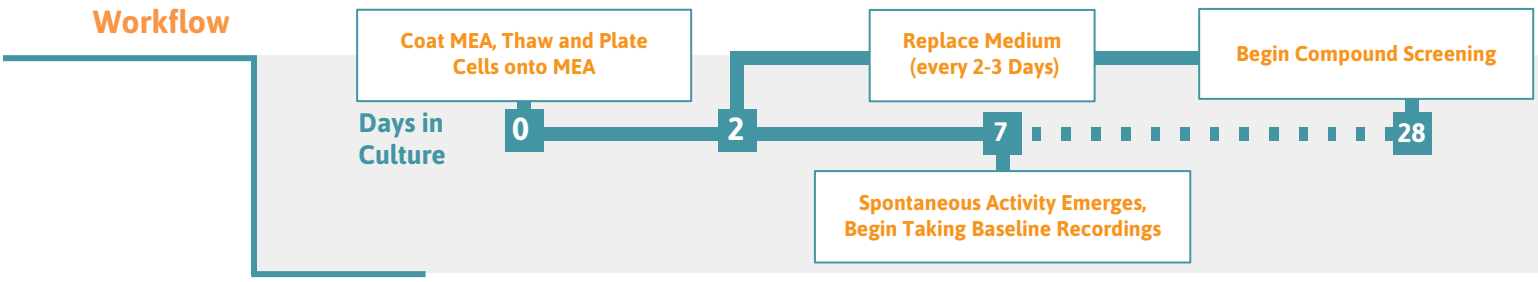
Cell Culture Protocol

QBM Cell Science Rat Cortical Neurons



Preparing the MEA Plate

1. Place an 5 µl droplet of 0.1 % PEI solution over the recording electrode area of each well in the MEA plate. See Figure 1 on page 2 for appropriate drop placement.
2. Incubate the PEI-coated MEA plate in a cell culture incubator at 37°C, 5% CO_2 for at least 60 minutes.
3. Rinse PEI from the culture surface with 200 µL of sterile DI water 4 times, then allow the MEA plate to air dry overnight.

Tip

Recommended to add 6-8 mL of sterile water to the on-plate reservoirs to increase humidity.

Culturing Rat Cortical Neurons

4. Prepare complete media with Neurobasal media, 5% fetal bovine serum, 2% B27, 2mM L-glutamine and 1% penstrep.
5. Thaw Rat Cortical Neurons by holding the tube partially submerged in a 37°C water bath for 3 minutes, making sure to not submerge the cap. Spray the outside with 70% ethanol and transfer to a biosafety cabinet. Dilute the cells with 37°C complete media.
6. Remove a sample of the cell suspension and count the neurons using a hemocytometer to determine both the viability and total number of viable cells. Transfer the cell suspension to a 15 ml conical tube.
7. Centrifuge the cell suspension at 100 x g for 1 minute max.
8. Aspirate the supernatant, being careful not to disturb the cell pellet.
9. Dilute the cell suspension in complete medium with laminin (20 µg/ml) to at least 16,000,000 plated neurons/ml.

Tip

Ensure the neurons are evenly suspended before removing an aliquot to count.

Tip

The rat 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.

Tip

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

Plating QBM Rat Cortical Neurons onto the MEA

10. Place an 5 µl droplet of Rat Cortical Neuron suspension over the recording electrode area of each well of the MEA. See Figure 1 on page 2 for appropriate drop placement.
11. Incubate the MEA plate with the seeded neurons in a cell culture incubator at 37°C, 5% CO_2 for 1 hour.

12. Gently add 1/2 of the final volume of Culture Medium to each well of the MEA. Adding the medium too quickly will dislodge the adhered neurons. Recommended well volumes for each plate type are: 6- and 12-well = 1000 µL, 24-well = 500 µL, 48-well = 300 µL, 96 well = 200 µL.
13. Repeat step 12 a second time to reach the final recommended volume of complete medium.
14. Incubate in a cell culture incubator at 37°C, 5% CO_2 .
15. For optimal cell health, be sure to exchange 50% of the medium every 2-3 days. Though neural spikes may be detectable on the Maestro within 7 days, optimal neural network structure is typically achieved after 28-30 days in culture.

