



## Cell Culture on Microelectrode Arrays

Cell Type: Cryopreserved Human iPSC-Derived Cardiomyocytes (Direct Plating)

Protocol

# Human iPSC-derived Cardiomyocytes from Cellular Dynamics



## Introduction

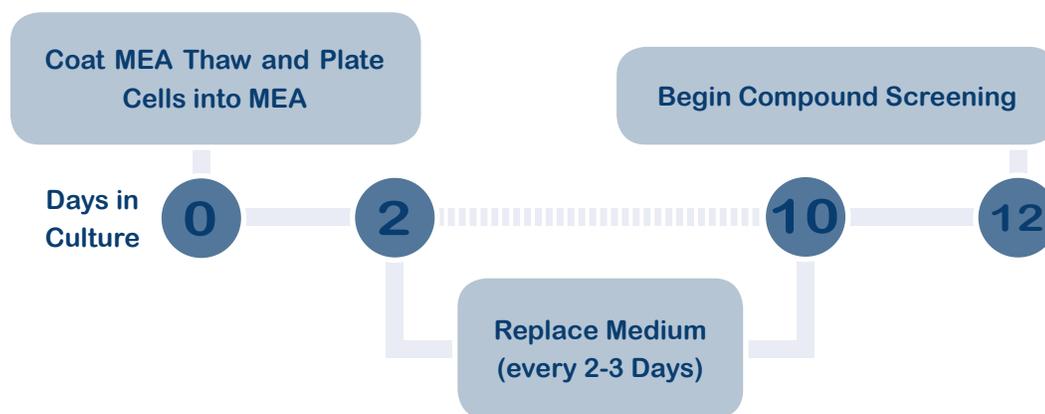
iCell Cardiomyocytes are human induced pluripotent stem cell-derived cardiomyocytes that exhibit typical biochemical, electrophysiological, mechanical, and pathophysiological characteristics of native human cardiac myocytes. Due to their human origin, high-purity, functional relevance, and ease of use, iCell Cardiomyocytes represent an optimal test system for interrogating cardiomyocyte biology in basic research and many areas of drug development.

The Maestro multielectrode array (MEA) system from Axion BioSystems is a non-invasive, label-free platform that measures local field potentials of electrically active cells, representing summed activity of underlying ion channels. iCell Cardiomyocytes can be cultured on MEAs to form an electrically and mechanically active stable syncytium amenable to electrophysiological interrogation. Together, iCell Cardiomyocytes and Axion's MEA technology form an excellent, non-invasive platform for *in vitro* screening of compound effects on human cardiomyocyte physiology.

This Application Protocol describes how to handle iCell Cardiomyocytes for use on The Maestro MEA system and provides basic instructions for compound treatments, data acquisition, and analysis.

## Workflow

iCell Cardiomyocytes are thawed into Axion 12- or 48-well MEA plates previously coated with fibronectin. On day 2 post-plating, replace the spent medium with iCell Cardiomyocytes Maintenance Medium (Maintenance Medium), and replace medium every 2 - 3 days thereafter until day 10. From day 10-14 post-plating, baseline activity is recorded, cells can be treated with compounds, and the cardiac activity recorded on the MEA plate.



## Links to Resources

Axion BioSystems  
Technical Support  
Applications

## Methods

### Preparing the MEA Plate

1. Place a 8  $\mu$ l droplet of fibronectin (1:20 in D-PBS, final concentration of 50  $\mu$ g/ml) over the recording electrode area of each well in the MEA plate. See Figure 1 for appropriate drop placement.



Reconstitute fibronectin in sterile water at 1 mg/ml according to the manufacturer's instructions. Aliquot and store at -20°C, and dilute to 50  $\mu$ g/ml the day of use.

2. Add 2-6 mL of sterile deionized water to the area surrounding the wells (MEA reservoirs) of the MEA plate to prevent substrate evaporation. Do not allow the water into the wells of the MEA plate.



MEA reservoir water is no longer required following the media addition in Step 30.



