Cell Culture on Microelectrode Arrays

Cell Type: GE Healthcare - Cytiva™ Plus Cardiomyocytes

Protocol
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Origin

Axion BioSystems Microelectrode Arrays are manufactured in the United States of America.

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Acknowledgement

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Before You Begin

1. Read this entire manual before using cells or the microelectrode arrays.

2. Check the Axion Maestro™ system for correct performance. Contact Axion at support@axion-biosystems.com with any issues.

3. Consult with Axion about untested experimental variables if there is concern with the safety of the equipment.
Introduction

Cytiva™ Plus Cardiomyocytes, which are human embryonic stem cell-derived, exhibit typical biochemical, electrophysiological, mechanical, and pathophysiological characteristics of native human cardiomyocytes. Due to their human origin, functional relevance and ease of use, Cytiva Plus Cardiomyocytes represent an optimal test system for interrogating cardiomyocyte biology in basic research and drug development.

The Maestro Multielectrode Array (MEA) system from Axion BioSystems is a non-invasive, label-free platform that measures local field potentials of electrically active cells. The field potential measurement represents the summed electrical activity of the individual cells in the culture and their underlying ion channels. Cytiva Plus Cardiomyocytes can be cultured on MEAs to form an electrically and mechanically active syncytium amenable to electrophysiological interrogation. Together, Cytiva Plus Cardiomyocytes and Axion’s MEA technology form a rapid, non-invasive platform for in vitro screening of compound effects on human cardiomyocyte physiology.

This Application Protocol describes how to handle Cytiva Plus Cardiomyocytes for use with a Maestro MEA system and provides basic instructions for compound treatments, data acquisition, and analysis. This protocol will outline the direct from vial seeding technique that can be used with Cytiva Plus Cardiomyocytes, reducing transfer steps for more efficient cardiomyocyte seeding.
Technical Support

For any questions about cell plating or Maestro system operation, please contact Axion BioSystems Support using the information below.

Telephone: (404) 477-2557
Fax: (404) 385-4638
E-mail: support@axion-biosystems.com
## Required Materials

### Consumables

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<th>Catalog Number</th>
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<td>GE Healthcare</td>
<td>29091880 or 29091881</td>
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<tr>
<td>FBS (at +4°C)</td>
<td>GIBCO/Life Technologies</td>
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<td>Fibronectin</td>
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<td>RPMI 1640 Medium with L-Glutamine</td>
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### Equipment

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<tr>
<td>Liquid Nitrogen Storage</td>
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Methods

Preparing Complete Medium

1. Using a 10 mL sterile serological pipette, add 10 mL of B-27 supplement to 500 mL bottle of RPMI1640 + L-Glutamine medium. Invert bottle several times to mix.

2. Filter the RPMI/B-27 medium by vacuum filtration into a sterile container for long term storage.

MEA Surface Pretreatment

3. Wipe the package of the sealed MEA plate with 70% ethanol (EtOH), then place it in a biological tissue culture hood.

4. Pull the MEA from the sealed package and wipe the top, bottom, and sides of the plate with a Kimwipe soaked in 70% EtOH.

5. Add ~6 mL of sterile distilled water 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.

6. Place a 4 µL bead of FBS over the recording electrode area located in the center of each well of the MEA plate.

7. Put the lid on the MEA plate and incubate the MEA plate for 1.5 hrs at room temperature.

8. Prepare a 1 mg/mL solution of fibronectin by adding 1 mL sterile distilled water to 1 mg fibronectin.

9. Take 12.5 µL of this 1 mg/mL fibronectin solution and add it to 987.5 µL D-PBS in a 50 mL tube for a final concentration of 12.5 µg/mL fibronectin.

10. Aspirate the FBS bead from each well of the MEA plate (use a pipette set to dispense 4 µL) and immediately replace with a 4 µL bead of 12.5 µg/mL fibronectin solution over the recording electrode area in the center of the well.

