axion BioSystems

Cell Culture on Microelectrode Arrays

Cell Type: Cryopreserved Human iPSC-Derived Neurons

Protocol

v. 1.1

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Origin

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

Copyright Notice

Acknowledgement

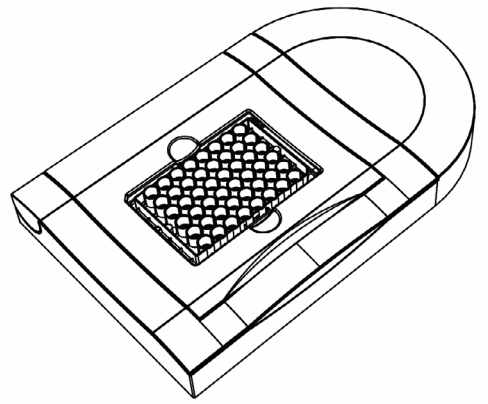
Axion BioSystems would like to thank Cellular Dynamics International, Inc. for providing their experience and resources toward the creation of this application note.

Table of Contents

Notes:

Before You Begin

1. Read this entire manual before using cells or the microelectrode arrays.
2. Check the Axion Maestro system for correct performance. Contact Axion at support@axion-biosystems.com with any issues.
3. Consult with Axion about untested experimental variables if there is concern with the safety of the equipment.
4. The User Guide created by Cellular Dynamics International, Inc. is referenced throughout this manual, please refer to this document for further information: CDI iCell Neurons User Guide



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Introduction

iCell® Neurons are human induced pluripotent stem cell (iPSC)-derived neurons that exhibit biochemical, electrophysiological, and pathophysiological properties characteristic of native human neurons. Due to their high purity, functional relevance, and ease of use, iCell Neurons represent an optimal *in vitro* test system for neurobiology interrogations in basic research and in 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 reflecting underlying activity of ion channels and synapses. With proper handling, iCell Neurons can be thawed and cultured directly on MEAs to form neuronal networks amenable to electrophysiological interrogation. Together, iCell Neurons and Axion Biosystems MEA technology provide an excellent, non-invasive platform for assessing effects of compounds on neuronal excitability and neurotransmission.

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

Notes:

Technical Support

For any questions about cell plating or Maestro system operation, please contact Axion BioSystems Support using the information below.

Telephone: (404) 477-2557

Fax: (404) 385-4638

E-mail: support@axion-biosystems.com

Required Materials

Consumables

Item	Vendor	Catalog Number
iCell Neurons Kit	Cellular Dynamics International	NRC-100-010-001
HBSS	Life Technologies	14170-112
50% Polyethylenimine Solution (PEI)	Sigma-Aldrich	P3143
Boric Acid	Fisher Scientific	A73-500
Sodium Tetraborate	Sigma-Aldrich	221732
Hydrochloric Acid	Sigma-Aldrich	H1758
Laminin	Sigma-Aldrich	L2020
Kimwipes	Various	
Pipettes and Pipettors	Various	
15 mL and 50 mL Centrifuge Tubes	Various	
Pipette Aid and Sterile Pipettes	Various	
Sterile 70% Ethanol	Various	

