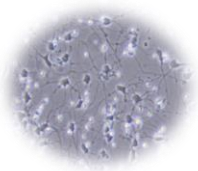
Application Note

Neural Spheroid Formation Using Neurosight®-S



Introduction

3D cell culture aims to mimic living cells and organs. By creating an *in-vivo* like environment, not only basic *in-vitro* research, but also research on neurosensory deafness, Parkinson's disease, and Huntington's disease are able to be carried out, and are actively conducted around the world. The advantages of creating an *in-vivo* like environment have led to the majority of research conducted today experimenting in 3D culture.

In line with this trend, we present a method for neural spheroids formation using Neurosight®-S.

Neurosight®-S have gone through the differentiation process, which eases control than working with iPSCs. Furthermore, spheroid formation proceeds simultaneously and forms stable spheroids following cell thawing. Please refer to the **Neurosight®-S User Guide** when performing experiments following this protocol.

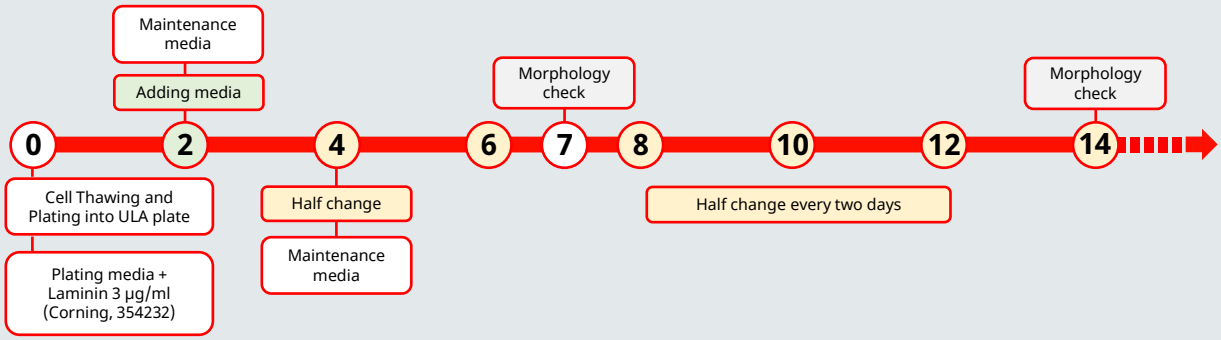


Table 1. Timeline of the Workflow From Day 0.

Workflow

Neurosight®-S is plated in ULA plates with a media-cell mixture supplemented with 3 µg/ml Laminin. Stable spheroid morphology can be observed starting at Day 2. 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 on Day 7 and proceed with the experiment after Day 7 at earliest.

Required Consumables

Item	Vendor	Catalog number
Neurosight® -S Neurons	NEXEL	N-001 N-002
Neurosight® -S Media	NEXEL	NMS-001 NMS-002
Laminin, mouse, 1mg	Corning	354232
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.

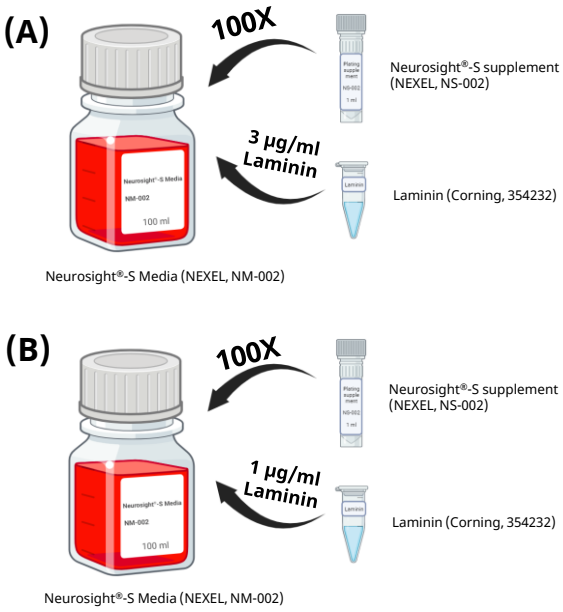


Figure 1. Plating and Maintenance medium preparation. **(A)** Plating medium preparation: Add supplement (NEXEL, NS-002) and 3 µg/ml Laminin (Corning, 354232) to the Neurosight®-S media (NEXEL, NM-002). **(B)** Maintenance medium preparation: Add supplement (NEXEL, NS-002) and 1 µg/ml Laminin (Corning, 354232) to the Neurosight®-S media (NEXEL, NM-002).

