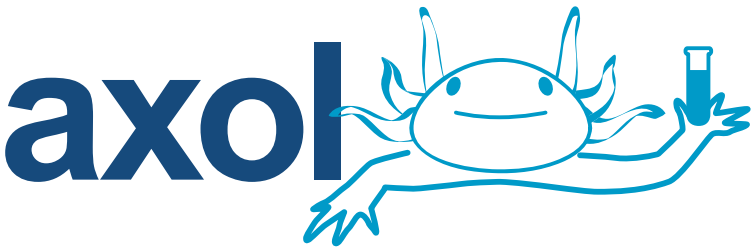
Axol Guide to Culturing Axol human Cerebral Cortical Neurons (hyCCNs) on Axion BioSystems Microelectrode Arrays

Application Protocol

Version 2.0



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Before You Begin

1. Read this entire manual before using cells or microelectrode arrays.

2. Check the Axion Maestro system for correct performance. Contact Axion at support@axion-biosystems.com with any issues.

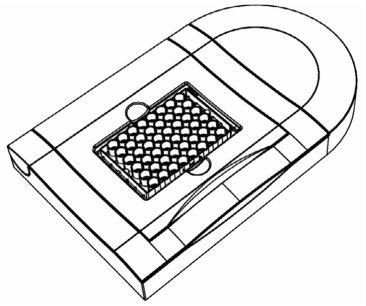
3. Consult with Axion about untested experimental variables if there is concern with the safety of the equipment.

- This user guide will aid you in growing your Axol hyCCNs on Axion's MEA plates and dishes.

- Axol Bioscience would like to thank Axion BioSystems for providing their experience and resources towards the generation of this protocol.

- Axion BioSystems MEA wells are 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 12-well, 48-well, or 96-well area, providing concurrent access to both single-cell and network- level activity.

Neurons cultured using this protocol should show spike activity detectable in Axion's Integrated Studio software by day 7 *in vitro*.



axion BioSystems

Required Materials

Consumables

Item	Vendor	Catalog Number
Axol human cerebral cortical neurons (hyCCNs)	Axol Bioscience	ax0023F-ax0026F
Axol Neural Maintenance Media Kit	Axol Bioscience	**ax0031(a&b)**
Axol Sure Bond	Axol Bioscience	**ax0041**
Axol Sure Mix	Axol Bioscience	**ax0043-2**
Axol Sure Boost	Axol Bioscience	**ax0045**
Axol Neural Enhance	Axol Bioscience	**ax0046**
50% Polyethlenimine Solution (PEI)	Sigma-Aldrich	P3143
Boric Acid	Fisher Scientific	A73-500
Sodium Tetraborate	Sigma-Aldrich	221732
Hydrochloric Acid	Sigma-Aldrich	H1758
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	
37ºC Water Bath	Various	
Cell Culture Incubator	Various	
Hemocytometer or Automated Cell Culture	Various	
Biological Safety Cabinet	Various	
Tabletop Centrifuge	Various	
Phase Contrast Microscope	Various	
Liquid Nitrogen Storage	Various	
1.5 and 15 mL Centrifuge Tubes	Various	

Preparing Complete Axol Neural Maintenance Medium

1. Remove the **Axol Neural Maintenance Medium Supplement** from the -80ºC freezer the day before seeding and allow to thaw overnight at 4ºC.

2. Inside a biological safety cabinet, make the 500 mL complete medium by combining 7.5 mL of the thawed **Axol Neural Maintenance Medium Supplement** with the **Axol Neural Maintenance Basal Medium** and thoroughly mixing.

MEA Surface Pre-Treatment

1. Wipe the packaged and sealed MEA plate with 70% EtOH, then place the MEA in a biosafety cabinet.

2. Pull the MEA from the sealed package and wipe the top, bottom and sides of the plate with a Kimwipe soaked in 70% EtOH.

3. While the plate is drying, prepare a 0.1% PEI solution for initial coating.

    a) Prepare 1L 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 through a 0.22 µm filter.

4. 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 water into the wells of the MEA.

