axoCells™

Human iPSC-Derived Atrial and Ventricular Cardiomyocytes on the Axion Maestro MEA

User Guide

Version 4

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This protocol and its associated products are for research use only.

Products are not for diagnostic or therapeutic use and are not to be administered to humans or animals.

Updates to protocol:

Part	Change	Rationale	Previous version	New version
Human Fibronectin	New part	Material no longer supplied	1ml of ax0049 at 100x stock, diluted for use to 1:100 solution	ax0050 diluted entirely in 20 ml D-PBS.
Human iPSC-Derived Atrial Cardiomyocyte Kit - Male	New kit	New kit	NA	New atrial cardiomyocyte kit ax2510 added to document.
MEA recording	New section	Expanded protocol	NA	Additional section of protocol added

Product Information

Catalog No.	Product Name	Format	Stock Conc.	Storage on Arrival	Thawing Instructions	Storage Once Thawed
ax2508	Human iPSC-Derived Ventricular Cardiomyocytes (Male)	1.0 million cells/vial	N/A	Liquid Nitrogen	Follow protocol	N/A
ax2558	Human iPSC-Derived Ventricular Cardiomyocytes (Male)	5.0 million cells/vial	N/A	Liquid Nitrogen	Follow protocol	N/A
ax2518	Human iPSC-Derived Atrial Cardiomyocytes (Male)	1.0 million cells/vial	N/A	Liquid Nitrogen	Follow protocol	N/A
ax2568	Human iPSC-Derived Atrial Cardiomyocytes (Male)	5.0 million cells/vial	N/A	Liquid Nitrogen	Follow protocol	N/A
ax2530-500	Cardiomyocyte Maintenance Medium	1 x 500 mL Basal Medium 1 x 10 mL Supplement	1x 50x	Store the Basal Medium at 4°C and the Supplement at -80°C	Thaw the Supplement overnight at 4°C	Once thawed store at 4°C for 2 weeks. If required, the medium can be aliquoted and stored at -80°C for later use
ax0050	Human Fibronectin	1 vial	N/A	Store at -80°C	Thaw at 4°C	Once diluted, use immediately
ax2500	Human iPSC-Derived Ventricular Cardiomyocyte Kit - Male	Kit Components: 1 million cells; Cardiomyocyte Maintenance Medium; Human Fibronectin	See above for component details			
ax2510	Human iPSC-Derived Atrial Cardiomyocyte Kit - Male	Kit Components: 1 million cells; Cardiomyocyte Maintenance Medium; Human Fibronectin	See above for component details			

Additional Reagents		
Product Name	**Supplier**	**Product Code**
Y-27632 2HCl (ROCK inhibitor)	Focus Biomolecules	10-2301
Fetal bovine serum (FBS)-EU Approved heat inactivated	Sigma Adrich	F9665-500mL

Individual experimental results may vary depending on the supplier and batch of FBS used. For Japan, we recommend the use of heat-inactivated FBS (Netherland origin) #S-FBS-NL-025 from Serana.

Lot-specific information such as specifications and quality control details are stated in the Certificate of Analysis. Expiry dates for Axol-supplied components are stated on the label. Consult the manufacturer's guidelines for expiry dates of any additional reagents.

Introduction

axoCells Cardiomyocytes exhibit typical biochemical, electrophysiological, mechanical, and pathophysiological characteristics of native human cardiac myocytes. Due to their human origin, high-purity, functional relevance, and ease of use, axoCells Cardiomyocytes represent an optimal test system for investigating cardiomyocyte biology for basic research and drug development.

The Maestro Edge and Pro multielectrode array (MEA) systems from Axion BioSystems are a non-invasive, label-free platforms that measures local field potentials of electrically active cells, representing the summed activity of the underlying ion channels and the contractility of the syncytium.

Together, axoCells Cardiomyocytes and Axion's MEA technology form an excellent, non-invasive platform for *in vitro* screening of compound effects on human cardiomyocyte physiology.

This user guide describes how to handle axoCells Atrial and Ventricular Cardiomyocytes for use on the Maestro MEA system and provides basic instructions for compound treatments, data acquisition, and analysis.

