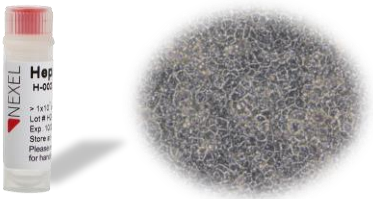
Application note

Hepatic Spheroid Formation Using Hepatosight®-S



Introduction

Drug screening and *in vitro* toxicology research on liver cells is being actively conducted worldwide. In case of primary human hepatocytes, there exist significant limitations such as short life span and experiment difficulty. As a result, iPSC-derived hepatocytes receive great attention as an alternative to compensate for above limitations. Although research is actively conducted in 2D hepatocytes, 3D hepatic spheroids are also widely used due to its similarity to human livers. Currently, 3D hepatic spheroids are mainly produced through a protocol aided by the addition of ECM (Extracellular matrix) material.

However, when ECM material is added, it becomes difficult to decompose the spheroid, which not only makes additional experiments challenging, but also has the potential to interfere with drug screening and *in vitro* toxicology research. Therefore, we would like to introduce a spheroid production method using Hepatosight®-S that does not use ECM material or serum.

In addition, experiment time and process can be minimized through NEXEL's method that allows immediate spheroid formation after cell thawing without the need for replating. Please refer to the **Hepatosight®-S User Guide** when performing experiments following this protocol.

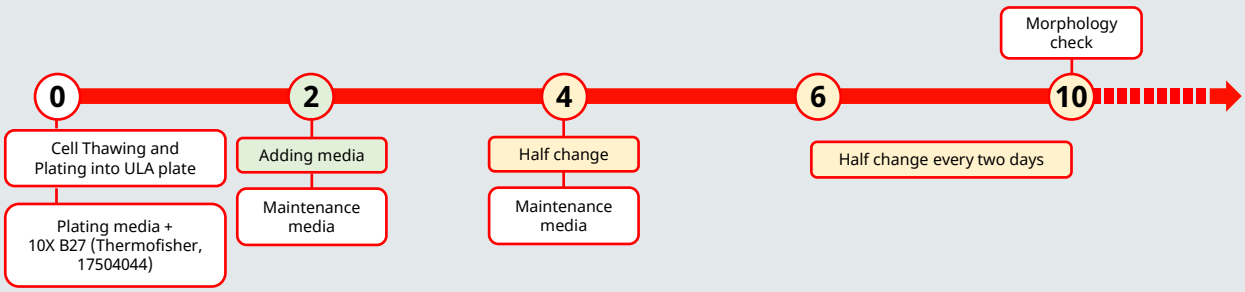


Table 1. Timeline of the Workflow From Day 0.

Workflow

The Hepatosight®-S is plated in ULA plates with a media-cell mixture supplemented with 10X B27. Stable spheroid morphology can be observed starting at Day 2 or Day 4. Add, not change, media to the ULA plates on Day 2.

From Day 4 and onward, replace half of the media every other day. It is recommended to perform an interim morphology check and proceed with the experiment between Day 6 and Day 10.

Required Consumables

Item	Vendor	Catalog number
Hepatosight® -S Hepatocytes	NEXEL	H-002
Hepatosight® -S Media	NEXEL	HMS-001 HMS-002
B-27™ supplement (50X), serum free	ThermoFisher	17504044
D-PBS – 1X	Welgene	LB001-02
PrimeSurface® 3D culture: Ultra-low Attachment Plates: 96 well, V bottom, Clear plates	S-bio	#MS-9096VZ
PrimeSurface® 3D culture: Ultra-low Attachment Plates: 384 well, U bottom, Clear plates	S-bio	#MS-9384UZ

* The application note written here is an optimized method using PrimeSurface® 3D culture: Ultra-low Attachment Plates.

