



All-inclusive 3D Human Blood Brain Barrier Spheroid Formation Kit with HUVEC
3D-HBBBSF-HUVEC
 Cat. #3D-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. In ScienCell™'s 3-dimensional blood brain barrier spheroid model, HUVEC, pericytes, and astrocytes are co-cultured in low adhesion conditions, and self-assembled into 3D spheroids. Within spheroids, cells are able to interact with one another to recapitulate the key aspects of BBB, including the expression of ZO1 tight junction marker on the surface of the spheroids.

Kit Components (Included)

3D Cell Culture Components				
Cat #	# of vials	Product Name	Quantity	Storage
8000	1	Human Umbilical Vein Endothelial Cells (HUVEC)	5 x 10 ⁵ cells	Liquid nitrogen
1200-3D	1	Human Brain Vascular Pericytes (HBVP-3D)	1 x 10 ⁵ cells	Liquid nitrogen
1800	1	Human Astrocytes (HA)	1 x 10 ⁶ cells	Liquid nitrogen
3D-8701	1	3D-BBB Spheroid Medium – basal (3D-BBBSpM)	200 mL	2-8 °C
3D-8752	1	3D-BBB Spheroid Supplement (3D-BBBSpS)	2 mL	-20 °C
0010	1	Fetal Bovine Serum (FBS)	10 mL	-20 °C
0583	1	Penicillin/Streptomycin Solution (P/S)	2 mL	-20 °C
0343 (or) 0353 (or) 0383	2	Ultra-Low Binding Culture Plates (24-, 48-, or 96- well plate)	2 plates	RT
2D Cell Culture Components				
Cat #	# of vials	Product Name	Quantity	Storage
8701	1	2D-BBB Co-culture Medium – basal (2D-BBBCM)	500 mL	2-8 °C
8752	1	2D-BBB Co-culture Growth Supplement (2D-BBBCGS)	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 (PLL) (10 mg/mL)
8248	Bovine Plasma Fibronectin

Quality Control

3D-HBBBSF-HUVEC is tested for the formation of functional and uniform 3D BBB spheroids according to the included protocol. All components are negative for bacterial and fungal contamination.

Product Use

3D-HBBBSF-HUVEC are 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, 3D-8752, 0010, 0583, 8752, 0025, and 0503 are shipped on dry ice. 3D-8701, 8701, and [0343 (or) 0353 (or) 0383] are shipped at room temperature.

References

- [1] Bernacki J, Dobrowolska A, Nierwińska 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 co-culture medium

1. Thaw 2D-BBB co-culture growth supplement (2D-BBBCGS; Cat. #8752), fetal bovine serum (FBS; Cat. #0025), and penicillin/streptomycin solution (P/S solution; Cat. #0503) at 37°C. Add 2D-BBBCGS, FBS and P/S solution to the 2D-BBB co-culture medium-basal (2D-BBBCM; Cat. #8701) and mix well.
 - a. Warm the complete BBB co-culture medium only to room temperature 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 co-culture 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 co-culture 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 the complete 2D-BBB co-culture 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 spheroid culture

Step III: Prepare the complete 3D spheroid medium

5. Thaw 3D-BBB spheroid supplement (3D-BBBSpS; Cat. #3D-8752), fetal bovine serum (FBS; Cat. #0010), and penicillin/streptomycin solution (P/S solution; Cat. #0583) at 37°C. Mix 3D-BBBSpS, FBS and P/S solution into the 3D-BBB spheroid medium (3D-BBBSpM; Cat. #3D-8701) by gently swirling the medium bottle around.
 - a. 3D-BBBSpM medium is **viscous** and optimized for homogenous spheroid formation.
 - b. Warm the complete 3D-BBBSpM medium only **to room temperature** before use.
 - c. 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 Culture Volume per Sample

1	2	3	4	5
Plate formats	HUVEC cell number	HBVP cell number	HA cell number	3D Culture Volume per well
24-well	1.5×10^5 cells	1.5×10^5 cells	1.5×10^5 cells	~ 1000 μ L
48-well	7.5×10^4 cells	7.5×10^4 cells	7.5×10^4 cells	~ 500 μ L
96-well	3.2×10^4 cells	3.2×10^4 cells	3.2×10^4 cells	~ 200 μ L

- Please see **Table A** for the suggested cell densities for different plate formats. A confluent T-75 and T-175 flasks should yield about 5×10^6 and 1×10^7 cells, respectively.
- When desired amount of cells have been achieved in 2D monolayer culture, you can begin setting up 3D spheroid culture as described below.
- Rinse the cells with DPBS.
- Add 5 mL of DPBS and 5 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.
- 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).
- 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.
- Add 5 ml of TNS solution to the flask and transfer detached cells to the 50 ml centrifuge tube. Rinse the flask with another 5 ml of TNS to collect the residual cells.

Step III: Resuspend and seed cells in 3D cell culture medium

- Count cells using a hemacytometer. Please see **Table A** for the suggested cell numbers for HUVEC, HBVP, and HA for different plate formats.
- Aliquot the suggested number of cells for HUVEC, HBVP, and HA into a fresh conical tube.

Note: It is recommended to make a minimum of 5 mL cell suspension in 3D medium for easier pipetting due to the viscosity of 3D medium.

- Centrifuge the tube at 1000 rpm for 5 minutes.
- Aspirate the supernatant while leaving behind the 100-200 μ L supernatant above the pellet in the tube.
- Resuspend cells in the residual supernatant by pipetting up and down for ~ 10 times to obtain a single cell suspension.

18. Next, add the appropriate volume of the 3D-BBBSpM medium to obtain the suggested density of cell suspension (see **Table A; column 5**).
19. Slowly pipette up and down for ~ 10 times and make sure you have uniform cell suspension in 3D medium before proceeding to next step.

