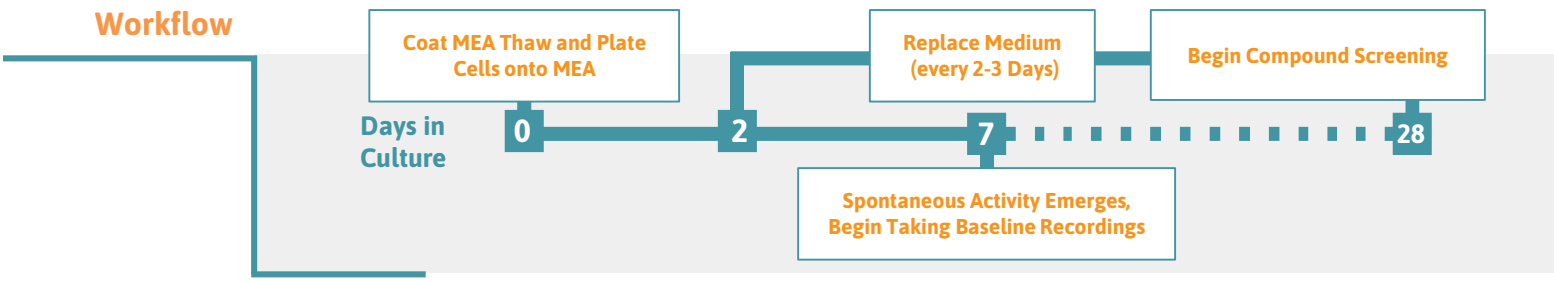
Cell Culture Protocol

Gibco Primary Rat Cortical Neurons



Preparing the MEA Plate

1. Place a 5 µl droplet of PDL solution (4.5 µg/cm^2) to each well in the MEA plate.
2. Incubate the PDL-coated MEA plate at 37°C, 5% CO_2 for 1 hour.
3. Rinse PDL 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 Neurobasal Plus Complete Media according to the Gibco Primary Rat Cortex and Hippocampus Neurons User Guide.
5. Thaw primary rat cortical neurons according to the Gibco Primary Rat Cortex and Hippocampus Neurons User Guide.
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 5 minutes.
8. Aspirate the supernatant, being careful not to disturb the cell pellet.
9. Dilute the cell suspension in Neurobasal Plus Complete Medium to 16,000,000 neurons/ml.

Tip

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

Tip

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.

Plating Gibco Rat Cortical Neurons onto the MEA

10. Place an 5 µl droplet of the 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 the medium to each well of the MEA. Adding the medium too quickly will dislodge the adhered neurons. Recommended final 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 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 within 4 days, optimal neural network structure is typically achieved after 28 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

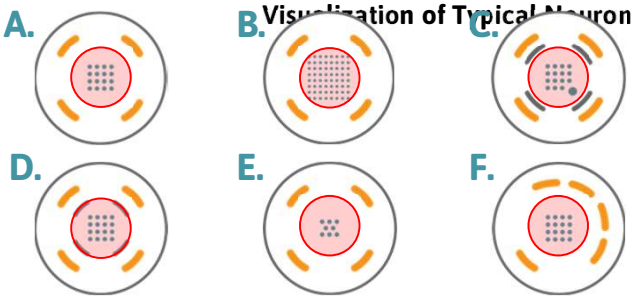


Figure 1: Drop Placement Diagram
The layouts above represent the bottom surfaces of wells in (A) a 48-well MEA, (B) a 6- or 12-well MEA, (C) a 24-well MEA or 48 well E-Stim+ MEA, (D) a 48-well AccuSpot MEA, (E) a 96-well MEA, and (F) a 48-well CytoView MEA. The number of electrodes per well is different 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 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

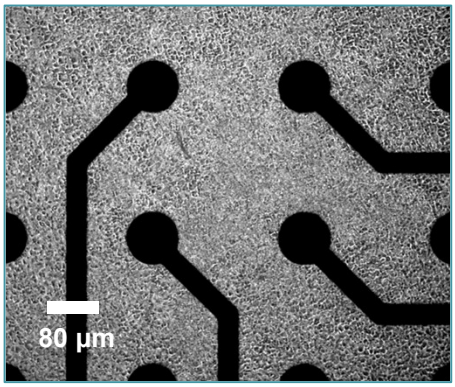


Figure 2: Gibco Rat Cortical Neuron Morphology
Gibco Rat Cortical Neurons at day 2 *in vitro* in a 48-well CytoView MEA, 160K cells, 10x magnification. Notice that neuron morphology is easily recognizable.

Required Materials

Consumables

Item	Vendor	Catalog #
Axion MEA (6, 12, 24, 48, or 96-Well)	Axion BioSystems	
Gibco™ Rat Cortical Neurons	Thermo Fisher	A1084002
B-27™ Plus Neuronal Culture System	Thermo Fisher	A3653401
GlutaMAX™ I Supplement (100X)	Thermo Fisher	35050
Poly-D-Lysine (PDL)	Thermo Fisher	A3890401
Dulbecco's PBS without Ca2+/Mg 2+	Thermo Fisher	14040
Gentamicin (50 mg/mL)	Thermo Fisher	15750-060
15 mL and 50 mL Centrifuge Tubes	Various	

Equipment

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