All-inclusive 3D Rat Blood Brain Barrier Spheroid Formation Kit

3D-RBBBSF

Cat. #3D-R8738

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 rat blood brain barrier spheroid model, rat brain microvascular endothelial cells, 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**
R1000	1	Rat Brain Microvascular Endothelial Cells (RBMEC)	5 x 10^5 cells	Liquid nitrogen
R1200	1	Rat Brain Vascular Pericytes (RBVP)	5 x 10^5 cells	Liquid nitrogen
R1800	1	Rat Astrocytes (RA)	5 x 10^5 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-cs	1	Calf Serum (CS)	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-cs	1	Calf Serum (CS)	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-RBBBSF 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-RBBBSF is for research use only. It is not approved for human or animal use, or application in clinical or *in vitro* diagnostic procedures.

Shipping

R1000, R1200, R1800, 3D-8752, 0010-cs, 0583, 8752, 0025-cs, 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, 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 co-culture medium

1. Thaw 2D-BBB co-culture growth supplement (2D-BBBCGS; Cat. #8752), Calf serum (CS; Cat. #0025-cs), 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 rat brain microvascular endothelial cells (RBMEC; Cat. #R1000), 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 rat brain vascular pericytes (RBVP; Cat. #R1200), one cryopreserved vial contains 5×10^5. It is recommended to plate directly into one poly-L-lysine-coated **T-75** flask using the complete 2D-BBB co-culture medium.

4. For the rat astrocytes (RA; Cat. #R1800), one cryopreserved vial contains 5×10^5. It is recommended to plate directly into one poly-L-lysine-coated **T-75** flask using the complete 2D-BBB co-culture medium.

    Note: For detailed instructions on thawing and maintaining the RBMEC, RBVP, and RA in 2D culture, please see the product sheets Cat. #R1000, #R1200, and #R1800, 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), calf serum (CS; Cat. #0010-cs), and penicillin/streptomycin solution (P/S solution; Cat. #0583) at 37°C. Mix 3D-BBBSpS, CS and P/S solution into the 3D-BBB spheroid medium (3D-BBBSpM; Cat. #3D-8701) by 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	**RBMEC cell number**	**RBVP cell number**	**RA cell number**	**3D Culture Volume per well**
24-well	1.3×10^5 cells	1.3×10^5 cells	1.3×10^5 cells	~ 1000 µL
48-well	6.0×10^4 cells	6.0×10^4 cells	6.0×10^4 cells	~ 500 µL
96-well	2.6×10^4 cells	2.6×10^4 cells	2.6×10^4 cells	~ 200 µL

Note: The ratio of RBMEC to RBVP to RA must be maintained to achieve the compact spheroid formation.

6. 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 x 10^6 and 1 x 10^7 cells, respectively.

7. When desired amount of cells have been achieved in 2D monolayer culture, you can begin setting up 3D spheroid culture as described below.

8. Rinse the cells with DPBS.

9. 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.

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 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 culture medium

13. Count cells using hemacytometer. Please see **Table A** for the suggested cell numbers for RBMEC, RBVP, and RA for different plate formats.

14. Aliquot the suggested number of cells for RBMEC, RBVP, and RA into a fresh 50 ml 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.

15. Centrifuge the tube at 1000 rpm for 5 minutes.

16. Aspirate the supernatant while leaving behind the 100-200 µl supernatant above the pellet in the tube.

17. 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.

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

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. Do not use the serological pipette to minimize pipette errors while adding small volumes to the wells.

21. Incubate the cells at 37°C in a 5% CO_2 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).

    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.

