All-Inclusive 3D Human Cortical Spheroid Formation Kit

3D-HCSF
Cat. #3D-1520

Product Description

Damage to the central nervous system (CNS) has a life-threatening impact on humans, and there is a high demand for relevant models to study CNS diseases and therapeutic strategies. Mature neurons and astrocytes in the brain together form a complex three-dimensional network system [1]. Astrocytes *in vivo* regulate blood flow, provide energy to neurons, and supply the building blocks of neurotransmitters, which support the formation of functional synapses [2]. The complex cellular arrangement of the 3D central nervous system are unlikely to be recapitulated in the 2D cultures in which cells display the planar morphology and have the cellular interactions only in the lateral direction. To create a more biologically relevant *in vitro* brain model, ScienCell has developed the 3D human cortical spheroid model which contains human primary neurons, and astrocytes. Immunofluorescence analysis revealed that the 3D human cortical spheroids maintain direct cell-cell interactions between neurons and astrocytes and form functional synapses throughout the spheroids (Figures 1 and 2). Importantly, in the defined, serum-free spheroid medium, the neurons are networked with quiescent astrocytes, a phenotype closely resembling in vivo astrocytes. The quiescent astrocytes are critical for synapse function and neural development. Therefore, ScienCell's 3D human cortical spheroids provide a simple and highly functional 3D brain model for the study of CNS functions, diseases, and therapeutics.

Kit Components (Included)

3D Cell Culture Components				
Cat #	**# of vials**	**Product Name**	**Quantity**	**Storage**
1520	1	Human Neurons (HN)	1 x 10^6 cells	Liquid nitrogen
1800	1	Human Astrocytes (HA)	1 x 10^6 cells	Liquid nitrogen
3D-1521	1	3D-Neuronal Spheroid Medium – basal (3D-NSpM)	200 mL	2-8 °C
3D-1562	1	3D-Neuronal Spheroid Supplement (3D-NSpS)	4 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**
1801	1	Astrocyte Medium – basal (AM)	500 mL	2-8 °C
1852	1	Astrocyte Growth Supplement (AGS)	5 mL	-20 °C
0010	1	Fetal Bovine Serum (FBS)	10 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)

Quality Control

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

Product Use

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

Shipping

1520, 1800, 3D-1562, 0583, 1852, 0010, and 0503 are shipped on dry ice. 3D-1521, 1801, and [0343 (or) 0353 (or) 0383] are shipped at room temperature.

References

[1] Zhuang P, Sun AX, An J, Chua CK, Chew SY. (2018) "3D Neural Tissue Models: From Spheroids to Bioprinting." *Biomaterials.* 154: 113-133.

[2] Eroglu C, Barres BA. (2010) "Regulation of synaptic connectivity by glia." *Nature.* 468(7321): 223-231.

Procedure:

IMPORTANT NOTE: For human neurons (Cat. #1520), ***do not plate*** them in 2D culture. Thaw and use them directly in 3D culture.

A. Initiating astrocytes in 2D culture

Step I: Prepare the complete astrocyte medium

1. Thaw astrocyte growth supplement (AGS; Cat. #1852), fetal bovine serum (FBS; Cat. #0010), and penicillin/streptomycin solution (P/S solution; Cat. #0503) at 37°C. Add AGS, FBS and P/S solution to the astrocyte medium-basal (AM; Cat. #1801) and mix well.

    a. Warm the complete 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 astrocytes in 2D cell culture

2. 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 astrocyte medium.

    a. For detailed instructions on thawing and maintaining the HA in 2D culture, please see the product sheets **Cat. ##1800**.

    b. Thaw human astrocytes **3-4 days** prior to performing the 3D culture. When they become confluent, astrocytes can be cultured together with neurons in 3D culture.

B. Establishing 3D spheroid culture

Step III: Prepare the complete 3D spheroid medium

3. Thaw 3D-neuronal spheroid supplement (3D-NSpS; Cat. #3D-1562), and penicillin/streptomycin solution (P/S solution; Cat. #0583) at 37°C. Mix 3D-NSpS and P/S solution into the 3D-neuronal spheroid medium (3D-NSpM; Cat. #3D-1521) by gently swirling the medium bottle around.

    a. 3D-NSpM medium is **viscous** and optimized for homogenous spheroid formation.
    b. Warm the complete 3D-NSpM 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
Plate formats	**HN cell number**	**HA cell number**	**3D Culture Volume per well**
24-well	8.1×10^4 cells	5.7×10^5 cells	~ 1000 µL
48-well	3.2×10^4 cells	2.3×10^5 cells	~ 500 µL
96-well	1.4×10^4 cells	9.5×10^4 cells	~ 200 µL

4. Please see **Table A** for the suggested cell numbers for 3D culture.

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

    a. Note: for human neurons, ***do not plate*** them in 2D cell culture. Instead, <u>thaw and use them directly</u> in 3D culture.

6. To harvest astrocytes from 2D culture, rinse the cells with DPBS.

7. 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.

8. 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^{o}C for another minute (no solution in the flask at this time).

9. 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.

10. 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. Count astrocytes using a hemocytometer.

11. After harvesting astrocytes, **thaw neurons** by placing the frozen vial in a 37°C water bath.

    a. 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. Carefully remove the cap without touching the interior threads.
    b. Count neurons using a hemocytometer.

