axion BioSystems

Cell Culture on Microelectrode Arrays

Cell Type: QBM Cell Science - Neonatal (P1) Rat DRG 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

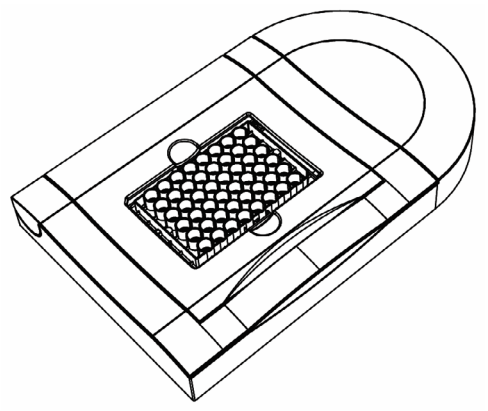
Axion BioSystems would like to thank QBM Cell Science for providing their experience and resources toward the creation of this protocol.

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 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.

The dorsal root ganglion (DRG) contains the cell bodies of sensory neurons, whose afferents transmit signals from sensory nerve endings to the central nervous system. DRG cell bodies can be dissociated into primary culture, where they extend neurites and maintain sensitivity to some sensory modalities, thus representing an important *in vitro* model for applications such as neuropathic pain. This user guide will aid you in growing your neonatal (P1) rat dorsal root ganglion (DRG) neuron cultures on Axion's MEA plates and dishes. Neurons cultured using this protocol should show spike activity detectable in AxIS software by day 4 *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
Cyropreserved Rat DRG Neurons	QBM Cell Science	R-DRG-505
PNGM™ BulletKit™	Lonza	CC-4461
NGF-β Nerve Growth Factor Beta	Sigma-Aldrich	N2513
Uridine	Sigma-Aldrich	U3003-5G
5-Fluoro-2'-Deoxyuridine	Sigma-Aldrich	F0503-100MG
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	
1.5 and 15 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. Remove the components of the PNGM™ BulletKit™; L-glutamine, gentamycin/amphotericin (GA), NSF-1, B-27 supplement, and the NGF-β from the -80°C freezer and allow to thaw.
2. Inside a biological safety cabinet, make the complete medium by adding the following components to the Neuron Basal medium (Bulletkit™) to achieve indicated concentrations: 2 mM L-glutamine, 1% gentamycin/amphotericin (GA), 2% NSF-1, 2% B-27 supplement, and 100 ng/mL NGF-β.

For DRG neurons cultured beyond DIV 7, antimitotics should be added to the medium 4 hours after seeding to prevent Schwann cell proliferation and detachment of the DRG neurons.

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 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.

MEA reservoir water is no longer required following the media addition in Steps 28 and 29.

7. Add a 5 µL droplet of PEI 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 or 2 on page 7 for placement.

Notes:

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

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

11. Add a 5 µL droplet of laminin over the MEA electrode area in a bio-safety cabinet. Look to Figure 1. on page 8 for placement.
12. Incubate for 2 hours at 37°C. Do not allow the laminin droplet to dry.

Thawing Cryopreserved Rat DRG Neurons

13. Remove the cryopreserved rat DRG neurons cryovial from the liquid nitrogen storage container.
14. Hold the cryovial (avoid submerging above cap) in a 37°C water bath for **exactly 1.5 minutes.**
15. Quickly remove the cryovial from the water bath following the 1.5 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 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 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 medium is critical in limiting the osmotic shock and maximizing viability and attachment to the MEA.

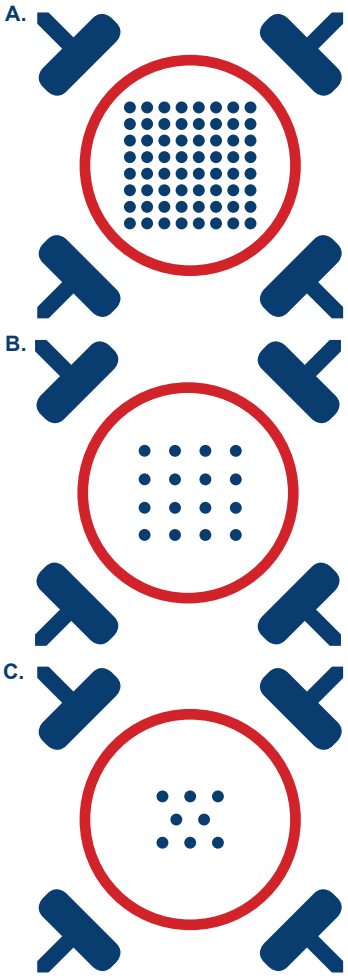


Figure 1: Drop Placement Diagram

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.