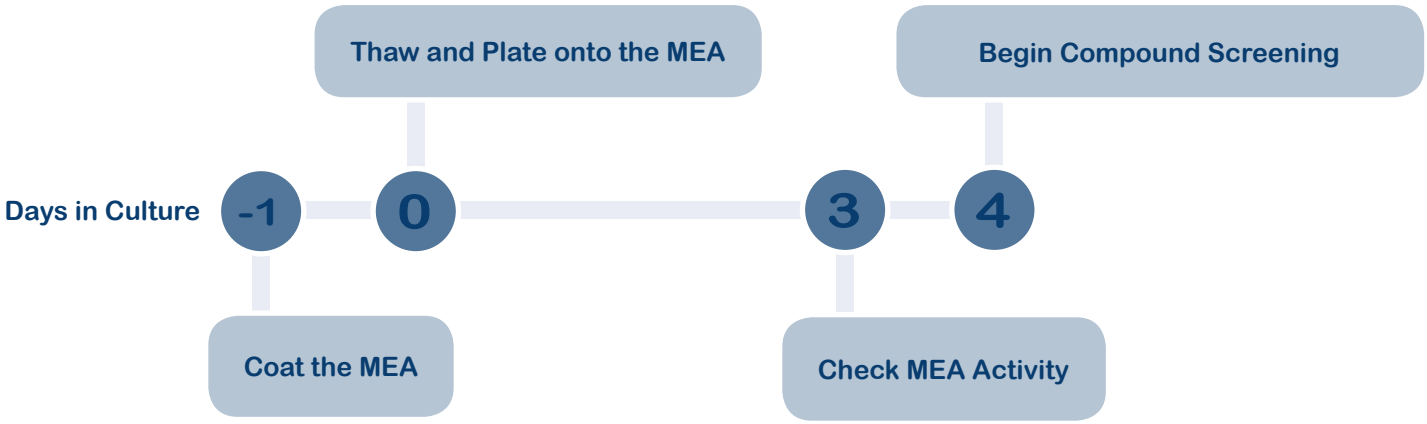
Equipment

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	

Notes:

Workflow

iCell Neurons are thawed and plated onto the MEA plates coated with polyethylenimine (PEI). On day 3 post-plating, electrical activity can first be checked using the Maestro. On day 4, cells can be used for experimentation such as compound screening.



Notes:

Methods

Preparing the MEA Plates

1. Prepare 1 L of borate buffer by dissolving 3.10 g of boric acid and 4.75 g of sodium tetraborate in distilled water. Adjust the pH to 8.4.
2. Prepare a 0.1% PEI solution by diluting 50% PEI solution in borate buffer. Filter the 0.1% PEI solution through a 0.22 µm filter.

Tip **0.05 - 0.1% PEI solution can be stored at 4°C for up to 1 month.**

3. Add 200 µl of 0.1% PEI solution to each well of the MEA plate. Incubate at room temperature for 1 hour.
4. Aspirate the PEI solution from each well of the MEA plate. Do not allow the wells to dry.
5. Rinse each well 4 times with 300 µl of sterile water.
6. Air-dry the MEA plate with the lid off in a sterile biological safety cabinet overnight.

It is essential to allow the MEA plate to air-dry overnight to achieve optimal cell adhesion.

Thawing Cryopreserved iCell Neurons

7. Prepare Complete iCell Neurons Maintenance Medium (Complete Maintenance Medium) according to the iCell Neurons User's Guide*.
8. Dilute the laminin solution (1 mg/ml) in 15 ml Complete Maintenance Medium to a final concentration of 20 µg/ml. Gently mix by inverting the tube.

Thaw stock laminin solution at room temperature or overnight at 4°C. Do not thaw stock laminin solution in a 37°C water bath. Do not vortex laminin solution.

9. Thaw cryopreserved iCell Neurons according to the iCell Neurons User's Guide* to a final volume of 5 ml in Complete Maintenance Medium.
10. Remove a sample of the cell suspension and count the viable neurons using a hemocytometer.

* Refer to: http://www.cellulardynamics.com/products/lit/CDI_iCellNeuronsUsersGuide121106.pdf