Method

Thawing and Plating Neurosight®-S for spheroid formation

1. Thaw the cells according to the Neurosight®-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 3 µg/ml Laminin (Corning, 354232) by adding to the plating media. Prepare enough volume to seed 50 µl per well.
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	690 µm	620 µm	400 µm	320 µm	290 µm	190 µm

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

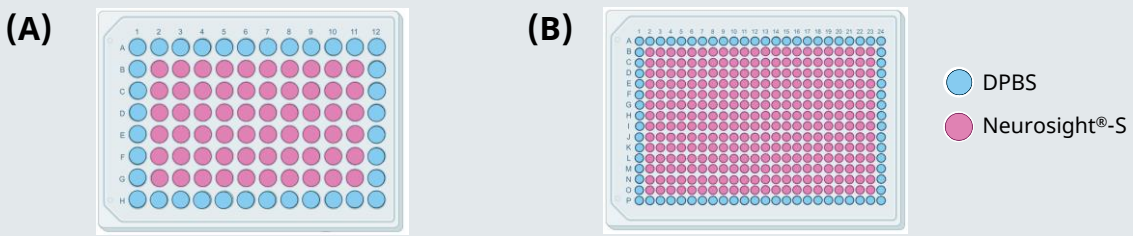


Figure 2. Recommended Plate design.
(A) PrimeSurface® ULA 96 well V bottom plate (S-bio, #MS-9096VZ).
(B) PrimeSurface® ULA 384 well U bottom plate (S-bio, . #MS-9384UZ).

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.