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

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

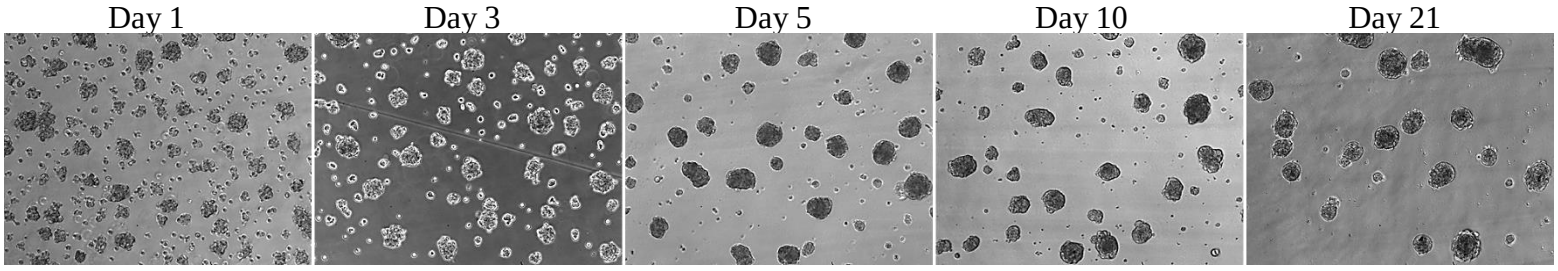
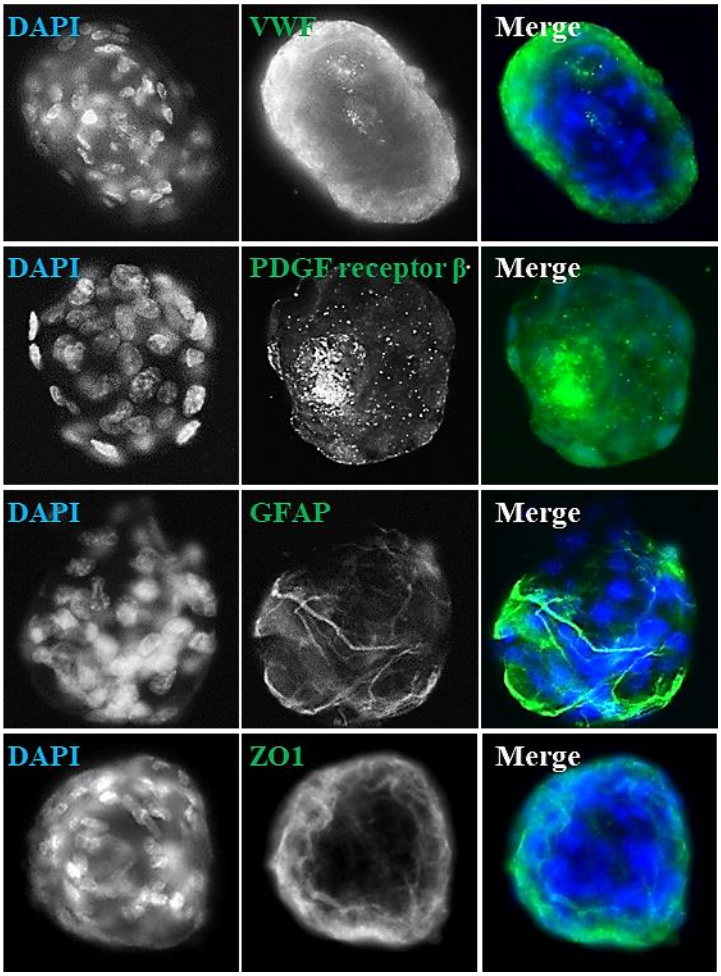


Fig. 2 – Day 7; Immunofluorescence analysis of rat blood brain barrier spheroids. (A) VWF+ rat brain microvascular endothelial cells are localized on the surface of the BBB spheroids. (B) Next, PDGF receptor β+ rat pericytes are enriched on the outer rim and inner core of the spheroids. (C) GFAP+ rat astrocytes are present throughout the spheroids. (D) Tight junction marker ZO-1 is present on the surface of the spheroids, recapitulating the key barrier function of BBB.



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.	- 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).
	2. Shelves in the cell culture incubator are not level.	- Level your shelves of the CO_2 incubators.