Tip

Using a pipettor, add medium first in a semi-circle along the outer edge of the well. Progressively add medium to either side of the well so it fills evenly towards the center. The goal is to prevent a rush of medium in either direction that might dislodge the neurons.

Drop Placement

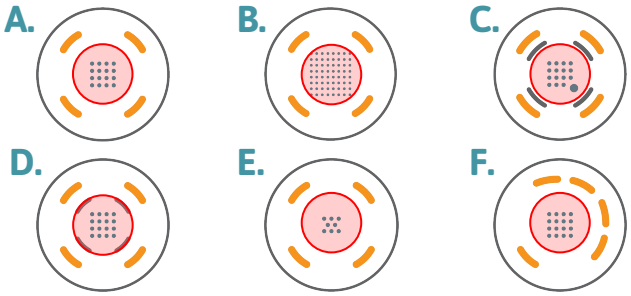


Figure 1: Drop Placement Diagram
The layouts above represent the bottom surfaces of wells in (A) a 48-well MEA, (B) a 6- or 12-well MEA, (C) a 24-well MEA or 48 well E-Stim+ MEA, (D) a 48-well AccuSpot MEA, (E) a 96-well MEA, and (F) a 48-well CytoView MEA. The number of electrodes per well is different across the plate formats, however the drop placement is the same, with the drop (red circle) centered on the recording electrodes and staying within the ground electrodes. On plate types (C) with the addition of the stim-paddle in the lower right corner of the array, it is important to make sure the droplet covers this feature. The droplet may need to be manipulated after placement of the pre-treatment to ensure stim-paddle coverage.

Visualization of Typical Neuron Seeding Results

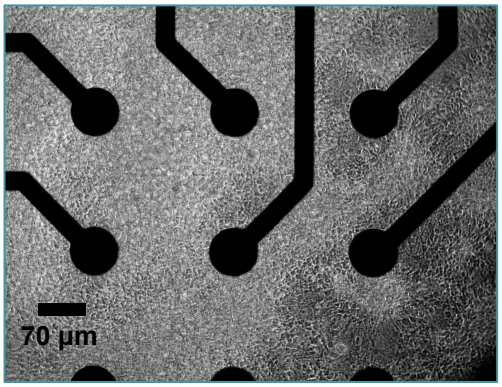


Figure 2: QBM Rat Cortical Neuron Morphology
Primary Rat Cortical Neurons (80,000) at day 7 *in vitro* in a 48-well CytoView MEA, 10x magnification. Notice that neuron morphology is easily recognizable.

Required Materials

Consumables

Item	Vendor	Catalog #
Axion MEA (6, 12, 24, 48, or 96-Well)	Axion BioSystems	
QBM Rat Cortical Neurons	Lonza	R-CX-500
Neurobasal Medium	Thermo Fisher	21103049
GlutaMAX™ Supplement	Thermo Fisher	35050061
Fetal Bovine Serum	Various	
B-27 Supplement	Thermo Fisher	17504044
50% Polyethylenimine Solution (PEI)	Sigma-Aldrich	P3143
Dulbecco's PBS without Ca2+/Mg 2+	Thermo Fisher	14040
Laminin	Sigma-Aldrich	L2020
Pipettes and Pipettors	Various	
15 mL and 50 mL Centrifuge Tubes	Various	

Equipment

Item	Vendor	Catalog #
Maestro MEA System	Axion BioSystems	
Axion Integrated Studio (AxIS)	Axion BioSystems	
37°C Water Bath	Various	
Cell Culture Incubator	Various	
Hemocytometer or Cell Counter	Various	
Biological Safety Cabinet	Various	
Tabletop Centrifuge	Various	
Phase Contrast Microscope	Various	
Liquid Nitrogen Storage	Various	