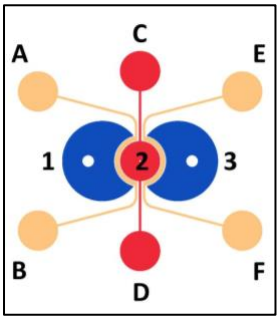
Complete removal of air from the SynBBB chips is critical for both the successful culture of cells and accurate measurement of resistance using the Cell Impedance Analyzer. The Pneumatic Primer (SynVivo 205001) works by pressurizing the SynBBB chip and forcing trapped air bubbles through the pores in the PDMS. This method requires a source of inert gas, as well as a pressure regulator to control the flow of gas into the chip.

Equipment:

- SynVivo SynBBB Impedance (TEER) Chips (SynVivo 102015-SB), coated with ECM of choice
- Tygon tubing (0.02 inch ID x 0.06 inch OD, or 0.05 cm ID x 0.15 cm OD; SynVivo 201005)
- Blue slide clamps (SynVivo 202001)
- 1 mL syringe with Luer-Lok tip (25 pack; SynVivo 203005)
- 24-gauge blunt-tipped needles (0.5 inches or 1.27 cm long; SynVivo 204003)
- Pneumatic Primer (SynVivo 205001)
- Forceps
- Scissors
- Multiple port manifold for the Pneumatic Primer (SynVivo 207001)
- Source of inert compressed gas

Reagents:

- 1X PBS without calcium or magnesium
  or
- Serum-free endothelial cell basal medium



Protocol:

Before beginning this protocol, remove the 1-inch segments of Tygon tubing from Ports A, C, and E, and remove all clamps from the chip tubing.

1. Attach a 24-gauge blunt-tipped needle to a 1ml syringe. Fill the syringe with either 1X PBS or serum-free basal medium.
2. Cut and attach a 5-8 inch segment of Tygon tubing to the end of the needle, and using gentle pressure, fully perfuse the attached tubing.
3. Connect the tubing to **Port A**. Gently push liquid into the chip until 1-2 drops of liquid come out the tubing of **Port B**. Clamp the **Port B** tubing below the liquid line. Continue to gently push liquid into the chip until tubing attached to electrode **Port 1** is filled with liquid. Clamp **Port 1** tubing below the liquid line.
4. Cut the tubing attached to the syringe to approximately 1-inch from **Port A** and clamp.
5. Connect the tubing to **Port E**. Gently push liquid into the chip until 1-2 drops of liquid come out the tubing of **Port F**. Clamp the **Port B** tubing below the liquid line. Continue to gently push liquid into the chip until tubing attached to electrode **Port 3** is filled with liquid. Clamp **Port 3** tubing below the liquid line.
6. Cut the tubing attached to the syringe to approximately 1-inch from **Port E** and clamp.
7. Connect the tubing to **Port C**. Gently push liquid into the chip until 1-2 drops of liquid come out the tubing of electrode **Port 2**. Clamp the **Port 2** tubing below the liquid line. Continue to gently push liquid into the chip until tubing attached to Port D is filled with liquid. Clamp **Port D** tubing below the liquid line.
8. With one end of Tygon tubing still attached to **Port C**, slip the other end of the tubing off the needle. **All port tubes except Port C should be clamped.**
9. Connect the chip to the Pneumatic Primer by attaching the Port C tubing to the needle on the LuerLock connector on the manifold. *Note: Multiple chips can be primed simultaneously using the multiple port manifold, available from SynVivo (cat # 207001)*
10. Turn the knob on the controller box and adjust the pressure to 7.5 psi. Apply the pressure for ~20-25 minutes.
11. Turn off the pressure and cut the Tygon tubing connected to the Pneumatic Primer.
12. Allow the chip to incubate at 37° for a minimum of 20 minutes before introducing cells.

