axion BioSystems

Cell Culture on Microelectrode Arrays

Cell Type: QBM Cell Science - E14,15 Embryonic C57 Mouse Cortical 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

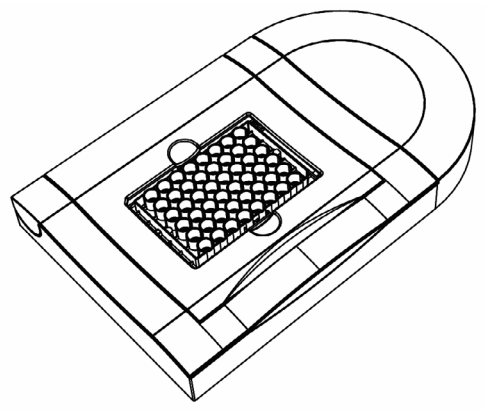
Axion BioSystems would like to thank QBM Cell Science for providing their experience and resources toward the creation of this protocol.

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.



Notes:

Introduction

Axion BioSystems multi-well and single-well microelectrode arrays (MEAs) are ideally suited for investigation of electroactive cells and tissue. The MEA wells are organized in an ANSI-SBS compliant format, compatible with traditional plate readers and automated instrumentation. Within each well, multiple substrate-embedded microelectrodes are each capable of monitoring the activity of numerous individual cells. The arrangement of these electrodes into a grid extends the recording range across a 1.43x1.43 mm (12-well), a 1.05x1.05 mm (48-well), or a 0.7x0.6 mm (96-well) 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 E14,15 embryonic C57 mouse cortical neuron cultures on the microelectrode array (MEA). Neurons cultured using this protocol should show spike activity detectable in AxIS software by day 10 *in vitro*.

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
Cryopreserved C57 Mouse Cortical Neurons	QBM Cell Science	M-CX-300
Neurobasal Medium	Life Technologies	21103-049
L-Glutamine	Life Technologies	25030-081
Fetal Bovine Serum (cell culture grade)	Various	
B-27 Supplement	Life Technologies	17504-044
Penicillin-Streptomycin	Life Technologies	15140-122
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:

Methods

Preparing Complete Medium

1. Take the fetal bovine serum, L-glutamine, penicillin/streptomycin, and B-27 supplement from -80°C freezer and allow to thaw.
2. Inside a biological safety cabinet, make the complete medium by adding the following reagents to Neurobasal medium to achieve indicated concentrations: 5% fetal bovine serum*, 2 mM L-glutamine, 1% penicillin/streptomycin and 2% B-27 supplement.

MEA Surface Pretreatment

3. Wipe the packaged and sealed MEA plate with 70% EtOH, then place the MEA in a bio-safety cabinet.
4. Pull the MEA from the sealed package and wipe the top, bottom, and sides of the plate with a Kimwipe soaked in 70% EtOH.
5. While the plate is drying, prepare a 0.1% PEI solution for initial coating.
    a. Prepare 1 L of borate buffer by dissolving 3.10 g boric acid and 4.75 g of sodium tetraborate in distilled water. Adjust the pH to 8.4 using HCl.
    b. Prepare 0.1% PEI solution in borate buffer using 50% PEI.
    c. Filter solution through a 0.22 µm filter.
6. Add a 5 µL droplet of solution over the MEA electrode area in a bio-safety cabinet. Incubate for 1 hour at 37°C in a cell culture incubator. Look to Figure 1 page 7 for placement.

Droplet volume can range from 5 to 10 uL as long as the droplet volume remains consistent throughout all steps.

7. Rinse PEI from the culture surface with 200 µL of sterile deionized water 4 times.
8. Air dry the MEA plate in a biological safety cabinet overnight.
9. Prepare fresh laminin solution in the Neurobasal medium (20 µg/mL).

Prepare the laminin fresh from frozen aliquots for every cell culture.

* Fetal bovine serum can be removed after initial seeding or altogether if preferred.

Notes:

10. Add 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.

Tip MEA reservoir water is no longer required following the media addition in Steps 27 and 28.

11. Add a 5 µL droplet of laminin over the MEA electrode area in a bio-safety cabinet. Look to Figure 1 on page 7 for placement.