Figures adapted from Axion Biosystems user guides available at **https://www.axionbiosystems.com/**

Recommendations

- Recommended culture vessel: **Axion CytoView MEA 48 #M768-tMEA-48B**
- Recommended culture vessel coating: **Fibronectin**
- Recommended cell culture medium: **Cardiomyocyte Maintenance Medium**
- Recommended seeding density for assay: **100,000 – 200,000 cells/cm²**
- Recommended centrifugation speed: **200 x g for 5 minutes**
- Recommended days in culture before assay: **7 to 10 days**

Important! Cardiomyocyte Maintenance Medium = Basal Medium + Supplement DOES NOT contain antibiotics or antifungal agents.

Axol Bioscience does not recommend the use of antimicrobial agents such as penicillin, streptomycin, and amphotericin. Antimicrobial agents should not be necessary if proper aseptic techniques are adopted.

Preparing the MEA Plate

- Calculate the total volume of fibronectin that is required for coating.
- Dilute the stock Human Fibronectin in 20 ml D-PBS (without magnesium and calcium) to make a 1x working solution.
- At least 4 hours before plating cardiomyocytes, place an 8 µL droplet of the 1x **fibronectin** working solution over the recording electrode area of each well in the MEA plate. Do not let the tip touch the electrodes. See Figure 1 and 2 for appropriate drop placement.
- Add 1 mL of DPBS -/- to the area surrounding the wells (MEA reservoirs) of the MEA plate to prevent substrate evaporation. Do not allow the water into the wells of the MEA plate.
- Incubate the fibronectin-coated MEA plate in a cell culture incubator at 37°C, for at least 4 hours.

Care should be taken when using higher volumes of water in the MEA reservoirs to prevent sloshing and spilling.

Preparation of Reagents

Cardiomyocyte Maintenance Medium

- Upon receipt store **Cardiomyocyte Maintenance Basal Medium** at 4°C - 8°C and **Supplement** at -80°C.
- Remove 10mL of the basal medium and add the **Supplement** to the **Cardiomyocyte Maintenance Basal Medium**.
- For long-term storage, prepare aliquots of **Cardiomyocyte Maintenance Medium** and store at -80°C. The **Cardiomyocyte Maintenance Medium** is then stable from the first expiry date of media/supplement (please refer to the Certificate of Analysis).

Plating Medium

- When ready to use, thaw an aliquot of **Cardiomyocyte Maintenance Medium** overnight in the dark at 4°C.
- Take an aliquot of **Cardiomyocyte Maintenance Medium** and add 10% fetal bovine serum (FBS) and Y-27632 2HCl (ROCK inhibitor) to a final concentration of 10 µM to make the **Plating Medium**. Complete plating media must be used fresh and cannot be stored once supplemented.
- Before use, pre-warm an aliquot of **Plating Medium** at 37°C.

Plating Medium			
Supplement	**Stock Concentration**	**Final Concentration**	**Final Volume in 45 mL of Medium**
Y-27632 dihydrochloride (ROCK inhibitor)	10 mM	10 µM	50 µl
Fetal bovine serum (FBS)	NA	10%	5 ml

Thawing of axoCells Atrial & Ventricular Cardiomyocytes

- On the day of thawing **axoCells Atrial or Ventricular Cardiomyocytes**, prepare the **Cardiomyocyte Maintenance Medium** and **Plating Medium** and pre warm before use.
- To thaw the cells, transfer the cells from liquid nitrogen storage by carrying cells buried in dry ice. Remove the cells from dry ice and transfer them immediately to a 37°C water bath.
- Quickly thaw the vial of cells in a 37°C water bath, taking care not to completely

submerge the vial (only up to two thirds should be placed in the water). Remove the vial before the last bit of ice has melted, ~ 2 minutes.

