Rev. 0

# Ready-to-use 3D Human Mini Liver Spheroids (SP3D-HMLS)

Catalog #SP3D-5108

## Product Description

The liver is a vital metabolic organ and its functional unit is termed hepatic lobule, which is comprised of hexagonally-arranged hepatocytes innervated by the hepatic sinusoids. The liver has a multi-dimensional role, including protein synthesis, nutrient storage, bile production and bilirubin metabolism. Another key hepatic function is the clearance of first pass doses of orally-absorbed substances, such as drugs and xenobiotics. Drug-induced hepatoxicity remains a major concern for pharmaceutical industries. Presently, 2D culture of primary hepatocytes is the gold standard for hepatotoxicity testing. This approach, however, is limited by factors such as poor proliferative capacity of primary hepatocytes and their tendency to rapidly dedifferentiate in 2D culture [1]. Furthermore, the cellular diversity and liver architecture are not taken into consideration. The crosstalk between hepatocytes and the non-parenchymal cells in the liver, such as sinusoidal endothelial cells and hepatic stellate cells, significantly influences hepatic function and the accuracy of predictive hepatotoxicity testing.

To provide an *in vitro* liver model that better reflects hepatic function physiologically, ScienCell has developed ready-to-use human mini liver spheroids comprised of human hepatocytes, hepatic stellate cells, and sinusoidal endothelial cells. The co-culture of these main hepatic cell types allows the functional maintenance of cells over time due to a more native, three-dimensional (3D) architecture. These spheroids are ready for experiments within 24-48 hours of thawing, and are an excellent *in vitro* model for studying liver function and for the examination of hepatotoxicity.

## Kit Components (Included)

| 3D Cell Culture Components | | | | |
|---|---|---|---|---|
| **Cat #** | **# of vials** | **Product Name** | **Quantity** | **Storage** |
| SP-5108 | 1 | Human Mini Liver Spheroids (SP-HMLS) | $4 \times 10^3$ spheroids | Liquid nitrogen |
| 3D-5201 | 1 | 3D-Liver Spheroid Medium (3D-LSpM) | 200 mL | 2-8 °C |
| 3D-5252 | 1 | 3D-Liver Spheroid Supplement (3D-LSpS) | 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-HMLS are tested for the formation of functional and uniform 3D human mini liver spheroids according to the included protocol. All components are negative for bacterial and fungal contamination.

Rev. 0

**Product Use**
SP3D-HMLS 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-5108, 3D-5252, 0583 are shipped on dry ice. 3D-5201 and (0343 or 0353 or 0383) are shipped at room temperature.

**References**
[1] Elaut G, Henkens T, Papeleu P, Snykers S, Vinken M, Vanhaecke T, Rogiers V. (2006)) "Molecular mechanisms underlying the dedifferentiation process of isolated hepatocytes and their cultures." *Curr Drug Metab*. 7: 629-660.

**Procedure:**

Step I: Preparing the complete 3D culture medium

1. Thaw 3D-liver spheroid supplement (3D-LSpS; Cat. #3D-5252), and penicillin/streptomycin solution (P/S solution; Cat. #0583) at 37°C. Mix 3D-LSpS and P/S solution into the 3D-liver spheroid medium (3D-LSpM; Cat. #3D-5201) by gently swirling the medium bottle around.

    a. 3D-LSpM is **viscous** and optimized for homogenous spheroid formation.
    b. Warm the complete 3D-LSpM 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 $\geq 4 \times 10^3$ 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 mini liver 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 mini liver spheroids after thawing (taken at 100X magnification).**