12. Incubate for 2 hours at 37°C. Do not allow the laminin droplet to dry.

Thawing Cryopreserved C57 Mouse Cortical Neurons

13. Remove the cryopreserved C57 mouse neurons cryovial from the liquid nitrogen storage container.

14. Hold the cryovial (avoid submerging above cap) in a 37°C water bath for **exactly 2.5 minutes.**

15. Quickly remove the cryovial from the water bath following the 2.5 minute incubation, then spray the outside with 70% ethanol, wipe dry, and place in a bio-safety cabinet.

16. Carefully transfer the contents of the cryovial to a 50 mL centrifuge tube using a 1 mL pipettor.

! Avoid repeatedly pipetting the thawed neurons.

17. Wash the inside of the cryovial with 1 mL of room temperature Neurobasal medium to recover what residual cells are left in the vial. Add this 1 mL of media from the cryovial drop-wise (~1 drop/sec) to the 50 mL centrifuge tube with the neural cell suspension. Gently swirl the tube while also adding the medium to completely mix the solution and to limit the chances of osmotic shock to the thawed cells.

! Drop-wise transfer of Neurobasal medium is critical in limiting the osmotic shock and maximizing viability and attachment to the MEA.

18. Slowly add 3 mL of room temperature Neurobasal medium to the 50 mL centrifuge tube (~1-2 drops/sec). Carefully swirl the centrifuge tube while transferring the medium.

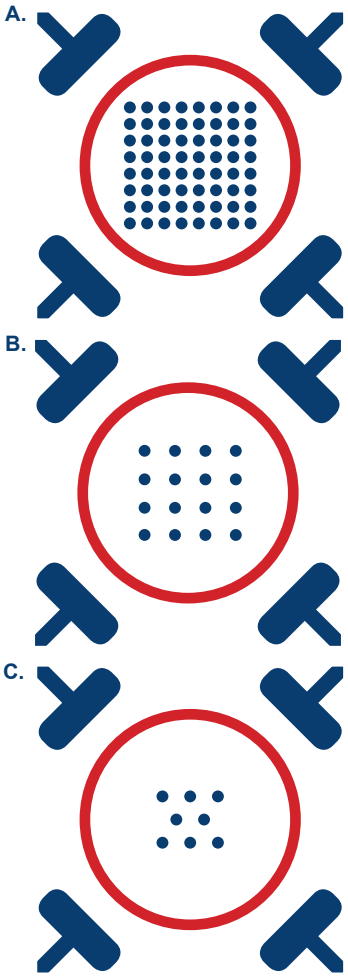


Figure 1: Drop Placement Diagram

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.

19. Carefully mix the contents of the 50 mL centrifuge tube by inverting it 2-3 times. Careful mixing is key to ensuring maximal viability. Take care to avoid any vigorous shaking or vortexing of the cell suspension.

Seeding C57 Mouse Cortical Neurons onto the MEA

20. Determine the total number of cells in suspension via hemocytometer count.

21. Concentrate the neurons by centrifuging at 380 x g for 5 minutes.

The mouse cortical neurons are sensitive to centrifugation, so care should be taken to monitor speed and duration during this step. *The cell provider does not recommend centrifugation and is not responsible for cell death induced by centrifugation.*

22. Calculate the cell concentration to a 5 µL volume for each well and re-suspend the cells accordingly.

23. Remove, from a single row or column, most of the laminin on the MEA surface, but do not let MEA surface dry before seeding the cells onto the surface (the surface will dry in ~2-3 minutes).

24. Seed a 5 µL droplet of the suspension (80,000 neurons) directly over the array of electrodes in each pre-treated well. See Figure 1 on the left for an example of drop placement.

25. Repeat Steps 23 and 24 until all rows or columns have been seeded.

26. Incubate the MEA with seeded neurons in a cell culture incubator at 37°C, 5% CO_2 for 1 hour.

27. Remove the MEA plate after 1 hour and carefully add 150 µL of Neurobasal medium to each well using a multi-channel pipette in a bio-safety cabinet. Adding the medium too quickly will detach the adhered neurons.

The timing of the media addition is critical as performance of the neurons degrades if the droplets begin to dry (~1-2 minutes).

