All-inclusive 3D Human Hepatic Stellate Cell Monoculture Spheroid Formation Kit

3D-HHSteCMSF

Cat #3D-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**
5300	1	Human Hepatic Stellate Cells (HHSteC)	5×10^5 cells	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	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**
5301	1	Stellate Cell Medium – Basal (SteCM)	500 mL	2-8 °C
5352	1	Stellate Cell Growth Supplement (SteCGS)	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
0113	Trypsin Neutralization Solution
0183	0.05% Trypsin/EDTA (T/E)
0303	Dulbecco's Phosphate-Buffered Saline (DPBS)
0413	Poly-L-Lysine (10 mg/mL)

Quality Control

3D-HHSteCMSF is tested for the uniform formation of 3D hepatic stellate cell spheroids according to the included protocol. All components are negative for bacterial and fungal contamination.

Product Use

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

Shipping

5300, 3D-5352, 5352, 0004, 0010, 0583, and 0503 are shipped on dry ice. 3D-5201, 5301, 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:

A. Initiating cells in 2D culture

1. Please see the product sheet Cat. #5300 for thawing and maintaining ScienCell's human primary hepatic stellate cells (Cat. #5300) in 2D monolayer culture.

2. For expansion in 2D monolayer culture, use the included 2D culture kit components (Cat. #5301, 5352, 0010, 0503).

B. Establishing 3D spheroid culture

Step I: Prepare the complete 3D culture medium

3. 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; 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 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: Harvest cells for 3D culture

Table A: An Example of Suggested Cell Suspension Density, and Medium Volumes

1	2	3
Plate formats	**Concentration of cell suspension (cells/mL)**	**Volume per well**
24-well	3.4×10^5 cells/mL	~ 1000 µL
48-well	3.0×10^5 cells/mL	~ 500 µL
96-well	3.2×10^5 cells/mL	~ 200 µL

4. 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.

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

6. 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 conical 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 conical 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

11. Count cells using hemocytometer, and aliquot the appropriate volume of cell suspension into a fresh 50 mL conical tube. Please see **Table A** for the suggested cell densities for different plate formats.

    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.

12. Centrifuge the 50 ml conical tube at 1000 rpm for 5 minutes.

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

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

15. Next, add the appropriate volume of the 3D-HSpM medium to obtain the suggested density of cell suspension (see **Table A; column 2**).

16. 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.

17. Add the suggested volume of cell mixture (see **Table A; column 3**) 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.

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

19. Change the 3D culture medium every four 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.

20. Monitor the growth and formation of spheroid every day under the microscope. Mature hepatic stellate cell spheroids develop at ~ 1-2 days post seeding (Figure 1).

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Fig. 1 – At 100x magnification, brightfield images of 3D human hepatic stellate cell monoculture spheroids at different days in 3D cell culture.

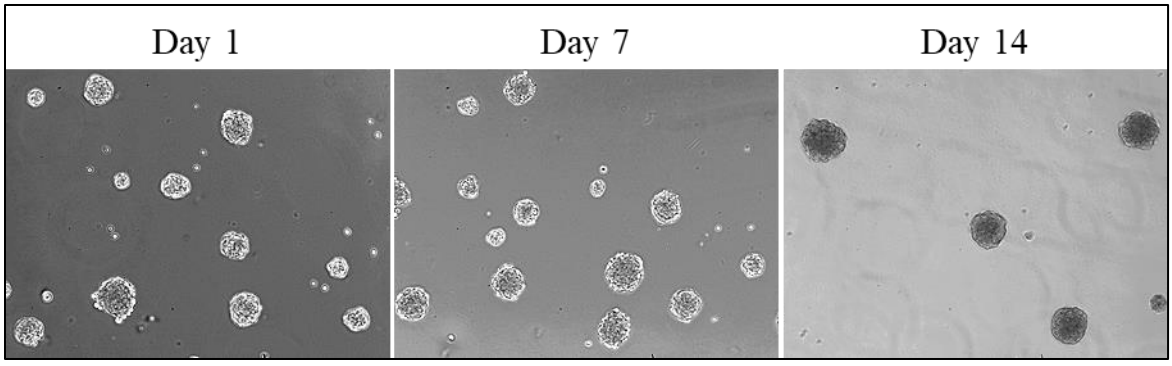


Fig. 2 –Immunostaining of the human hepatic stellate cell monoculture spheroids with antibody against type I collagen (200x magnification).

