Rev. 1

Ready-to-use 3D Human Blood Brain Barrier Spheroid Kit with human brain microvascular endothelial cells
SP3D-HBBBS-HBMEC

Cat. #SP3D-8768

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 ready-to-use 3D human blood brain barrier spheroid kit offers cryopreserved 3D multicellular BBB spheroids comprised of **human brain microvascular endothelial cells, brain vascular pericytes, and astrocytes** at a 1:1:1 ratio, recapitulating the intracellular interactions at BBB. A highly unique feature of this kit is that researchers can achieve the functional and homogenous 3D BBB spheroids in 24-48 hours after thawing (Fig. 1-3), without encountering the long and complex procedures involved in 3D cell culture.

Kit Components (Included)

3D Cell Culture Components				
Cat #	**# of vials**	**Product Name**	**Quantity**	**Storage**
SP-8768	1	Human Blood Brain Barrier Spheroids (SP-HBBBS)	1 x 10^4 spheroids	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	1	Ultra-Low Binding Culture Plates (24-, 48-, or 96- well plate)	1 plate	RT

Quality Control

SP3D-HBBBS-HBMEC 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

SP3D-HBBBS-HBMEC are for research use only. It is not approved for human or animal use, or application in clinical or *in vitro* diagnostic procedures.

Shipping

SP-8768, 3D-8752, 0010, and 0583 are shipped on dry ice. 3D-8701, and [0343 (or) 0353 (or) 0383] 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:

Step I: Preparing the complete 3D spheroid medium

1. 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 II: Thawing and maintaining the ready-to-use 3D spheroids

2. One frozen vial contains $\geq 1 \times 10^4$ spheroids, which is sufficient for plating into **half of a multiwell plate** (e.g. 24-, 48-, and 96-well ultra-low binding plate).

3. Place the frozen vial in a 37°C water bath. Hold and rotate the vial gently until the contents completely thaw. Promptly remove the vial from the water bath, wipe it down with 70% ethanol, and transfer it to the sterile field.

4. Carefully remove the cap without touching the interior threads. GENTLY pipette spheroid suspension up and down **one time** to disperse potential spheroid aggregates.

5. Gently transfer the spheroid suspension into a fresh 50 mL conical tube.

6. Add 12 mL of 3D culture medium to the above 50 mL conical tube.

7. Resuspend spheroids in 3D culture medium by gently pipetting up and down for ~ 5-7 times using a serological pipette.

    Note: 3D culture medium has a high viscosity; thus, pipetting slowly is important to avoid the formation of bubbles.

8. Aliquot the suggested volumes (see **Table A, column 2**) of spheroid suspension into each well of the included ultra-low binding plate (24-, or 48- or 96-well plate).

Table A: An Example of Suggested Medium Volumes Per Well

1	2	3
Plate formats	**Volume per well**	**Total number of wells**
24-well	~ 1000 µL	12 wells
48-well	~ 500 µL	24 wells
96-well	~ 250 µL	48 wells

9. Incubate spheroids at 37°C in a 5 % CO_2 incubator.

10. For best results, do not disturb the culture for at least 16 hours after the culture has been initiated.

11. Next day, change 60-70 % of the top layer of the medium using a pipette by hand to remove the residual DMSO. (Do not use a vacuum aspirator). After 1st medium change, change 60-70% of the top layer of the medium every 3-4 days.

    Note: Spheroids are situated at the bottom of the well due to the viscosity of the 3D culture medium. Thus, centrifugation of the plates is not necessary, and spheroid loss will not occur by changing 60-70 % of the top layer of the medium by pipetting.

12. Monitor the health of spheroids every day under the microscope. Human BBB spheroids are recovered and ready for your experiment around 1-2 days post thawing (see Figure 1).

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Figure 1 – At 100x magnification, brightfield images of ready-to-use 3D human BBB spheroids at after thawing.

