



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-Hepatic Spheroid Medium (3D-HSpM)	200 mL	2-8 °C
3D-5352	1	3D-Hepatic Stellate Spheroid Supplement (3D-HSteSpS)	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-hepatic stellate spheroid supplement (3D-HSteSpS; Cat. #3D-5352), fetal bovine serum (FBS; Cat #0004), and penicillin/streptomycin solution (P/S solution; Cat. #0583) at 37°C. Mix 3D-HSteSpS, FBS, and P/S solution into the 3D-hepatic spheroid medium (3D-HSpM medium; Cat. #3D-5401) by gently swirling the medium bottle around.
 - a. 3D-HSpM medium is **viscous** and optimized for homogenous spheroid formation.
 - b. Warm the complete 3D-HSpM 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.

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

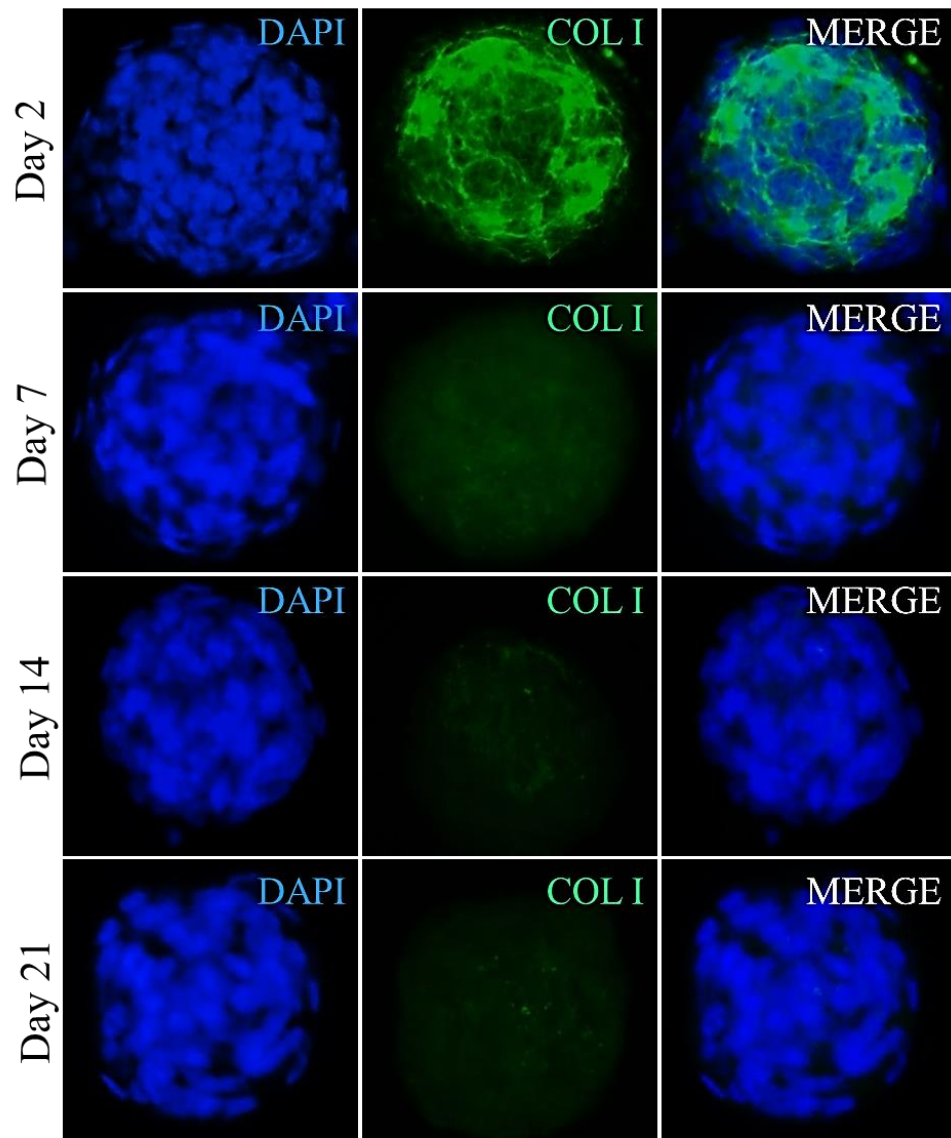


Fig. 3 –Immunostaining of the human hepatic stellate cell monoculture spheroids with antibody against α -SMA (200x magnification). Data revealed that culturing hepatic stellate cells (HSC) in 3D culture results in the clearance of α -SMA, suggesting that HSC returns to their quiescent state when cultured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

