axion BioSystems

Cell Culture on 12-Well Microelectrode Arrays

Cell Type: E18 Embryonic Rat Cortical Neurons

User's Guide

v. 1.0

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Origin

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

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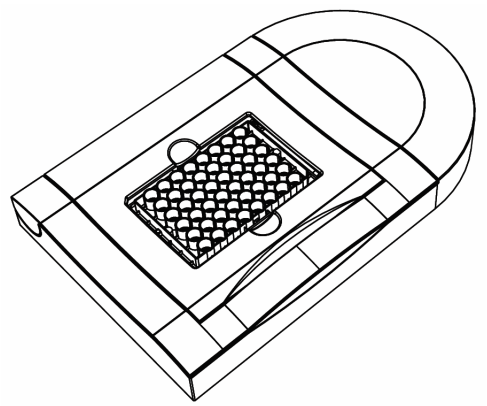
Notes:

Before You Begin

1. Read this entire manual before using cells or the MEA.
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.



Notes:

Introduction

Axion BioSystems's multi-well and single-well microelectrode arrays are ideally suited for investigation of electroactive cells and tissue (e.g., neural, cardiac, muscle, and spinal tissue). The MEA-wells are organized in an ANSI-SBS compliant format, compatible with traditional plate readers and automated instrumentation. Within each well, individual embedded microelectrodes with integrated ground electrodes are capable of simultaneously monitoring the activity of individual cells. The arrangement of these electrodes into a grid extends the recording range across a 2×2 mm area, providing concurrent access to both single-cell and network-level activity.

Axion's Integrated Studio (AxIS) software simplifies the process of performing MEA cell culture experiments. Our easy to use software provides complete access to critical information and total control of experimental parameters. AxIS allows concurrent monitoring of channel recordings, digital and analog filter adjustments, electrode assignment, and stimulus waveform design, all within the same application in an easy to use modular layout.

This user guide will aid you in growing your E18 embryonic rat cortical neuron cultures on the microelectrode array (MEA). With fully functional and healthy cultures, you will be able to collect electrophysiological spike data from your neurons. It is important to follow these guidelines closely for the greatest chance of obtaining a successful culture system. By day 5-7 in vitro the user will find it possible to begin recording electrophysiological data from their cultures. This user guide will help the user obtain useful data through AxIS to be compared with expected results after collection.

Notes:

Technical Support

If there is an issue or question at anytime before, after, or during any Maestro or Muse use; please contact Axion BioSystems. The Axion BioSystems support team can help with any issues related to the equipment, software or cell culture.

Please use one of the contact options below and our team will start looking into your concern.

Telephone: (404) 477-2557

Fax: (404) 385-4638

E-mail: support@axion-biosystems.com

Required Materials (as suggested by Axion BioSystems)

Consumables

Item	Vendor	Catalog Number
E18 Rat Cortical Neurons	BrainBits LLC	cx
NbActiv4 Medium	BrainBits LLC	NRM-100-121-001
g5 Supplement	Life Technologies	NRM-100-031-001
50% Polyethylenimine Solution (PEI)	Sigma-Aldrich	P3143
DNase I	Sigma-Aldrich	D5319
Trypsin/ETDA	Life Technologies	25200056
HBSS	Life Technologies	14170112
Boric Acid	Fisher Scientific	A73-500
Sodium Tetraborate	Sigma-Aldrich	221732
Laminin	Sigma-Aldrich	L2020
Poly-D-Lysine	Sigma-Aldrich	P6407
Poly-L-Lysine	Sigma-Aldrich	P6282
Trypan Blue	Gibco	15250
Isopropanol Wipes	Dynarex	1106
KimWipes	Various	
Pipettes and Pipettors	Various	
15mL and 50mL Centrifuge Tubes	Various	
Pipet 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
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:

Methods

MEA Surface Pretreatment

1. Wipe the packaged and sealed MEA with 70% EtOH, then place the MEA in a biological tissue culture hood.
2. Pull the MEA from the sealed package and wipe the outside of the MEA with 70% EtOH.
3. While the MEA is drying, prepare a 0.05% PEI* solution for initial coating.
4. Prepare 1L of borate buffer by dissolving 3.10g boric acid and 4.75g of sodium tetraborate in distilled water. Adjust the pH to 8.4.
5. Prepare 0.05% PEI solution in borate buffer using 50% PEI.
6. Filter solution through a 22µm filter.
7. Add 500µL of solution to each well of the MEA and incubate for 2 hours at 37°C in a cell culture incubator.
8. Rinse PEI from the culture surface with 750 µL of sterile deionized water 4 times.
9. Air dry the MEA in a biological safety cabinet over night.
10. Prepare fresh laminin solution in cell culture medium (20µg/mL).

Prepare the laminin fresh for every cell culture.

Dotting Method

>> Dotting method provides greater cell conservation and noise reduction, but is more time consuming and involved. Skip to Whole Area Method if it is not a priority.

11. Add sterile deionized water to the area surrounding the wells of the MEA to prevent substrate evaporation. Do not allow the water into the wells of the MEA.

The exact volume of the water is not important as long as the MEA maintains a moist environment.

12. Add a 10µL droplet of laminin over the MEA electrode area in a biosafety cabinet.
13. Incubate for 1 hour at 37°C. Do not allow the laminin droplet to dry.

* Poly-L-Lysine (PLL) or Poly-D-Lysine (PDL), can be used as alternatives to PEI with varying success.

Notes:

Whole Area Method

14. Add 100µL of laminin over the MEA electrode area in a bio-safety cabinet.
15. Incubate for 1 hour at 37°C.

For increased adhesion, laminin can be incubated up to overnight with care taken to prevent the droplet from drying.

