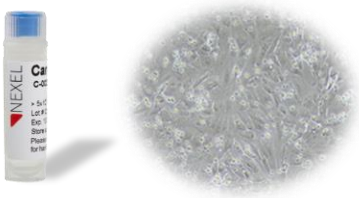
Application note

Cardiac Spheroid Formation Using Cardiosight®-S



Introduction

3D culture methods have been developed to reduce the gap between 2D culture and native tissue. In practice, there exists a difference in experimental results between 2D and 3D culture experiments, and results using 3D culture have found to be more reliable than that of the 2D culture. Currently, various experiments are being conducted centered around 3D models with increasing interest in 3D culture models. Therefore, we present a method for spheroid formation using Cardiosight®-S without the need for pre-culture.

Spheroids are formed immediately without pre-culture, allowing high live cell counts and fast experimentation. Cardiosight®-S is a product composed of highly purified hiPSC-derived cardiomyocytes, produced through NEXEL's proprietary myocardial differentiation technology that allows for purity above 90%. In addition to the extremely low percentage of fibroblasts or other unrelated cells, the use of serum-free media allows end users to design experiments to their specification. Please refer to the **Cardiosight®-S User Guide** when performing experiments using this application note.

0	2	4	6
Cell Thawing and Plating into ULA plate	Adding media	Half change	Half change
Plating media + Collagen 10 µg/ml (ThermoFisher, A1048301)	Maintenance media	Maintenance media	Maintenance media

Table 1. Timeline of the Workflow From Day 0.

Workflow

The Cardiosight®-S is plated in ULA plates with collagen I in Day 0, then forms spheroids and begin beating in Day 2. Only the maintenance media is added in Day 2 to ensure stable spheroid formation. No media removal is necessary. From Day 4 and onward, replace half of the media every other day. It is recommended to proceed with spheroid experiments between Day 5 and Day 7.

Required Consumables

Item	Vendor	Catalog number
Cardiosight® -S Cardiomyocytes	NEXEL	C-001 C-002
Cardiosight® -S Advanced Media	NEXEL	CMS-001A CMS-002A
Collagen I, rat tail	ThermoFisher	A1048301
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.

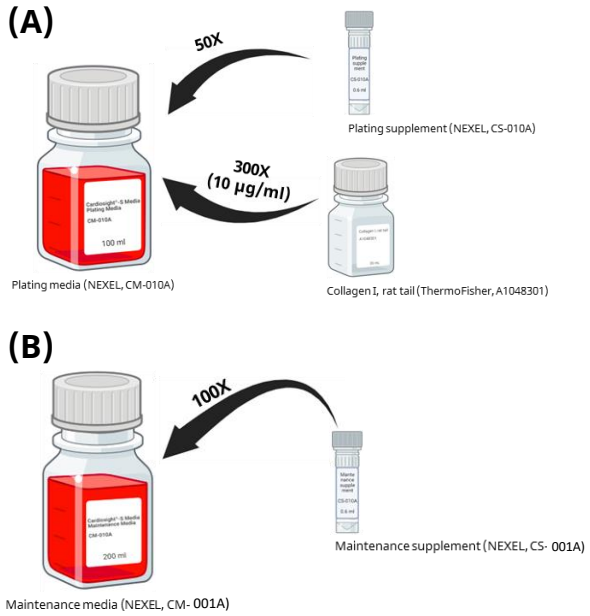


Figure 1. Plating and Maintenance medium preparation.
(A) Plating medium preparation: Add Plating supplement (NEXEL, CS-010A) and Collagen I, rat tail (ThermoFisher, A1048301) into the Plating media (NEXEL, CM-010A).
(B) Maintenance medium preparation: Add Maintenance supplement (NEXEL, CS-001A) and into the Maintenance media (NEXEL, CM-001A).

Method

Thawing and Plating Cardiosight®-S for spheroid formation

1. Thaw the cells according to the Cardiosight®-S User Guide.
2. Select and prepare an appropriate plate to form spheroids of the desired size.
   (Based on a 96-well plate)
3. Add collagen I, rat tail (ThermoFisher, A1048301) to the plating media by 300X dilution to a concentration of 10 µg/ml.
4. Count the number of live cells and add an appropriate amount according to the desired size of spheroids to the prepared plating media.
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	900 µm	730 µm	500 µm	380 µm	300 µm	190 µm

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

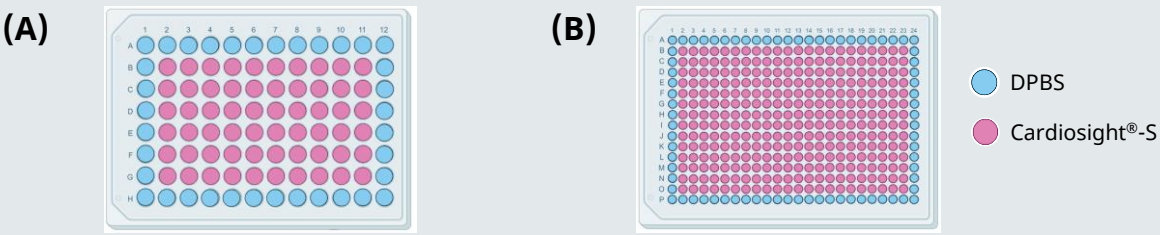


Figure 2. Recommended Plate design.
(A) PrimeSurface® ULA 96 well V bottom plate (S-bio, #MS-9096VZ).
(B) PrimeSurface® ULA 384 well U bottom plate (S-bio, #MS-9384UZ).