18. Carefully mix the contents of the 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.
19. Concentrate the neurons by centrifuging at 380 x g for 5 minutes.
20. Remove the supernatant and resuspend the neurons in a 1 mL or greater volume for counting purposes.

Seeding Rat DRG Neurons onto the MEA

21. Determine the total number of cells in suspension via hemocytometer count.
22. Concentrate the neurons by centrifuging at 380 x g for 5 minutes.
23. Calculate the cell concentration to a 5 µL volume for each well and resuspend the cells accordingly. For cell densities yielding application specific options, look to Figure 3 in the Data Acquisition section.
24. 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).
25. 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.
26. Repeat Steps 23 and 24 until all rows or columns have been seeded.
27. Incubate the MEA with seeded neurons in a cell culture incubator at 37°C, 5% CO_2 for 1 hour.
28. Remove the MEA plate after 1 hour and carefully add 150 µL of Neuron Basal medium to each well using a multi-channel pipette in a bio-safety cabinet. Addition of the medium too quickly will detach the adhered neurons.

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

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

If culturing beyond DIV 7, add antimitotics 4 hours after seeding into medium, 17.5 µg/mL uridine and 7.5 µg/mL of 5-fluoro-2'-deoxyuridine. Continue the antimitotic addition to the complete medium with each maintenance medium change.

Notes:

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

Maintaining Rat DRG Neurons

31. Immediately before use, warm the medium in a 37°C water bath.
32. 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 media color change as indicator of pH).
33. Continue to culture the cells in a cell culture incubator at 37°C, 5% CO_2.
34. Perform the initial baseline MEA recording on day 4 after seeding, and experiments involving compound addition on DIV 7.

Tip

Compound addition of capsaicin has been shown to elicit a robust firing response from the DRG neurons on the MEA. For more information on this application see Figure 3 below and look online at www.axion-biosystems.com

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 characterize electrical activity of QBM Cell Science DRG neurons, cells at several plating densities were assayed for spike activity before and after addition of capsaicin, an agonist of the heat sensitive TRPV1 ion channel. Fig 3 demonstrates that capsaicin causes a robust density-dependent increase in spike activity.