5. Add a 5 µL droplet of PEI solution over the MEA electrode area in a biosafety cabinet. Incubate for 1 hour at 37ºC in a cell culture incubator. See **Figure 1** for placement.

6. Rinse PEI from the culture surface with 200 µL of sterile de-ionized water 4 times.

7. Air dry the MEA plate in a biological safety cabinet overnight.

8. Prepare fresh **Axol Sure Bond** (50X) working solution in complete Axol Neural Maintenance Medium.

9. Add a 5 µL droplet of **Axol Sure Bond** working solution over the MEA electrode area in a biosafety cabinet. See **Figure 1** for placement.

10. Incubate for 2 hours at 37ºC. Do not allow the **Axol Sure Bond** droplet to dry.

Warning: Prepare the **Axol Sure Bond** fresh from frozen aliquots every time. Never reuse **Axol Sure Bond**!

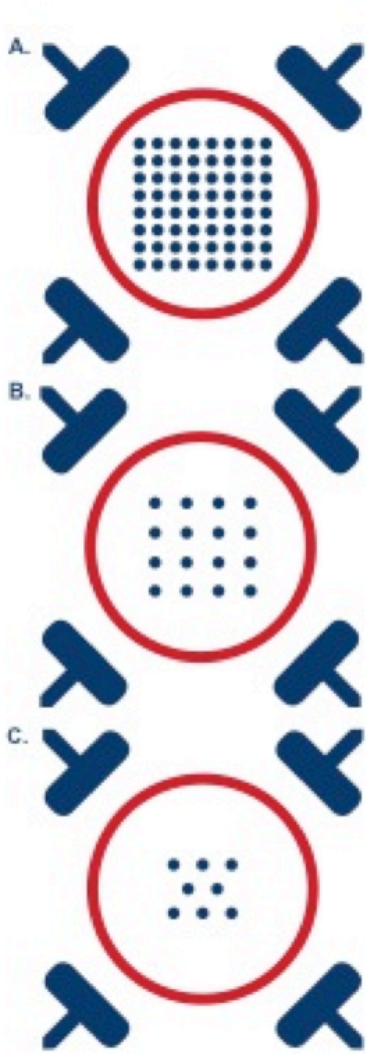


Figure 1: Drop Placement Diagram.

The layouts on the left 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.

Thawing Cryopreserved Axol hyCCNs for MEA Attachment

1. Remove the cryopreserved hyCCNs from the liquid nitrogen storage container.

2. Hold the cryovial (avoid submerging above the cap) in a 37ºC water bath for **exactly 1.5 minutes**.

3. Quickly remove the cryovial from the water bath. Spray the outside with 70% ethanol, wipe dry and place in a biosafety cabinet.

Top Tip: Avoid repeatedly pipetting the thawed neurons and be very gentle to promote cell viability.

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

5. Wash the inside of the cryovial with 1 mL of room temperature **complete Axol Neural Maintenance 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.

Top Tip: Drop-wise transfer of **Complete Axol Neural Maintenance Medium** is critical in this step. It limits the osmotic shock and maximizes viability and attachment to the MEA.

6. Add an additional 8 mL of **Complete Axol Neural Maintenance Medium**.

7. 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.

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

9. Resuspend the cell pellet in **Complete Axol Neural Maintenance Medium** supplemented with **Axol Sure Boost** (1000X stock solution; 1 µL per mL of medium).

Top Tip: After centrifugation be mindful of the pellet as the final volume will be very small to accommodate the 5 µL droplet. Resuspending the cortical neurons in this small-scale volume will require some finesse and careful pipetting up and down.