11. Put the lid on the MEA plate and incubate for 2 hours in a standard cell culture incubator at 37°C.

Notes:

MEA reservoir water is no longer required following the media addition in Steps 25 and 26.
Notes:

**Thawing Cryopreserved Cytiva Plus Cardiomyocytes**

12. Remove the cryopreserved Cytiva Plus Cardiomyocytes cryovial from the liquid nitrogen storage container.

13. Thaw cell suspension in a 37°C water bath with gentle agitation until ice crystals disappear.

**Tip**

*Take care not to immerse the whole cryovial into the water bath. Avoid extended incubation at 37°C.*

14. Wipe the outside of the cryovial with 70% ethanol and transfer to a biological safety cabinet.

15. Carefully transfer the cell suspension into a sterile 50 mL centrifuge tube using a 1000 µL pipette.

**Note:**

*Avoid repeatedly pipetting the thawed cardiomyocytes.*

16. Wash the inside of the cryovial with 1 mL of room temperature RPMI 1640/B27 medium to recover residual cells left in the vial. Add this 1 mL of media from the cryovial drop-wise (~1 drop/sec) to the centrifuge tube with the cardiomyocyte cell suspension. Gently swirl the tube while adding the medium to completely mix the solution and limit the chances of osmotic shock to the thawed cells.

**Tip**

*Drop-wise transfer of medium is critical in limiting the osmotic shock and maximizing viability and attachment to the MEA.*

17. Slowly (over the course of 2 minutes) add 8 mL of RPMI 1640/B27 to the centrifuge tube containing the cells.

18. Concentrate the cardiomyocytes by centrifuging at 1000 rpm for 5 minutes at 20°C.

19. Remove the supernatant using a 1000 µL pipette and resuspend the cardiomyocytes in 1 mL RPMI 1640/B27 by gently rasping the tip of the centrifuge tube against the grate at the front of the biological safety cabinet.
Seeding Cytiva Plus Cardiomyocytes onto the MEA

20. Determine the total cell number and cell viability using preferred method of choice.

21. Produce a cell suspension of 1.5x10^7 viable cells/mL by concentrating the cardiomyocytes via centrifugation at 300 g for 5 minutes at 20°C.

22. Aspirate the fibronectin beads from the MEA surface pre-treatment, but do not let MEA surface dry before seeding the cells onto the surface (the surface will dry in ~2-3 minutes).

23. Seed 6x10^4 cells in a 4 µL droplet (i.e. 1.5x10^7 viable cells/mL) directly over the array of electrodes in each pre-treated well of the MEA. See Figure 1 for examples of drop placement and Figure 2 for magnified drop appearance.

24. Incubate the MEA with seeded cardiomyocytes in a cell culture incubator at 37°C, 5% CO_2 for 2-3 hours.

25. Remove the MEA plate after 2-3 hours. Carefully add 500 µL (12-well) or 150 µL (48-well) of RPMI/B-27 medium to each well by pipetting gently down the wall of the well, using a multi-channel pipette in a biological safety cabinet. Addition of the medium too quickly will detach the adhered cardiomyocytes.

26. Repeat step 25 a second time, adding another 500 µL (12-well) or 150 µL (48-well) of medium.

27. Make a final addition of 1000 µL (12-well) or 300 µL (48-well) to bring the well volume to a total of 2 mL (12-well) or 600 µL (48-well) of medium.

28. Incubate the MEA plate in a cell culture incubator at 37°C, 5% CO_2.

Maintaining Cytiva Plus Cardiomyocytes

29. Immediately before use, warm the medium in a 37°C water bath.

30. Feed cells 4 days after thawing by replacing approximately 1/2 of the media. For subsequent media changes, replace 1/2 the media every 3 days.

31. Continue to store the cells in a cell culture incubator at 37°C, 5% CO_2.

32. For optimal Cytiva Plus Cardiomyocyte results, perform MEA recordings 5-7 days after thawing.

The timing of the medium addition is critical as performance of the cardiomyocytes degrades if the droplets begin to dry (~1-2 minutes).
Visualization of Typical Cardiomyocyte Seeding Results

Figure 2: Cytiva Plus Cardiomyocytes During Seeding on 12-well MEA
Representation of cardiomyocytes in a 4 µL droplet following seeding on a 12-well MEA electrode grid.

Figure 3: Cytiva Plus Cardiomyocyte Morphology
Cytiva Plus Cardiomyocytes at day 5 *in vitro* on a 12-well MEA.*

*Axion can provide transparent 48-Well blank plates with no electrodes to confirm cellular adhesion.