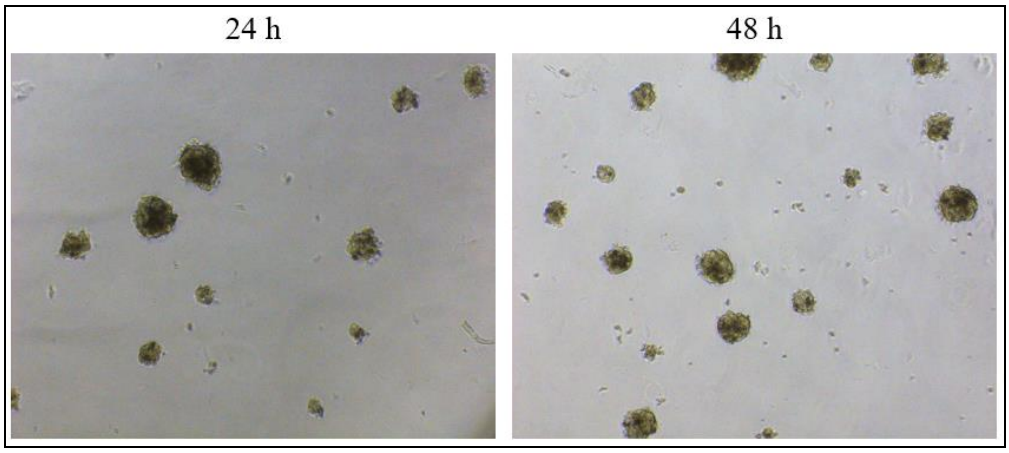
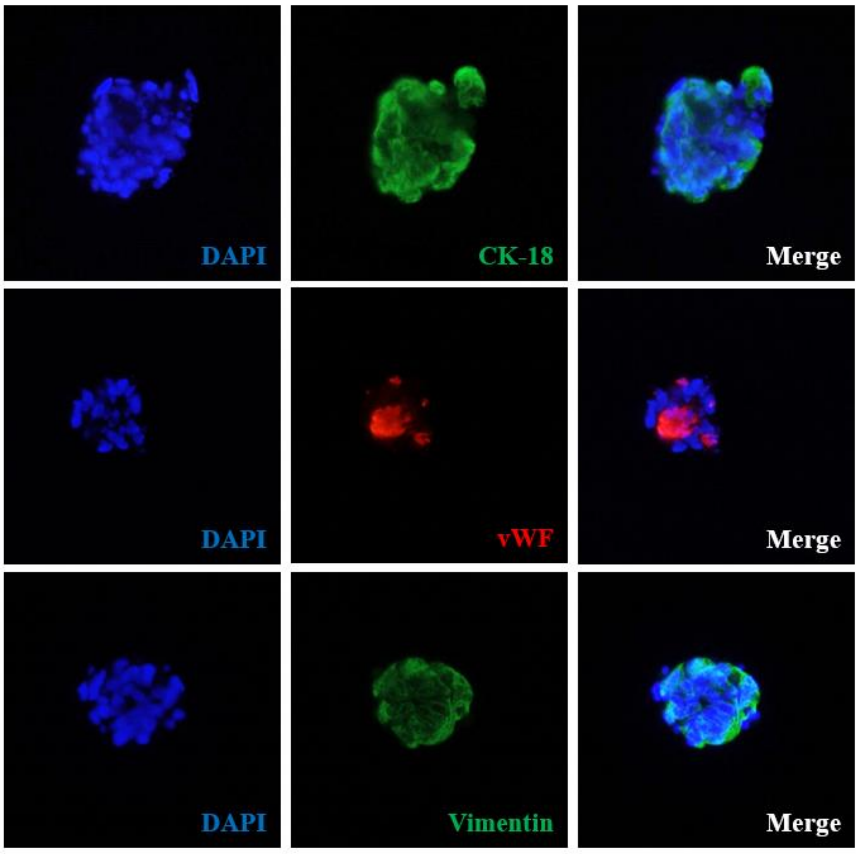



**Fig. 2 – Human mini liver spheroids express the hepatocyte markers cytokeratin 18 (CK-18), stellate cell marker vimentin and sinusoidal endothelial cell marker von Willebrand factor (vWF) (at 200x magnification).**


DAPI
CK-18
Merge
DAPI
vWF
Merge
DAPI
Vimentin
Merge