- Do not shake the vial whilst thawing.
- Take the vial of cells to a biological cabinet, spraying it thoroughly with 70% ethanol and wipe with an autoclaved paper towel before placing in the culture hood.
- Once thawed, use a P1000 pipette to immediately transfer the cells to a 15 ml sterile conical tube.
- Using a P1000, wash the now empty cryovial with 1 ml of room temperature **Plating Medium**. Add the 1 ml of **Plating Medium** to the conical tube containing the Cardiomyocytes.
- **Important** – the **Plating Medium** should be added to the cells drop-wise whilst gently swirling the conical tube. This should take ~ 60 seconds to dispense the 1 ml of medium. This is a necessary step to prevent osmotic shock to the cells and improve post thaw viability.
- Using a 10 ml stripette, slowly add 8 ml of room temperature **Plating Medium** to conical tube containing the cells. This should take ~ 60 seconds to dispense the 8 ml of medium.
- Centrifuge the cells at 200 g for 5 minutes at room temperature.
- Aspirate and discard the supernatant, taking care not to disturb the cell pellet.
- Using a P1000 pipette, resuspend the pellet in 1 ml of warm (37°C) **Plating Medium**. Gently resuspend the cell pellet until a single cell suspension is obtained.
- Perform a cell count to ensure optimal seeding density. Remove 10 µl of cell suspension and mix it with 10 µl of trypan blue solution. Count the cells.
- Using warm, 37°C **Plating Medium**, resuspend the cells to give to 6,250,000 plated cardiomyocytes/mL. Alternative seeding densities and volumes can be calculated at https://www.axionbiosystems.com/cell-plating-calculator. Refer to the Axion guide at https://www.axionbiosystems.com/resources/product-videos/cell-plating-calculator-how-guide.

Plating Cardiomyocytes onto the MEA

- Aspirate, from a single row or column, most of the **fibronectin** solution from the MEA surface.
- Place an 8 µl droplet of **cardiomyocyte** suspension (approx. 50,000 cardiomyocytes) over the recording electrode area of each well. See **Figure 1** and **Figure 2** for appropriate drop placement.

Timing is critical in this step. Cardiomyocyte attachment is compromised if the fibronectin is allowed to dry. Under typical conditions, the well will begin to dry within a few minutes after aspiration of the excess fibronectin solution. At this point the residual fibronectin in the well will begin to crystallize, turn white, and the well should then be ignored as cardiomyocyte attachment will be suboptimal.

- Repeat the previous steps until all rows or columns have been plated.
- Incubate the MEA plate with the seeded cardiomyocytes in a cell culture incubator at 37°C, 5% CO_2 for 1 hour.
- Gently add 150 µL of **Plating Medium** to each well of the MEA. Dispense into alternating sides of the well as adding medium too quickly will dislodge the adhered cardiomyocytes.
- Using a pipettor, add medium first in a semi-circle along the outer edge of the flat bottom area. Progressively add medium so it fills evenly towards the centre, stopping before contact is made with the droplet in the centre. Gently bridge the gap with additional medium. The goal is to prevent a rush of medium in either direction that might dislodge the cardiomyocytes.
- Slowly add the remaining volume to reach the plate recommended media volume. Recommended well volumes for each plate type: 12-well = 500 µL, 48-well = 300 µL, 96 well = 200 µL. At this point, MEA reservoir water is no longer required.
- Incubate in a cell culture incubator at 37°C, 5% CO_2 for 24 hours.