Maintaining Spheroid

1. Prepare a 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 the initial plating 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.

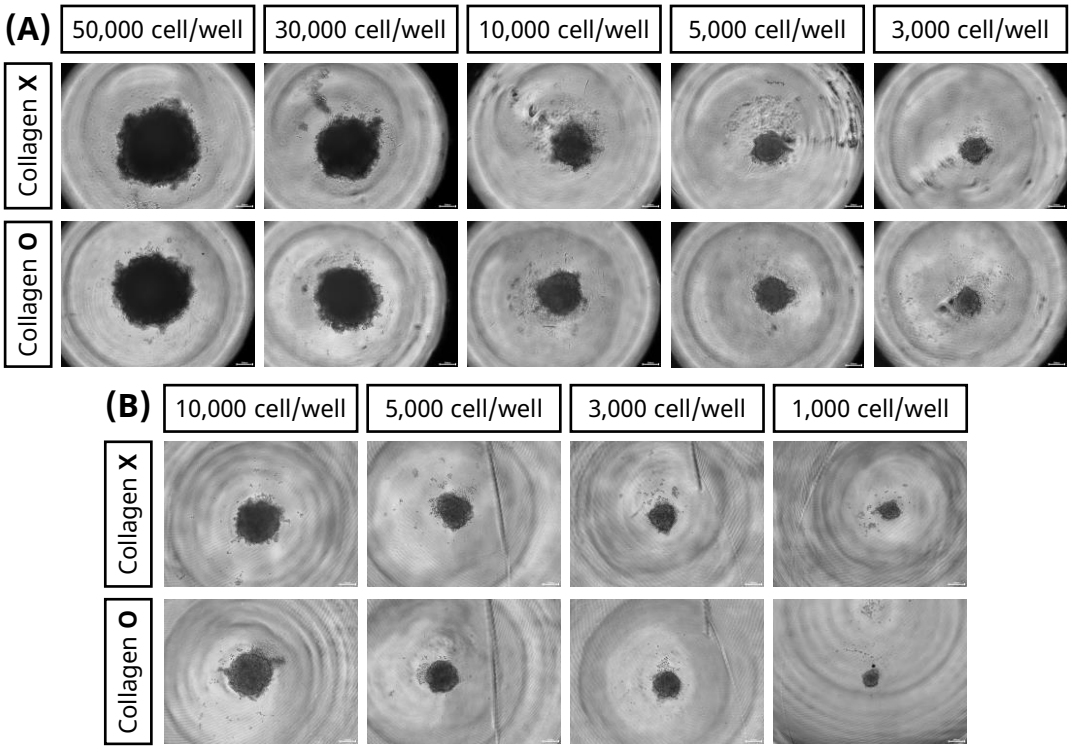


Figure 3. Size-controlled cardiac spheroids using ULA plate.
Images in the top row exhibit spheroid formation on Day 7 without the addition of collagen to the plating media. In contrast, the images in the bottom row are that of spheroids formed on Day 7 with the addition of collagen to the plating media. **(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.

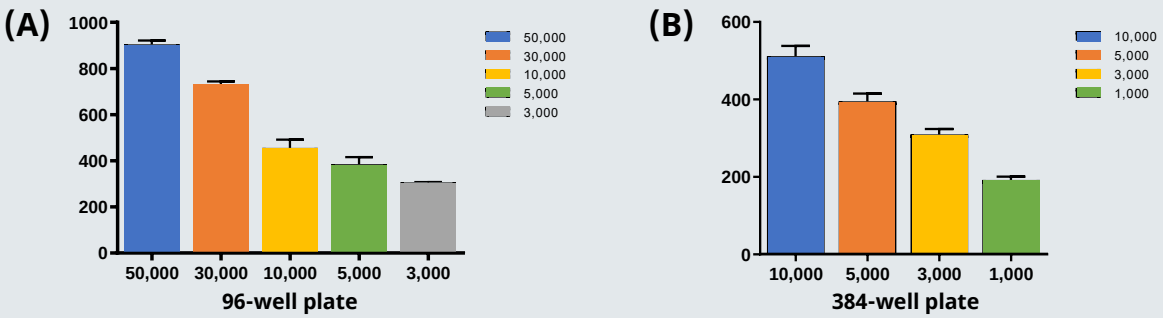


Figure 4. Graph of spheroid size.
The Y axis represents the spheroid size, and the X axis the number of cells added per well. **(A)** Graph of spheroid size formed using a PrimeSurface® ULA 96-well V bottom plate on Day 7. **(B)** Graph of spheroid size formed using a PrimeSurface® ULA 384-well U bottom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

Caution: All experimental steps described in this application note are optimized for Cardiosight®-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.