Seeding Axol hyCCNs onto the MEA

1. Determine the total number of cells in suspension via hemocytometer count.
2. Remove, from a single row or column, most of the Axol Sure Bond on the MEA surface, but **do not let the MEA surface dry** before seeding the cells onto the surface (the surface will dry in ~2-3 minutes).
3. Seed a 5 µL droplet of the suspension (60,000 neurons) directly over the array of electrodes in each pre-treated well. See **Figure 1** for an example of drop placement.
4. Repeat the previous 2 steps until all rows or columns have been seeded.
5. Incubate the MEA with seeded neurons in a cell culture incubator at 37ºC, 5% CO_2 for 1 hour.
6. Remove the MEA plate after 1 hour and carefully add 150 µL of **Complete Axol Neural Maintenance Medium** to each well using a multi-channel pipette in a biosafety cabinet. Addition of the medium too quickly will detach the adhered neurons.
7. Repeat the previous step a second time to reach a volume of 300 µL per well.
8. Incubate the MEA plate in a cell culture incubator at 37ºC, 5% CO_2.

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

Maintaining Axol hyCCNs on MEAs

1. Pre-warm **Complete Axol Neural Maintenance Medium** in a 37ºC water bath immediately before use.

2. A day after seeding the hyCCNs on the MEA, replace the medium with fresh **Complete Axol Neural Maintenance Medium** supplemented with **Axol Neural Enhance** (1000X stock solution; 1 µL per mL medium) and **Axol Sure Mix** (500X stock solution; 2 µL per mL medium).

3. Culture the hyCCNs for **two days**, after which the medium should be replaced with **Complete Axol Neural Maintenance Medium** supplemented with **Axol Sure Mix**.

4. Feed cells every 2 days by replacing approximately half of the medium. Continue to culture the cells in a cell culture incubator at 37ºC, 5% CO_2.

Top Tip: Axol Sure Mix should be added once every 4 days during a media change to prevent neuronal clumping.

5. Perform the initial baseline MEA recording on day 7 after seeding, and conduct any compound testing after DIV 34.

Top Tip: Using larger cell densities, such as 60,000 hyCCNS per well, has been shown to elicit increasing firing responses from the cortical neurons on the MEA. See **Figure 2** for more information.

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 http://www.axionbiosystems.com/ for more comprehensive instructions. To characterize the use of Axol hyCCNs, several densities were tested to determine a minimal cell density required for robust spike activity. The data displayed in **Figure 2** demonstrates activity at 60,000 cells per well. **Figure 3** shows the morphology of hyCCNs in a 12-well MEA when plated at different densities.

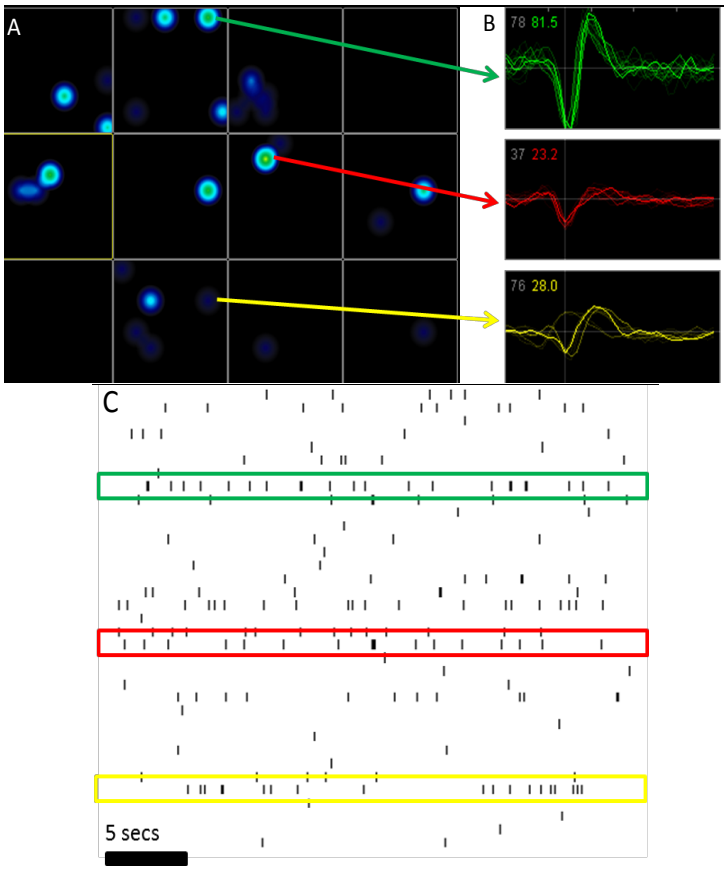


Figure 2: Neural spike activity at 5 weeks on the MEA for hyCCN cells plated at 60,000 per well.

