Cell Culture on Microelectrode Arrays

Cell Type: E18 Embryonic Rat Cortical Neurons

Protocol
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Origin

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

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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.
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 E18 embryonic rat cortical neuron cultures on the microelectrode array (MEA). Neurons cultured using this protocol should show spike activity detectable in AxIS software by day 7 in vitro.
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 (as suggested by Axion BioSystems)

## Consumables

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<td>NbActiv4 Medium</td>
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## Equipment

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<td>Liquid Nitrogen Storage</td>
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Methods

Preparing Complete Medium

1. Take g5 supplement from -80°C freezer and allow to thaw.

2. Inside a biological safety cabinet, combine the g5 supplement and NbAc-tiv4 media (1:100) to make complete media.

MEA Surface Pretreatment

3. Wipe the packaged and sealed MEA 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.

Dotting Method

>> The Dotting Method uses fewer cells and reduces electrical noise, but is more time consuming. Skip to Whole Area Method if it is not a priority.

6. Add 5 µL of PEI solution to each well of the MEA and incubate for 1 hour at 37°C in a cell culture incubator. See Figure 1 or 2 on page 7 for placement.

7. Rinse PEI from the culture surface with 100 µL of sterile deionized water 4 times.

8. Air dry the MEA in a biological safety cabinet over night.

9. Prepare fresh laminin solution in cell culture medium (20 µg/mL).

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

* Poly-L-Lysine (PLL) or Poly-D-Lysine (PDL), can be used as alternatives to PEI with varying success.
11. Add a 5 µL droplet of laminin over the MEA electrode area in a bio-
   safety cabinet.

12. Incubate for 1 hour at 37°C. Do not allow the laminin droplet to dry.

**Whole Area Method**

13. Add 150 µL of PEI solution to each well of the MEA plate and incubate for 1 hour at 37°C in a cell culture incubator.

14. Rinse PEI from the culture surface with 200 µL of sterile deionized water 4 times.

15. Air dry the MEA in a biological safety cabinet over night.

16. Prepare fresh laminin solution in cell culture medium (20 µg/mL).

   **Prepare the laminin fresh for every cell culture.**

17. Add 100 µL of laminin to each well of the MEA plate in a bio-
   safety cabinet.

18. Incubate for 1 hour at 37°C.

   **For increased adhesion, laminin can be incubated up to overnight with care taken to prevent drying by adding additional laminin.**

**Seeding E18 Rat Cortical Neurons onto the MEA**

19. Transfer the cortical tissue in Hibernate solution to a 15 mL conical tube.

20. Remove the Hibernate solution while taking care to avoid disturbing the tissue.

21. Rinse cortical tissue with 2 mL of HBSS (Ca⁺, Mg⁺ free) 2 times.

22. Add 5 mL of 0.25% trypsin (pre-warmed to 37°C) to the tube with the rat cortical tissue.

23. Place this tube in water bath for 5-10 minutes. Around 7 minutes, the previously free floating cortices should be clumping together and
exhibiting a slightly flocculated appearance. At this point the tissue is adequately digested.

24. Remove the trypsin while taking care to avoid disturbing the tissue.

25. Gently wash 2 times with HBSS.

26. Add 2 mL 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).

27. Centrifuge the cells at 1100 rpm (200 x G), 4 minutes.

28. Remove DNase and re-suspend pellet in 1 mL of growth media.

29. Determine the total number of cells in suspension via hemocytometer count using Trypan Blue.

30. Remove most of the laminin from 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).

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

31. Seed 8x10⁴ cells per dot in each well of the MEA for high density, or at 3x10⁴ for a lower density. Dot by placing a small droplet 5-10 µL over the electrode area, see Figure 1 for placement.

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

Whole Area Method

33. Seed 5x10⁵ cells per well in each MEA for a high density, or at 2x10⁵ cells per MEA for a lower density. The area for single and 12-well MEAs is 0.6 cm², while the area for 48-well MEAs is 0.3 cm².

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% CO₂.
Visualization of Typical Neuron Seeding Results

Whole Area Method on 12-Well MEA

Figure 3: Whole Area Method on 12-Well MEA
E18 rat cortical neurons in a 12-well MEA under serum-free media culture conditions imaged at 4x magnification. The cell culture density is at 5x10^5/per well. The cells are present across the entire surface area.

Dotting Method on 12-Well MEA

Figure 4: Dotting Method on 12-Well MEA
E18 rat cortical neurons in a 12-well MEA under serum-free media culture conditions imaged at 4x magnification. Approximately 3x10^4 total cells are confined to only the area around the electrode grid by using the dotting method described above.

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