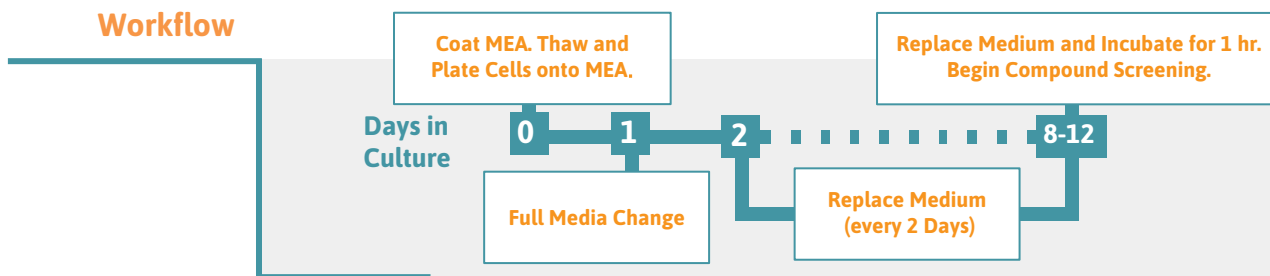


Cell Culture Protocol

Pluricyte® Human iPSC Cardiomyocytes



Preparing the MEA Plate

1. Place an 8 µl droplet of fibronectin 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 fibronectin-coated MEA plate in a cell culture incubator at 37°C, 5% CO₂ for at least 3 hours.

Tip

Reconstitute fibronectin in sterile PBS at 1 mg/ml. Aliquot and store at -20° C, and dilute to 50 µg/ml the day of use.

Tip

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

Culturing Pluricyte Cardiomyocytes

3. Thaw Pluricyte Cardiomyocytes according to the Pluricyte Cardiomyocytes User's Guide.
4. Remove a sample of the cell suspension and count the cardiomyocytes using a hemocytometer to determine both the viability and total number of viable cells. Transfer the cell suspension to a 15 ml conical tube.
5. Centrifuge the cell suspension at 250 x g for 5 minutes.
6. Aspirate the supernatant, being careful not to disturb the cell pellet.
7. Dilute the cell suspension in Pluricyte Cardiomyocytes Medium to 3,750,000 viable cardiomyocytes/ml. See the Pluricyte User's Guide for instructions to calculate the Target Plating Density based on Plating Efficiency.

Tip

Ensure the cardiomyocytes are evenly suspended before removing an aliquot to count.

Tip

Timing is critical in this step. Cardiomyocyte attachment is compromised if the fibronectin is allowed to dry after aspiration. Therefore, best practice is to aspirate and plate cells one row at a time. If the residual fibronectin has turned white and crystallized, the well should be ignored.

Plating Pluricyte Cardiomyocytes onto the MEA

8. Aspirate the fibronectin solution from the MEA.
9. Place an 8 µl droplet of Pluricyte Cardiomyocyte suspension (approx. 30,000 cells) over the recording electrode area of each well of the MEA. See Figure 1 on page 2 for appropriate drop placement.
10. Incubate the MEA plate with the seeded cardiomyocytes in a cell culture incubator at 37°C, 5% CO₂ for 1 hour.

11. Gently add 1/2 of the final volume of culture medium to each well of the MEA. Adding the medium too quickly will dislodge the adhered cardiomyocytes. Recommended 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.
12. Repeat step 11 a second time to reach the final recommended volume of culture medium.
13. Incubate in a cell culture incubator at 37°C, 5% CO₂.
14. For optimal cell health, the media should be changed after 24 hours and then 50% of the medium every 2 days. Though cardiomyocyte beats may be detectable within 24 hours, optimal Na⁺ spike and T-wave amplitudes are typically achieved after 8-12 days in culture.

Tip

Using a pipette, add medium first in a semi-circle along the outer edge of the well. Progressively add medium to either side of the well so it fills evenly towards the center. The goal is to prevent a rush of medium in either direction that might dislodge the cardiomyocytes.

Drop Placement

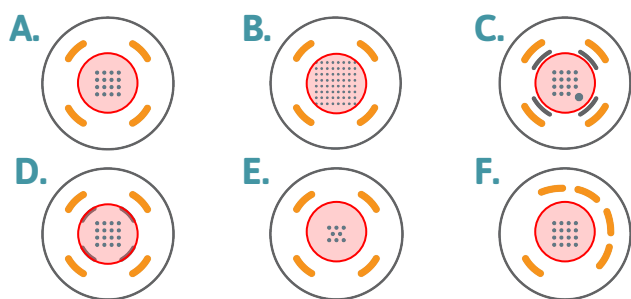


Figure 1: Drop Placement Diagram

The layouts above represent the bottom surfaces of wells in (A) a 48-well MEA, (B) a 6- or 12-well MEA, (C) a 24-well MEA or 48 well E-Stim+ MEA, (D) a 48-well AccuSpot MEA, (E) a 96-well MEA, and (F) a 48-well CytoView MEA. The number of electrodes per well is different across the plate formats, however the drop placement is the same, with the drop (red circle) centered on the recording electrodes and staying within the ground electrodes. On plate types (C) with the addition of the stim-paddle in the lower right corner of the array, it is important to make sure the droplet covers this feature. The droplet may need to be manipulated by a pipette tip after placement of the pre-treatment to ensure stim-paddle coverage.

Visualization of Typical Cardiomyocyte Seeding Results

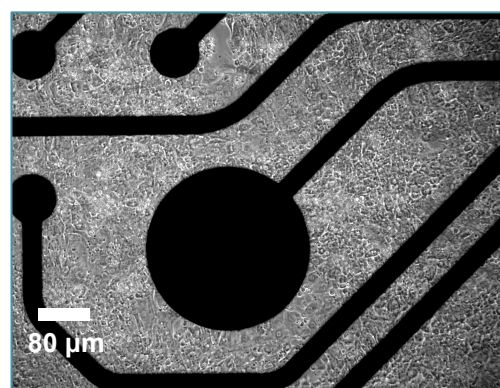


Figure 2: Pluricyte Cardiomyocyte Morphology

Human iPSC-Derived Cardiomyocytes (30,000) at day 4 *in vitro* in a 24-well CytoView MEA, 10x magnification. Notice that cardiomyocyte morphology is easily recognizable.

Required Materials

Consumables

Item	Vendor	Catalog #
Axion MEA (12, 24, 48, or 96-Well)	Axion BioSystems	
Pluricyte Cardiomyocytes	Ncardia	PCK-1.5
Pluricyte Cardiomyocyte Medium	Ncardia	PCK-1.5
Dulbecco's PBS without Ca2+/Mg 2+	Thermo Fisher	14040
Fibronectin	Roche	11051407001
KimWipes	Various	
Pipettes and Pipettors	Various	
15 mL and 50 mL Centrifuge Tubes	Various	

Equipment

Item	Vendor	Catalog #
Maestro MEA System	Axion BioSystems	
Axion Integrated Studio (AxIS)	Axion BioSystems	
37°C Water Bath	Various	
Cell Culture Incubator	Various	
Hemocytometer or Cell Counter	Various	
Biological Safety Cabinet	Various	
Tabletop Centrifuge	Various	
Phase Contrast Microscope	Various	
Liquid Nitrogen Storage	Various	