TECHNICAL MANUAL

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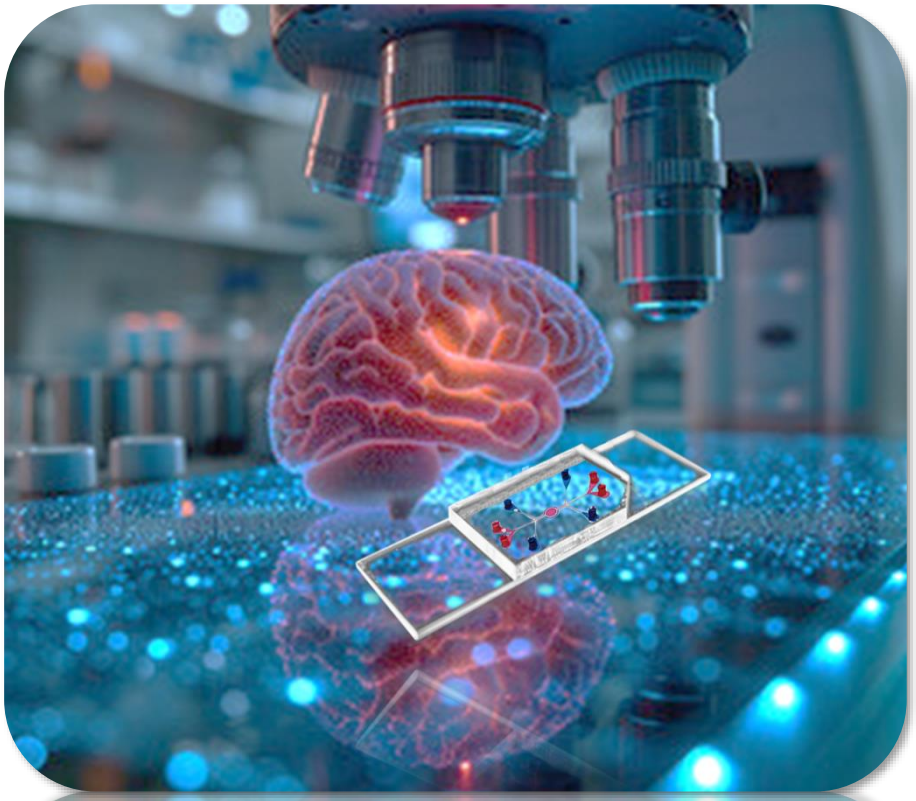
Organ-on-Chip Models



Primary SynBBB Blood-Brain-Barrier Model

3PLEX CONFIGURATION

For Chips and Starter Kits:
Catalog #s: 402014, 102003-SB3

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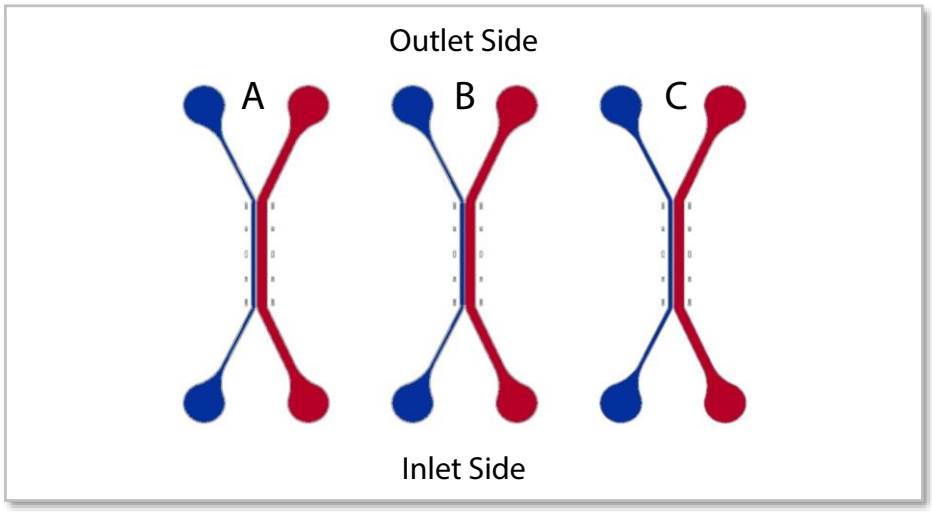
INTRODUCTION

Introduction

SynVivo's 3PLEX SynBBB 3D blood brain barrier model recreates the in vivo microenvironment by emulating a histological slice of brain tissue cells in communication with endothelial cells across the blood brain barrier (BBB). Shear-induced endothelial cell tight junctions are easily achieved in the SynBBB model using physiological fluid flow. Side-by-side architecture makes visualization of the interactions between brain tissue cells and endothelial cells possible. Each device contains three sets of channels for three experiments per slide. The SynBBB Protocol can be modified to accommodate cell sources alternative to the ones presented in these instructions.