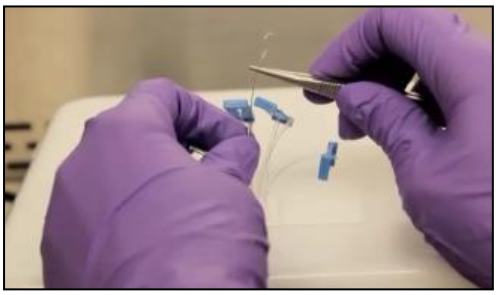
Measuring Resistance Z

Principle:

The SynVivo™ Cell Impedance Analyzer is used measure changes in electrical resistance between cell layers as a method of monitoring cellular tight junctions. **To measure the resistance of the cell layer, acquire measurements before and after culturing cells in the primed SynBBB chip.** Calculated the cell layer Z by subtracting the baseline resistance of the primed SynBBB chip (no cells) from the resistance of a SynBBB chip functionalized with cells. This is equivalent to subtracting the TEER of a blank cell culture insert from TEER of a functionalized insert in a 2-D cell culture system.

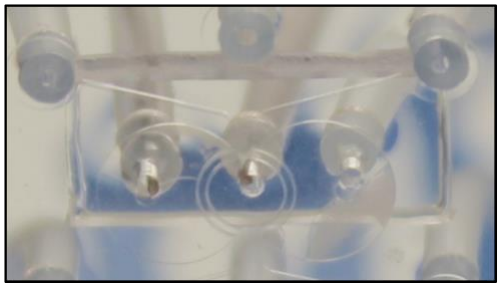
Equipment:

- SynBBB Impedance (TEER) Chips (Catalog # 102015-SB)
- SynVivo Impedance Analyzer (Catalog # 304001)
- AgCl Electrodes
- Hotplate (optional)

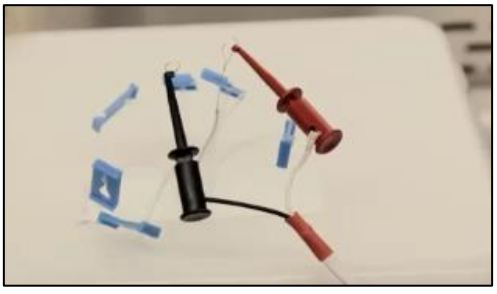


Protocol:

1. Place a 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. Clean two AgCl electrodes with 70% ethanol or isopropanol.



3. Using forceps, thread an electrode through electrode **Port 2** on the chip, pushing the electrode tip down in contact with the glass slide.
4. Thread the 2nd electrode into electrode **Port 1**. Ensure that both electrode tips are touching the glass slide.
5. Turn on the SynVivo Cell Impedance Analyzer by plugging in the power cord. The Analyzer will calibrate automatically.



6. Attach a micro-grabber clip to each electrode, being sure not to pull the electrodes out of place.
7. Select the desired operating frequency using the toggle located on the right side of the Analyzer (see Choosing an Operating Frequency on Page 4). Press the measurement button to display the impedance (Z) and phase values on the screen.
8. 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.
9. Repeat steps 3 through 7 on electrode **Port 3**.

Establishing Monoculture

Principle:

Shear-induced endothelial cell tight junctions, which cannot be achieved in a 2-D model, are readily achieved in the SynBBB model using fluid perfusion. Formation of tight junction changes can be measured using biochemical or electrical analysis (assessing changes in electrical resistance Z) with the SynVivo Cell Impedance Analyzer (SynVivo 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.

