All-inclusive 3D Human Hepatic Stellate-Endothelial Cell Spheroid Formation Kit
3D-HHSteECSF
Cat. #3D-5000

Product Description

Liver fibrogenesis and angiogenesis are closely linked processes. For example, in physiological conditions, hepatic sinusoidal endothelial cells (HSEC) maintain hepatic stellate cell (HSteC) quiescence, thereby inhibiting intrahepatic vasoconstriction and fibrosis development [1, 2]. When capillarized, however, hepatic sinusoidal endothelial cells lose their capacity to inactivate hepatic stellate cells, thus promoting fibrogenesis and intrahepatic vasoconstriction [1, 2]. To investigate the signaling crosstalk between these cellular events, ScienCell has developed the all-inclusive 3D human hepatic stellate-endothelial cell spheroid formation kit (3D-HHSteECSF) comprised of human hepatic stellate cells and sinusoidal endothelial cells. Furthermore, immunostaining of the co-culture spheroids reveals the presence of vimentin-positive stellate cells and von willebrand factor (VWF)-positive sinusoidal endothelial cells. 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**
5000	1	Human Hepatic Sinusoidal Endothelial Cells (HHSEC)	5×10^5 cells	Liquid nitrogen
5300	1	Human Hepatic Stellate Cells (HHSteC)	5×10^5 cells	Liquid nitrogen
3D-5201	1	3D-Hepatic Spheroid Medium (3D-HSpM)	200 mL	2-8 °C
3D-5452	1	3D-Hepatic Stellate-Endothelial Spheroid Supplement (3D-HSteESpS)	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**
8711	1	2D-Hepatic Stellate-Endothelial Cell Co-culture Medium (2D-HSteECM)	500 mL	2-8 °C
8762	1	2D-Hepatic Stellate-Endothelial Cell Co-culture Growth Supplement (2D-HSteECGS)	5 mL	-20 °C
0025	1	Fetal Bovine Serum (FBS)	25 mL	-20 °C
0503	1	Penicillin/streptomycin Solution (P/S)	5 mL	-20 °C

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

Shipping
5000, 5300, 3D-5452, 0583, 8762, 0025, and 0503 are shipped on dry ice. 3D-5201, 8711, 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:

A. Initiating cells in 2D culture
Step I: Prepare the complete 2D-hepatic stellate-endothelial cell co-culture medium

1. Thaw 2D-hepatic stellate-endothelial cell co-culture growth supplement (2D-HSteECGS; Cat. #8762), fetal bovine serum (FBS; Cat. #0025), and penicillin/streptomycin solution (P/S solution; Cat. #0503) at 37°C. Add 2D-HSteECGS, FBS and P/S solution to the 2D-co-culture medium-basal (2D-HSteECM; Cat. #8711) and mix well.
    a. Warm the 2D-complete co-culture medium only to room temperature prior to use.
    b. When stored in the dark at 4°C, the complete medium is stable for one month.

Step II: Thaw, maintain and sub-culture cells in 2D cell culture

2. For the human hepatic sinusoidal endothelial cells (HHSEC; Cat. #5000), one cryopreserved vial contains 5×10^5. It is recommended to plate directly into one fibronectin-coated **T-75** flask using the complete 2D co-culture medium (2D-HSteECM).

3. For the human hepatic stellate cells (HHSteC; Cat. #5300), one cryopreserved vial contains 5×10^5. It is recommended to plate directly into one pLL-coated **T-75** flask using the complete 2D co-culture medium (2D-HSteECM).

    Note: *HHSEC are guaranteed to further expand for* ***ONLY 5 population doublings*** *under the conditions provided by ScienCell Research Laboratories. Therefore, please use cells for experiments* ***at earliest passage after initial plating.***

    Note: *For detailed instructions on thawing and maintaining the HHSEC and HHSteC in 2D culture, please see the product sheets* ***Cat #5000, and #5300,*** *respectively.*

B. Establishing 3D spheroid culture

Step III: Prepare the complete 3D spheroid medium

4. Thaw 3D-Hepatic Stellate-Endothelial Spheroid Supplement (3D-HSteESpS; Cat. #3D-5452), and penicillin/streptomycin solution (P/S solution; Cat. #0583) at 37°C. Mix 3D-HSteESpS, and P/S solution into the 3D-Hepatic spheroid medium (3D-HSpM; Cat. #3D-5201) 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 only **to room temperature** before use.
    c. When stored in the dark at 4°C, the complete medium is stable for one month.