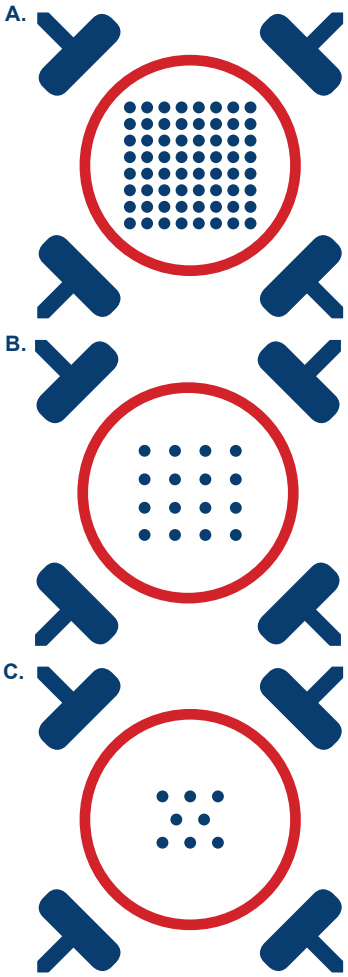


Figure 1: Drop Placement Diagram

The layouts above represent the bottom surfaces of wells in a 12-well MEA (A), a 48-well MEA (B), and a 96-well MEA (C). Diagram A represents a 12-well MEA and the inner 64 dots of the electrode array with the 4 ground electrodes located in the corners. Diagram B represents a 48-well MEA and the inner 16 dots of the electrode array with the 4 ground electrodes located in the corners. Diagram C represents a 96-well MEA and the inner 8 dots of the electrode array with the 4 ground electrodes located in the corners. The red circles indicate the approximate size and location for the drop placement.

11. Further dilute the cell suspension with 5 ml of 20 µg/ml laminin solution to achieve a final laminin concentration of 10 µg/ml.
12. Transfer the cell suspension to a 15 ml centrifuge tube.
13. Concentrate the neurons by centrifuging at 380 x g for 5 minutes.
14. Dilute 500 µl of 20 µg/ml laminin solution in additional 500 µl of Complete Maintenance Medium to achieve a 10 µg/ml laminin solution.
15. Aspirate the supernatant to just above the cell pellet, being careful not to disturb the pellet.
16. Resuspend the cell pellet in 10 µg/ml laminin solution to a final concentration of 28,000 viable neurons/µl.
17. Transfer the cell suspension to a 1.5 ml centrifuge tube.

Plating iCell Neurons onto the MEA

18. Dispense a 5 µl droplet of the cell suspension (140,000 neurons) directly over the recording electrode area of each well of the MEA precoated with PEI solution. See Figure 1 for appropriate drop placement and size.

Do not allow the cell suspension droplets to incubate in the MEA plate for longer than 1 hour before beginning addition of Complete Maintenance Medium.

19. Add 6 ml of sterile water to the area surrounding the wells (MEA reservoirs) of the MEA plate to prevent substrate evaporation. Do not allow water into the wells of the MEA plate.

Tip **MEA reservoir water is no longer required following the media addition in Steps 21 and 22.**

20. Incubate the MEA plate with the seeded neurons in a cell culture incubator at 37°C, 5% CO_2 for 1 hour.
21. Gently add 100 µl of Complete Maintenance Medium down the side of each well of the MEA one row at a time using a multi-channel pipettor. Adding the medium too quickly will dislodge the adhered neurons.
22. Repeat step 21 two times to reach a final volume of 300 µl/well.

Notes:

Timing is critical in this step. The performance is compromised if the droplets are allowed to dry. CDI recommends adding a small volume of medium to all wells first rather than adding the total volume in each well at once.

23. Incubate in a cell culture incubator at 37°C, 5% CO_2.
24. Exchange 50% of medium every 7 days by aspirating 150 µl of spent medium from each well and adding 150 µl of Complete Maintenance Medium.
25. Test electrical activity of iCell Neurons using the Maestro MEA system on day 3 post-plating.

