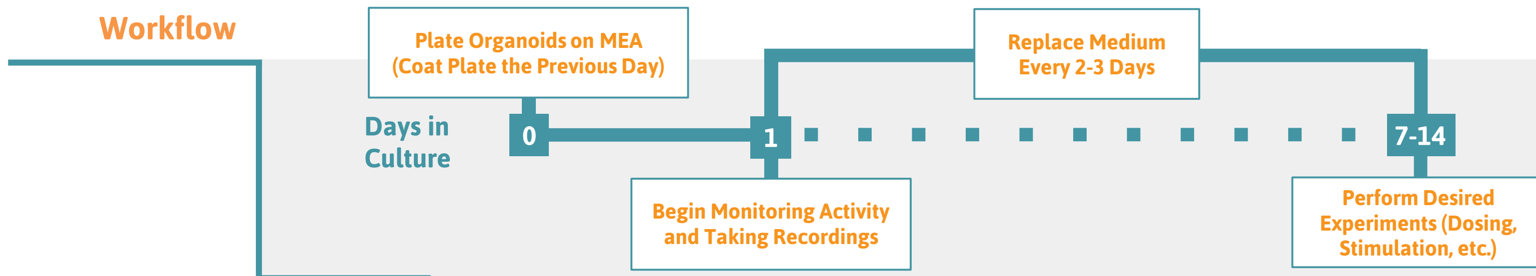
Cell Culture Protocol

Neural Organoids



Preparing the MEA Plate

1. Prepare 0.1% PEI solution by diluting 50% PEI solution into borate buffer and filtering with a 0.22 µm filter.

   Note: 1L of borate buffer can be prepared by dissolving 3.10 g of boric acid and 4.75 g of sodium tetraborate. Adjust pH to 8.4.

2. Place a 10 µl drop of 0.1% PEI solution over the electrode array of each well in the MEA plate. See Figure 1 for appropriate drop placement.
3. Incubate the PEI-coated MEA plate in a cell culture incubator at 37°C, 5% CO_2 for at least 60 minutes.
4. Rinse PEI from the culture surface with 200 µl of sterile DI water 4 times, and then allow the MEA plate to air dry overnight.

Tip: Add 6-8 mL of sterile water to the on-plate reservoirs to increase humidity.

Culturing and Plating Neural Organoids

5. Add laminin (10 µg/mL) to the organoid medium for plating.
6. Use a 1000 µL pipette with a wide-bore pipette tip to aspirate the desired number of organoids for a single well. The wide-bore tip will provide an opening adequate for pulling one or more organoids into the pipette tip with minimal shear stress.
7. Allow the organoid(s) to settle to the end of the pipette tip by holding the pipette vertically and gently tapping the upper portion of the pipette tip with your finger.
8. Once the organoid(s) have accumulated at the pipette tip end, dispense in a small droplet over the array.
9. Check positioning of the organoids over the electrode array by carefully transferring the MEA plate to a microscope. Ensure that electrically-active cells on the outside of the organoid are in contact with the electrodes.
10. If organoids are not in contact with the electrodes, they can be repositioned by carefully moving them with a new 10-100 µL pipette tip. Gently, physically push the organoid with the side of the pipette tip, taking care not to push the organoid up into the pipette tip end.
11. Once the organoids are in place, incubate the MEA plate in a cell culture incubator at 37°C, 5% CO_2 for 3-4 hours.

Tip: Prepare the laminin fresh from frozen aliquots for every plating.

Tip: Don't tap the tip too hard as the liquid may escape from the large bore.

Tip: Try to keep the volume 10-20 µl so as to confine the organoid(s) to the array.

Tip: If you are having trouble keeping the organoids in place, try gently aspirating the medium from the droplet using a single channel pipettor. This can help force the organoids to the bottom of the well. However this should only be a temporary step - the well can not dry out or viability of the organoids will decrease

12. Gently add 1/2 of the final volume of the medium to each well of the MEA. Adding the medium too quickly may dislodge the adhered organoids. 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 to reach the recommended final well volume.
14. Incubate in a cell culture incubator at 37°C, 5% CO_2.
15. For optimal cell health, replace medium every 2-3 days.

Recording Activity

While some organoids may show activity after 24 hours, organoids typically require 1-2 weeks of culture before showing robust activity. Begin monitoring after 24 hours to identify the optimal analysis window when peak activity and stability is observed.

Drop Placement

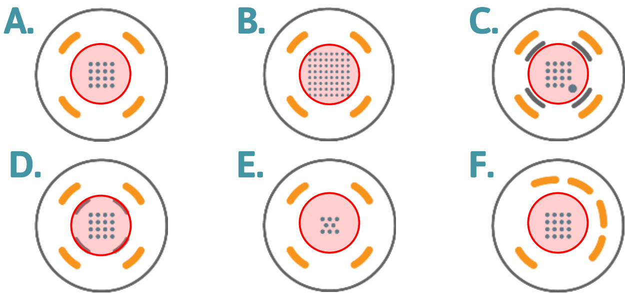


Figure 1: Drop Placement Diagram
The layouts above represent the bottom surfaces of wells in (A) a 48-well MEA with 1.1 x1.1 mm recording area, (B) a 6-well MEA with 2.1 x 2.1 mm recording area, (C) a 24-well MEA or 48 well E-Stim+ MEA with 1.1 x 1.1 mm recording area, (D) a 48-well AccuSpot MEA with 1.1 x 1.1 mm recording area, (E) a 96-well MEA with 0.8 x 0.8 mm recording area, and (F) a 48-well CytoView MEA with 1.1 x 1.1 mm recording area. The number of electrodes per well and recording area size 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.

Visualization of Typical Organoid Seeding Results

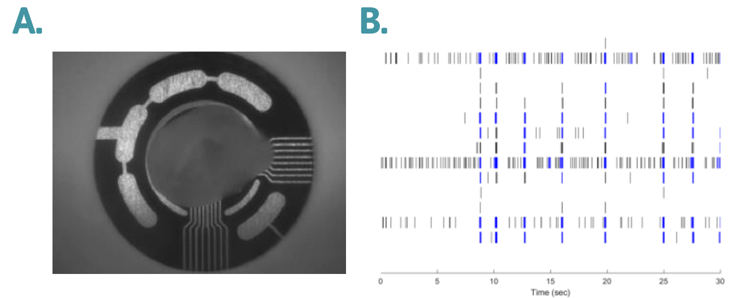


Figure 2: Neural Organoid Morphology and Activity
A) Cerebral organoids generated from human induced pluripotent stem cells (hiPSCs) on a CytoView MEA 24-well plate. B) Representative well-wide raster plot illustrating spikes generated by the organoid. By day 30 in culture, the organoid exhibited bursts of network activity.

Required Materials

Consumables

Item	Vendor	Catalog #
Axion MEA (6, 12, 24, 48, or 96-Well)	Axion BioSystems	
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
Finntip 1000 Wide sterile	Thermo Fisher	9405163
15 mL and 50 mL Centrifuge Tubes	Various	

Equipment

Item	Vendor	Catalog #
Maestro Pro or Edge MEA System	Axion BioSystems	
AxIS Navigator	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	