NEXEL

Cardiosight®-S Application Protocol

for the Axion Maestro MEA

Contents

1. Product Information

Unpacking & Handling

- Upon receiving the shipment of Cardiosight®-S, check whether all temperature-sensitive components are correctly stored. 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.

Components & Description

COMPONENTS	Cat#	Storage on arrival
Cardiosight®-S Cardiomyocytes		
Cryopreserved, frozen vial		LN2
>2.5 million cells	C-001	
>5 million cells	C-002	
Cardiosight®-S Media Kit : Electrophysiology Kit		
CMS-001A : Small		
CMS-002A : Large		
Cardiosight®-S Advanced Plating Media		
30 ml	CM-010A	4° C
45 ml	CM-020A	
Cardiosight®-S Advanced Media		
100 ml	CM-001A	4° C
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 Guide[1]		
Certificate Of Analysis (COA)		
MSDS[2]		

[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 Advanced Media & Media Supplements

- The Cardiosight®-S Advanced Media need to be combined with corresponding supplements before use, after which it should be used within 1 month. **DO NOT FREEZE** the Cardiosight®-S Advanced Media, aliquot into smaller quantities for best results.
- The Cardiosight®-S Advanced Media are serum-free. For additional information on the composition, please contact our technical support team.
- The Cardiosight®-S Advanced 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.

The Cardiosight®-S has been validated as a functional population of human cardiomyocytes derived from induced pluripotent stem cells, able to be used in a variety of functional assays quickly after thawing, reducing culture times, and accelerating research for our users.

Here, we provide an application protocol for the use of the Cardiosight®-S on the Axion Maestro MEA platform for users looking to test cardiac electrophysiology by local field potentials. The Cardiosight®-S, used in combination with the Maestro MEA technology, can reliably detect potential arrhythmic risks using the same protocols as in the Comprehensive in vitro Proarrhythmia Assay (CiPA) myocyte study (Blinova *et al.* 2018). An example is shown in the Figure 1 below.

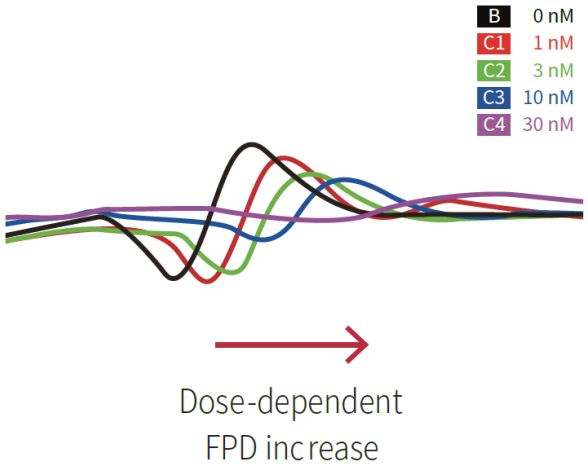

Figure 1. Effect of E-4031 on the Cardiosight®-S measured using the Axion Maestro.

In this Application Protocol, we hope to provide our users guidance on how to plate and culture the Cardiosight®-S for cardiac safety screening applications. This is not a standalone document and should be used together with the **Cardiosight®-S User Guide**.

For better understanding, we highly recommend watching the video on YouTube (https://www.youtube.com/watch?v=XBIZq31qEAI&t=56s).

3. Preparing for Cell Culture

Required Equipment and Consumables (Not Provided)

ITEM	Cat#	Vendor
Equipment		
Maestro MEA	-	Axion Biosystems
48-well MEA plate	M768-tMEA-48w	Axion Biosystems
Multichannel pipettor: 8 or 12 channels	-	Multiple providers
Software		
AxIS Navigator		Axion Biosystems
Cardiac Analysis Tool		Axion Biosystems
Coating Material		
Fibronectin	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		
Dulbecco’s Phosphate Buffered Saline (DPBS)		

Preparing Cardiosight®-S Media

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

Table 1. Media type and designation used in the User Guide

MEDIA	COMPONENTS	EXAMPLE
Cardiosight®-S Media Kit : Electrophysiology Kit **CMS-001A : Small** **CMS-002A : Large**		
P Media	**Cardiosight®-S Advanced Plating Media** **Cardiosight®-S Advanced Plating Supplement (50X)**	**980 µl** **20 µl**
M Media	**Cardiosight®-S Advanced Maintenance Media** **Cardiosight®-S Advanced Maintenance Supplement (100X)**	**990 µl** **10 µl**

When making each media, discard the calculated volume of supplements then add the supplement to the media to maintain an appropriate dilution factor.

For example, to make 10 mL of M Media, discard 100 µl of media from 10 mL then add 100 µl of the Advanced Maintenance Supplement (100X) into the media.

Coating the MEA plate

1. Calculate the amount of coating solution to be used, with **5 µl** of Fibronectin diluted in DPBS at a final concentration of **50 µg/ml (1:20 ratio)** used for each well. To coat an Axion 48-well MEA plate, prepare a total of 250 µl (Including excess to account for pipetting error).

Table 2. Recommended coating material and concentrations

Coating type	Stock concentration	Working concentration
Fibronectin	**1 mg/ml**	**50 µg/ml (1:20 dilution)**

- It is not recommended to use any other coating material than Fibronectin.

2. Pipette **5 µl of Fibronectin coating solution** to the center of the wells in a manner that forms a **drop over the measurement electrodes** as described in **Figure 2**.

- This step determines the seeding placement of the cells – covering all the measurement electrodes is best. It is preferable to avoid covering reference electrodes on the perimeter. Please refer to figure 2 for droplet placement.

- **DO NOT SWIRL** the MEA plate after adding the coating solution.