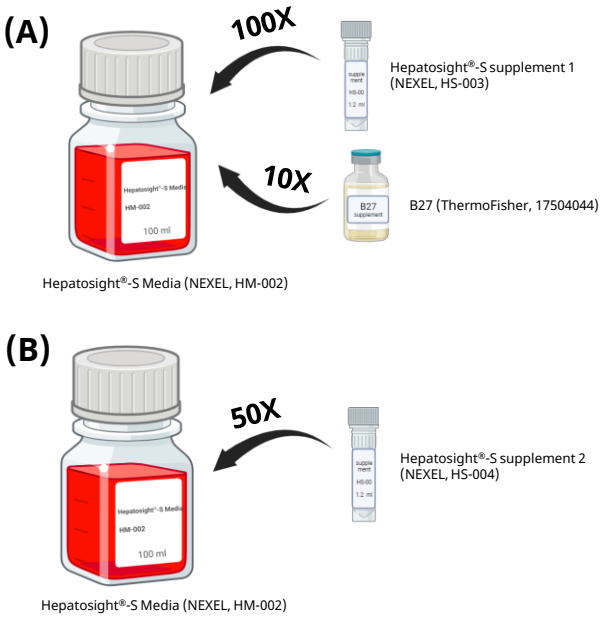


Figure 1. Plating and Maintenance medium preparation.
(A) Plating medium preparation: Add supplement (NEXEL, HS-003) and 10X B27 (Thermofisher, 17504044) to the Heaptosight®-S media (NEXEL, HM-002).
(B) Maintenance medium preparation: Add supplement (NEXEL, HS-004) and 10X B27 (Thermofisher, 17504044) to the Hepatosight®-S media (NEXEL, HM-002).

Method

Thawing and Plating Hepatosight®-S for spheroid formation

1. Thaw the cells according to the Hepatosight®-S User Guide.
2. Select and prepare an appropriate plate to form spheroids of the desired size.
   (Based on a 96-well plate)
3. Prepare 10X B27 (ThermoFisher, 17504044) by adding to the plating media. Prepare enough volume to seed 50 µl per well.
   Note: ECM can result in improved spheroid formation. If ECM is preferred, it is recommended to add Collagen I to the plating media at 600X.
4. Prepare a cell suspension by counting the number of live cells and adding them to the prepared plating media according to the desired size of the spheroids.
5. Plate 50 µl of plating media loaded with cells per well and add 200 µl of DPBS in the outer perimeter wells to prevent cells from drying out.
6. Centrifuge the plate at 180 g for 3 minutes.
   Note: If 384-well plate has been opted instead of 96-well plate, adjust the plating media volume to hold 20 µl per well and plate 20 µl of cell suspension per well.

	50,000 cells/well	30,000 cells/well	10,000 cells/well	5,000 cells/well	3,000 cells/well	1,000 cells/well
Recommended Plate	96-well		Both			384-well
Approximate Size	670 µm	560 µm	430 µm	330 µm	300 µm	250 µm

Table 2. Recommended Plate and Average spheroid size according to cell number

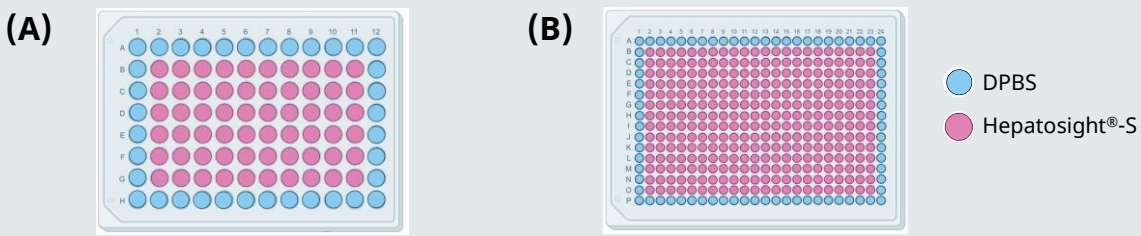


Figure 2. Recommended Plate design.
(A) PrimeSurface® ULA 96 well V bottom plate (S-bio, #MS-9096VZ).
(B) PrimeSurface® ULA 384 well U bottom plate (S-bio, . #MS-9384UZ).