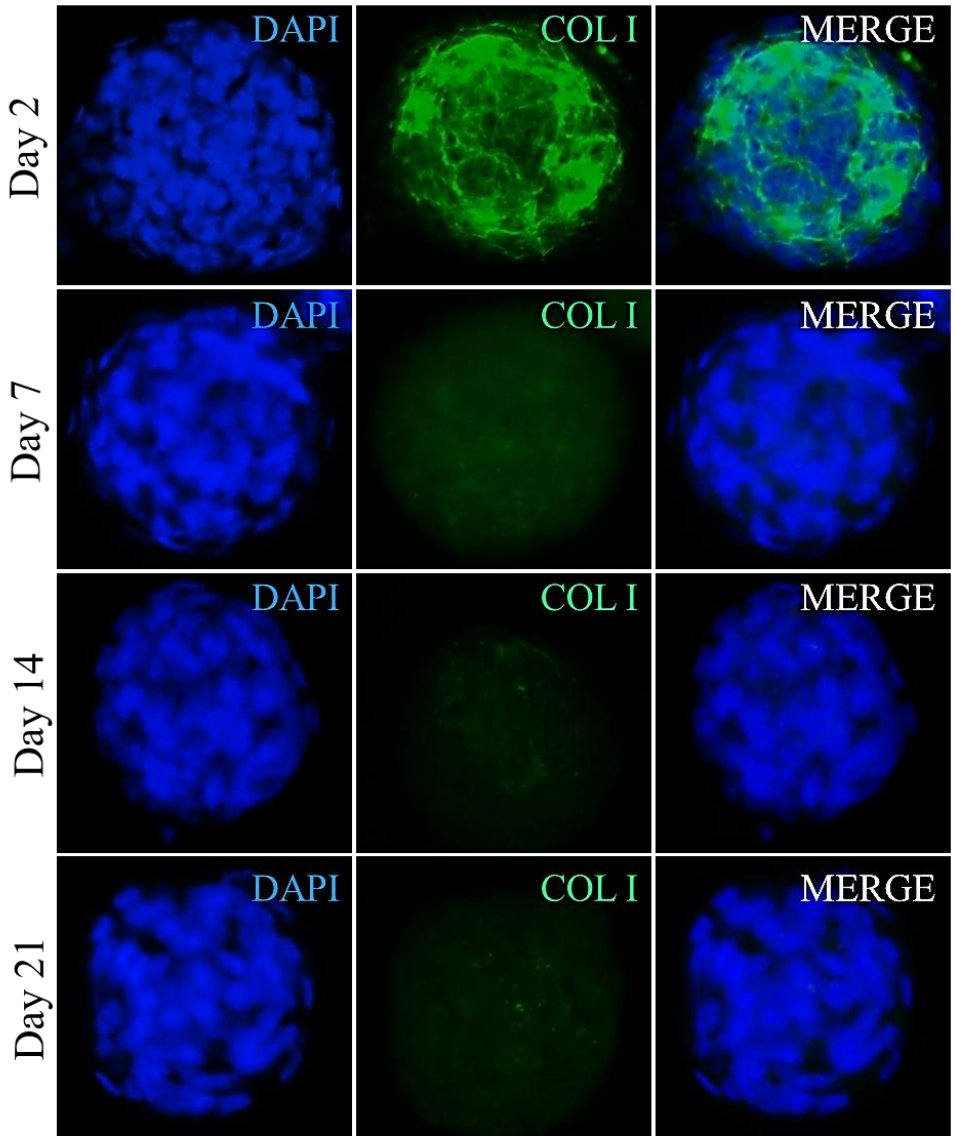
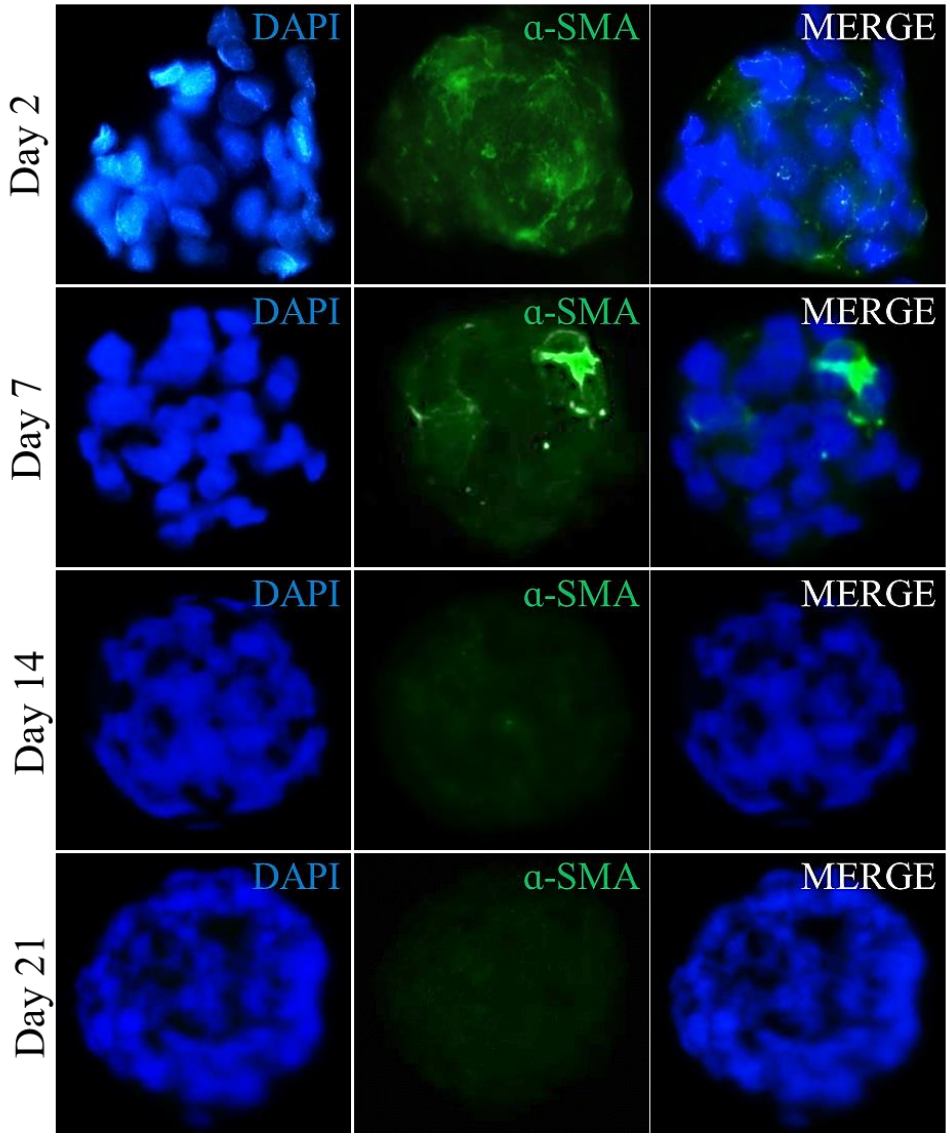


Fig. 3 –Immunostaining of the human hepatic stellate cell monoculture spheroids with antibody against α-SMA (200x magnification). Data revealed that culturing hepatic stellate cells (HSC) in 3D culture results in the clearance of α-SMA, suggesting that HSC returns to their quiescent state when cultured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



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 14). - 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 16).
	2. Shelves in the cell culture incubator are not level.	- Level your shelves of the CO_2 incubators.