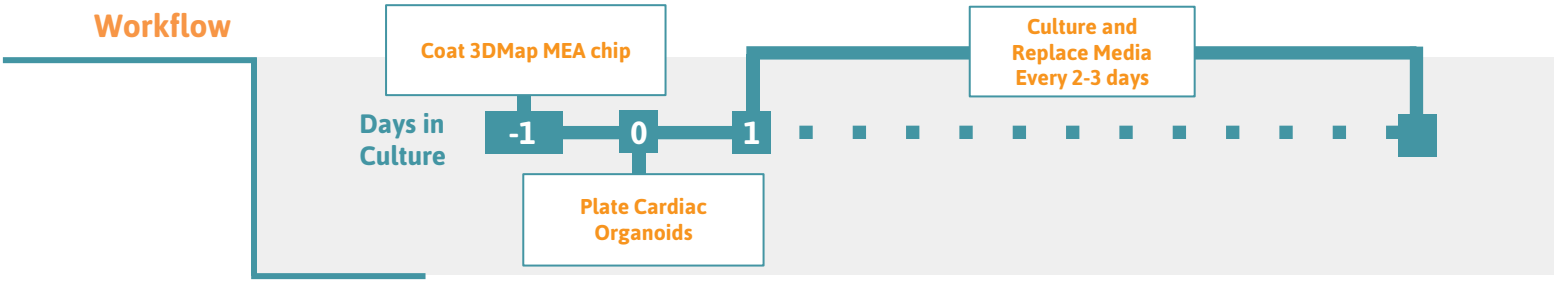
3DMap MEA Protocol

Cardiac Organoids



Preparing the MEA Plate

1. Place a 10-20 µl of stock Biolaminin 332 (or neural coating of choice) over the recording electrode area of each well in the MEA plate.
2. Incubate the LN332-coated 3DMap chip overnight at 4°C or for at least 60 minutes at 37°C.

Culturing and Plating Cardiac Organoids

3. Aspirate the LN332 coating.
4. Use a 1mL pipette and cut off the tip of the pipette tip using a sterile razor blade or scissors, between 10-15 mm from the end of the tip. The aperture should be approximately 2-3mm inner diameter to provide an opening adequate for pulling one or more organoids into the pipette tip with minimal shear stress.
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.
6. Once they have accumulated at the pipette tip end, dispense in a small volume over the array and position the organoid over the array.
7. Organoids can be repositioned if misplaced by carefully moving them with a new pipette tip. This can be achieved with either of 2 methods:

   a) use the pipette tip to gently squirt media at the organoids or drop it near them to maneuver them

   b) gently physically push them with a pipette tip
8. Once the organoids are in place, add a 30 µl drop of Matrigel on top of the organoid and incubate the MEA plate in a cell culture incubator at 37°C, 5% CO_2 for 20 mintues.

Tip

Try to keep the volume low so as the confine the organoids to the array.

Tip

Before Matrigel addition, gently draining the media from the well and a short incubation (5 minutes) at 37°C can aid in attachment. However this should only be a temporary step – if incubated dry for too long, viability of the organoids may decrease.

9. Gently add medium to 3DMap chip. Working volume for the chip is generally 1 mL, and adding media 200 µl at time to reach 1 mL total volume is recommended.
10. Incubate in a cell culture incubator at 37°C, 5% CO_2.
11. Replace media every 2-3 days, or at whatever interval is optimal for your organoid's health.
12. Record cardiac activity on the Maestro using the MEA Creator Kit. Some organoids may show activity shortly after plating, but many organoids require 1-2 weeks of culture on the MEA before robust activity is present.

3DMap MEA Chip and Electrode Layout

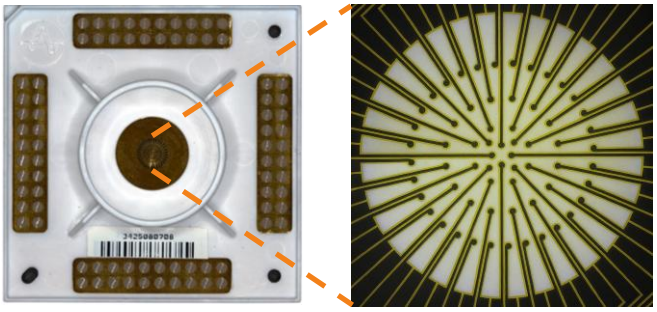


Figure 1: 3DMap MEA Chip and Electrode Layout
The 3DMap MEA chip (left) and the electrode layout (right) are shown above. The electrodes are positioned at the end of flexible cantilevers that conform to the organoid shape and allow for recording in multiple z-planes. A total of 64 electrodes are arranged in concentric circles.

Visualization of Typical Organoid Seeding Results

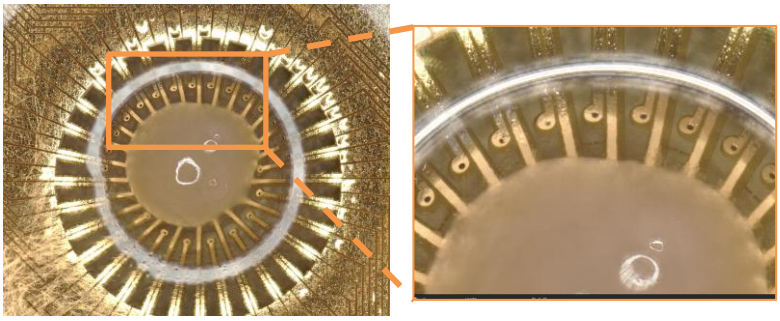


Figure 2: Cardiac Organoid Morphology
A cardiac organoid plated on a 3D Map MEA. A whole-well (left) and magnified (right) view are shown, illustrating the engagement of the organoid with the flexible electrodes.

Required Materials

Consumables

Item	Vendor	Catalog #
3D Map MEA	Axion BioSystems	
Organoid Culture Media	Various	
Matrigel	Corning	354277
Biolaminin 332	Biolamina	LN332-0202

Equipment

Item	Vendor	Catalog #
Maestro Pro or Edge MEA System	Axion BioSystems	
MEA Creator Kit	Axion BioSystems	
AxIS Navigator	Axion BioSystems	
Cell Culture Incubator	Various	
Biological Safety Cabinet	Various	
Phase Contrast Microscope	Various	