Maintaining Spheroid

1. Prepare the required amount of maintenance media and equilibrate at room temperature for at least 30 minutes.
2. On Day 2, add 3 times the volume of maintenance media seeded on Day 0 to each well.
   Note: Add 150 µl for 96-well plates and 60 µl for 384-well plates.
3. Starting on Day 4, replace half of the total media to maintain the spheroid.
   Note: 100 µl for 96-well plates and 40 µl for 384-well plates.

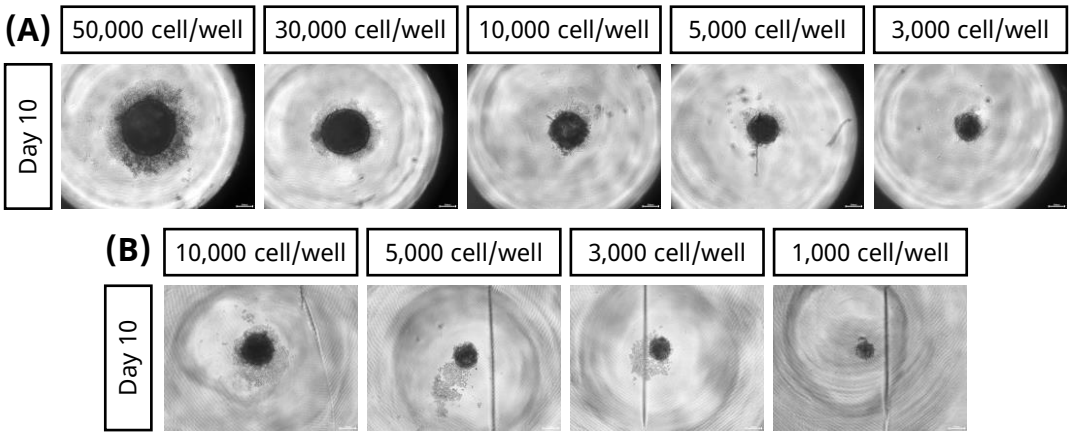


Figure 3. Size-controlled hepatic spheroids using ULA plate.
Images is that of spheroids formed on Day 10. **(A)** Images of spheroid formation using a PrimeSurface® ULA 96 well V bottom plate. **(B)** Images of spheroid formation using a PrimeSurface® ULA 384 well U bottom plate.

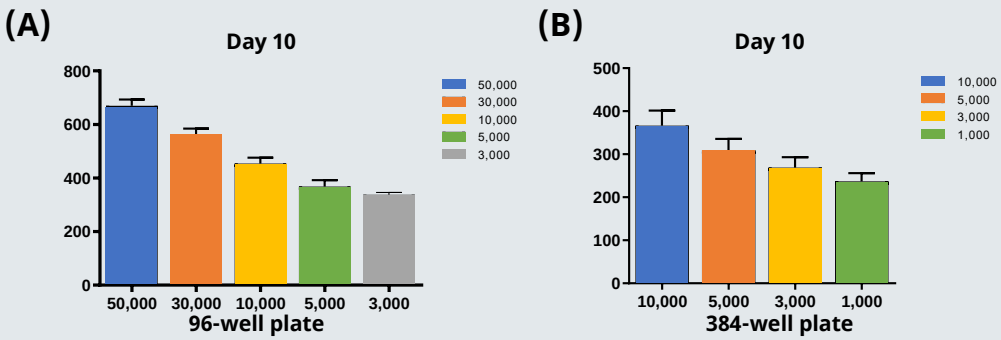


Figure 4. Graph of spheroid size.
The Y axis is the spheroid size, and the X axis is the number of cells added per well. **(A)** Graph of spheroid size formed using a PrimeSurface® ULA 96-well V bottom plate on Day 10. **(B)** Graph of spheroid size formed using a PrimeSurface® ULA 384-well U bottom plate on Day 10.

Caution: All experimental steps described in this application note are optimized for Hepatosight®-S. Results cannot be guaranteed when carried out with different cells. NEXEL recommends the use of media and reagents listed in the application note, otherwise results may not be replicable and further technical support may be difficult.