Human Blood Brain Barrier Modeling Kit

3D-HBBB
Cat. #8738

Product Description

The blood brain barrier (BBB) is a specialized capillary bed that separates the brain from the circulatory system and protects the brain from most pathogens [1]. Endothelial tight junctions supported by pericytes and astrocytes are primarily responsible for the highly selective nature of the BBB, restricting the passage of numerous solutes, most antibodies, and some antibiotics [2]. As such, efforts to understand the mechanisms underlying BBB integrity have been critical to developing techniques that are able to penetrate the BBB to deliver therapeutic or diagnostic molecules to the brain. Due to the complexities of the BBB, it is difficult to study in a 2-dimensional *in vitro* system, which inherently lacks multiple aspects of the physiological microenvironment. ScienCell™'s 3-dimensional blood brain barrier modeling kit (3D-BBB) co-cultures endothelial cells, pericytes, and astrocytes in collagen I matrix with a defined serum-free medium to replicate intercellular interactions at the BBB.

Kit Components (Included)

3D Cell Culture Components				
Cat #	**# of vials**	**Product Name**	**Quantity**	**Storage**
8000	1	Human Umbilical Vein Endothelial Cells (HUVEC)	5×10^5 cells	Liquid nitrogen
1200-3D	1	Human Brain Vascular Pericytes (HBVP-3D)	1×10^5 cells	Liquid nitrogen
1800	1	Human Astrocytes (HA)	1×10^6 cells	Liquid nitrogen
8001	2	3D Medium – basal – serum free (3D-M-SF)	100 mL	2-8 °C
8052	2	3D Growth Supplement (3D-GS)	1 mL	- 20 °C
0573	2	Penicillin/streptomycin Solution (P/S)	1 mL	- 20 °C
8708-a	1	Collagen I from rat tail, 4 mg/mL	10 mL	2-8 °C
8708-b	1	Buffer A, 10X	1.5 mL	2-8 °C
8708-c	1	Buffer B	1 mL	2-8 °C
8708-d	1	Sterile H_2O	5 mL	2-8 °C
2D Cell Culture Components				
Cat #	**# of vials**	**Product Name**	**Quantity**	**Storage**
8701	1	2D-BBB Coculture Medium – basal (2D-BBBCM)	500 mL	2-8 °C
8752	1	2D-BBB Growth Supplement (2D-BBBGS)	5 mL	-20 °C
0025	1	Fetal Bovine Serum (FBS)	25 mL	-20 °C
0503	1	Penicillin/Streptomycin Solution (P/S)	5 mL	-20 °C

Additional Recommended Materials (Not Included)

Cat #	Product Name
0183	0.05% Trypsin/EDTA (T/E)
0113	Trypsin Neutralization Solution (TNS)
0303	Dulbecco's Phosphate-Buffered Saline (DPBS)
0413	Poly-L-Lysine (10 mg/mL)
8248	Bovine Plasma Fibronectin
N/A	Normal TC-treated 24-well plate

Quality Control

3D-HBBB is tested for the formation of lumen-containing HUVEC tubules according to the included protocol. All components are negative for bacterial and fungal contamination.

Product Use

3D-HBBB is for research use only. It is not approved for human or animal use, or application in clinical or *in vitro* diagnostic procedures.

Shipping

8000, 1200-3D, 1800, 8052, 0573, 8752, 0025, and 0503 are shipped on dry ice. 8708-a, 8708-b, 8708-c, and 8708-d are shipped on gel ice. 8001 and 8701 are shipped at room temperature.

References

[1] Bernacki J, Dobrowolska A, Nierwiñska K, Maecki A. (2008) "Physiology and pharmacological role of the blood-brain barrier." *Pharmacological Reports*. 60: 600-622.

[2] Daneman R, Zhou L, Kebede A, Barres B. (2010) "Pericytes are required for blood–brain barrier integrity during embryogenesis." *Nature*. 468(7323): 562-566.