Figure 2. Droplet Placement Diagram

Independent of the plate type, all Axion plates are shaped in a similar manner with smaller round measuring electrodes in the center and reference electrodes, as highlighted in **blue**, on the perimeter. **A 5 µl droplet will cover measurement electrodes as shown in red**.

3. Handle the plate gently and add 4~6 ml of DPBS around the wells to increase the humidity. Should the fibronectin droplet dry out, cells will not be able to attach properly.

4. **Incubate at 37°C for exactly 1 hour.**

- It is important to let the coating incubate for one hour but also to not let it dry. We recommend starting the thawing process of the cells with about 40 minutes after starting the incubation of the coating.

Plating onto the MEA plate

Prepare media and thaw the cells according to the Cardiosight®-S User Guide using the P Media.

1. Calculate the total number of cells required at a density of **50,000 cells/well**. To plate a whole 48-well plate, calculate the volume that corresponds to 2,500,000 cells (excess of 100,000 cells; 50 wells total).
2. Transfer the corresponding volume of cell suspension to a 1.5 ml tube.
3. **Centrifuge** the cell suspension at **180 x g for 3 minutes** at room temperature.
4. Prepare **250 µl** of **P Media** for resuspension at the plating density (**50,000 cells/ 5 µl**).
5. Discard the supernatant and resuspend the cells in **250 µl** of **P Media** prepared in step 4.
6. Discard the coating solution and pipette 5 µl of cell suspension to the coated area in a manner that forms a **drop over the area** (Figure 2).
    - We recommend discarding the coating solution in **4 wells** at a time at most. Drying out the fibronectin coating can lead to poor cell attachment.
7. Incubate for **exactly 1 hour** at room temperature in the biosafety cabinet.
8. After incubation, carefully add 300 µl of pre-warmed **P Media** to each well.

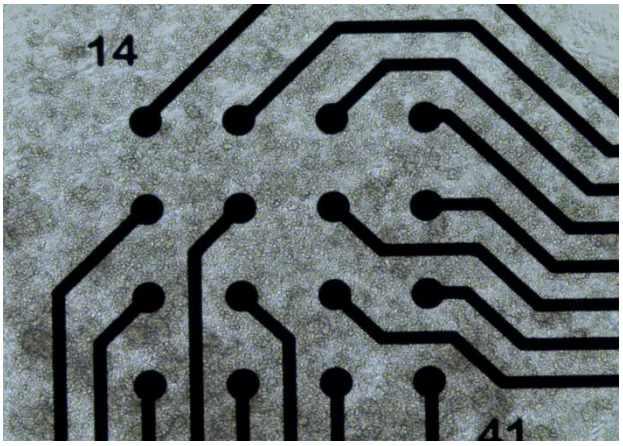

Figure 3. Morphology of the Cardiosight®-S on day 1 after thawing (40X).

The recommended density for the MEA plate is considerably higher than what is expected from the User Guide.

4. MEA Experimental Protocol

Cell culture maintenance of MEA plate

- **Prepare media and maintain the cells according to the Cardiosight®-S User Guide.**

Starting from 24 hours after plating cells, the media needs to be changed every 2-3 days. Ideally, the media should be changed at 48-hour intervals. If electrophysiological assay on the cells is to be performed in the **afternoon**, we recommend **completely 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 **M Media** at room temperature for at least 30 minutes. As for the preparation of the **M Media**, please refer to page 8 of this Application Protocol.
2. **One day post-plating**, perform a **full-media change** with the newly warmed 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 pre-warmed **M Media** from the side of the well to avoid disturbing the cardiomyocyte monolayer and to avoid touching the eletrodes.
    - Users should take extra care not to cause cell detachment during both removal and addition of media. Therefore, please avoid changing more than 3 wells at a time to avoid damage due to air contact.
3. Continue to culture the cardiomyocytes in a cell culture incubator.
4. **2 days after** the initial full-media change with **M Media**, maintain the cells by performing a **half-media change** with the newly warmed media in a biosafety cabinet. Pipette softly on to the cell culture plate walls to avoid any damage to the cell culture.
5. Repeat 1 to 3 every 2~3 days.

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

Data acquisition, compound application, and analysis

1. On the day of MEA measurement, perform a **full media change** with 300 µl pre-warmed **M Media** for each well **at least 3 hour prior** to the main experiment, then leave the MEA plate mounted on the MEA equipment under controlled environment conditions. If the DPBS outside the wells has evaporated since the day of thawing, refill as necessary
2. Prepare the drugs to be treated and buffer (vehicle) controls at a concentration 10X higher than the desired final concentration in **M Media**.

    The amount required per well is 30 µl and we recommend n=3 at the very least and n=5 to match CiPA standards. It is possible to perform sequential increases of concentrations in the same well and solutions should be prepared accordingly. An example calculation is provided in **Table 3**.

Table 3. Example of drug treatment calculation

Final Concentration (nM)	**3**	**10**	**30**	**100**
Concentration in Drug Treat Solution for Single Treat (nM)	30	100	300	1000
Concentration in Drug Treat Solution for Sequential Increases (nM)	30	73	210	730

3. Map the plate inside the Axis software according to your experimental plan.
4. Perform a baseline measurement by placing the plate in the Axion Maestro and pressing play.
5. Let the plate **equilibrate** for **30 minutes** and then record for 5 minutes.
6. Treat the drug by pipetting out 30 µl of media from each well and then adding 30 µl of the prepared drug solution.
7. Let the plate equilibrate for 1 hour (at least 30 minutes) and then record for 5 minutes.
8. For sequential treatments, repeat steps 6 and 7. Typically, at least 4 different concentrations (number of conditions supported in the Axion Cardiac Analysis Tool) are measured in each well with no adverse effects.