Each device contains experimental units A, B, and C. Apical channels (blue) are for culture of endothelial cells while basolateral chambers (red) are for culture of astrocytes and pericytes. A porous barrier between basolateral and apical enables communication between the vascular and tissue cells.

The SynBBB system is a highly versatile platform for investigation of:

- Drug permeability: Evaluate real-time permeability of therapeutics and small molecules across the endothelium of the BBB.
- Omic changes: Perform genomic, proteomic and metabolic analysis on the BBB under normal conditions or in neurological disease.
- Neurotoxicity: Analyze toxicity effects of chemical, biological and physical agents on the cells of the BBB.

Primary Human Brain Microvascular Model Protocols

MONOCULTURE AND TRI-CULTURE

Workflow

The following workflow chart shows the basic steps of setting up the monoculture or tri-culture SynBBB model:

Monoculture SynBBB Model:

- Human brain microvascular endothelial cells (iXCells Biotech 10HU-051, Passage 1)

Tri-culture SynBBB Model:

- Human brain microvascular endothelial cells (iXCells Biotech 10HU-051, Passage 1)
- Human astrocytes (HA; ScienCell 1800, Passages 2 to 4)
- Human brain vascular pericytes (HBVP; ScienCell 1200, Passages 2 to 4)

Media and Reagents:

- 1X PBS without calcium or magnesium (Corning 21040CV)
- Poly-L-lysine (Sigma Aldrich P4707)
- Astrocyte medium (ScienCell 1801)
- Pericyte medium (ScienCell 1201)
- Endothelial cell growth medium (R&D Systems CCM027)
- 0.05% and 0.25% Trypsin-EDTA (ScienCell 0103)
- Trypsin neutralization solution (ScienCell 0113)
- Fibronectin, human plasma (Millipore Sigma FC010-1MG)

Equipment:

- Pneumatic Primer with Multiple Port Manifold (Synvivo 205001)
- Inverted Microscope
- Syringe pumps: remote-head and multichannel syringe pumps
- Cell Culture Incubator
- Scissors
- Forceps

Establishing the Basement Membrane

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

1. Place approximately 1-inch (2.54 cm) long segments of Tygon tubing into all the outlet ports.
2. Dilute fibronectin in sterile 1X PBS to a final concentration of 200 µg/ml.
3. Using a 1-mL syringe with approximately 5 inches of tubing attached, draw up the fibronectin solution into tubing.
4. Place the liquid-filled tubing into one inlet port.
5. Fill the channel with fibronectin.
6. Cut the inlet tubing.
7. Repeat process for the remaining inlet ports until all channels on the slide are perfused with fibronectin.
8. Place device in a 37° C incubator for 1 hour before priming with serum free media.
9. After incubation, completely perfuse the device with serum-free cell media using the Pneumatic Primer (see Pneumatic Primer-Technical Manual for details).

Seeding the Vascular Channel with Endothelial Cells

Dissociating endothelial cells from the flask:

1. Remove the T75 flask of human brain microvascular endothelial cells (HBMVECs; 85 to 95% confluent) from the incubator and aspirate the cell media from the flask.
2. Rinse the HBMVE with 5 mL of room temperature 1X PBS and aspirate.
3. Add 3 mL of 0.05% trypsin-EDTA to the T75 flask and place the flask in a 37° C incubator for 2 minutes.
4. After 2 minutes, check the cells under a microscope for cell rounding and detachment. If the cells are not rounded, place the flask into the incubator for 1 more minute.
5. Gently knock the flask to detach cells and add 6 mL of trypsin neutralizing solution then transfer the contents of the flask to a 15 mL conical tube.
6. Centrifuge the cells for 5 minutes at 200 g at room temperature.
7. Aspirate the supernatant and re-suspend the cell pellet in 2 mL of HBMVEC media.
8. Take a sample to count, and during counting, centrifuge the cells for 5 minutes at 200 g.
9. Resuspend the cell pellet in the volume of complete HBMVEC media to achieve $3x10^7$ cells/ml (typically 40 to 80 uL)
10. The cell solution should have the appearance of frosted glass, which will indicate a dense cell concentration.

