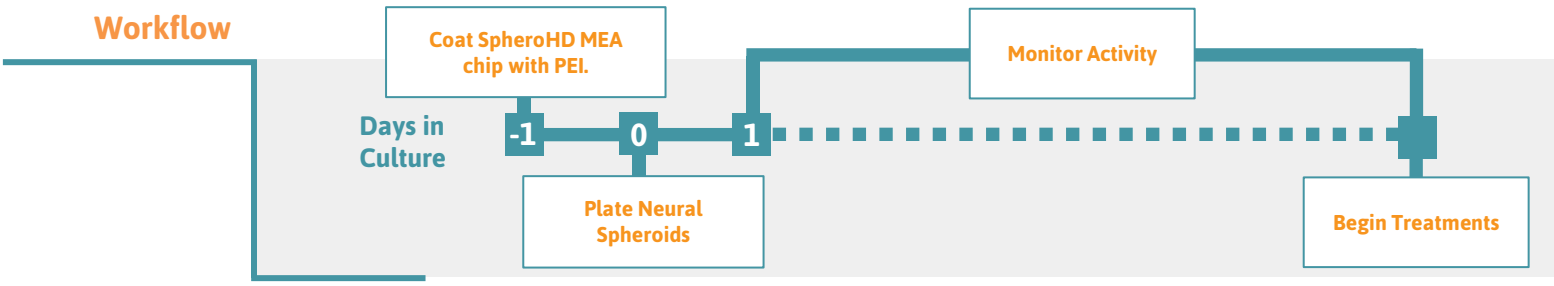
SpheroHD Protocol

Neural Spheroids



Preparing the SpheroHD Chip

1. Pipette 10 µl of 0.1% PEI in borate buffer (or neural coating of choice) into each of the four chambers of the Ibidi insert on the SpheroHD chip.
2. Incubate the PEI-coated SpheroHD chip in a cell culture incubator at 37°C, 5% CO_2 for at least 60 minutes
3. Aspirate PEI from the chambers and rinse with 10 µl of sterile DI water 4 times, then allow the SpheroHD chip to air dry in a biosafety cabinet overnight at room temperature.

Culturing and Plating Neural Spheroids

4. Add 10 µl of neural spheroid media to each chamber of the Ibidi insert on the SpheroHD chip.
5. Centrifuge at 1500 x g for 1 minute using the provided single-well centrifuge holder to remove bubbles. Remove the media from each chamber.
6. Using a 200 µl wide-bore pipette tip, add one neural spheroid from culture to each chamber of the Ibidi insert on the SpheroHD.
7. Centrifuge at 100 x g for 1 minute using the provided single-well centrifuge holder to bring the spheroids to the bottom of the chambers. Check under the microscope to ensure that all spheroids are positioned over the electrodes at the bottom of the well. Repeat or increase speed if necessary.
8. Once the spheroids are in place, incubate the SpheroHD MEA chip in a cell culture incubator at 37°C, 5% CO_2 for 1 hour.
9. Add enough neural spheroid media (about 1.75 mL) to cover the top surface of the Ibidi insert. Maximum volume for the SpheroHD chip is 2 mL.
10. Take recordings using the MEA Creator Kit on the Maestro Pro, Edge, or Volt as desired.

Tip

More information on proper handling of the Ibidi 4 well FulTrac mico insert can be found on Ibidi's website (Cat. No: 80486).

Tip

Transferring spheroids in small volumes (10 µl, e.g.) can be difficult. Alternatively, spheroids can be transferred in larger volumes by pipetting up the spheroid, allowing it to fall to the bottom of the wide bore tip, and touching the tip to media in the chamber to transfer the spheroid.

SpheroHD Well Diagram

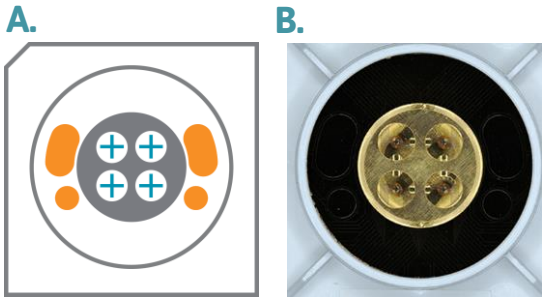


Figure 1: Well Diagram
A) A diagram of a SpheroHD chip, including the four microwells in the Ibidi insert. A total of 16 electrodes are arranged in a tightly spaced plus configuration in each microwell. B) A whole-well view of the SpheroHD chip. The Ibidi microinsert sits atop the MEA, with each chamber in the insert containing one 16-electrode array.

Visualization of Typical Spheroid Seeding Results

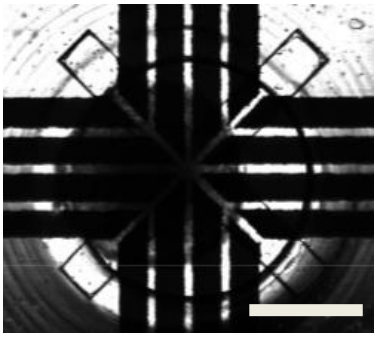


Figure 2: Neural Spheroid Morphology
Neural Spheroid at the bottom of a SpheroHD chamber after centrifugation. Scale bar 200 µm.

Required Materials

Consumables

Item	Vendor	Catalog #
Axion SpheroHD MEA Chip	Axion BioSystems	
Neural Spheroid Media	Various	
50% Polyethylenimine solution (PEI)	Sigma-Aldrich	P3143
Sterile DI water	Thermo Fisher	14040

Equipment

Item	Vendor	Catalog #
Maestro Pro MEA System	Axion BioSystems	
MEA Creator Kit	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	