Care should be taken when using higher volumes of water in the MEA reservoirs to prevent sloshing and spilling.

3. Incubate the fibronectin-coated MEA plate in a cell culture incubator at 37°C, for at least 60 minutes.

### Culturing iCell Cardiomyocytes

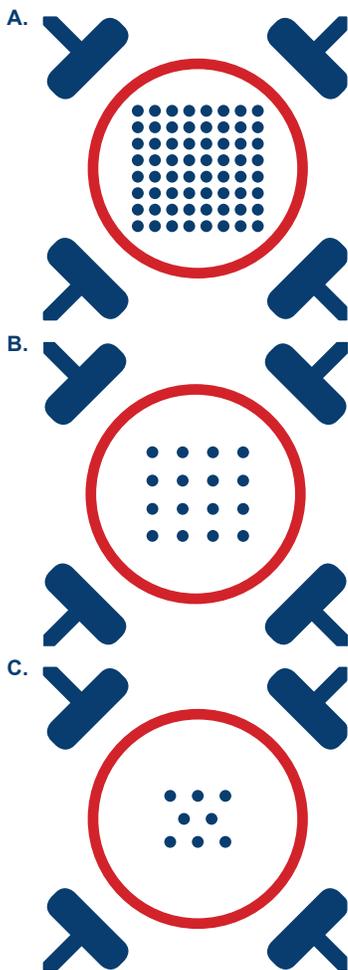
4. Thaw iCell Cardiomyocytes according to the iCell Cardiomyocytes User's Guide.
5. Remove a sample of the cell suspension and count the cardiomyocytes using a hemocytometer to determine both the viability and total number of viable cells. Transfer the cell suspension to a 15 ml conical tube.



Ensure the cardiomyocytes are evenly suspended before removing an aliquot to count.

6. Centrifuge the cell suspension at 180 x g for 5 minutes.
7. Aspirate the supernatant, being careful not to disturb the cell pellet.
8. Dilute the cell suspension in iCell Cardiomyocytes Plating Medium (Plating Medium) to 2,000,000 plated cardiomyocytes/ml. See the iCell Cardiomyocytes User's Guide for instructions to calculate the Target Plating Density based on Plating Efficiency.

Notes:



**Figure 1: Drop Placement Diagram**

The layouts above represent the bottom surfaces of wells in (A) a 12-well MEA, (B) a 48-well MEA, and (C) a 96-well MEA. The number of electrodes per well is different across the plate formats, however the drop placement is the same, with the drop (red circle) centered on the recording electrodes and staying within the ground electrodes.

9. Transfer the cell suspension to a sterile 1.5 ml Eppendorf tube.

### Plating iCell Cardiomyocytes onto the MEA

10. Aspirate, from a single row or column, most of the fibronectin solution from the MEA surface.

11. Place a 8  $\mu$ l droplet of iCell Cardiomyocytes suspension (approx. 16,000 cardiomyocytes) over the recording electrode area of each well. See Figure 1 for appropriate drop placement.



Timing is critical in this step. Cardiomyocyte attachment is compromised if the fibronectin is allowed to dry. Under typical conditions, the well will begin to dry within a few minutes after aspiration of the excess fibronectin solution. At this point the residual fibronectin in the well will begin to crystallize, turn white, and the well should then be ignored as cardiomyocyte attachment will be suboptimal.

12. Repeat Steps 10 and 11 until all rows or columns have been plated.

13. Incubate the MEA plate with the seeded cardiomyocytes in a cell culture incubator at 37°C, 5% CO<sub>2</sub> for 1 hour.

14. Gently add 150  $\mu$ l of Maintenance Medium to each well of the MEA. Adding medium too quickly will dislodge the adhered cardiomyocytes.



Using a pipettor, add medium first in a semi-circle along the outer edge of the flat bottom area. Progressively add medium so it fills evenly towards the center, stopping before contact is made with the droplet in the center. Gently bridge the gap with additional medium. The goal is to prevent a rush of medium in either direction that might dislodge the cardiomyocytes.

