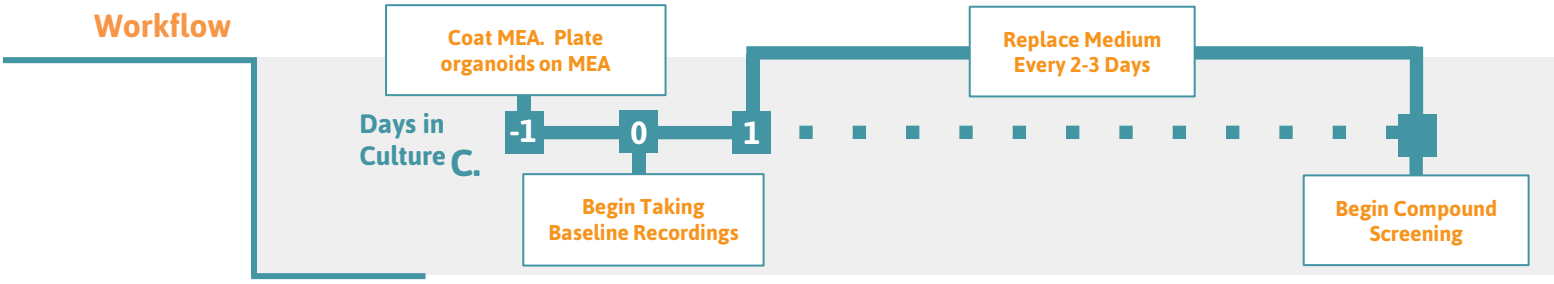
Cell Culture Protocol

Neural Organoids



Preparing the MEA Plate

1. Place a 5-10 µl drop of stock Biolaminin 332 LN solution directly from the vial over the recording electrode area of each well in the MEA plate. See **Figure 1** on for appropriate drop placement.

Tip

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

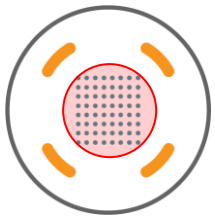


Figure 1. Droplet placement over array

2. Incubate the LN332-coated plate overnight at 4°C or for at least 60 minutes at 37°C.

Culturing and Plating Neural Organoids

3. Aspirate the Biolaminin 332 LN from the well. Prepare enough 100% Matrigel to add at least 30 µL to each well of the MEA plate that will contain an organoid.
4. Using a wide-bore pipette tip or a pipette tip that has been cut to a wide-bore size, gently aspirate some media from the organoid prep wells, and then remove one organoid via pipetting (**Figure 2**).

Figure 2. Organoid collection

Deposit the Organoids

5. Allow the organoid(s) to settle to the end of the pipette tip by holding the pipette vertically and gently tapping the pipette tip (**Figure 3A**).
6. Place the organoid in the center of a well in the MEA plate by holding the pipette vertically.
7. The MEA plate should be flat on the surface during this transfer step. Repeat steps 4-6 until all wells in the plate have an organoid placed.

Tip

Use your other hand to stabilize the pipette tip during the dispense. See Figure **3c-d** for examples of stabilizing techniques.

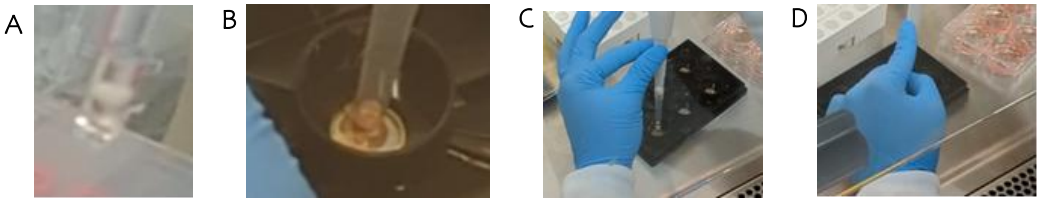


Figure 3. Organoid deposition and stabilization techniques

Position the Organoid

8. Using the pipette of choice (10 µL or 200 µL), gently aspirate media from the region surrounding each organoid, using that suction to reposition the organoid in the well (e.g., if the organoid is placed too far to the right use suction from the left to reposition the organoid toward the center). See **Figure 4** for positioning examples.

Tip

The organoids will be sitting in only a small volume of media, but this should be sufficient to maintain hydration for the duration of the plating.

Figure 4. Organoid deposition and stabilization techniques

9. Once the Matrigel is at the appropriate temperature, use a 200 µL pipette tip to aspirate 30 µL. Hold the pipette vertically directly over the organoid and drip the Matrigel onto the organoid (**Figure 5**).

Tip

30 µL is optimal for 2-3 mm organoids, but this volume can be adjusted based on size.

Figure 5. Matrigel addition

Incubate the Organoid

10. Once Matrigel has been added on top of all plated organoids, close the plate lid and transfer to a 37°C/5% CO2 incubator for at least 30 minutes, allowing the Matrigel to solidify.
11. After 30 minutes, transfer the plate back to the biosafety cabinet, and add half volumes of media to each side of each well (**Figure 6**). If this is a 6-well plate, use 500 µL on either side, paying careful attention to add the media to the wall of the conical feature in the well, allowing the media to gently flow into the well and not disturb the organoids. The plate may be tilted at this stage to assist in media addition.

Figure 6. Media addition

Troubleshooting detachment

If an organoid becomes detached, which can occur, simply aspirate the media and repeat from step 8. Consider incubation with Matrigel for longer OR using a larger volume of Matrigel if the organoid is larger.

Maintenance

Once the wells on the plate have media added and organoids are secure (**Figure 7**), you may proceed with maintaining the culture by performing half media changes regularly. Record from the plate via the Maestro periodically to observe culture maturation over time. You may be able to observe signals within hours of plating the organoids on the MEA. However, the onset of activity will vary based on the maturity of the organoids, the differentiation protocol used, and, in general, time on the MEA.

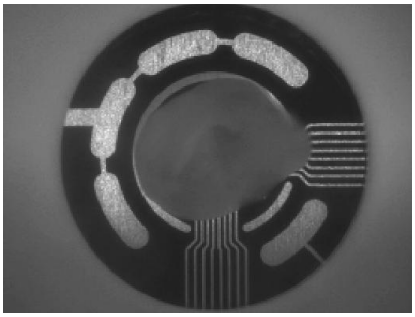


Figure 7. Organoid plated in CytoView Plate

Recording activity

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

Required Materials

Consumables

Item	Vendor	Catalog #
Axion MEA (6, 12, 24, 48, or 96-Well)	Axion BioSystems	
Biolaminin 332 LN	Biolamina	LN332-0202
Organoid Culture Media	Various	
Matrigel	Corning	354277
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	
Biological Safety Cabinet	Various	
Phase Contrast Microscope	Various	