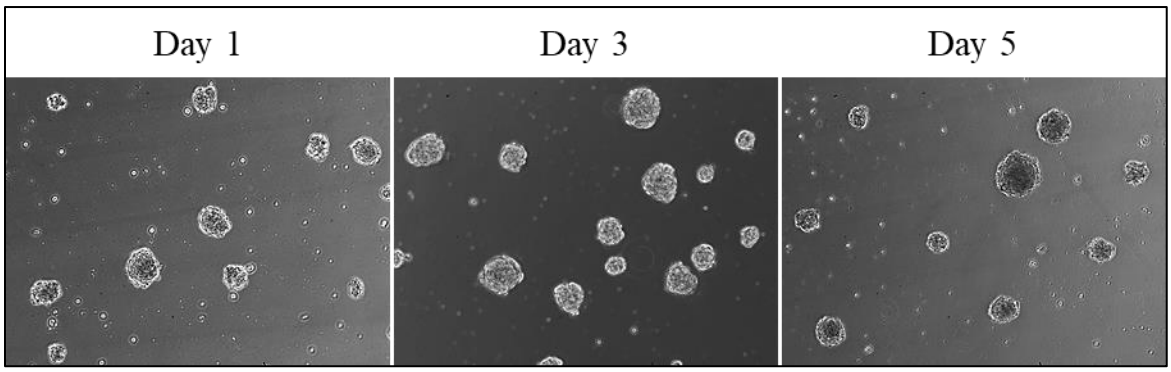

Figure 2 – Day 2; Top row – Nuclear stain DAPI (blue) and endothelial cell marker VWF (green). Middle row – Nuclear stain DAPI (blue) and astrocyte marker GFAP (green). Bottom row – Nuclear stain DAPI (blue) and pericyte marker PDGF receptor β (green) (taken at 200x magnification).

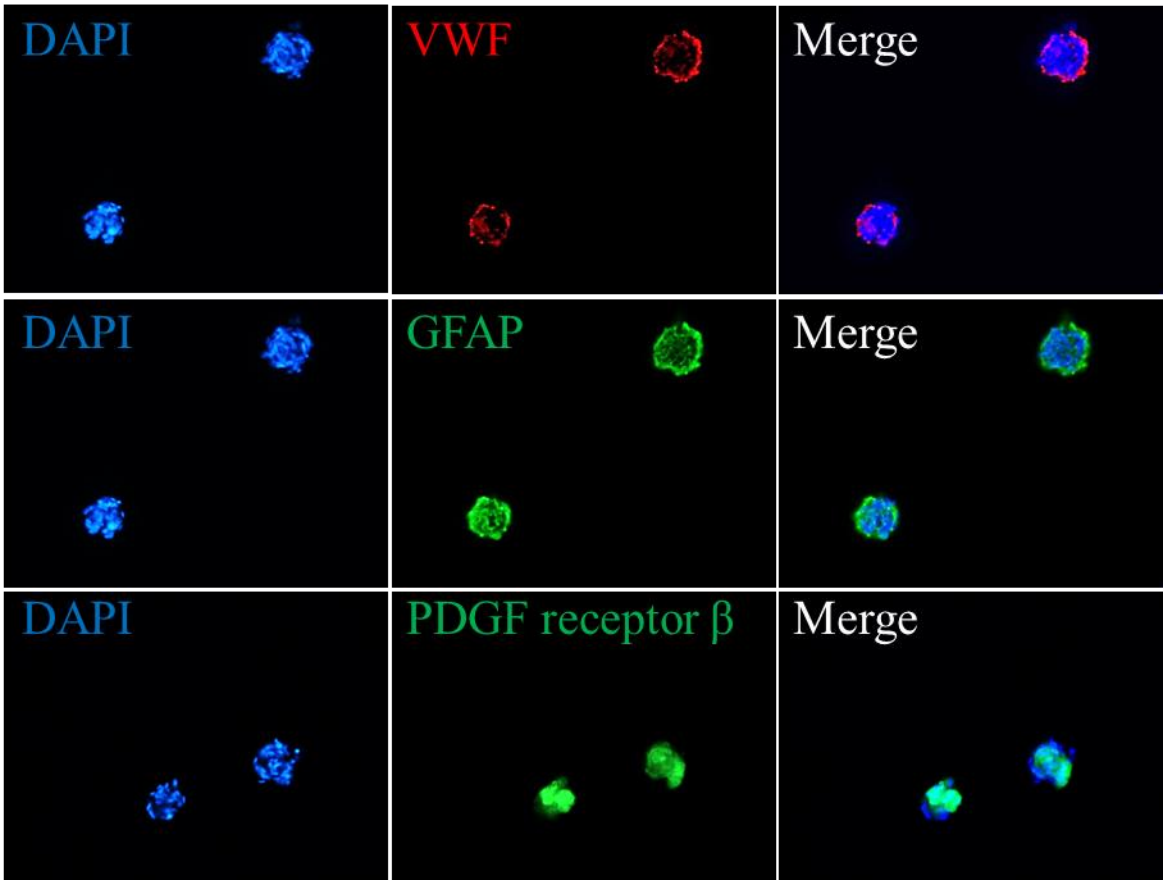

Figure 3 – Day 2; Expression of the tight junction marker ZO1 (red) on the surface on the human BBB spheroids (taken at 200x magnification).