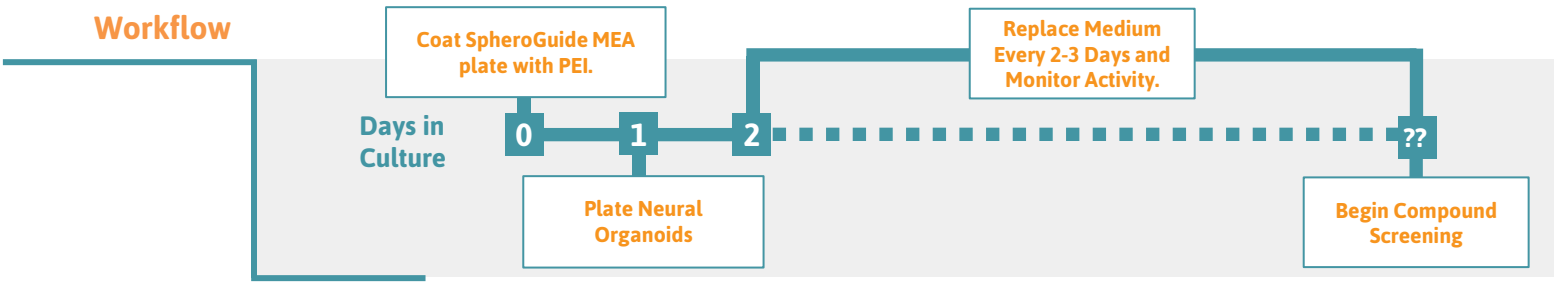
Cell Culture Protocol

Neural Organoids on SpheroGuide MEA



Preparing the MEA Plate

1. Place a 5-8 µl drop 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 SpheroGuide 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 100 µl of sterile DI water 4 times, then allow the MEA plate to air dry in a biosafety cabinet overnight at room temperature.

Tip

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

Culturing and Plating Neural Organoids

4. Add 200 µl of neural organoid media to each well of a SpheroGuide MEA plate.
5. Centrifuge at 300 x g for 5 minutes to remove any bubbles.
6. Using a 1mL wide-bore pipette tip, add one neural organoid from culture to each well of the SpheroGuide MEA plate in 100 µl of media. The total well volume will now be 300 µl.
7. Check positioning of the organoids by carefully transferring the MEA plate to a microscope. Positioning of the organoid and its contact with the electrodes can also be confirmed via the MEA Viability module on the Maestro Pro.
8. Once the organoids are in place, incubate the SpheroGuide MEA plate in a cell culture incubator at 37°C, 5% CO_2.
9. Record activity on the Maestro Pro as needed.
10. For optimal cell health, replace half of the media every 2-3 days. Half-volume (150 µl) media changes are recommended to avoid dislodging the organoid.

Tip

Any volume can be used to aspirate the organoid and pipette it into the well. However, it is recommended to not exceed 300 µl per well. It is also recommended to pipette one organoid at a time and to keep the total volume of each well consistent.

Tip

Some organoid/media compositions may require centrifugation to attach to the bottom of the plate. We recommend starting at 50 x g for 30 seconds and optimizing speed and time as needed.

SpheroGuide Well Diagram and Drop Placement

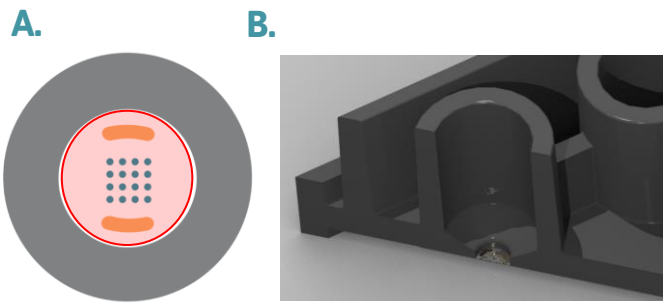


Figure 1: Well Diagram and Drop Placement
The layout represents the typical well of a SpheroGuide MEA 48-well plate (A). The gray exterior represents a funnel that helps to position organoids over the grid of planar electrodes. The red circle represents a typical 5 µl liquid (PEI) drop, which will cover most of or all of the well area. A cross-section of the well highlighting the funnel design is also shown (B). The well area that contains the electrodes and is available for organoid attachment is 2.5 mm in diameter.

Visualization of Typical Organoid Seeding Results

Figure 2: Neural Organoid Morphology
Day 52 neural organoid on a SpheroGuide MEA plate, 3 hours after initial platng (4x magnification). The organoid is roughly 1.5 mm in diameter.

Required Materials

Consumables

Item	Vendor	Catalog #
Axion SpheroGuide MEA 48-well plate	Axion BioSystems	
Neural Organoid Medium	Various	
50% Polyethylenimine solution (PEI)	Sigma-Aldrich	P3143
Dulbecco's PBS without Ca2+/Mg 2+	Thermo Fisher	14040

Equipment

Item	Vendor	Catalog #
Maestro Pro MEA System	Axion BioSystems	
AxIS Navigator	Axion BioSystems	
37°C Water Bath	Various	
Cell Culture Incubator	Various	
Biological Safety Cabinet	Various	
Tabletop Centrifuge	Various	
Phase Contrast Microscope	Various	