Rev. 2

Ready-to-use 3D Hepatocyte-Stellate Cell Spheroids
SP3D-HSteCS

Cat. #SP3D-5300

Product Description

Liver is a complex unit formed by parenchymal cells (hepatocytes) and non-parenchymal cells (e.g. hepatic stellate cells, endothelial cells, immune cells, etc.) [1]. Hepatocytes are primarily responsible for drug metabolism and a range of functions, while hepatic stellate cells support hepatocytes by producing extracellular matrix, and mediate inflammatory responses during liver injury [2]. The contribution of non-parenchymal cells, however, is not accounted for in monocultures of hepatocytes. Recent studies have highlighted the importance of hepatic stellate cells and their contribution to drug toxicity and overall liver responses using the hepatocyte/stellate cell co-culture models [1 and 2]. To more closely mimic the cellular complexity of the liver, ScienCell has developed the ready-to-use 3D liver co-culture spheroids composed of hepatocytes and hepatic stellate cells at a 2:1 ratio. In 3D culture, hepatic stellate cells not only support the formation of compact hepatocyte spheroids by matrix remodeling, but also significantly improve the liver-specific functions (Figures 1 and 2). For example, the mRNA expression levels of phase I and II enzymes (CYP3A4, CYP2D6, GSTT1, and ABCB11), and hepatic markers (CASR, PPARA, HNF4A, and ALB) are significantly higher in hepatocyte-stellate cell co-culture spheroids (SP3D-HSteCS) versus the 3D hepatocyte monocultures (Figure 2). SP3D-HSteCS, therefore, are a more integrated co-culture system designed to study the complex cellular crosstalk or can be used as a tool to prolong hepatocyte function in a defined, serum-free 3D medium.

Kit Components (Included)

3D Cell Culture Components				
Cat #	**# of vials**	**Product Name**	**Quantity**	**Storage**
SP-5300	1	Human Hepatocyte-Stellate Cell Coculture Spheroids (SP-HHSCS)	1×10^4 spheroids	Liquid nitrogen
3D-5201	1	3D-Liver Spheroid Medium (3D-LSpM)	200 mL	2-8 °C
3D-5252	1	3D-Liver Spheroid Supplement (3D-LSpS)	4 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-HSteCS is tested for the formation of functional and uniform 3D human hepatocyte-stellate cell co-culture spheroids according to the included protocol. All components are negative for bacterial and fungal contamination.

Product Use
SP3D-HSteCS 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-5300, 3D-5252, 0583 are shipped on dry ice. 3D-5201, and (0343 or 0353 or 0383) are shipped at room temperature.

References
[1] Abu-Absi SF, Hansen LK, and Hu W. (2004) "Three-dimensional co-culture of hepatocytes and stellate cells." *Cytotechnology* 45: 125-140.
[2] Baze A. et. al. (2018) "Three-Dimensional Spheroid Primary Human Hepatocytes in Monoculture and Coculture with Nonparenchymal Cells." *Tissue Engineering.* 24(9): 534-545.

Procedure:

Step I: Preparing the complete 3D culture medium

1. Thaw 3D-liver spheroid supplement (3D-LSpS; Cat. #3D-5252), and penicillin/streptomycin solution (P/S solution; Cat. #0583) at 37°C. Mix 3D-LSpS, and P/S solution into the 3D-liver spheroid medium (3D-LSpM medium; Cat. #3D-5201) by gently swirling the medium bottle around.

    a. 3D-LSpM medium is **viscous** and optimized for homogenous spheroid formation.
    b. Warm the complete 3D-LSpM medium 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 multi-well plate (e.g. 24-, 48-, and 96-well ultra-low binding culture 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 the spheroid suspension up and down **two times** to disperse potential spheroid aggregates.

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

6. Add the 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 bubble formation.

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

Table A: An Example of Suggested Medium Volumes

1	2
Plate formats	**Volume per well**
24-well	~ 1000 µL
48-well	~ 500 µL
96-well	~ 250 µL

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, no additional medium changes are necessary.

