NEXEL

Cardiosight®-S Application Protocol

for the Axion Maestro MEA

Contents

1. Introduction

MEA Electrophysiology with the Cardiosight®-S

The Cardiosight®-S has been validated as a functional population of human cardiomyocytes derived from induced pluripotent stem cell (iPSC) 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 Figure 1 below.

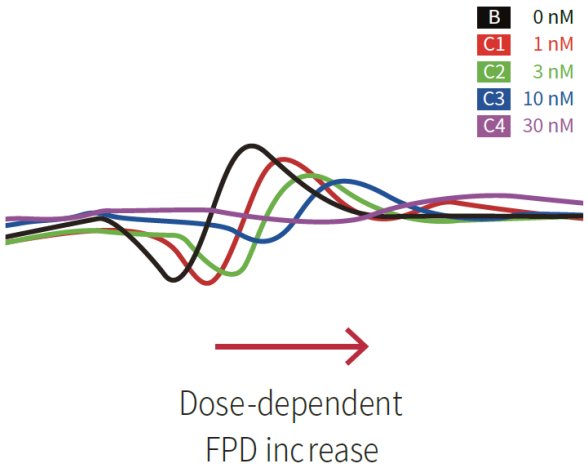

Figure 1. Effect of E-4031 on the Cardiosight®-S measured using the Axion Maestro.

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).

Required Equipment, Consumables and Software

Item	Provider	Catalog number
Equipment		
Multichannel pipettor: 8 or 12 channels	Multiple providers	
MEA system	Axion	
Consumables		
Cardiosight®-S, cryopreserved hiPSC-derived cardiomyocytes	NEXEL Co., Ltd.	C-001/C-002
Cardiosight®-S Media kit	NEXEL Co., Ltd.	CMS-001/002
Cardiosight®-S Advanced Media kit	NEXEL Co., Ltd.	CMS-001A/002A
CytoView MEA 24	Axion	M384-tMEA-24w
CytoView MEA 48	Axion	M768-tMEA-48w
Fibronectin	Sigma-Aldrich	F0895
Sterile Reagent Reservoirs	Multiple providers	
Software		
AxIS Navigator	Axion	
Cardiac Analysis Tool	Axion	

2. Plating Protocol

Coating the MEA plate

1. Calculate the amount of coating media to be prepared to keep in mind that 5 µl is required for each well. E.g., To coat a whole 48-well plate, prepare a total of 250 µl (excess of 10 µl to account for pipetting error).
2. Prepare the coating media to working concentrations immediately before use as described in the following table. The coating solution can be kept at 4°C for a short period of time (~1 hour) but this is not recommended.

Coating Type	Stock Concentration	Working Concentration
Fibronectin	**1 mg/ml**	**50 µl (1:20 dilution)**

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

3. Hold the plate at an angle which allows us to see the electrode grid in each well. Pipette the correct amount of coating solution (5 µl) to the center of the wells in a manner that forms a drop over the measurement electrodes. This step determines the seeding placement of the cells – covering all the measurement electrodes is best. It is preferable to avoid covering the T-shaped reference electrodes.

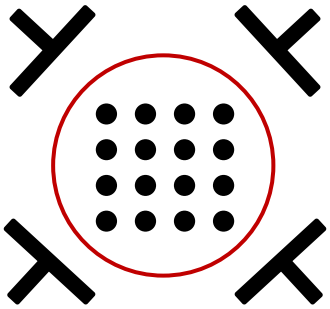

Figure 2. Droplet Placement Diagram

Independently of the plate type, all Axion plates are shaped in a similar manner with smaller round measuring electrodes in the center and reference electrodes on the outside. A 5 µl droplet will cover the measurement area appropriately as shown in red.

4. Handle the plate gently and add 6~8 ml of D-PBS around the wells to increase the humidity. Should the droplet dry out, cells will not be able to attach properly.
5. 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 about 40 minutes after starting the incubation of the coating.

Plating onto the MEA plate

1. Thaw the cells according to the Cardiosight®-S User Guide.
2. Calculate the number of total cells keeping in mind that 50,000 cells are required for each well. E.g., 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).
3. Transfer the corresponding volume to a 1.5 ml tube.
4. Centrifuge the suspended cells at 180 g for 3 minutes at room temperature.
5. During the centrifugation, remove the D-PBS which had been added around the wells. This will allow for easier handling of the plate when seeding the cells.
6. Resuspend the cells using Cardiosight®-S Plating Media to match the plating density (5 µl per 50,000 cells). E.g., For 50 wells, 250 µl is the correct volume.
7. Discard the coating solution and add 5 µl of the cell suspension 6 wells at a time at most. Drying out of the fibronectin can lead to poor cell attachment. The droplet of cells should not be able to spread out of the coating area due to surface tension.
8. Handle the plate gently and add 3~5 ml of D-PBS around the wells to increase the humidity. Should the droplet dry out, cells will not be able to attach properly.
9. Incubate at room temperature for exactly 1 hour.
10. Add 300 µl of pre-warmed Cardiosight®-S Plating Media to each well.