Procedure:

A. Initiating cells in 2D culture

Step I: Prepare the complete 2D-BBB coculture medium

1. Thaw 2D-BBB growth supplement (2D-BBBGS; Cat. #8752), fetal bovine serum (FBS; Cat. #0025), and penicillin/streptomycin solution (P/S solution; Cat. #0503) at 37°C. Add 2D-BBB GS, FBS and P/S solution to the 2D-BBB coculture medium-basal (Cat. #8701) and mix well.
    a. Warm the complete BBB coculture medium only to room temperature (instead of 37°C) prior to use.
    b. When stored in the dark at 4°C, the complete medium is stable for one month.

Step II: Thaw, maintain and sub-culture cells in 2D cell culture

2. For the human umbilical vein endothelial cells (HUVEC; Cat. #8000), one cryopreserved vial contains 5×10^5. It is recommended to plate directly into one fibronectin-coated **T-75** flask using the complete 2D-BBB coculture medium.
3. For the human brain vascular pericytes (HBVP-3D; Cat. #1200-3D), one cryopreserved vial contains 1×10^5. It is recommended to plate directly into one poly-L-lysine-coated **T-25** flask using the complete 2D-BBB coculture medium.
4. For the human astrocytes (HA; Cat. #1800), one cryopreserved vial contains 1×10^6. It is recommended to plate directly into one poly-L-lysine-coated **T-75** flask using the complete 2D-BBB coculture medium.

    Note: For detailed instructions on thawing and maintaining the HUVEC, HBVP, and HA in 2D culture, please see the product sheets Cat. #8000, #1200, and #1800, respectively.

B. Establishing 3D culture in type I collagen gel

Step III: Prepare the complete 3D medium

5. Thaw 3D-GS (Cat. #8052), and P/S solution (Cat. #0573) at 37°C. Add 3D-GS, and P/S solution to the 3D medium-basal (Cat. #8001) and mix well.
    a. Warm the complete 3D medium to room temperature (instead of 37°C) prior to use.
    b. When stored in the dark at 4°C, the complete medium is stable for one month.

Step IV: Harvest cells for 3D culture

Table A: An Example of Suggested Cell Number, and Collagen Gel Volume

# of samples in 24 well plate	HUVEC cell number	HBVP cell number	HA cell number	Collagen gel volume
1 sample	7.5×10^4	1.5×10^4	3.6×10^4	75 µL
10 samples	7.5×10^5	1.5×10^5	3.6×10^5	750 µL
24 samples	1.8×10^6	3.6×10^5	8.6×10^5	1.8 mL

Note: The ratio of HUVEC to HBVP to HA must be maintained to achieve the optimal tubule formation.

6. Please see Table A for the required cell number for different sample sizes. A confluent T-75 and T-25 flask should yield about 4 x 10^6 and 2 x 10^6 cells, respectively.
7. When the desired amount of cells have been achieved in 2D monolayer culture, you can prepare cells for 3D culture as described below.
8. Rinse the cells with DPBS.
9. Add 10 ml 0.05% T/E solution (Cat. #0183) into flask (in the case of a T-75 flask). Gently rock the flask to ensure complete coverage of cells by T/E solution. Use a microscope to monitor the change in cell morphology.
10. Transfer T/E solution from the flask to the 50 ml centrifuge tube (a small percent of cells may detach) and continue to incubate the flask at 37°C for another minute (no solution in the flask at this time).
11. At the end of incubation, gently tap the side of the flask to dislodge cells from the surface. Check under a microscope to make sure that all cells detach.
12. Add 5 ml of TNS solution to the flask (can alternatively add 10% FBS) and transfer detached cells to the 50 ml centrifuge tube. Rinse the flask with another 5 ml of TNS to collect the residual cells.
13. Count cells and aliquot the appropriate number of cells (for HUVEC, HBVP, and HA). Please refer to **Table A** to scale up/down appropriately for your experiments.
14. Centrifuge at 1,000 rpm for 5 minutes and remove the supernatant.
15. Save the pelleted cells and proceed to Step IV.