15. Slowly add the remaining volume to reach the plate recommended media volume. Recommended well volumes for each plate type: **12-well = 500 $\mu$ L, 48-well = 300 $\mu$ L, 96 well = 200 $\mu$ L.**

16. Incubate in a cell culture incubator at 37°C, 5% CO<sub>2</sub>.

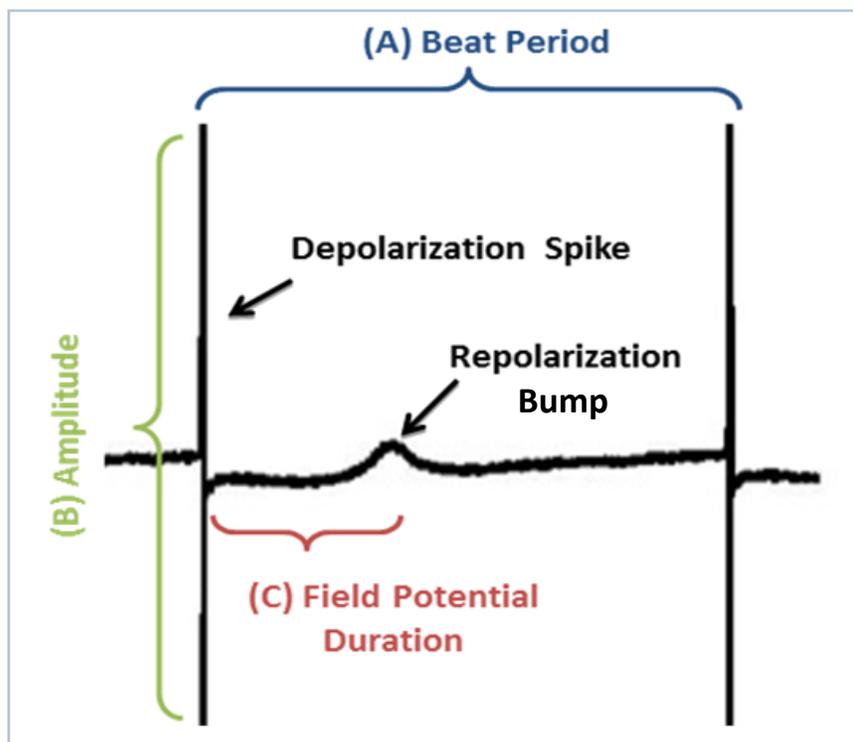
17. On day 2 post-plating, replace 100% of the Plating Medium with iCell-Cardiomyocytes Maintenance Medium (Maintenance Medium).

18. Culture iCell Cardiomyocytes on the MEA plate replacing 50% of the spent medium with Maintenance Medium every 2-3 days.