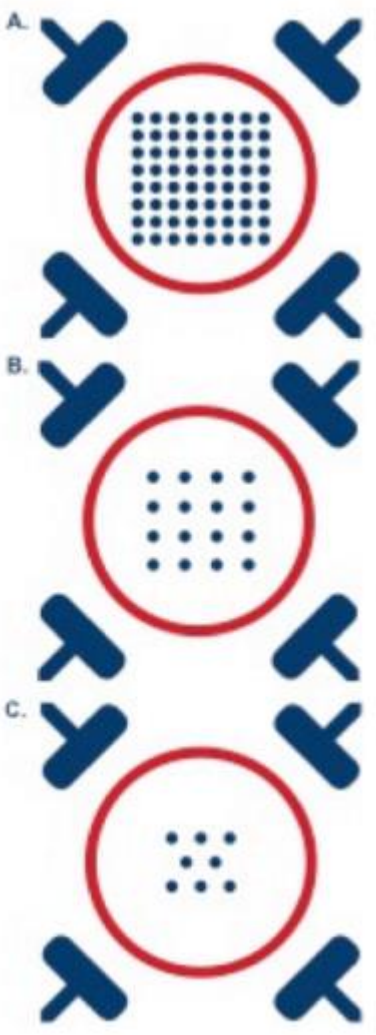

Figure 1: Drop Placement Diagram

The layouts on the left represent the bottom surfaces of wells in a 12-well MEA (A), a 48-well MEA (B) and a 96-well MEA (C). Diagram A represents a 12-well MEA and the inner 64 dots of the electrode array with the 4 ground electrodes located in the corners. Diagram B represents a 48-well MEA and the inner 16 dots of the electrode array with the 4 ground electrodes located in the corners. Diagram C represents a 96-well MEA and the inner 8 dots of the electrode array with the 4 ground electrodes located in the corners. The red circles indicate the approximate size and location for the drop placement.

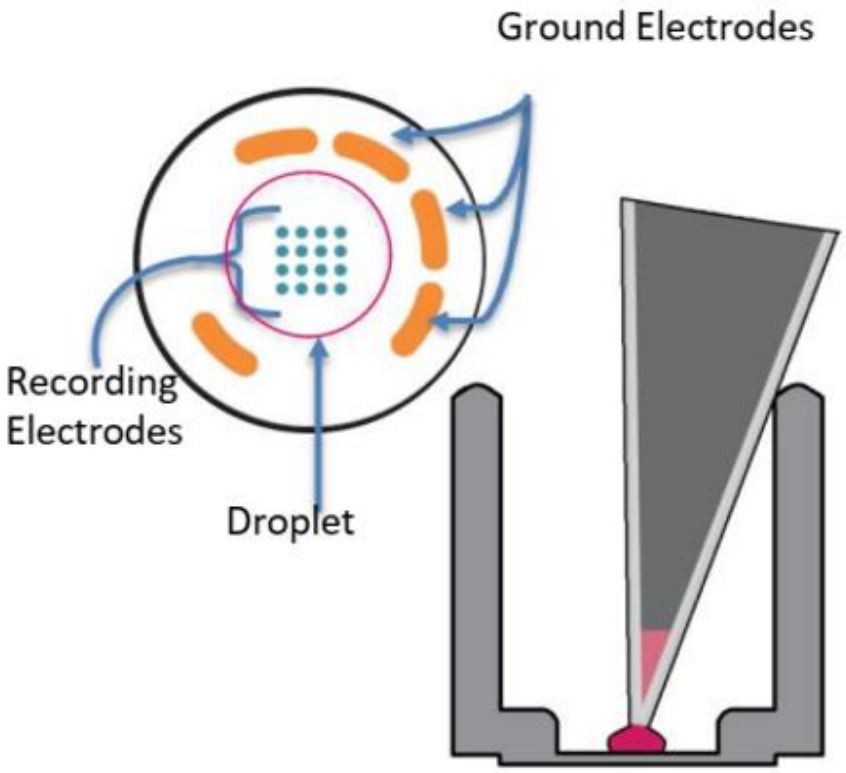

Figure 2: Drop Placement guide for both fibronectin coating and cardiomyocyte seeding on the Axion 48-well MEA plate.

- Orient tip just above the recording electrodes.
- Eject droplet without touching the surface.
- If droplet does not release gently, touch it down to the surface.
- Cover recording electrodes completely while avoiding ground electrodes.

Maintenance of axoCells Cardiomyocytes on the MEA

- On the day after plating, replace 100% of the **Plating Medium** with **Cardiomyocyte Maintenance Medium.**
- Culture cardiomyocytes on the MEA plate replacing 50% of the spent medium with **Maintenance Medium** every 2 days (see **Figure 3**).
- After 7 days in culture, the **Human iPSC-Derived Atrial or Ventricular Cardiomyocytes** should beat spontaneously (this can occur within 72 hours).
- For optimal results, perform MEA recordings 7-18 days after plating.

Please note that 50,000 cells/well is a recommended seeding density and that density may need to be optimized by the user to suit their culture conditions and final assay requirements.

