Rev. 0

Ready-to-use 3D Human Sertoli Cell Spheroids

SP3D-HSerC
Catalog #SP3D-4520

Product Description

The basolateral region of the seminiferous tubules is lined with large epithelial supporting cells known as Sertoli cells. This cell type plays a significant role in the process of spermatogenesis. Sertoli cells facilitate various aspects of germ cell progression into mature spermatozoa, including providing nutritional and energy support to the developing germ cells (1). Tight junctions formed between adjacent Sertoli cell make up the blood-testis barrier (BTB) or Sertoli cell barrier that is unique to the testis. This physical barrier plays an immunoprotective role in the testis by sequestering the spermatocytes and spermatids (2). The BTB also serves as a crucial barrier to prevent the introduction of external factors into the seminiferous tubules. Validation of the capability for male contraceptives to reach its target germ cells, as well as potential interference with male fertility as a side-effect of certain drugs necessitates a model that delineates the blood-testis-barrier *in vitro*. To provide a more native *in vitro* blood-testis-barrier model, ScienCell offers ready-to-use human Sertoli cell spheroids (SP3D-HSerC), which expresses junction markers such as Claudin-5. These spheroids are ready for experiments at 24-48 hours post thawing, and are an excellent *in vitro* model for studying the male fertility and drug penetration.

Kit Components (Included)

3D Cell Culture Components				
Cat #	**# of vials**	**Product Name**	**Quantity**	**Storage**
SP-4520	1	Human Sertoli Cell Monoculture Spheroids (SP-HSerC)	1 x 10^4 spheroids	Liquid nitrogen
3D-4521	1	3D-Sertoli Spheroid Medium – basal (3D-SerSpM)	200 mL	2-8 °C
3D-4572	1	3D-Sertoli Spheroid Supplement (3D-SerSpS)	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-HSerC is tested for the formation of functional and uniform 3D Sertoli Cell spheroids according to the included protocol. All components are negative for bacterial and fungal contamination.

Product Use

SP3D-HSerC 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-4520, 3D-4572, 0010, and 0583 are shipped on dry ice. 3D-4521, and [0343 (or) 0353 (or) 0383] are shipped at room temperature.

References

[1] Shi JF, Li YK, Ren K, Xie YJ, Yin WD, Mo ZC. (2017) "Characterization of cholesterol metabolism in Sertoli cells and spermatogenesis." *Molecular Medicine Reports*. 17 (1): 705-713.
[2] Washburn RL, Hibler T, Kaur G, Dufour JM. (2022) "Sertoli Cell Immune Regulation: A Double-Edged Sword." *Front Immunol*. 13: 913502.

Procedure:

Step I: Preparing the complete 3D spheroid medium

1. Thaw 3D-Sertoli spheroid supplement (3D-SerSpS; Cat. #3D-4572), fetal bovine serum (FBS; Cat. #0010), and penicillin/streptomycin solution (P/S solution; Cat. #0583) at 37°C. Mix 3D-SerSpS, FBS and P/S solution into the 3D-Sertoli spheroid medium (3D-SerSpM; Cat. #3D-4521) by gently swirling the medium bottle around.

    a. 3D-SerSpM medium is **viscous** and optimized for homogenous spheroid formation.
    b. Warm the complete 3D-SerSpM 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 sertoli spheroids are recovered and ready for your experiment after 2-3 days post thawing (see Figure 1).

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Figure 1 – Ready-to-use human Sertoli spheroids at 24-72 hours after thawing (taken at 100x magnification)

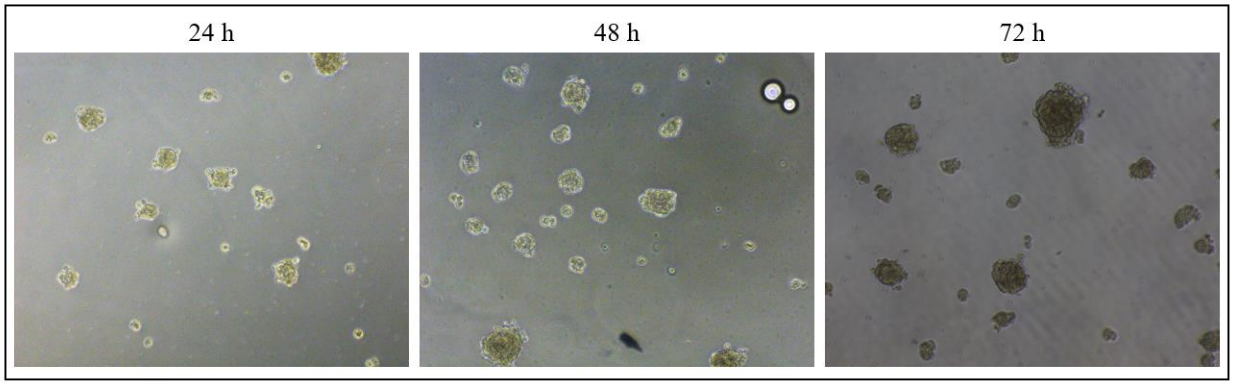

Figure 2 – At days 2; immunostaining of the human Sertoli spheroids with the Sertoli cell markers SOX9 and GATA4, and junction marker Claudin-5 (taken at 200x magnification).