Rev. 1

Ready-to-use 3D Human Hepatic Stellate Cell Monoculture Spheroids
SP3D-HHSteCMS

Cat. #SP3D-8750

Product Description

Liver fibrosis is the typical response to liver injuries. It is characterized by an excessive deposition of extracellular matrix (ECM) protein, which impairs normal liver function and can ultimately lead to cirrhosis and organ failure [1, 2]. Activated hepatic stellate cells (HSC) are the primary source of excess ECM in liver fibrosis [1, 2]. In the normal liver, HSC are in a quiescent state and store retinoids. Following liver injury, HSC are activated and transdifferentiate into a myofibroblast-like phenotype [1, 2]. At present, it is not yet clear which specific genes are responsible for initiating and maintaining the fibrotic response. The *in vitro* liver models are commonly used to study liver fibrosis. HSC, however, are always activated in 2D monolayer culture, impeding the investigation on the role of HSC during liver diseases. ScienCell has developed ready-to-use 3D hepatic stellate cell monoculture spheroids (SP3D-HHSteCMS) using human primary hepatic stellate cells (Fig. 1). Our immunostaining data shows the clearance of collagen deposition and the α-SMA expression after culturing HSC in 3D culture for a week (Fig. 2 and 3). Therefore, culturing HSC in a physiologically-relevant 3D environment brings these cells back to their native quiescent state. ScienCell's HSC spheroids are, therefore, great models for studying the signaling pathways that govern the hepatic stellate cell activation process during liver diseases.

Kit Components (Included)

3D Cell Culture Components				
Cat #	**# of vials**	**Product Name**	**Quantity**	**Storage**
SP-8750	1	Human Hepatic Stellate Cell Monoculture Spheroids (SP-HHSteCMS)	1×10^4 spheroids	Liquid nitrogen
3D-5201	1	3D-Liver Spheroid Medium (3D-LSpM)	200 mL	2-8 °C
3D-5352	1	3D-Stellate Cell Spheroid Supplement (3D-SteCSpS)	2 mL	-20 °C
0004	1	Fetal Bovine Serum	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-HHSteCMS is tested for the formation of functional and uniform 3D human hepatic stellate cell monoculture spheroids according to the included protocol. All components are negative for bacterial and fungal contamination.

Product Use
SP3D-HHSteCMS 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-8750, 3D-5352, 0004, and 0583 are shipped on dry ice. 3D-5201, and (0343 or 0353 or 0383) are shipped at room temperature.

References
[1] Coll et al. (2018) "Generation of Hepatic Stellate Cells from Human Pluripotent Stem Cells Enables In Vitro Modeling of Liver Fibrosis." *Cell Stem Cell* 23: 1-13.
[2] Gutiérrez-Ruiz M.C. and Gómez-Quiroz L.E. (2017) "Liver fibrosis: searching for cell model answers." *Liver International:* 434-439.

Procedure:

Step I: Preparing the complete 3D culture medium

1. Thaw 3D-stellate cell spheroid supplement (3D-SteCSpS; Cat. #3D-5352), fetal bovine serum (FBS; Cat #0004), and penicillin/streptomycin solution (P/S solution; Cat. #0583) at 37°C. Mix 3D-SteCSpS, FBS, and P/S solution into the 3D-liver spheroid medium (3D-LSpM medium; Cat. #3D-5401) 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 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. Hepatic stellate cell monoculture spheroids are recovered and ready for experiments after 24 hours post thawing (see Figure 1).

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Fig. 1 – At 100x magnification, phase contrast images of 3D human hepatic stellate cell monoculture spheroids at different days after thawing.

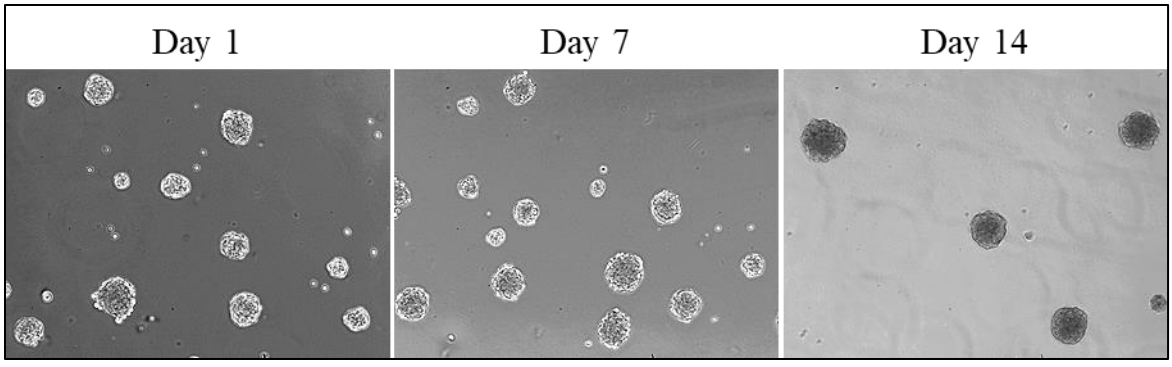

Fig. 2 –Immunostaining of the human hepatic stellate cell monoculture spheroids with antibody against type I collagen (200x magnification).