<u>Step V: Embed cells in collagen I gel</u>

16. Keep all components shown in Table B on ice.

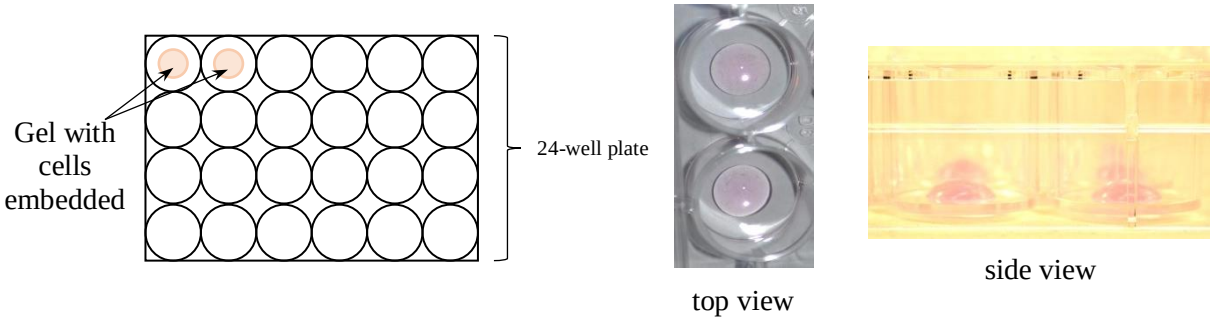
Table B: Preparation of ~1mL Collagen Gel (for 10 samples)

Components	Volume
Collagen I from rat tail, 4 mg/mL	625 µl
Buffer A, 10X	100 µl
Sterile H_2O	225 µl
Buffer B	50 µl

17. On ice, mix all components shown in Table B by adding **Buffer B** as the **LAST** component.
18. Immediately after the addition of Buffer B, resuspend cell pellets (from step 14) in 75 µL of collagen I gel (for one sample) by gently pipetting up and down for ~ 15 times using a p200 pipette.

    Note: Scale up cell number and collagen gel volume accordingly for your experiment (see Table A).

19. Slowly add 75 µl of cells/collagen gel mixture to the center of each well in the normal TC-treated 24 well plates as a dome.
    a. Once gel dots have been plated, do not tilt or move plate in hood for 5 minutes.
    b. The gel dots with embedded cells will approximate this diagram (left) and photos of gel dots properly plated from the top (middle) and viewed from the side (right).



Step VI: Collagen Gel Polymerization and Sample Incubation

20. Incubate the plate at 37°C for 1 hour to allow collagen gel polymerization.
21. After 1 hour, slowly add 750 µl of the complete 3D medium to the side of each well in the cell culture hood.
22. Incubate the plates at 37°C in a 5% CO_2 incubator.
23. Change the 3D medium every other day by removing the media using a pipette. (Do not use a vacuum aspirator to prevent disrupting the gel)
24. Observe cells; assay typically peaks around day 6 post seeding. Please see Figures 1 and 2 highlighting the direct cell-cell interactions among astrocytes, pericytes and endothelial tubules in 3D BBB model.

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Fig. 1 – Day 6; Endothelial marker CD31 (red), and pericyte marker PDGF receptor β (green). Purple arrows indicate PDGF receptor β+ pericyte investment on CD31+ endothelial tubules (at 400x magnification).