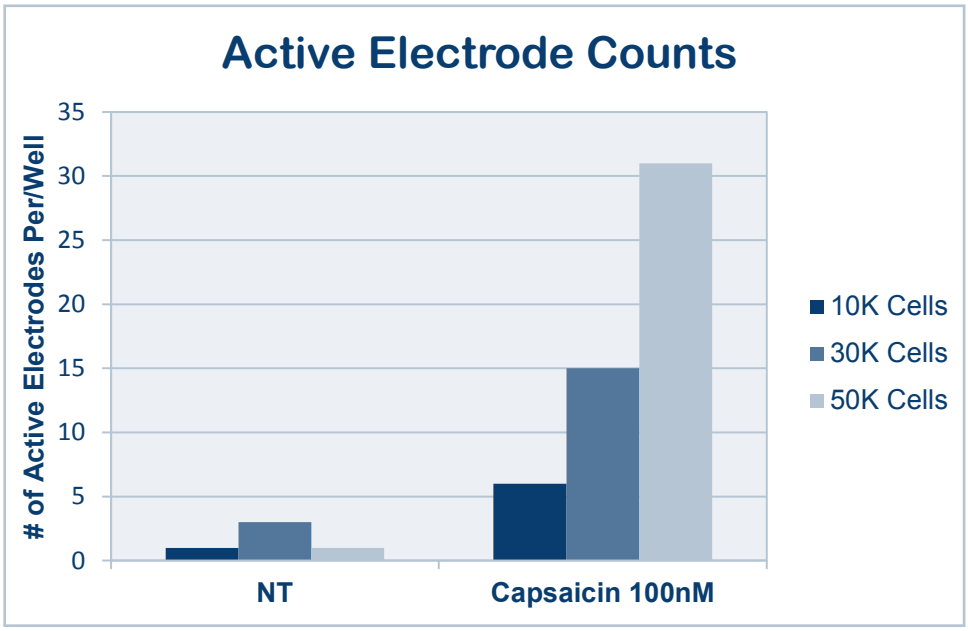


Figure 3: Effect of Capsaicin on Active Electrode Count in DRG Neurons Cultured for 7 Days in an Axion 12-well MEA Plate.
Active Electrode Counts (AE) were calculated for recordings pre and post-dose, and an AE change was calculated. AE was calculated as total electrodes with spike rates > 5 spikes/minute. NT = no treatment, K implies 'times 1000', e.g. 10K = 10,000 cells. All cells plated using 3 µL dot size. Values represent means ± SEM for n=1 wells.

Notes:

Visualization of Typical Neuron Seeding Results

Rat DRG Neurons on 12-Well MEA

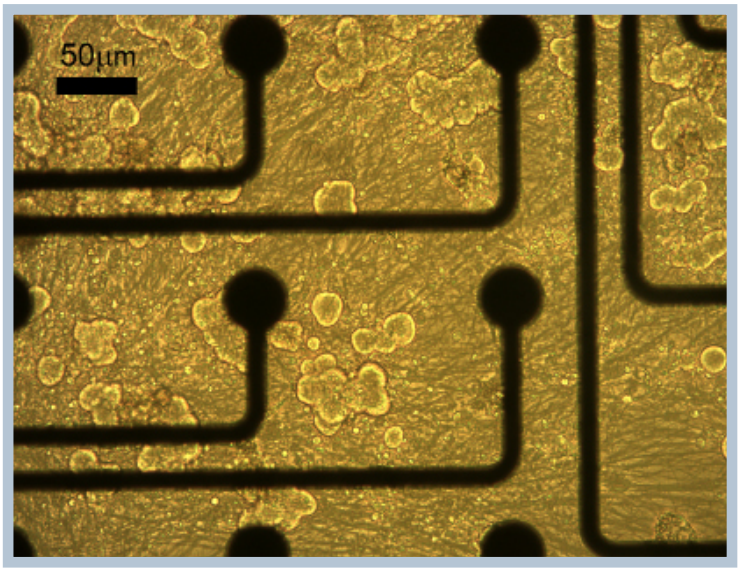


Figure 4: Rat DRG Neuron Morphology
Neonatal (P1) rat DRG neurons (10,000) at day 6 *in vitro* in a 12-well MEA, 20x magnification. Notice that neurite outgrowth and cell morphology are easily recognizable.

Rat DRG Neurons on 12-Well MEA

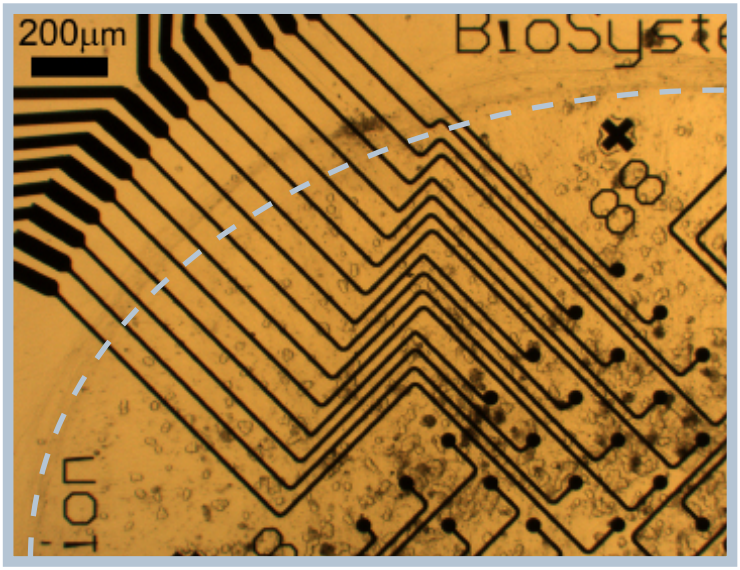


Figure 5: Rat DRG Neuron Morphology
Neonatal (P1) rat DRG neurons (10,000 in a 3 µL dot) at day 6 *in vitro* in a 12-well MEA, 4x magnification. Notice the dotting method described above confines cells to the area indicated by the dotted blue line surrounding the grid of circular electrodes.