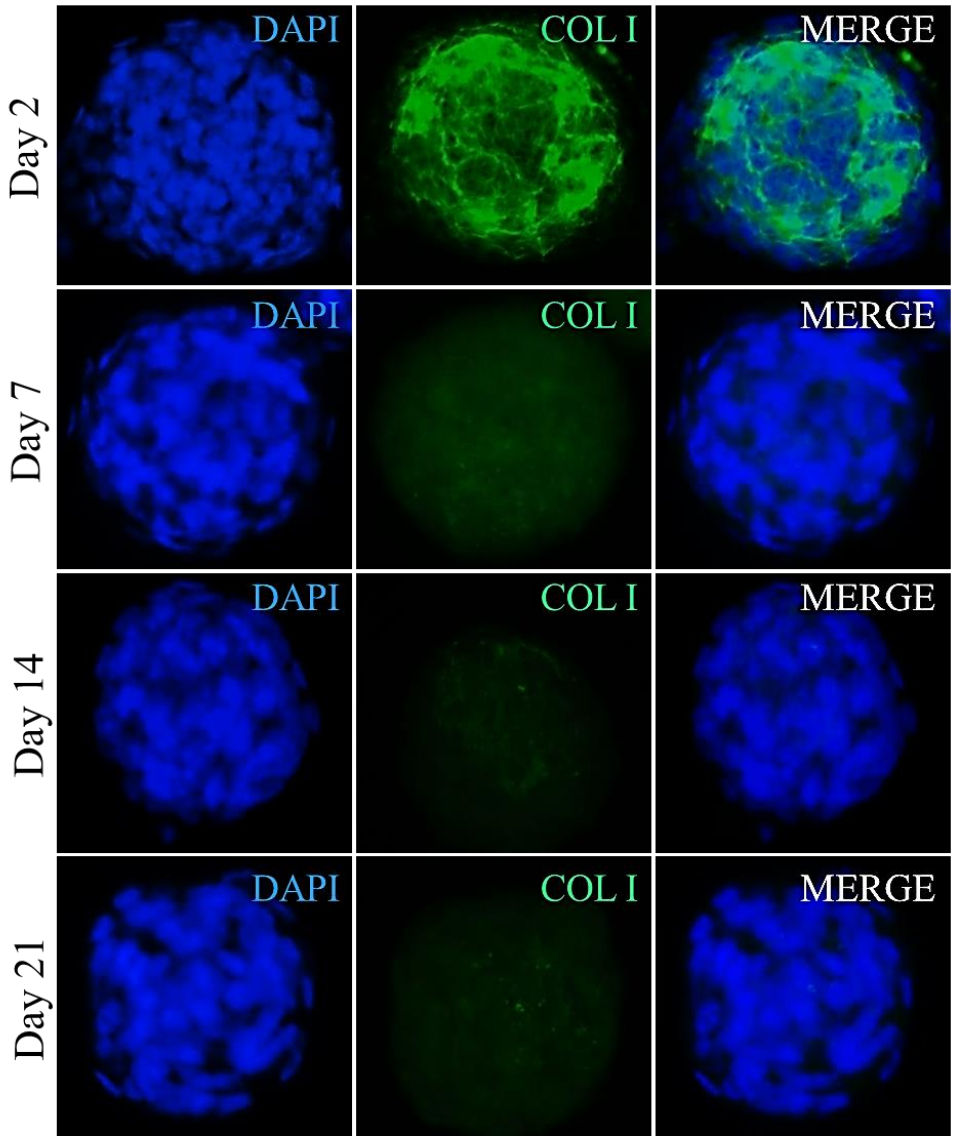

Fig. 3 –Immunostaining of the human hepatic stellate cell monoculture spheroids with antibody against α-SMA (200x magnification). Data revealed that culturing hepatic stellate cells (HSC) in 3D culture results in the clearance of α-SMA, suggesting that HSC returns to their quiescent state when cultured in 3D environment.