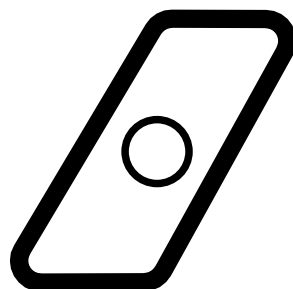




Cardiosight[®]-S
User Guide
(Advanced media)



Contents

1. Product Information.....	2
Unpacking & Handling	2
Components & Description	3
Safety Precaution & User Notice.....	5
2. Introduction	6
3. Preparing for Cell Culture.....	7
Required Equipment and Consumables (Not Provided)	7
Preparing Cardiosight®-S Advanced Media	8
Preparing Cell Culture Surfaces	9
4. Cardiosight®-S Thawing and Plating.....	10
Thawing	10
Plating.....	12
5. Cardiosight®-S Maintenance	13

1. Product Information

Unpacking & Handling

- ▼ Upon receiving the shipment of Cardiosight®-S, check whether all temperature-sensitive components are correctly frozen. If this is not the case, please contact our support team immediately.
- ▼ Immediately transfer each of the components to the appropriate storage conditions.
- ▼ Please check the catalog number, lot number, and expiry date. The basal media expiration date is the shortest (**date of expiration on label**) so experiments should be planned accordingly.
- ▼ The Cardiosight®-S should be handled by technically qualified individuals complying with good laboratory practices, applicable laboratory regulations, and the MSDS. Following the User Guide herein is recommended for best results.
- ▼ The Cardiosight®-S is intended for research use only, not intended for any type of use in animals or humans.

Cardiosight®-S User Guide (Advanced media)



Components & Description

COMPONENTS	CAT#	STORAGE ON ARRIVAL
Cardiosight®-S Cardiomyocytes		
Cryopreserved, frozen vial		Liquid Nitrogen
>2.5 million cells	C-001	
>5 million cells	C-002	
Cardiosight®-S Advanced Media		
Advanced Plating medium		
30 ml	CM-010A	
45 ml	CM-020A	4°C
Advanced Maintenance medium		
100 ml	CM-001A	
200 ml	CM-002A	
Cardiosight®-S Advanced Plating Supplement (50X)		
0.6 ml	CS-010A	-20°C
0.9 ml	CS-020A	
Cardiosight®-S Advanced Maintenance Supplement (100X)		
1 ml	CS-001A	-20°C
2 ml	CS-002A	
Cardiosight®-S User Guide¹		
Certificate of Analysis (CoA)		
MSDS²		

¹ Also available online at www.nexel.co.kr

² Enclosed with shipping documents.

▼ Should any of the above components be missing from your shipment, please contact us at NEXEL Co., Ltd. or the distributor in your country upon which our support team will provide the necessary assistance.

Cardiosight®-S Cardiomyocytes

Cell Type	Human Induced Pluripotent Stem Cell (hiPSC) derived cardiomyocytes
Cell Line of Origin	hiPSC cell line reprogrammed from commercially available normal donor fibroblast cell line
Quality Control	Please refer to the CoA for lot-specific information. Virus clearance & STR analysis data is available upon request.

Cardiosight®-S User Guide (Advanced media)



Cardiosight®-S Advanced Media & Media Supplements

- ▼ The Cardiosight®-S Advanced Medium & Supplements need to be combined to make the Cardiosight®-S Advanced Plating or Maintenance Media, after which it should be used within 1 month. DO NOT FREEZE the Cardiosight®-S Advanced Plating or Maintenance Media, aliquot into smaller quantities for best results.
- ▼ The Cardiosight®-S Advanced Plating or Maintenance Media are serum-free. For additional information on the composition, please contact our technical support team.
- ▼ The Cardiosight®-S Advanced Plating or Maintenance Media are antibiotic and antifungal free as they are not necessary if proper conditions are kept. NEXEL does not recommend the use of such agents for accurate results, but they should be used if aseptic cell culture conditions are not possible.

Safety Precaution & User Notice



Biosafety Level: 1

For research use only, not intended for any type of use in animal or humans. Appropriate safety procedures should always be used with this material. Please refer to the MSDS for detailed instructions.

User Notice & Restrictions:

- ▼ User may use the Product (Cardiosight®-S) for internal research including but not limited to screening potential drug compounds for efficacy and safety, and for the provision of such services to third parties. No other right is granted to User whether expressly, by implication, by estoppel or otherwise. In particular, the purchase of the Product does not include nor carry any right or license to use, develop or otherwise exploit the Product commercially, and no rights are conveyed to User to use the Product for any other purpose.
- ▼ User agrees to use the Product in compliance with all applicable statutes and regulations, but not to use the Product for any administration or application to humans. Moreover, User agrees not to use the Product in human subjects for human clinical use for therapeutic, diagnostic or prophylactic purposes, or in animals for veterinary use for therapeutic, diagnostic or prophylactic purposes, including but not limited to clinical applications, cell therapy, transplantation, and/or regenerative medicine without an appropriate license.
- ▼ In the case that User transfers Product to a third party, User shall convey the User Restrictions set forth herein to such third party.

