



Ready-to-use 3D Hepatic Stellate-Endothelial Cell Spheroids

SP3D-HStECS
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-HStECS) 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-HHStECS)	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-HStECS 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-HStECS 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.

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).

