Ready-to-use 3D Hepatic Stellate-Endothelial Cell Spheroids
SP3D-HSteECS
Cat. #SP3D-5000

Product Description

Activation of the quiescent hepatic stellate cell (HSteC) to a phenotype characterized by increased proliferation, migration, and synthesis of extracellular matrix is considered the pivotal event leading to fibrosis [1]. In addition to HSteC activation, another change that precedes fibrosis is capillarization of the hepatic sinusoidal endothelial cells (HSEC) [2]. In physiological conditions, HSEC maintain hepatic stellate cell quiescence, thereby inhibiting intrahepatic vasoconstriction and fibrosis development [2]. When capillarized, however, hepatic sinusoidal endothelial cells lose their capacity to inactivate hepatic stellate cells, thus promoting fibrogenesis and intrahepatic vasoconstriction [2]. Liver fibrogenesis and angiogenesis, therefore, are closely linked. For example, liver fibrosis enhances angiogenesis, and in turn, liver angiogenesis aggravates liver fibrosis. To investigate the signaling crosstalk between these cellular events, ScienCell has developed ready-to-use 3D hepatic stellate-endothelial cell spheroids (SP3D-HSteECS) comprised of hepatic stellate cells and sinusoidal endothelial cells at a 1:4 ratio. These spheroids are ready for experiments at 24 hours after thawing. Furthermore, immunostaining of the co-culture spheroids reveals the presence of vimentin-positive stellate cells and von willebrand factor (VWF)-positive sinusoidal endothelial cells. Furthermore, the spheroids also contain the activated stellate cell population marked by the smooth muscle actin (SMA) staining.

Kit Components (Included)

3D Cell Culture Components				
Cat #	**# of vials**	**Product Name**	**Quantity**	**Storage**
SP-5000	1	Human Hepatic Stellate Cell – Sinusoidal Endothelial Cell Spheroids (SP-HHSteECS)	1×10^4 spheroids	Liquid nitrogen
3D-5201	1	3D-Liver Spheroid Medium (3D-LSpM)	200 mL	2-8 °C
3D-5452	1	3D-Sinusoidal Endothelial Cell Spheroid Supplement (3D-SECSpS)	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-HSteECS is tested for the formation of functional and uniform 3D human hepatic stellate cell-sinusoidal endothelial cell co-culture spheroids according to the included protocol. All components are negative for bacterial and fungal contamination.

Product Use

SP3D-HSteECS 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-5000, 3D-5452, 0583 are shipped on dry ice. 3D-5201, and (0343 or 0353 or 0383) are shipped at room temperature.

References
[1] Yazdani S, Bansal R, Prakash J. (2017) "Drug targeting to myofibroblasts: Implications for fibrosis and cancer." *Adv Drug Deliv Rev.* 121: 101-116.
[2] Poisson J, Lemoinne S, Boulanger C, Durand F, Moreau R, Valla D, and Rautou P-E. (2017). "Liver sinusoidal endothelial cells: physiology and role in liver diseases." *Journal of Hepatology.* 66: 212-227.

Procedure:

Step I: Preparing the complete 3D culture medium

1. Thaw 3D-sinusoidal endothelial cell spheroid supplement (3D-SECSpS; Cat. #3D-5452), and penicillin/streptomycin solution (P/S solution; Cat. #0583) at 37°C. Mix 3D-SECSpS, 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 – sinusoidal endothelial cell co-culture spheroids are recovered and ready for experiments after 24 hours post thawing (see Figure 1).

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Fig. 1 – At 100x magnification, brightfield images of hepatic stellate cell–sinusoidal endothelial cell co-culture spheroids at 24 – 48 hours after thawing.

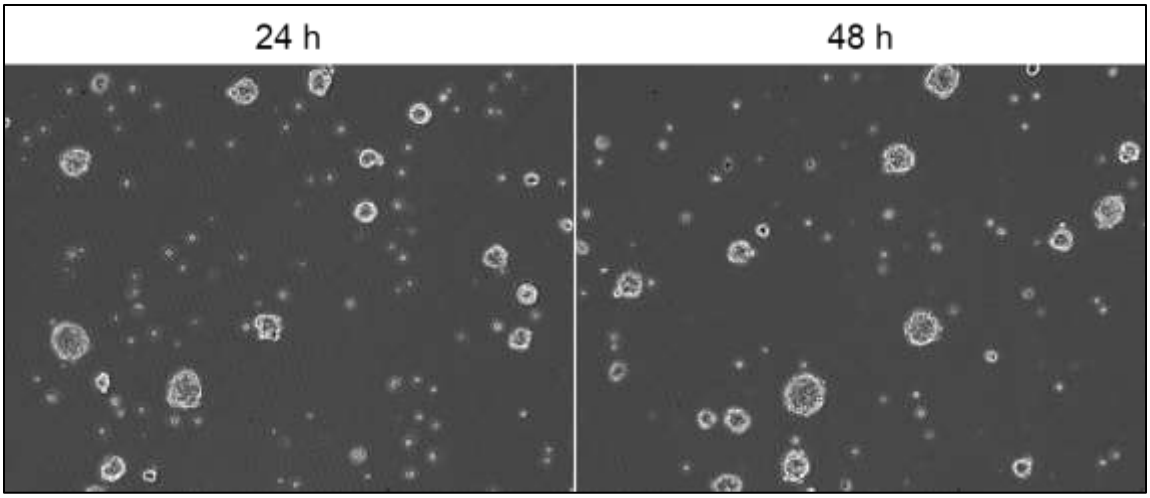

Fig. 2 –At day 7; immunostaining of the hepatic stellate cell – sinusoidal endothelial cell co-culture spheroids. Both the von willebrand factor (VWF)-positive sinusoidal endothelial cells, and the vimentin/smooth muscle actin (Vim/ SMA)-positive hepatic stellate cells are present within the spheroid (taken at 400x magnification).