Seeding HBMVECs

1. Attach a 24 G needle and 8 in Tygon tubing to a 1 mL syringe and mount the empty syringe onto the remote head syringe pump.
2. Unclamp the outlet ports of the three vascular channels. Leave the tissue channels clamped.
3. Place a drop of liquid beside the tubing for the first vascular inlet port.
4. Load the Tygon tubing of the mounted syringe with the prepared cell concentrate.
    a) Using the "Fast Reverse" button on the pump user interface, draw up approximately 1 inch of cell concentrate into the Tygon tubing of the mounted syringe.
    b) Using the "Fast Forward" function on the pump user interface, push the cell mixture forward until the concentrate liquid is flush with the end of the tubing.
5. Insert the tubing into the inlet port - the drop of water will prevent air entering the device as the tubing is inserted.
6. Inject the cells into the device at a flow rate of 3 µl/min.
7. Once the vascular channel is filled with cells, stop the flow and clamp the outlet tubing. Cell density should be consistent across the whole channel.
8. Carefully cut the inlet tubing, keeping the length of the inlet tubing as small as possible.
9. Repeat from step 13 for the remaining two vascular channels on the slide.
10. Place the device in a 37° C incubator for 4 hours before proceeding to flow.

Establishing Flow Model

After 4 hours of incubation at 37 °C, check the SynBBB device under the microscope for cell attachment.

1. For each experiment, prepare a 1-mL syringe with complete HBMVEC media (one slide will need 3 syringes). Attach a length of Tygon tubing to the syringe that is long enough to reach from the syringe pump to the device inside the tissue culture incubator.
2. Mount the syringes onto the syringe pump and perfuse the Tygon tubing completely, ensuring no air bubbles are in the tubing.
3. Place a drop of water at the base of the inlet port tubing to be removed and remove the tubing with forceps.
4. Remove the clamp on the outlet port of the vascular channel. Keep all other ports clamped. To capture the effluent during the media change, a small tube can be placed under the outlet tubing to serve as a waste reservoir.
5. While holding the end of the Tygon tubing above the level of the SynBBB device, use the "Fast Forward" function on the syringe pump to push the media forward until a droplet forms at the end of the tubing.
6. Insert the tubing into the inlet port. The drop of water will prevent air from entering the device as the tubing is inserted.
7. Clean the fluid from the surface of the device.
8. Start the syringe pump program (see Pump Programming below)
9. Incubate the device overnight in a 37° C incubator, 5% CO2.

Pump Programming

Monoculture SynBBB Instructions

If desired, perfuse the central tissue chamber with astrocyte conditioned media (ACM) by hand before beginning the flow program. The following program will establish a monoculture SynBBB model over 48 hours:

Table 1. Programming step increases in flow rate (0.02-0.1 µl/min)

STEP	Flow Rate (µl/min)	Time (h:m:s)	Direction
1	0.02	2:00:00	INFUSE
2	0.03	2:00:00	INFUSE
3	0.04	2:00:00	INFUSE
4	0.05	2:00:00	INFUSE
5	0.06	2:00:00	INFUSE
6	0.07	2:00:00	INFUSE
7	0.08	2:00:00	INFUSE
8	0.09	2:00:00	INFUSE
9	0.1	8:00:00	INFUSE

At the end of the program, the SynBBB device is ready for assay.

Pump Programming

Tri-culture SynBBB Instructions

The following program will begin flow overnight, then astrocytes and pericytes can be seeded into the tissue chamber the next day:

Table 2. Programming step increases in flow rate (0.02-0.0.05 µl/min)			
STEP	**Flow Rate (µl/min)**	**Time (h:m:s)**	**Direction**
1	0.02	2:00:00	INFUSE
2	0.03	2:00:00	INFUSE
3	0.04	2:00:00	INFUSE
4	0.05	12:00:00	INFUSE

When the flow program ends, continue to Seeding Astrocytes and Pericytes in the Tissue Chamber below.

Seeding Astrocytes and Pericytes in the Tissue Chamber

Dissociating astrocytes and/or pericytes from the flask:

1. Prepare two 50-mL conical tubes with 5mL of FBS in each tube (label one for astrocytes and one for pericytes). Prepare 20 mL of 0.025% trypsin-EDTA for dissociation.
2. Following the manufacturer's dissociation instructions, add 10 mL of 0.025% trypsin-EDTA solution to each T-75 flask and observe under the microscope until cells round up (approximately 1 minute).
3. Add 5 mL of trypsin neutralizing solution to each flask and transfer solutions into their respective 50-mL conical tubes with FBS.
4. Add an additional 5 mL of trypsin neutralizing solution to each flask, and transfer into their respective 50-mL conical tubes.
5. Centrifuge for 5 minutes at 200 g and remove supernatant.
6. Resuspend cells in 4 mL of either astrocyte or pericyte medium.
7. Take a sample from each tube to count, and during counting, centrifuge the cells for 5 minutes at 200 g.
8. Calculate the total number of cells and media to resuspend each cell pellet to a final concentration of 1 x 10^7 cells/mL (typically 200 to 800 uL)
9. Resuspend astrocytes at 1 x 10^7 cells/mL in astrocyte media. Resuspend pericytes at 1 x 10^7 cells/mL in pericyte media.
10. Mix astrocytes to pericytes in a ratio of 2:1 (typically 200 µl astrocytes to 100 µl pericytes should be adequate) into a 1.5 mL sample tube.