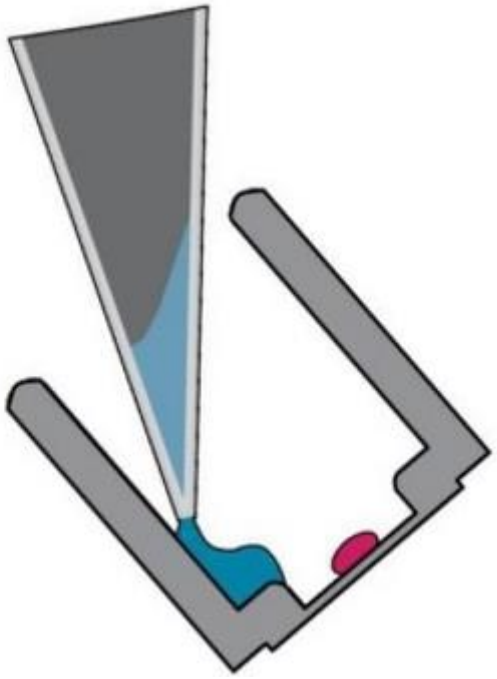

Figure 3: Media change for cardiomyocyte culture on the Axion 48-well MEA plate

- Expel 50-75 µl against the well wall.
- Break the surface tensions between the media and cell droplets with the tip.
- Continue adding a half volume of media slowly against the well wall.
- Add half volumes to each well to minimize time cells are exposed in droplets then add second half.

Axion MEA Recording

- Turn on the Axion MEA and check that the temperature is set to 37°C and the CO_2 to 5%.
- Allow the MEA's temperature and gas-mix to equilibrate for at least 30 minutes.
- Place the MEA plate onto the Axion and allow at least 10 minutes (and preferably over 30 minutes) to allow the plate to equilibrate.
- MEA recordings can be performed in the **Cardiomyocyte Maintenance Media.**
- **Note 1.** media changes can produce short-lived changes to the beating of the cardiomyocytes therefore Axol recommends allowing the cardiomyocytes to recover for at least 4 hours before commencing recording, and where possible perform the media change the day before, to allow stable, consistent beating to be re-established.
- **Note 2**. Similarly when looking at acute responses Axol recommends adding compounds of interest directly into the well and allowing the compound to dilute *in situ* rather than performing a full media change into the compound because of the media change artefact. 30µl of 10X compound into a 300µl volume on the 48wp format has been found to produce a rapid mixing and immediate compound effects. The media change artefact also makes washing-off of compounds problematic. In order to avoid this, we would recommend either only testing one concentration per well, performing an accumulating dose protocol without wash-off or allowing the wells to recover after wash-off before the subsequent dose.

- Axol would always recommend performing a vehicle control as a negative control with every experiment. DMSO loads greater than 0.1% has been shown to create vehicle artefacts.
- The choice of positive control will depend on the experiment.
- For acute cardiotoxicity studies, 100nM Dofetilide will rapidly induce significant FDP lengthening, the appearance of after-depolarisations and the appearance of arrhythmias (Figure 4).