A) An activity map from the AxIS Software suite showing positions of spike activity for each well of the MEA.

B) Extracted waveform for each raster and electrode referenced by the color arrows, and color coded to those arrows as examples of activity on plate.

C) Raster plots of spike activity recorded from the plate at the time shown in the activity map.

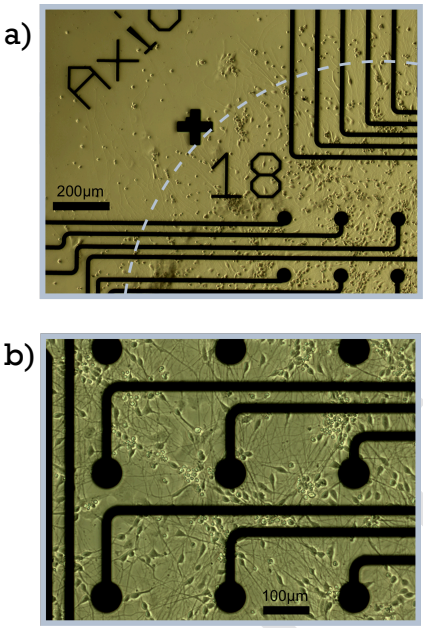


Figure 3: Human Cortical Neuron Morphology

a) hyCCNs (60,000) at day 4 *in vitro* in a 12-well MEA, 4X magnification. Notice the dotting method described above confines cells to the area indicated by the dotted line surrounding the grid of circular electrodes.
b) hyCCNs (20,000) at day 4 *in vitro* in a 12-well MEA, 10X magnification. Notice that branching and neurite outgrowth is easily recognizable.

Technical Support

- ## Online Resources

  Please visit our website at www.axolbio.com for additional product information and *Technical Resources*, including instruction manuals, application protocols, video guides, wall charts and webinars.

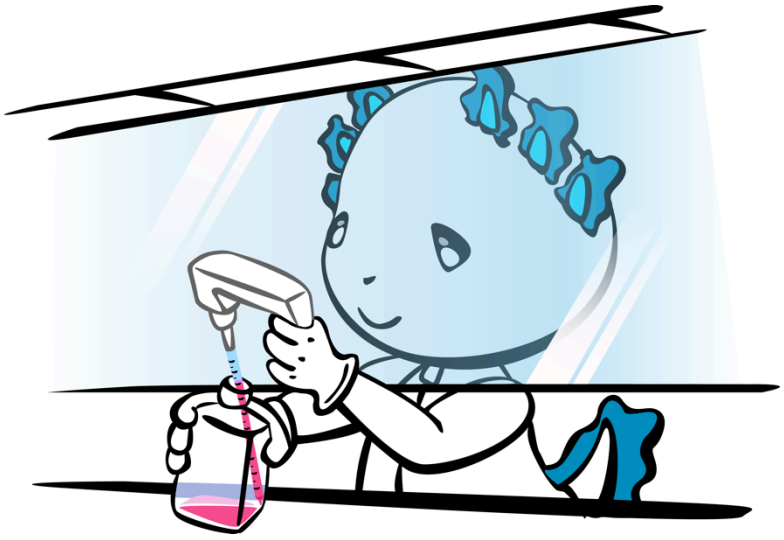
- ## Contact Us

  For more information or technical assistance, call +44 (0) 1223 497 119, or email support@axolbio.com. US Toll Free Tel: 1-800-678-2965 (1-800-678-AXOL), US Toll Free Fax: 1-800-861-2965 (1-800-861-AXOL).

- ## Certificate of Analysis

  The Certificate of Analysis provides detailed quality control information for each product. Certificates of Analysis are available on our website.

  Go to www.axolbio.com/certificate-of-analysis-lookup and search for the Certificate of Analysis with product lot number, which is printed on the cryovial label.



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