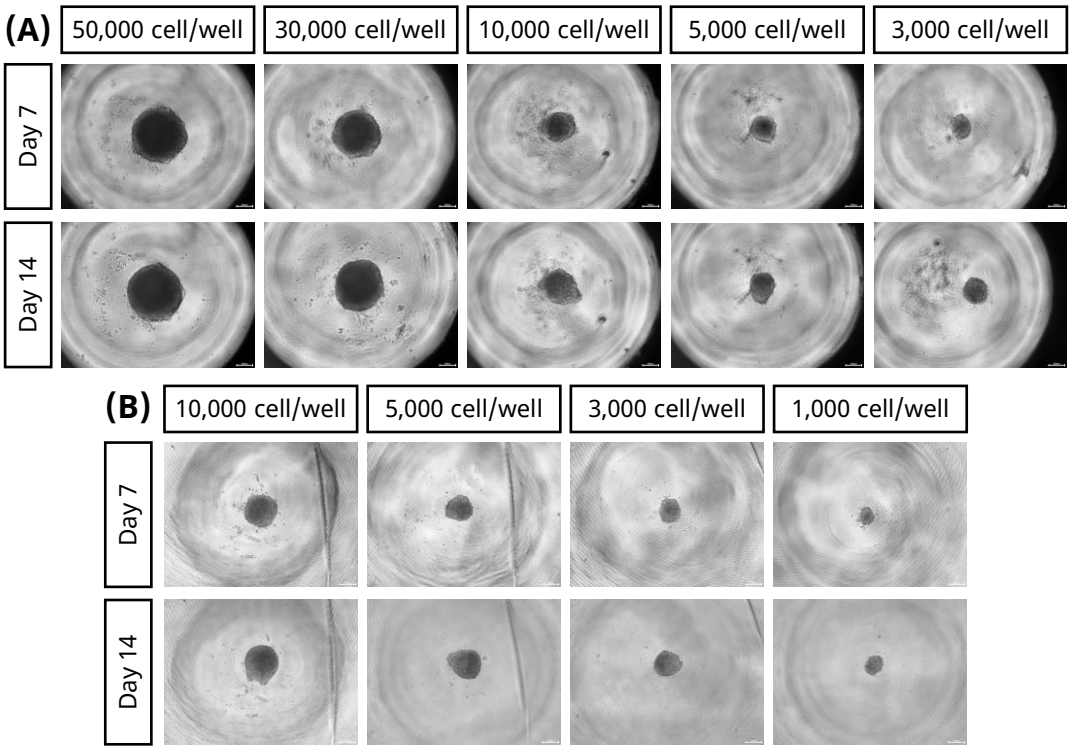


Figure 3. Size-controlled neural spheroids using ULA plate.
Images in the top row exhibit spheroid formation on Day 7 and images in the bottom row is that of spheroids formed on Day 14. **(A)** Images of spheroid formation using a PrimeSurface® ULA 96 well V bottom plate. **(B)** Images of spheroid formation using a PrimeSurface® ULA 384 well U bottom plate.

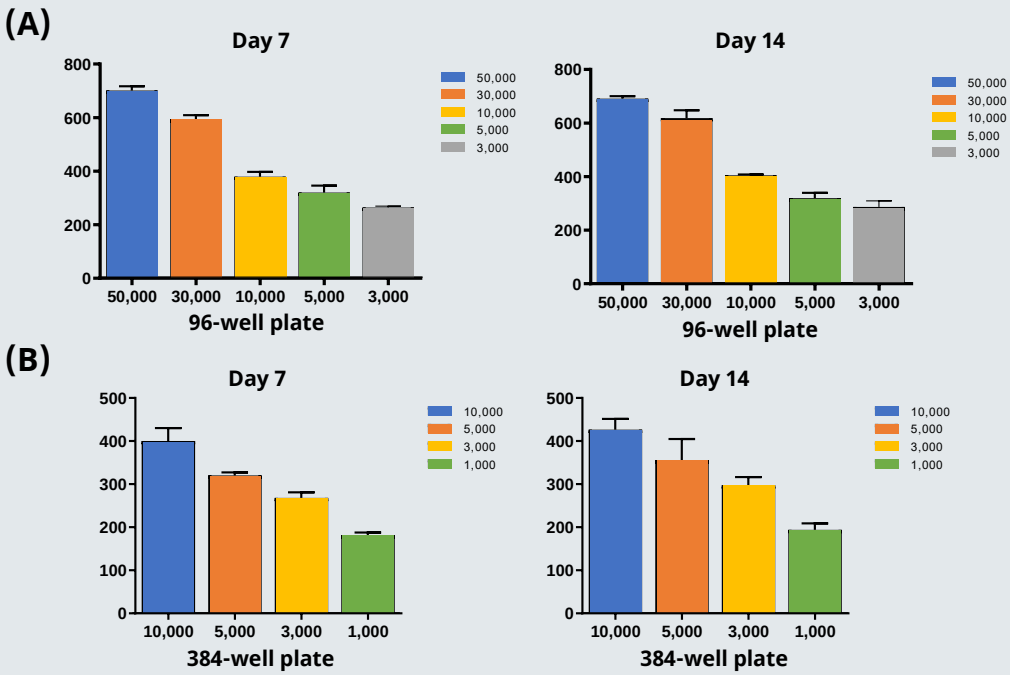


Figure 4. Graph of spheroid size.
The Y axis is the spheroid size and the X axis is the number of cells added per well. **(A)** Graph of spheroid size formed using a PrimeSurface® ULA 96-well V bottom plate on Day 7 and Day 14. **(B)** Graph of spheroid size formed using a PrimeSurface® ULA 384-well U bottom plate on Day 7 and Day 14.

Caution: Since all experimental steps described in this application note are optimized for Neurosight®-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.