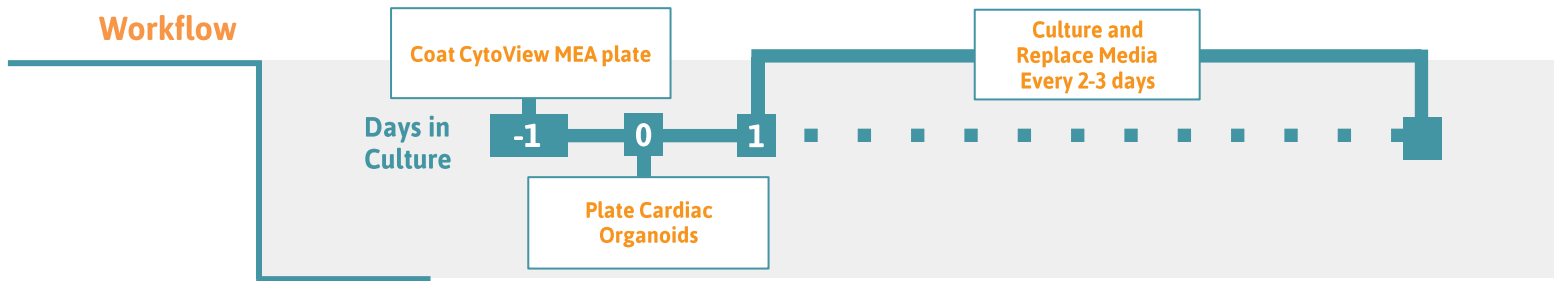


Cell Culture Protocol

Cardiac Organoids



Preparing the MEA Plate

1. Place a 10-20 μ l drop of stock Biolaminin 332 (or coating of choice) over the recording electrode area of each well in the MEA plate.
2. Incubate the LN332-coated MEA plate 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 μ l drop of Matrigel on top of the organoid and incubate the MEA plate in a cell culture incubator at 37°C, 5% CO₂ for 20 minutes.

Tip

Try to keep the volume low so as to 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 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 μ l, 24-well = 500 μ l, 48-well = 300 μ l, 96 well = 200 μ l.
10. Incubate in a cell culture incubator at 37°C, 5% CO₂.
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. 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.

Required Materials

Consumables

Item	Vendor	Catalog #
CytoView MEA Plate	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	
AxIS Navigator	Axion BioSystems	
Cell Culture Incubator	Various	
Biological Safety Cabinet	Various	
Phase Contrast Microscope	Various	