19. For optimal results, perform MEA recordings 10-14 days after plating.

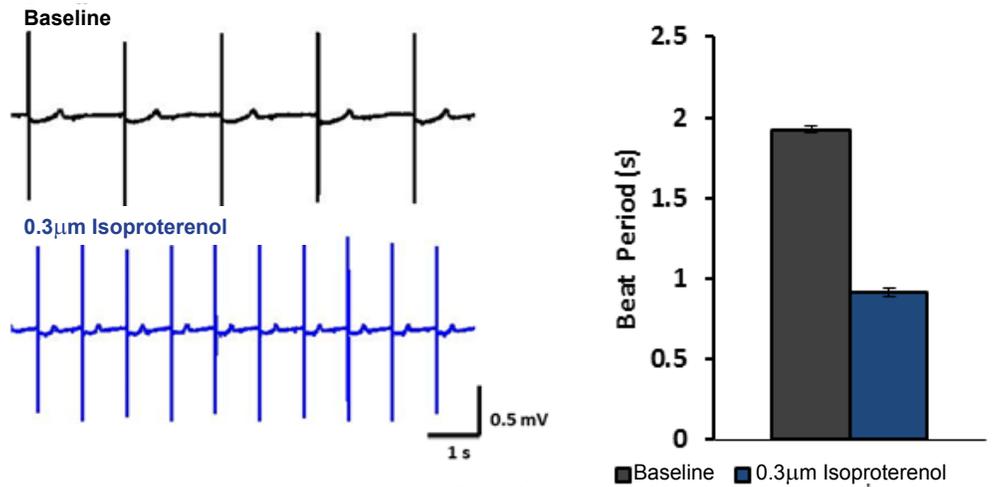
## Data Acquisition & Analysis

Electrical activity of iCell Cardiomyocytes in Axion MEA plates is acquired using the Maestro recording instrument and AxIS Software. Product manuals can be downloaded at [www.axion-biosystems.com](http://www.axion-biosystems.com). The waveform recorded by each electrode on the multi-well MEA plate reflects the field potential at that electrode relative to ground electrodes. Raw voltage signals from MEAs show easily-identifiable features corresponding to the depolarization and repolarization phases of the cardiomyocyte action potential. The following figures illustrate key characteristics of the MEA waveforms and performance of iCell Cardiomyocytes.



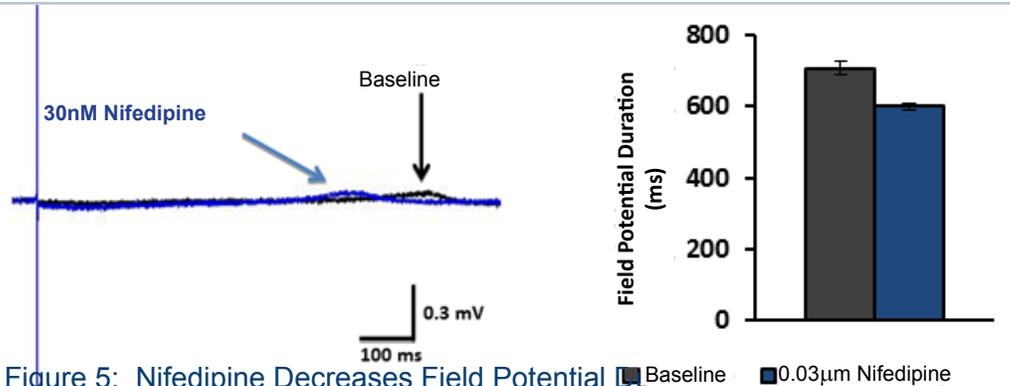
**Figure 3. Example Cardiac Field Potential and Analysis Parameters**

The depolarization and repolarization phases are labeled on the example cardiac spike complex in Fig. 3, along with corresponding regions for beat period (A), amplitude (B) and field potential duration (C). For the following figures MEA data were acquired and analyzed using AxIS Software and exported to Microsoft Excel or MATLAB for presentation. A 1 min baseline recording was made before the addition of compounds. Compounds were then added to the wells and the plate was placed in an incubator for 30 minutes before recording. For Figures 3, 4 and 5, a 1 minute period following the first 15 seconds of the recording after dosing was used for analysis in AxIS.



**Figure 4. Isoproterenol Decreases iCell Cardiomyocyte Beat Period**

On the left are example traces from iCell Cardiomyocytes for a baseline period (black) and a period of increased beat rate 30 minutes after addition of 0.3 μM isoproterenol. At right are the mean beat period ± SD for the two conditions.



**Figure 5: Nifedipine Decreases Field Potential**

On the left, an overlay of two spike traces from iCell Cardiomyocytes from a pre-dose baseline period (black) and a period 30 minutes after addition of 30 nM nifedipine. On the right, field potential duration (mean ± SD) from two wells before (gray bar) and after (blue bar) addition of 30 nM nifedipine.