    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. Hepatocyte-stellate cell co-culture spheroids are recovered and ready for experiments after 24 hours post thawing (see Figure 1).

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Fig. 1 – Brightfield images of human hepatocyte-stellate cell co-culture spheroids at 24 hours after thawing (at 100x magnification).

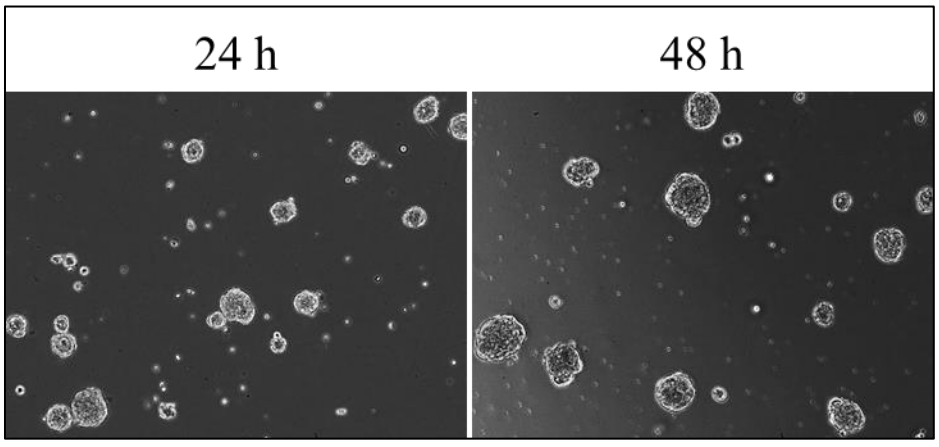

Fig. 2 – Day 7; Hepatocyte-stellate cell co-culture spheroids express the hepatocyte markers such as albumin (ALB) and cytokeratin 18 (CK18) (at 400X magnification).

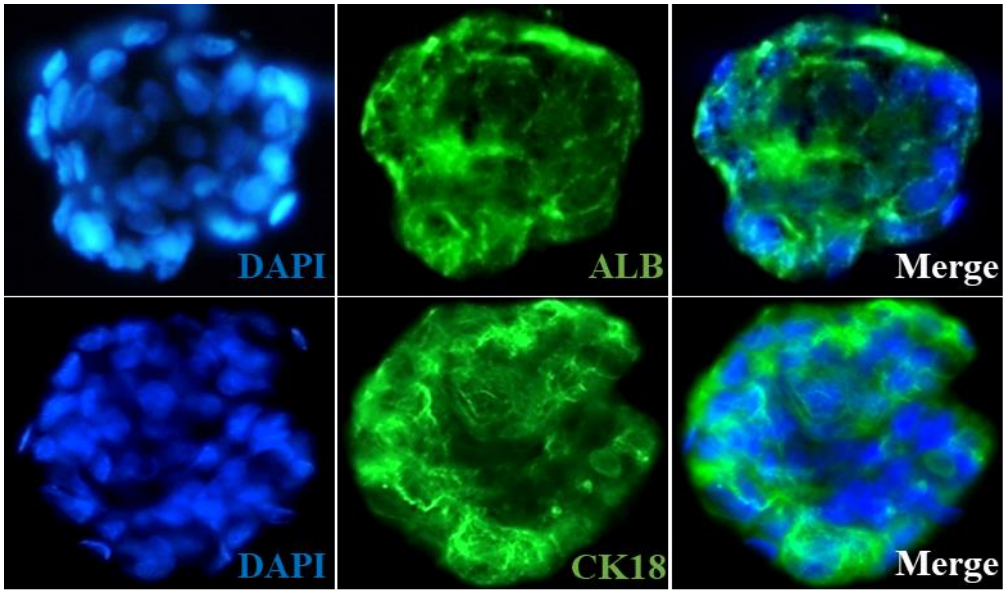

Fig. 3 – qPCR analysis shows that 3D hepatocyte-stellate cell co-culture spheroids are more functional and metabolically active, compared to 3D hepatocyte monoculture spheroids.