<u>Step V: Resuspend and seed cells in 3D cell culture medium</u>

12. Count astrocytes and neurons using a hemocytometer.

13. Aliquot and combine the suggested number of cells (shown in table A) for neurons and astrocytes into a fresh conical tube.

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

14. Centrifuge the tube at 1100 rpm for 5 minutes.

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

16. Resuspend cells in the residual supernatant by pipetting up and down for ~ 10 times to obtain a single cell suspension.

17. Next, add the appropriate volume of the 3D-NSpM medium to the cells.

18. 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.

19. Add the suggested volume of cell mixture (see **Table A; column 4**) 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.

20. Incubate the cells at 37°C in a 5% CO_2 humidity incubator.

21. 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.

22. Monitor the growth and formation of spheroids every day under the microscope. Mature cortical spheroids develop at ~ **4 days** post seeding (Figure 1).

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

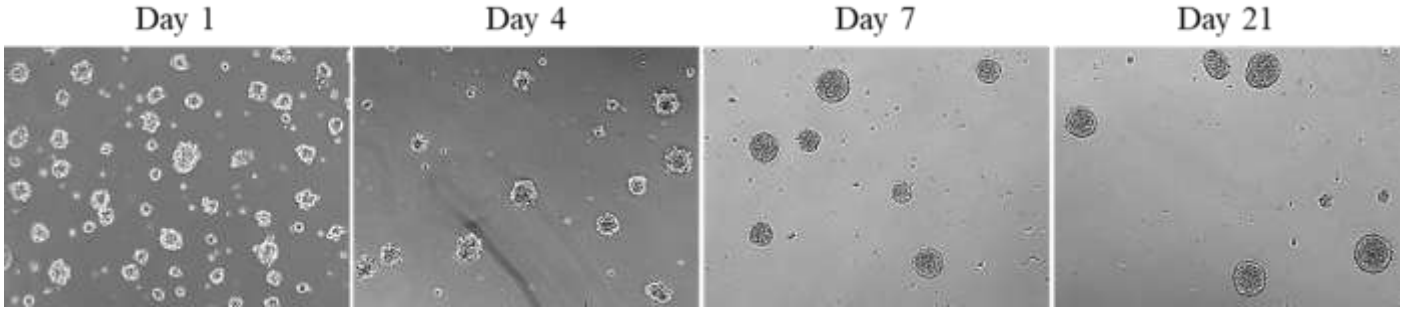
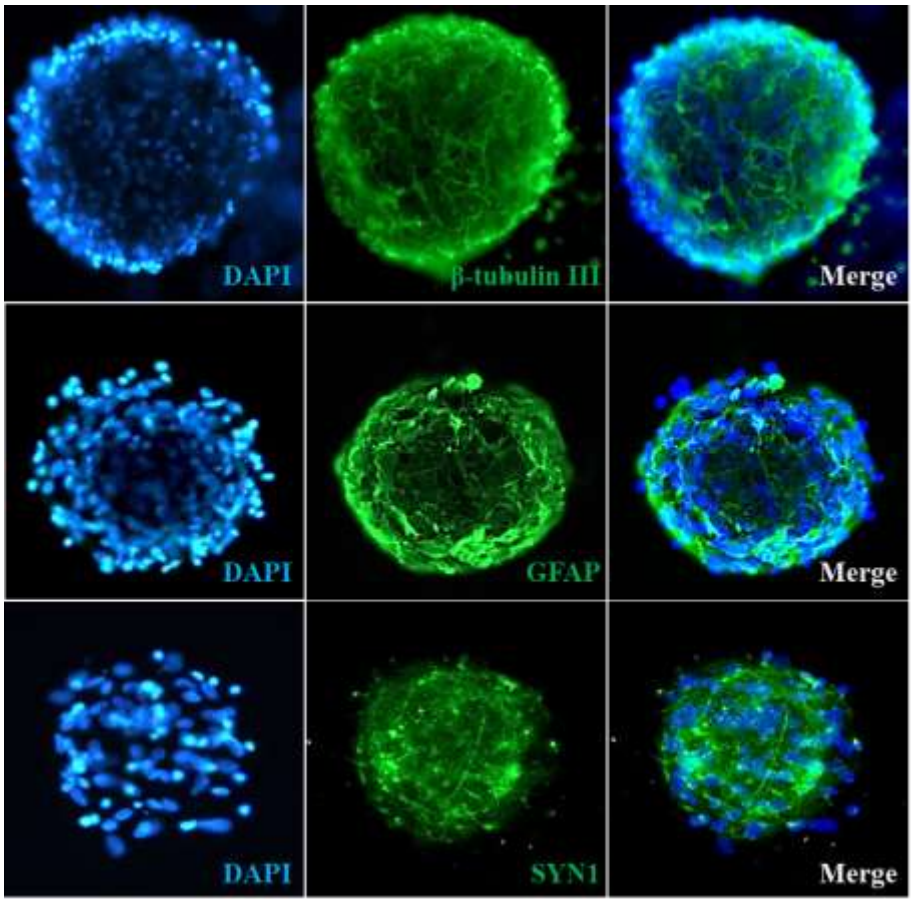


Fig. 2 – At days 7; immunostaining of the human cerebral cortical spheroids with the neuronal marker β-tubulin III, astrocyte marker GFAP and synaptic marker SYN1.



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.