Seeding Astrocytes and Pericytes in the Tissue Chamber

Seeding Astrocytes & Pericytes:

1. Attach a 24 G needle and 8 in Tygon tubing to a 1 mL syringe and mount the empty syringe onto the remote head syringe pump.
2. Unclamp outlet ports of each of the tissue channels.
3. Place a drop of liquid beside the tubing for the first tissue channel inlet port and remove the tubing.
4. Load the Tygon tubing of the mounted syringe with the prepared cell concentrate.
    a) Using the "Fast Reverse" button on the pump user interface, draw up approximately 1 inch of cell concentrate into the Tygon tubing of the mounted syringe.
    b) Using the "Fast Forward" function on the pump user interface, push the cell mixture forward until the concentrate liquid is flush with the end of the tubing.
5. Insert the tubing into the inlet port - the drop of water will prevent air entering the device as the tubing is inserted.
6. Inject the cells into the device at a flow rate of 3 µl/min.
7. Once the tissue channel is filled with cells, stop the flow and clamp the outlet tubing. Cell density should be consistent across the whole channel
8. Carefully and quickly cut the inlet tubing off the syringe.
9. Repeat from step 3 with the remaining two tissue channels on the slide.
10. Incubate the device for 1 hour in a tissue culture incubator at 37° C (5% CO2) to allow the cells to attach to the device.
11. Once the astrocytes and/or pericytes have adhered to the tissue chamber (for 1 hour), re-attach the vascular channel to the HBMVEC media on the syringe pump, and begin the following program:

Table 3. Programming step increases in flow rate (0.05-0.0.1 µl/min)

STEP	Flow Rate (µl/min)	Time (h:m:s)	Direction
1	0.05	2:00:00	INFUSE
2	0.06	2:00:00	INFUSE
3	0.07	2:00:00	INFUSE
4	0.08	2:00:00	INFUSE
5	0.09	2:00:00	INFUSE
6	0.1	32:00:00	INFUSE

At the end of the flow program (72 hours after seeding HBMVECs), the SynBBB tri-culture model is ready for assay.

Additional Resources – Peer-Reviewed Publications

For further information on how the SynBBB model has been used in research, see the following supporting information:

1. Greene C, Rebergue N, Fewell G, Damir Janigro, Yann Godfrin, Campbell M, Sighild Lemarchant. 2024. NX210c drug candidate peptide strengthens mouse and human blood-brain barriers. Fluids and Barriers of the CNS. 21(1). doi:https://doi.org/10.1186/s12987-024-00577-x.
2. Huang J, Li YB, Charlebois C, Nguyen T, Liu Z, Bloemberg D, Zafer A, Baumann E, Sodja C, Leclerc S, et al. 2022. Application of blood brain barrier models in pre-clinical assessment of glioblastoma-targeting CAR-T based immunotherapies. Fluids and Barriers of the CNS. 19(1). doi:https://doi.org/10.1186/s12987-022-00342-y.
3. Brown TS, Nowak M, Bayles AV, Balabhaskar Prabhakarpandian, Pankaj Karande, Lahann J, Helgeson ME, Samir Mitragotri. 2019. A microfluidic model of human brain (μHuB) for assessment of blood brain barrier. Bioengineering & translational medicine. 4(2). doi:https://doi.org/10.1002/btm2.10126.
4. Tang Y, Soroush F, Sun S, Liverani E, Langston JC, Yang Q, Kilpatrick LE, Kiani MF. 2018. Protein kinase C-delta inhibition protects blood-brain barrier from sepsis-induced vascular damage. Journal of Neuroinflammation. 15(1). doi:https://doi.org/10.1186/s12974-018-1342-y.
5. Terrell-Hall TB, Nounou MI, Fatema El-Amrawy, Griffith JIG, Lockman PR. 2017. Trastuzumab distribution in an in-vivo and in-vitro model of brain metastases of breast cancer. Oncotarget. 8(48):83734–83744. doi:https://doi.org/10.18632/oncotarget.19634.