Equipment:

- 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
- Hemacytometer

Reagents:

- Endothelial Cells (primary or cell line)
- Complete Endothelial Media
- Dissociation reagents and neutralizers
- 1X PBS without calcium or magnesium
  or
- Serum-free endothelial cell basal medium

Protocol:

1. Endothelial cells should be dissociated, centrifuged, and concentrated to approximately 2-5x10^7 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 PBS or serum-free basal media 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 chip.
4. Remove the clamp on the outlet **Port B**.
5. Attach a 24-gauge blunt-tip needle to a 1 mL syringe. Cut 5-8 inches of Tygon tubing and attach one end to the needle.
6. Draw the cells up only into the Tygon tubing and mount the syringe 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 A**—the meniscus and the drop of water will form a bridge to prevent air from entering the chip as the tubing is inserted.
9. Clean the fluid from the surface of the chip.
10. Begin the injection at 3 µL/min.
11. Observe the chip as the cells are flowing. Once the vascular channel is filled with cells, clamp the outlet tubing (**Port B**) and stop the flow.
12. Cut the inlet tubing to separate the chip from the syringe.
13. Repeat this process for the other vascular channels using **Port E** and **F**. Cells should be uniformly dispersed at this moment.
14. Place the chip in the incubator for a minimum of 4 hours before changing media.
15. Media can be gently exchanged by hand via syringe, or exchanged via programmable syringe pump overnight using the following steps:
    a. Prepare a 1 mL syringe with endothelial cell media and attach a 24-gauge blunt-tip needle and a 12 to 20-inch length of Tygon tubing.
    b. Mount the syringe to a programmable syringe pump and connect the Tygon tubing to the SynBBB chip in the incubator.
    c. Program the following:
        i. Step 1: infuse 3 µL/min, 0:05:00 (5 minutes)
        ii. Step 2: pause 6:00:00 hours
        iii. Step 3: Repeat from Step 1, 5 times
    d. Run the program overnight
16. For a co-culture model, continue with “Establishing Co-Culture”. For a monoculture model, skip to “3D Shear Model”

Establishing Co-Culture

Principle:

Brain endothelial cells cultured in alongside astrocytes will form tighter junctions than brain endothelial cells alone. The following protocol is an example of co-culture using brain endothelial cells and astrocytes. This protocol may be adapted for any vascular-tissue model.

Equipment:

- 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 10 nl/min to 10 µl/min

- Hemacytometer

Reagents:

- Astrocytes
- Complete Astrocyte Media
- Dissociation reagents and neutralizers
- 1X PBS without calcium or magnesium
  or
- Serum-free basal medium

Protocol:

1. All tubing should be clamped before beginning this procedure.
2. Dissociate and re-suspend astrocytes at a concentration of approximately $1x10^7$ cells/mL.
3. Place a drop of 1X PBS at the base of inlet **Port C.**
4. Gently remove the tubing - the PBS should cover the port once the tubing is removed and should remain there until new Tygon tubing is inserted into the port to prevent air from entering the chip.
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. Push the cell suspension to the end of the tubing to form convex meniscus.
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 chip as the tubing is inserted.
9. Begin the injection at 5 µL/min.
10. Observe the chip as the cells are flowing. Once the tissue chamber is filled with cells, stop the flow and clamp the outlet tubing (**Port D**).
11. Cut the inlet tubing and allow the chip to incubate at 37 °C overnight.
12. The following day, gently exchange astrocyte media by hand.

3D Shear Model

Principle:

Endothelial channels grown under shear conditions form a 3D morphology (REF) and increase in junction tightness REF. The following program is used to condition endothelial cells grown in SynVivo® SynBBB chips to withstand shear. 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.

Equipment:

- SynBBB chip with endothelial cells

- Programmable syringe pump
- 1 mL syringe
- 24-Gauge blunt-tip needle
- Tygon tubing

Reagents:

- Endothelial Cell Culture Media
- 1X PBS

1. Prepare a 1 mL syringe with endothelial cell media and attach a 24-gauge blunt-tip needle and a 12 to 20-inch length of Tygon tubing.
2. Mount the syringe to a programmable syringe pump and connect the Tygon tubing to the SynBBB chip in the incubator.
3. Program the following:
    a. Step 1: Ramp
    b. Step 2: Constant
4. The SynBBB model is ready for assay and resistance Z measurement (see “Measuring Resistance Z” on page 8).