Rev. 0

Ready-to-use 3D Human Hepatic Sinusoidal Endothelial Cell Spheroids (SP3D-HHECS)

Catalog #SP3D-5010

Product Description

Human Hepatic Sinusoidal Endothelial Cells (HHSEC) are one of the major non-parenchymal cell types in the liver. These cells make up the vascular wall of the hepatic sinusoids and in their differentiated state, are uniquely characterized by the presence of open fenestrae and lack of basement membrane [1]. Under normal physiological state, HHSEC play a key role in the maintenance of hepatic homeostasis. When stimulated by liver injury, however, HHSEC become rapidly dedifferentiated through a process termed 'capillarization', which contributes to the activation of other hepatic cells [1]. The maintenance of HHSEC in 2D culture can be challenging due to the rapid dedifferentiation of the cells *in vitro* [2]. To better understand the role of HHSEC physiologically, culture methods that promote the maintenance of these cells in their differentiated state is necessary.

To provide an *in vitro* model that better reflects HHSEC function physiologically, ScienCell has developed ready-to-use Human Hepatic Sinusoidal Endothelial Cell Spheroids (SP-HHECS) comprised of primary HHSEC. These cells are cultured in three-dimensional (3D) architecture and are embedded in their own ECM, which has been shown to alleviate the rapid dedifferentiation observed in 2D culture [2]. These spheroids are ready for experiments within 24-48 hours of thawing, and are an excellent *in vitro* model for studying HHSEC function and its contribution to hepatic homeostasis.

Kit Components (Included)

3D Cell Culture Components				
Cat #	**# of vials**	**Product Name**	**Quantity**	**Storage**
SP-5010	1	Human Hepatic Sinusoidal Endothelial Cell Spheroids (SP-HHECS)	1×10^4 spheroids	Liquid nitrogen
3D-5501	1	3D-Hepatic Sinusoidal Endothelial Cell Spheroid Medium (3D-HHECSpM)	200 mL	2-8 °C
3D-5552	1	3D-Hepatic Sinusoidal Endothelial Cell Spheroid Supplement (3D-HHECSpS)	4 mL	-20 °C
0010	1	Fetal Bovine Serum (FBS)	10 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-HHECS are tested for the formation of functional and uniform 3D human hepatic sinusoidal endothelial cell spheroids according to the included protocol. All components are negative for bacterial and fungal contamination.

Product Use
SP3D-HHECS are for research use only. It is not approved for human or animal use, or for application in clinical or *in vitro* diagnostic procedures.

Shipping
SP-5010, 3D-5552, 0010, and 0583 are shipped on dry ice. 3D-5501 and (0343 or 0353 or 0383) are shipped at room temperature.

References
[1] Gracia-Sancho J, Caparros E, Fernandez-Iglesias A and Frances R. (2021) "Role of liver sinusoidal endothelial cells in liver diseases." *Nat Rev Gastroenterol Hepatol*. 18: 411-431.
[2] Wang J, Zhao F, Brouwer L, Buist-Homan M, Wolters J, Moshage H, Harmsen M. (2024) "Collagen-rich liver-derived extracellular matrix hydrogels augment survival and function of primary rat liver sinusoidal endothelial cells and hepatocytes." *International Journal of Biological Macromolecules.* 134717.

Procedure:

Step I: Preparing the complete 3D culture medium

1. Thaw 3D-hepatic Sinusoidal Endothelial Cell Spheroid Supplement (3D-HHECSpS; Cat. #3D-5552), Fetal Bovine Serum (FBS; Cat. #0010) and Penicillin/Streptomycin solution (P/S solution; Cat. #0583) at 37°C. Mix 3D-HHECSpS, FBS and P/S solution into the 3D-Hepatic Sinusoidal Endothelial Cell Spheroid Medium (3D-HHECSpM; Cat. #3D-5501) by gently swirling the medium bottle around.

    a. 3D-HHECSpM is **viscous** and optimized for homogenous spheroid formation.
    b. Warm the complete 3D-HHECSpM 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 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. Monitor the health of spheroids every day under the microscope. Human hepatic sinusoidal endothelial cell spheroids are recovered and ready for experiments after 24-48 hours post thawing (see Figure 1).

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.

◆◆◆◆◆◆◆◆◆◆◆◆◆◆◆◆◆◆◆◆

Fig. 1 –Brightfield images of the ready-to-use Human Hepatic Sinusoidal Endothelial Cell Spheroids after thawing (taken at 100X magnification).

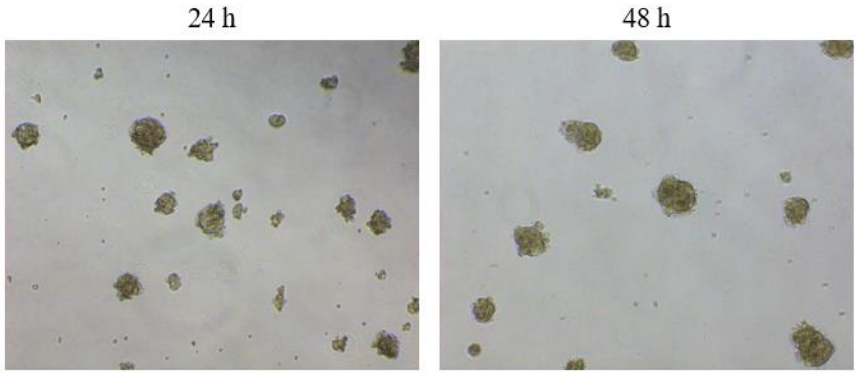

Fig. 2 – Human Hepatic Sinusoidal Endothelial Cell Spheroids express the sinusoidal endothelial cell marker von Willebrand factor (vWF) (at 200x magnification).