Step IV: Harvest cells for 3D culture

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 8 mL of DPBS and 2 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 centrifuge tube (a small percent of cells may detach) and continue to incubate the flask at 37°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 centrifuge tube. Rinse the flask with another 5 ml of TNS to collect the residual cells.

Step III: Resuspend and seed cells in 3D cell culture medium

11. Count cells using a hemacytometer. Please see **Table A** for the suggested cell numbers for HHSEC and HHSteC for different plate formats.

Table A: An Example of Suggested Cell Number and Culture Volume per Sample

1	2	3	4
Plate formats	**HHSEC cell number**	**HHSteC cell number**	**3D Culture Volume per well**
24-well	2.6 × 10^5 cells	6.4 × 10^4 cells	~ 1000 µL
48-well	9.7 × 10^4 cells	2.4 × 10^4 cells	~ 500 µL
96-well	4.1 × 10^4 cells	1.0 × 10^4 cells	~ 200 µL

12. Aliquot the suggested number of cells for HHSEC and HHSteC into a fresh conical tube.
    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.*

13. Centrifuge the tube at 1000 rpm for 5 minutes.

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

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

16. Next, add the appropriate volume of the complete 3D-HSpM medium to obtain the suggested density of cell suspension (see **Table A; column 4**).

17. Slowly pipette up and down for ~ 5-7 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.

18. Add the suggested volume of cell mixture (see **Table A; column 4**) to each well in the provided ultra-low binding plate by using a p1000 pipette. In order to minimize pipette errors, do not use a serological pipette.

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

20. Spheroids can be maintained in culture without changing the medium.

21. Monitor the growth and formation of spheroid every day under the microscope. Mature spheroids develop at ~ **4 days** post seeding (Figure 1 and 2).

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Fig. 1 – At 200x magnification, brightfield images of human hepatic stellate cell–sinusoidal endothelial cell co-culture spheroids at different days in 3D cell culture.

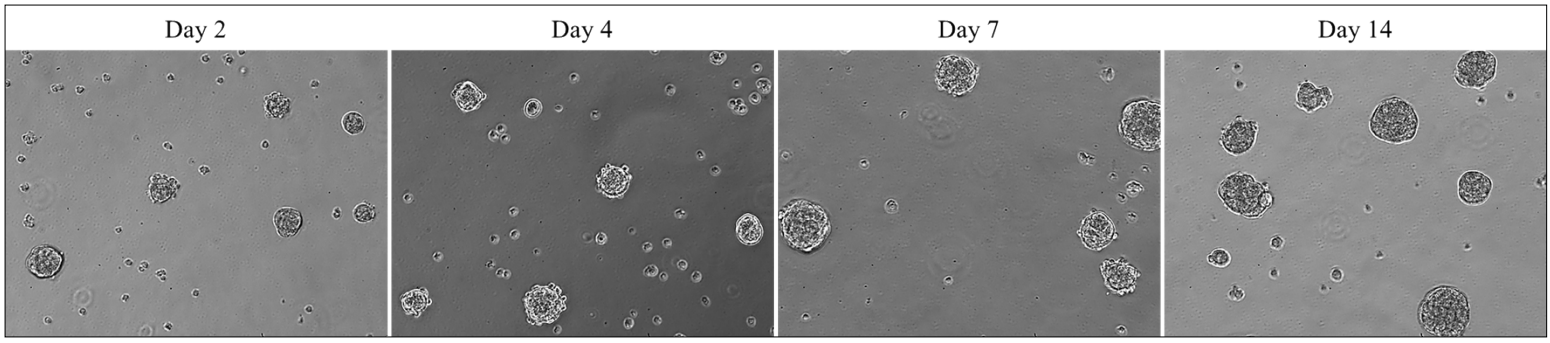

Fig. 2 –At day 7; immunostaining of the human hepatic stellate cell – sinusoidal endothelial cell co-culture spheroids. Both von willebrand factor (VWF)-positive sinusoidal endothelial cells, and vimentin/smooth muscle actin (Vim/ SMA)-positive hepatic stellate cells are present within the spheroid (taken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