20. Add the suggested volume of cell mixture (see **Table A; column 5**) to each well in the provided ultra-low binding plate by using a p1000 pipette. In order to minimize pipette errors, do not use a serological pipette.
21. Incubate the cells at 37°C in a 5% CO₂ humidity incubator.
22. Change the 3D culture medium every 4-5 days by only changing 60-70% of the top layer of the medium using a pipette by hand (Do not use a vacuum aspirator).

23. Monitor the growth and formation of spheroid every day under the microscope. Mature BBB spheroids develop at ~ **3-5 days** post seeding (Figure 1, 2, and 3).

Figure 1 – Development and maintenance of human BBB spheroids over 21 days (taken at 100x magnification).

Figure 2 – Day 7; Top – Endothelial cell marker VWF (green), and astrocyte marker GFAP (red). Bottom – Endothelial cell marker CD31 (red) and pericyte marker PDGF receptor β (green) (taken at 200x magnification).

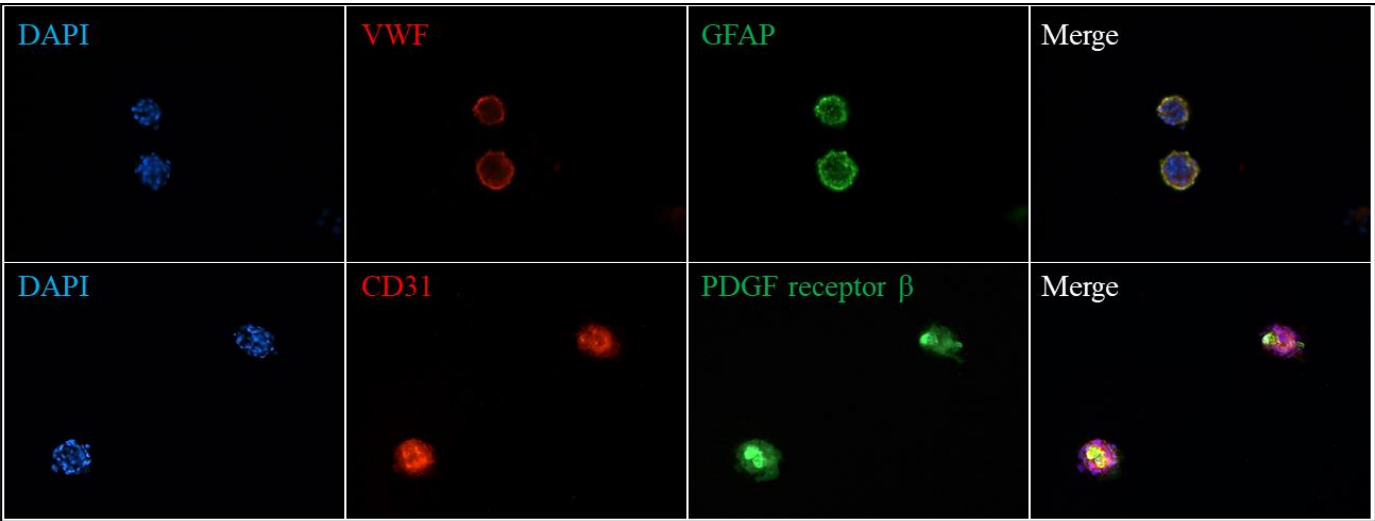
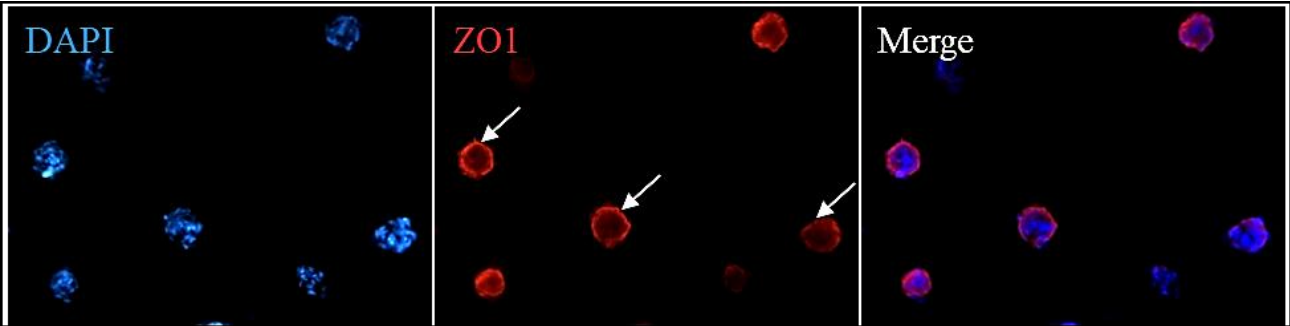


Figure 3 – Day 7; Expression of the tight junction marker ZO1 (red) on the surface on the human BBB spheroids (taken at 200x magnification).



Troubleshooting Guide

Problem	Possible Cause	Potential Solution
Cells do not form spheroids.	Cells are not healthy.	- Check cell viability (should be >90%) and cell proliferation using trypan blue. - Reduce extensive sub-culturing in 2D culture.
Spheroid formation is not homogenous.	1. Cells are not resuspended well. 2. Shelves in the cell culture incubator are not level.	- First, obtain single cell suspension in the residual supernatant by gently pipetting up and down for approximately 10-15 times (see step 17). - Next, obtain uniform cell suspension in 3D culture medium by pipetting up and down for approximately 10 – 15 times. Additionally, you can rotate the tube around to help mixing cells in 3D medium (see step 19). - Level your shelves of the CO₂ incubators.