2. Introduction

NEXEL Co., Ltd. strives to provide high quality human cardiomyocytes derived from induced pluripotent stem (iPS) cells using optimized proprietary protocols. The Cardiosight®-S is a highly pure and electrophysiologically active population of cells, suitable for all types of experiments in the field of cardiomyocytes. Advanced Media is newly designed to enhance the electrophysiological profiles of Cardiosight®-S, which adequately supports a robust maturation as well as excitation-contraction coupling. Together with Advanced Media, Cardiosight®-S have been validated on multiple electrophysiological platforms, resulting in synchronous beating with adequate FPD, higher calcium influx, and stronger contraction. As such, they are the perfect choice to test the advance of science in tissue-specific research, toxicity screening, efficacy testing, and drug discovery.

This User Guide will help you seed the Cardiosight®-S at the appropriate densities to create synchronous layers of cardiomyocytes appropriate for a variety of applications related to the electrophysiological behavior assays. However, please keep in mind that the best individual results will be obtained by close observation, care and optimization from the user.

3. Preparing for Cell Culture

Required Equipment and Consumables (Not Provided)

ITEM	CAT#	VENDOR
Coating Material		
Matrigel, Basal Membrane	356235	Corning
Fibronectin, Human	F0895	Sigma
Typical Cell Culture Equipment		
Liquid Nitrogen Storage Tank		
37°C Water Bath		
Tabletop Centrifuge		
Biological Safety Cabinet with UV Lamp		
Hemocytometer or Automated Cell Counter		
Phase Contrast Microscope		
Pipettes		
Cell Culture Incubator		
Typical Cell Culture Consumables		
Centrifuge Tubes		
Cell Culture Plates		
Pipette Tips		
Trypan Blue		
Phosphate Buffered Saline (PBS)		

Cardiosight®-S User Guide (Advanced media)



Preparing Cardiosight®-S Advanced Media

1. Thaw the Cardiosight®-S Advanced Media and Supplements by placing them at 4°C 24 hours prior to use.
2. In a biosafety cabinet, thaw and add the Cardiosight®-S Advanced Plating (50X) or Maintenance Supplement (100X) to the thawed medium to make the corresponding Media. Store at 4°C for up to 1 month after addition of the supplement. DO NOT FREEZE Cardiosight®-S Advanced Plating or Maintenance Media.

▼ To avoid oxidation of the media due to air contact and repeated warming/opening, it is recommended to aliquot the media into quantities enough for 2~3 media changes.

MEDIA TYPE	COMPONENTS
Cardiosight®-S Advanced Plating Media	Cardiosight®-S Advanced Plating medium Cardiosight®-S Advanced Plating Supplement
Cardiosight®-S Advanced Maintenance Media	Cardiosight®-S Advanced Maintenance medium Cardiosight®-S Advanced Maintenance Supplement

Preparing Cell Culture Surfaces

1. Calculate the amount of coating media required using the following table as a reference.

CELL CULTURE PLATE	6-well (9.6 cm ²)	12-well (3.8 cm ²)	24-well (1.9 cm ²)	48-well (1.0 cm ²)	96-well (0.33 cm ²)
COATING VOLUME	1 ml	500 µl	300 µl	100 µl	50 µl

2. Prepare the coating media to working concentrations immediately before use as described in the following table. Both coating solutions can be kept at 4°C for a short period of time but this is not recommended.

COATING TYPE	STOCK CONCENTRATION	WORKING CONCENTRATION
Matrigel	NA, 100X	1X
Fibronectin	1 mg/ml	50 µl (1:20 dilution)

▼ Fibronectin > Lamin > Matrigel

Fibronectin consistently gives the best results for Cardiosight®-S culture, but it is also the most expensive. Laminin (50 µg/ml) is a compromise between cost and quality. Whereas Matrigel is the most economic option at the cost of attachment quality. For long-term and any electrophysiological platform cultures, we strongly suggest using Fibronectin for consistent results.

3. Pipette the correct amount of coating solution to each well you intend to use.
4. Gently swirl the plate and check whether all the wells are completely covered.
5. Incubate at 37°C for at least an hour.

4. Cardiosight®-S Thawing and Plating

Thawing

The Cardiosight®-S can be thawed using typical cell culture thawing protocols. Here, we present NEXEL's optimized protocol and recommend our users to follow the instructions to maximize results. We strongly recommend thawing 1 vial at a time to minimize cell exposure to liquid DMSO.

1. Calculate the amount of Cardiosight®-S Advanced Plating Media required. For each vial, 10 ml of Advanced plating Media is required for resuspending the vial. The amount of Advanced Plating Media required can be calculated by the number of wells; recommendations for different cell culture plates can be found below.