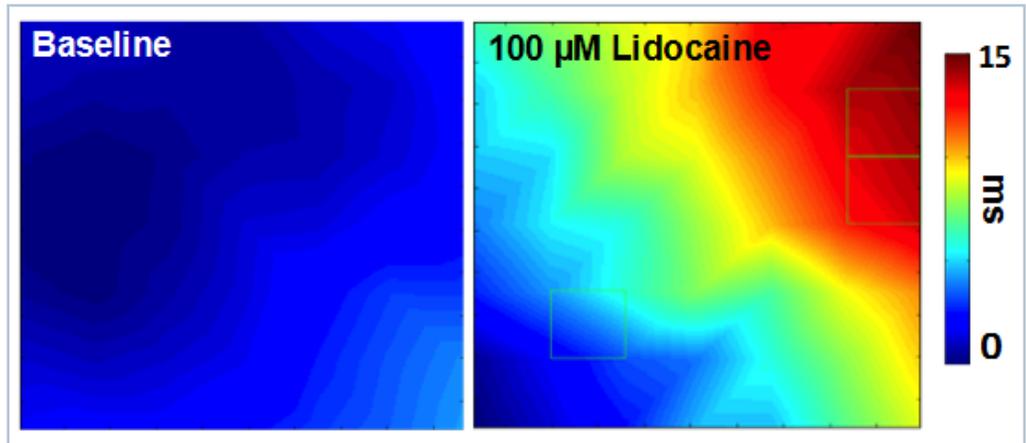


Figure 6: MEA Propagation Maps for Spontaneous Action Potentials in Cultured iCell Cardiomyocytes

For each beat, latency from the time at which an action potential is first detected is given a color-scale, illustrating signal conduction patterns. Boxes represent electrodes where action potential amplitude was sub-threshold, and latency is thus interpolated. In this example, application of 100  $\mu\text{M}$  lidocaine decreases the conduction velocity across the network compared to the pre-dose condition.

## Summary

iCell Cardiomyocytes can be cultured on MEAs where electrical activity corresponding to spontaneous beating can be monitored. The waveform characteristics representing cardiomyocyte depolarization, repolarization, automaticity, and propagation can be readily measured, and their modulation by cardioactive compounds can be robustly quantified. The methods and data presented here highlight the ease of using iCell Cardiomyocytes on The Maestro MEA platform from Axion BioSystems. Together, these products offer a high throughput in vitro system for gathering physiologically relevant data on the electrophysiological activity of human cardiac cells.

## Required Materials

### Consumables

| Item                                                         | Vendor                          | Catalog Number  |
|--------------------------------------------------------------|---------------------------------|-----------------|
| iCell Cardiomyocytes Kit                                     | Cellular Dynamics International | CMC-100-010-001 |
| Dulbecco's PBS without Ca <sup>2+</sup> and Mg <sup>2+</sup> | Life Technologies               | 14040           |
| Fibronectin                                                  | Roche                           | 11051407001     |
| KimWipes                                                     | Various                         |                 |
| Pipettes and Pipettors                                       | Various                         |                 |
| 1.5 mL Eppendorf Tubes                                       | Various                         |                 |
| 15 mL and 50 mL Centrifuge Tubes                             | Various                         |                 |
| Pipette Aid and Sterile Pipettes                             | Various                         |                 |
| Sterile 70% Ethanol                                          | Various                         |                 |

### Equipment

| Item                                    | Vendor           | Catalog Number |
|-----------------------------------------|------------------|----------------|
| Maestro MEA System                      | Axion BioSystems |                |
| 12-Well MEA                             | Axion BioSystems | M768-GLx       |
| 48-Well MEA                             | Axion BioSystems | M768-KAP       |
| 96-Well MEA                             | Axion BioSystems | M768-KAP-96    |
| Axion Integrated Studio (AxIS)          | Axion BioSystems |                |
| 37°C Water Bath                         | Various          |                |
| Cell Culture Incubator                  | Various          |                |
| Hemocytometer or Automated Cell Counter | Various          |                |
| Biological Safety Cabinet               | Various          |                |
| Tabletop Centrifuge                     | Various          |                |
| Phase Contrast Microscope               | Various          |                |
| Liquid Nitrogen Storage                 | Various          |                |

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## Origin

Axion BioSystems Microelectrode Arrays are manufactured in the United States of America.

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