Preparing Complete Medium

16. Take g5 supplement from -80°C freezer and allow to thaw.
17. Inside a biological safety cabinet, combine the g5 supplement and NbActiv4 media (1:100) to make complete medium.

Seeding E18 Rat Cortical Neurons onto the MEA

18. Transfer the cortical tissue in Hibernate solution to a 15mL conical tube.
19. Remove the Hibernate solution while taking care to avoid disturbing the tissue.
20. Rinse cortical tissue with 2mL of HBSS (Ca+, Mg+ free) 2 times.
21. Add 5mL of 0.25% trypsin (pre-warmed to 37°C) to the tube with the rat cortical tissue.
22. Place this tube in water bath for 5-10 minutes. Around 7 min, the previously free floating cortices should be clumping together and exhibiting a slightly flocculated appearance. At this point the tissue is adequately digested.
23. Remove the trypsin while taking care to avoid disturbing the tissue.
24. Gently wash 2 times with HBSS.
25. Add 2mL of DNase in HBSS (0.30 mg/ml) and apply vortex until the tissue is broken up (<5-10 seconds) into a suspension (a few small clumps may be present).
26. Centrifuge the cells at 1100rpm (200 x G), 4 minutes.
27. Remove DNase and re-suspend pellet in growth media.
28. Determine the total number of cells in suspension via hemocytometer count.

29. Remove most of the fluid volume from the MEA surface pre-treatment, but do not let MEA surface dry before plating cells onto the surface.

Dotting Method

>> Skip to Whole Area Method if dotting is not a priority.

30. Seed the 64-electrode MEAs (single and 12-well configurations) as if the plating area is 0.0314 cm^2 (diagonal of square array: 2mm) by placing a small droplet over the electrodes.

31. Seed $5x10^5$ cells per dot in each well of the MEA if you want a high concentration. Seed at $2.5x10^5$ for a lower concentration.

32. Allow the cells to settle and adhere to the substrate for 2 hrs, and then add gently 300-400 µl of media to the well.

Whole Area Method

33. Seed $5x10^5$ cells per well in each MEA for a high concentration, or at $2.5x10^5$ cells per MEA for a lower concentration (depending on cell yield). Seeding the cell suspension over the entire surface area should produce a total volume of 75-150 µl per well.

34. Allow the cells to settle and adhere to the substrate for 2 hrs, and then add gently 300-400 µl of media to the well.

Maintaining E18 Rat Cortical Neurons

35. Immediately before use, warm the media in a 37°C water bath.

36. Feed cells every 3 days by replacing approximately 2/3 of the media. As cultures grow, they may require feeding every other day (use pH change/ orange color change of media as an indicator).

37. Continue to culture the cells in a cell culture incubator at 37°C, 5% CO_2.

Please see a video demonstrating the cell plating procedure on our website at www.axionbiosystems.com

Notes:

Visualization of Typical Neuron Seeding Results

Whole Area Method - Cell Density 1x10^6

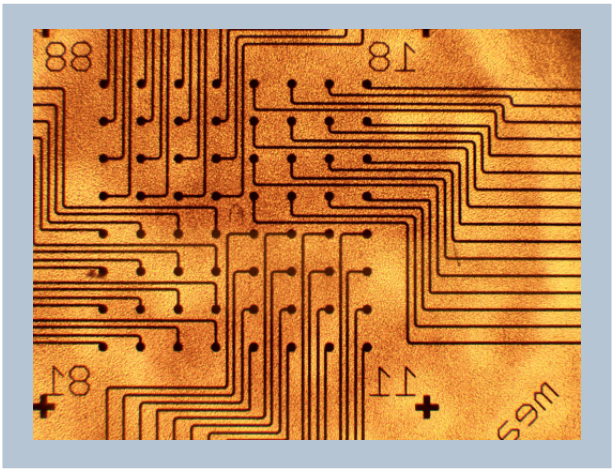


Figure 1: Whole Area Method - Cell Morphology
The above image shows a representation of the what a E18 rat cortical neurons in a 12-well MEA appear like with serum-free media culture conditions. The cell culture density is at 1x10^6, and this is represented in the monolayer across the entire surface area.

Dotting Method - Cell Density 1x10^6

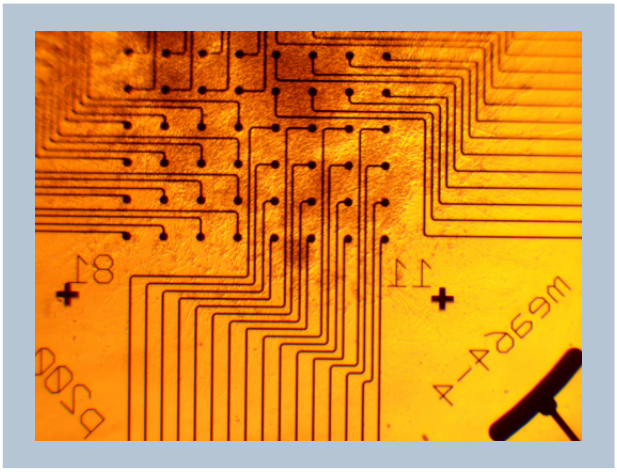


Figure 2: Dotting Method - Cell Morphology
The above image shows a representation of the what a E18 rat cortical neurons in a 12-well MEA appear like with serum-free media culture conditions. The cell culture density is at 1x10^6. The area only covers the section around the electrodes as it followed the dotting method using only a 10µL droplet for seeding.