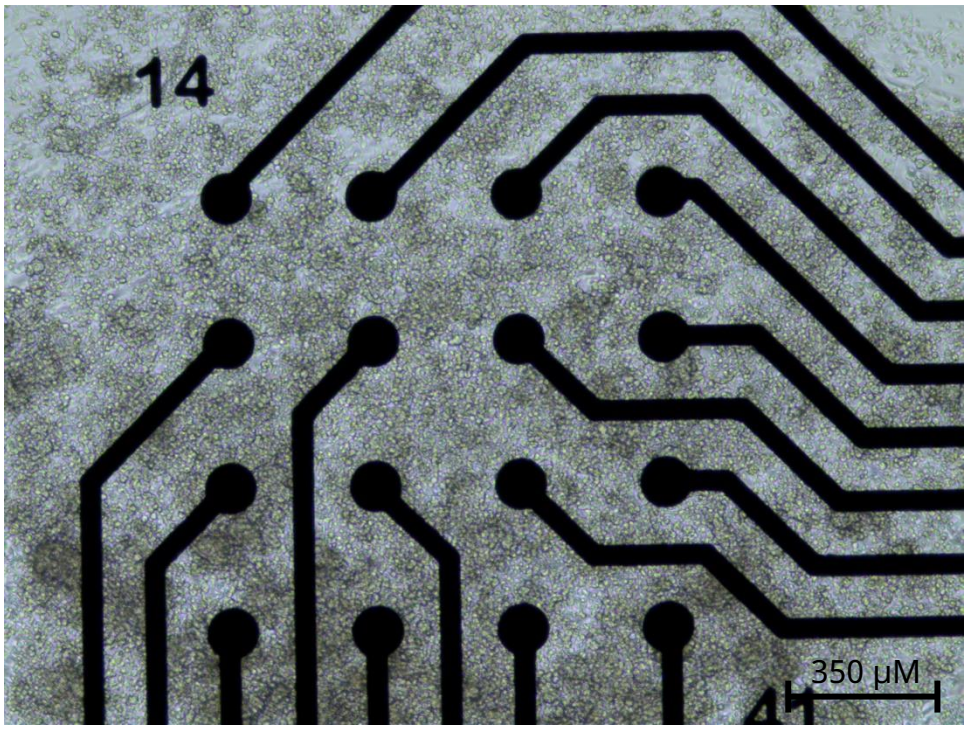

Figure 3. Morphology of the Cardiosight®-S on day 1 after thawing (40X).

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

3. MEA Experimental Protocol

Cell culture maintenance of MEA plate

1. Prepare the Cardiosight®-S Maintenance Media (or Advanced Maintenance Media) as described in Cardiosight®-S User Guide.
2. Immediately before use, equilibrate an aliquot of Maintenance Media (or Advanced Maintenance Media) at room temperature for 30 minutes.
3. One-day post-plating, replace the Plating Media with Maintenance Media. To remove the spent media, slightly tilt the MEA plate and aspirate the media using a pipette. Then, gently add 300 µl/well of pre-warm Manteinance Media to the from top of the well to avoid disturbing the cardiomyocyte monolayer and make sure not to touch the electrodes.
   - Avoid changing more than 6 wells at a time to avoid damage due to air contact.
4. Maintain the cardiomyocyte culture on the MEA plate by replacing 100% of the spent media with 300 µl/well of fresh pre-warm Maintenance Media (or Advanced Maintenance Media) every 48 hours.
5. Continue to culture the cardiomyocytes in a cell culture incubator at 37°C, with 5% CO_2.
6. We recommend performing an MEA assay from day 7 post-plating.

Data acquisition, compound application, and analysis

1. On day 7, change the media completely with 300 µl pre-warmed Cardiosight®-S Maintenance Media (or Advanced Maintenance Media) for each well at least 3 hours before the main experiment. If the D-PBS 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 Cardiosight®-S 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 below.

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. Open the lid and pipette 30 µl up and down two times to mimic treating the drug.
5. Let the plate equilibrate for 5 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. Pipette exactly twice for every well.
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.