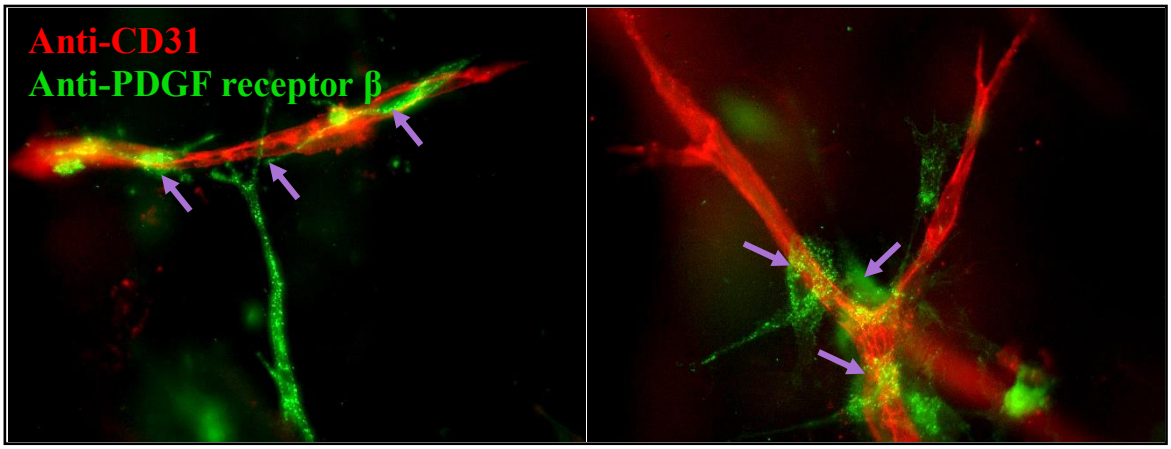
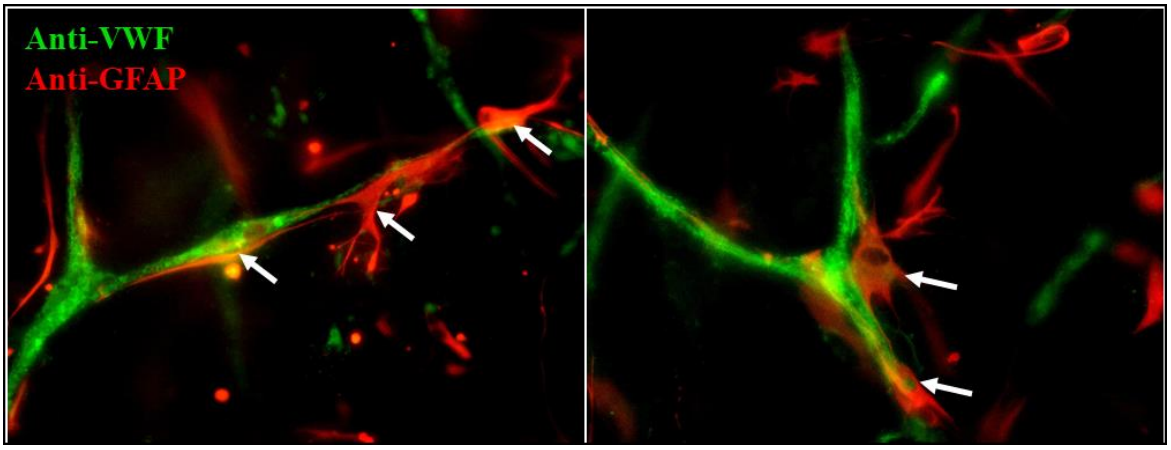


Fig. 2 – Day 6; Endothelial marker VWF (green), and astrocyte marker GFAP (red). White arrows indicate GFAP+ astrocyte investment on VWF+ endothelial tubules (at 400x magnification).



Troubleshooting Guide

Problem	Possible Cause	Potential Solution
Cells are not forming tubules.	Cells are not proliferating.	- Check cell viability (should be >90%) and cell population doubling. - Reduce extensive sub-culturing in 2D culture.
Cells are not forming tubules.	Collagen gel is floating or did not polymerize well.	- Avoid aggressive addition of complete 3D media to the gel. - Check the pH of the Collagen I solution (should be ~ 7.0 - 7.6).
Cells are assembling as clusters, instead of elongated tubules.	Cells are not resuspended well.	- Obtain single cell suspension in collagen gel by gently pipetting up and down for approximately 15 - 20 times.