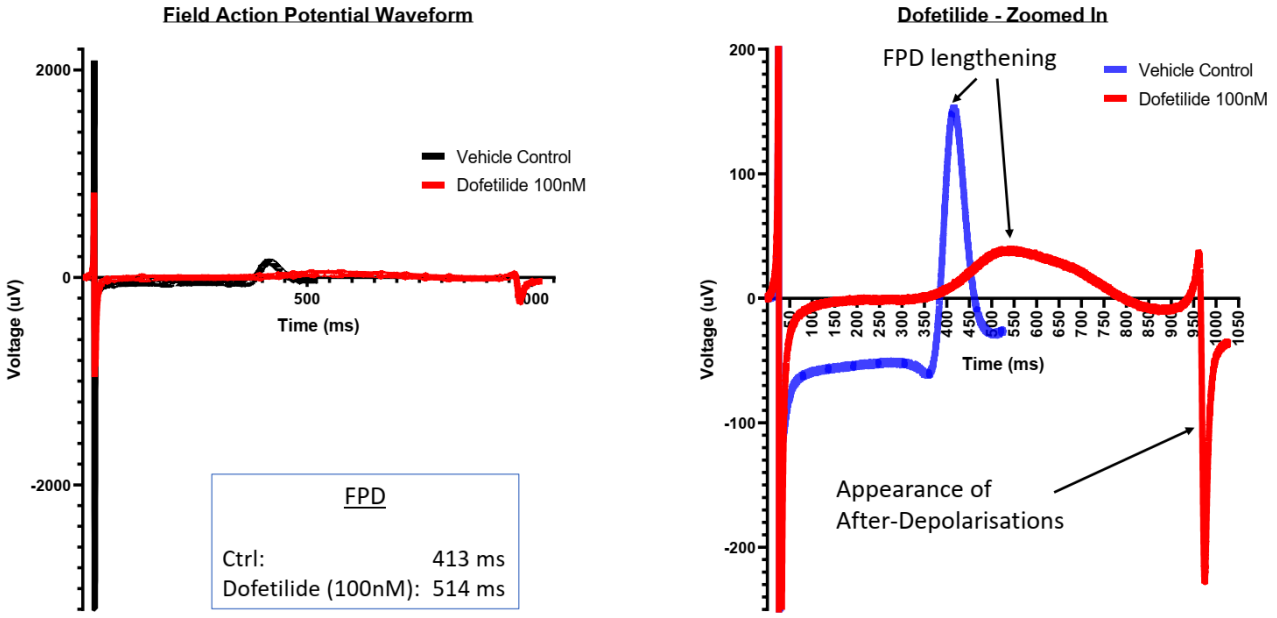

Figure 4 Acute Cardiotoxicity Example
An example of the effect on the fAP waveform of Axol's ventricular cardiomyocytes by Dofetilide (100nM).

- For chronic cardiotoxicity studies, 3µM Doxorubicin will produce significant and long-lasting cytotoxic within about 8 hours, producing significant changes in the contractility characteristics of the ventricular cardiomyocytes.

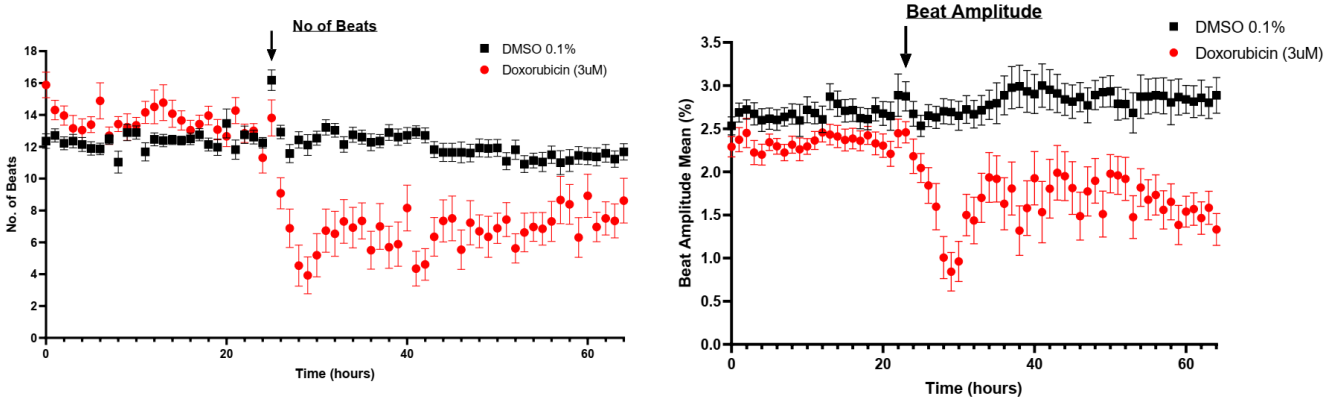

Figure 5 Chronic Cardiotoxicity Example
An example of the effect on contractility parameters of Axol's ventricular cardiomyocytes by Doxorubicin (3µM)

- For chamber-specific experiments, Carbachol (1-100µM) has been shown to have significant effects on Axol's atrial cardiomyocytes while only having limited effects on Axol's ventricular cardiomyocytes.

Got any questions? Need help with the protocol?

Contact Axol Technical Support

at support@axolbio.com

Or

Call +44 (0) 131 651 9710