CELL CULTURE PLATE	6-well (9.6 cm ²)	12-well (3.8 cm ²)	24-well (1.9 cm ²)	48-well (1.0 cm ²)	96-well (0.33 cm ²)
PLATING VOLUME	2 ml	1 ml	500 µl	300 µl	200 µl

2. Warm the Advanced Plating Media at Room Temperature (RT, 25°C) for at least 30 mins. For each vial to thaw, aliquot 8 ml of Advanced Plating Media in a 15 ml centrifuge tube.
3. Retrieve the Cardiosight®-S vial(s) from the liquid nitrogen storage tank.
4. Submerge the vial(s) 2/3 in a 37°C water bath so that the mouth of the vial does not come in contact with the water. Constantly check how much has thawed and once ~20% remain (~3 mins), spray the vial(s) with 70% Et-OH, wipe and place it in your biosafety cabinet. Ideally, the vial(s) should have completely thawed exactly when you start step 5.
5. Open the vial(s) and transfer the contents (~1 ml) using a 1 ml pipette to the aliquoted 8 ml of Advanced Plating Media dropwise while gently swirling the tube.
 - ▼ Dropwise pipetting while gently swirling the tube minimizes osmotic shock and maximizes mixing, which ensures high viability. Drops will remain on the surface for ~1 second and then drop towards the bottom of the tube (visible due to the DMSO content). For dropwise pipetting, simply pipette slowly into the air ~1 cm above the media surface. It should take approximately 1 min per 1 ml.
6. Use 1 ml of Advanced Plating Media to gently rinse the emptied vial and transfer dropwise to the centrifuge tube containing the cells from step 5 while gently swirling the tube.
7. Centrifuge the suspended cells at 180 x g for 3 minutes at room temperature.

Cardiosight®-S User Guide (Advanced media)



8. Carefully discard the supernatant.
9. Resuspend the cells gently using 1 ml of Advanced Plating Media and check the cell concentration using a hemacytometer or cell counter and Trypan Blue. Immediately move on to the Plating section.
 - ▼ Avoid rigorous pipetting of the cells to maximize viability. Single cell resuspension of the Cardiosight®-S during thawing should easily be achieved by gently pipetting 3~4 times.

Cardiosight®-S User Guide (Advanced media)



Plating

NEXEL recommends seeding the Cardiosight®-S at a density of ~150,000 cells/cm² for most standard applications. Application specific protocols are available upon request. The best results are obtained by the User's own optimization, for which NEXEL will try to provide as much assistance as possible.

1. Calculate the volume of Advanced Plating Media and cells required to match the correct density for the culture platform of choice. Below is a table with cell numbers.

CELL CULTURE PLATE	6-well (9.6 cm ²)	12-well (3.8 cm ²)	24-well (1.9 cm ²)	48-well (1.0 cm ²)	96-well (0.33 cm ²)
PLATING VOLUME	2 ml	1 ml	500 µl	300 µl	200 µl
CELL NUMBER	1,440,000	570,000	285,000	150,000	50,000

▼ Well area (cm²) can vary between different vendors, please check with your providers for exact calculations.

2. Combine the volumes as calculated above.
3. Remove the coating solution in the cell culture plates. Avoid drying out the coated wells as much as possible.
4. Gently mix by pipetting and evenly distribute the appropriate volumes of cells with Advanced Plating Media.
5. Move the cell culture plate to the incubator, shake the plate in perpendicular directions to evenly distribute the cells for attachment.
6. The day after, perform the first media change with Advanced Maintenance Media (Cardiosight®-S Maintenance Step 1).

5. Cardiosight[®]-S Maintenance

Starting 24 hours after plating the cells, the media needs to be changed every two days. Ideally, the media should be changed at 48-hour intervals. When performing electrophysiological assays on the cells, we recommend changing the media on the morning of the experiment to ensure there are enough nutrients for the cells and to deliver the desired drug concentration.

1. Warm the correct volume of Cardiosight[®]-S Advanced Maintenance Media volume (half of the volume for plating) at 37°C in a water bath or room temperature for at least 20 mins.
2. Perform a media change with the newly warmed media in a biosafety cabinet. Pipette softly onto the cell culture plate walls to avoid any damage to the cell culture.

▼ 24 hours after plating, replace a full volume of Plating Media with Maintenance Media. To remove the spent media, aspirate the media using a pipette without tilting the plate. Leave a small amount of media so that the cells do not come in contact with air. Then gently add 300 µl/well of pre-warmed Maintenance Media.

▼ After the initial full-media change, perform half-media changes on 48-hour interval.

▼ Performing half-media changes is crucial for cardiomyocyte cultures. Contact with air can damage cardiomyocyte cultures, which results in cell detachment from the plate, preventing long-term culture. An example of half-media change is: for 1 ml of media in the well, remove ~500 µl and add 550 µl.

▼ While performing half-media changes, it is critical to keep the plate flat on a surface. Tilting the plate during half-media changes can cause the cells to shift position and potentially induce aggregation towards the tilted direction.

3. Place the plate back in the incubator.
4. Repeat 1 to 3 every 2 days.

We recommend performing any planned assays with the Cardiosight[®]-S from Day 7 onwards. Long-term culture over 14 days can lead to aggregation or cell detachment, we recommend careful observation of borders and use of fibronectin coating for best results.