28. Repeat step 27 a second time to reach a volume of 300 µL per well.

29. Incubate the MEA in a cell culture incubator at 37°C, 5% CO_2.

Maintaining C57 Mouse Cortical Neurons

30. Immediately before use, warm the medium in a 37°C water bath.
31. Feed cells every 4-5 days by replacing approximately 1/2 of the media. As cultures grow, they may require feeding every 3-4 days (use pH change/ orange color change of media as an indicator).
32. Continue to culture the cells in a cell culture incubator at 37°C, 5% CO_2.
33. Perform the initial baseline MEA recording on day 7 after seeding, and expect spontaneous activity to emerge by approximately DIV 10.

Notes:

Visualization of Typical Neuron Seeding Results

C57 Mouse Cortical Neurons on 12-Well MEA

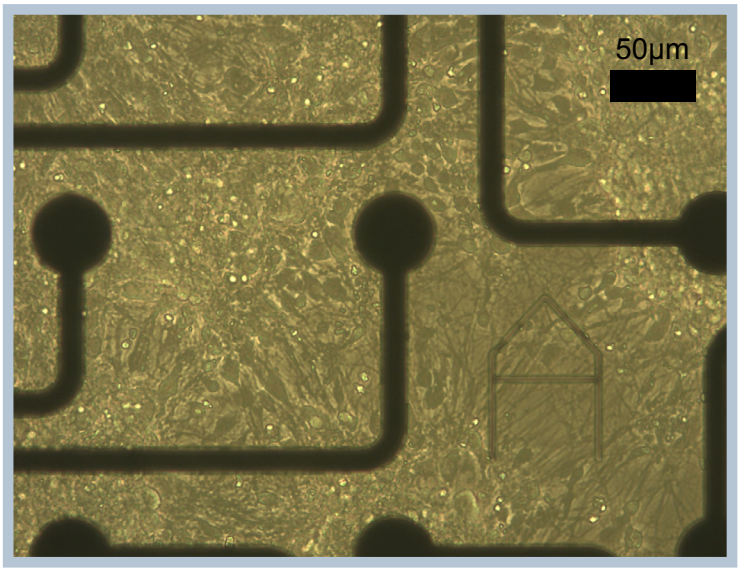


Figure 3: C57 Mouse Cortical Neuron Morphology
E14,15 mouse cortical neurons at day 7 in vitro, 20x magnification. Note the easily recognizable branching and network morphology.

C57 Mouse Cortical Neurons on 12-Well MEA

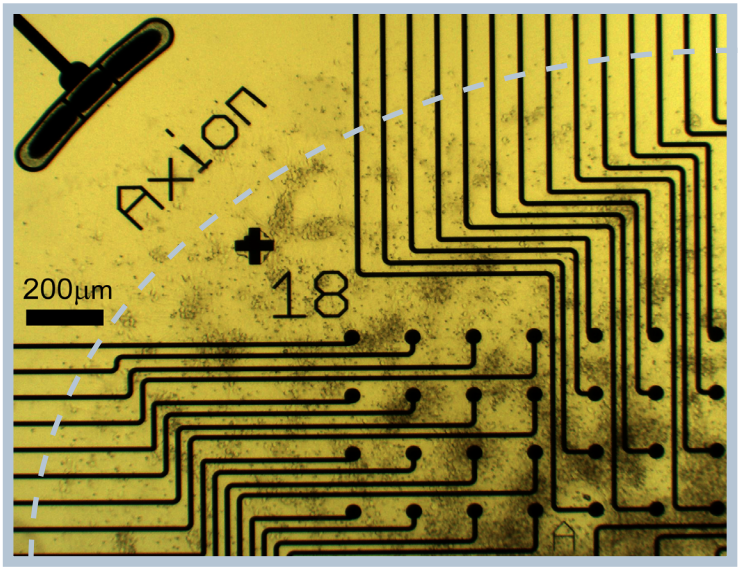


Figure 4: C57 Mouse Cortical Neuron Morphology
E14,15 mouse cortical neurons in a 12-well MEA imaged at 4x magnification. Note that the dotting method of plating described above confines cells mainly to the area around the signal electrode grid, and that few cells are found near the long reference electrode at upper left.

*Axion can provide transparent 48-Well blank plates with no electrodes to confirm cellular adhesion.