Peak activity will occur on day 4 to day 5 post-plating. For optimal experimental reproducibility, wait until these days to test effects of compounds

Notes:

Data Acquisition & Analysis

Axion AxIS Software is used to record raw voltage data and detect spikes for rate analysis. Product manuals can be downloaded at www.axion-biosystems.com for more comprehensive instructions. To test the sensitivity of iCell neurons to well-characterized neuroactive compounds, well-wide spontaneous spike activity was quantified pre and post-dose. Results are presented in Fig. 3. Substantial changes were observed for all compounds except negative controls. For example, the inhibitory neurotransmitter GABA reduces spontaneous activity, whereas disinhibition with the GABA antagonists PTX and gabazine increases activity.

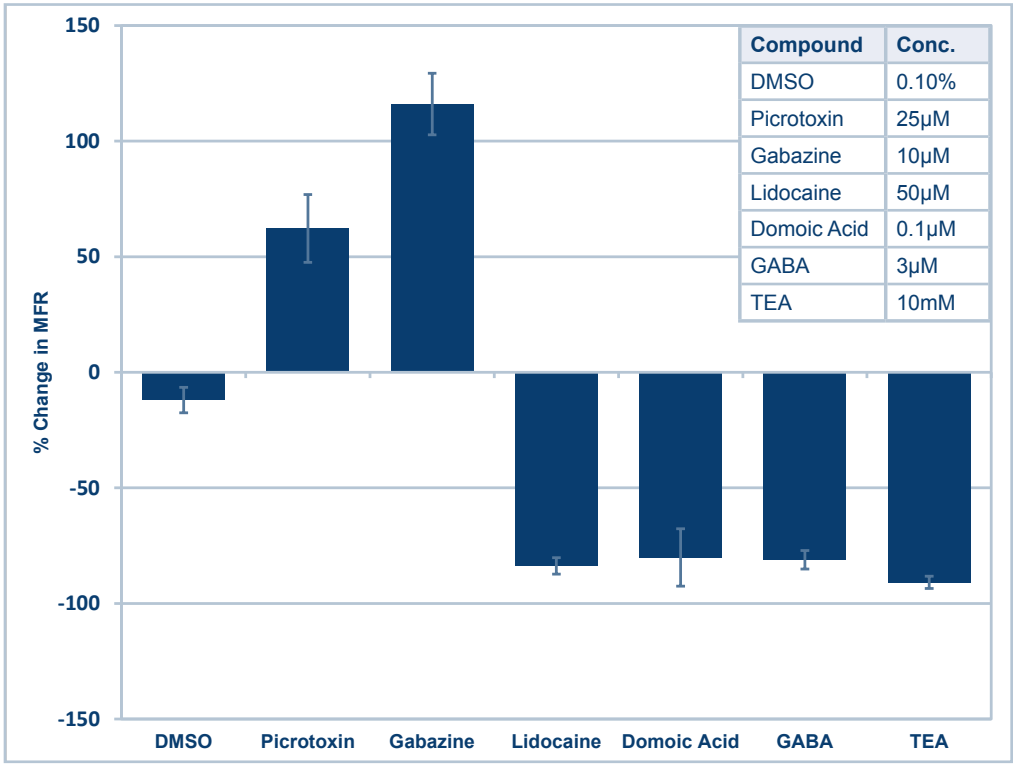


Figure 3: Effect of Neuroactive Compounds on Spontaneous Spike Activity in iCell Neurons Cultured for 4 Days in Axion 48-well MEA plates
Well-wide Mean Firing Rate (MFR) was calculated pre- and post-dose as total spikes/time. Electrodes with < 5 spikes/min were considered inactive and were not included in the calculations. Values represent means ± SEM for n=3 wells. The vehicle, DMSO, reduced MFR by only 12%.

Summary

iCell Neurons can be thawed and cultured directly on Axion multiwell MEAs, where neuronal networks are established and spontaneous electrical signals can be monitored. Neuronal spike activity can be modulated with compounds and functional responses can be robustly quantified. The methods and data presented here highlight the ease of using iCell Neurons on the Maestro MEA system from Axion BioSystems. Together, these products offer a high throughput *in vitro* platform for gathering relevant data on the electrophysiological activity of human neuronal cells.