Ncardia
Stem cell experts

Pluricyte® Cardiomyocytes

using the Multiwell MEA System from Multi Channel Systems

User Guide
Version 3.1 / March 2018

Contents

Getting Started

Please make sure to read the entire user guide carefully before you start thawing and culturing Pluricyte® Cardiomyocytes.

Pluricyte® Cardiomyocytes are for *in vitro* life science research use only.

A Material Safety Data Sheet (MSDS) for Pluricyte® Cardiomyocytes is available on our website.

Technical support and training

Our scientists are ready to help you with any questions you may have regarding this user guide or our Pluricyte® Cardiomyocytes. In addition, in-lab training is available upon request. For further information please visit our website www.ncardia.com, or contact us directly by e-mail (sci-support@ncardia).

1. Introduction

Pluricyte® Cardiomyocytes are highly suitable for Multi Channel Systems Multiwell-MEA-System assays

Pluricyte® Cardiomyocytes are fully functional human induced pluripotent stem cell (hiPSC) derived ventricular cardiomyocytes that are particularly suitable for electrophysiology-based multi-electrode array (MEA) assays for predictive safety pharmacology, toxicity testing and efficacy screening in early drug discovery. The combination of Pluricyte® Cardiomyocytes with the Multi Channel Systems Multiwell-MEA-System enables detailed electrophysiological detection of potential cardiotoxic/pro-arrhythmic effects of compounds in a 96-well plate format. The well-pronounced depolarization and repolarization peaks of Pluricyte® Cardiomyocytes field potential signals allow an easy detection of electrophysiological parameters (e.g. depolarization/repolarization peak amplitudes, beat rate, field potential duration) and facilitate efficient data analysis and interpretation of studies performed with the Multiwell-MEA-System from Multi Channel Systems.

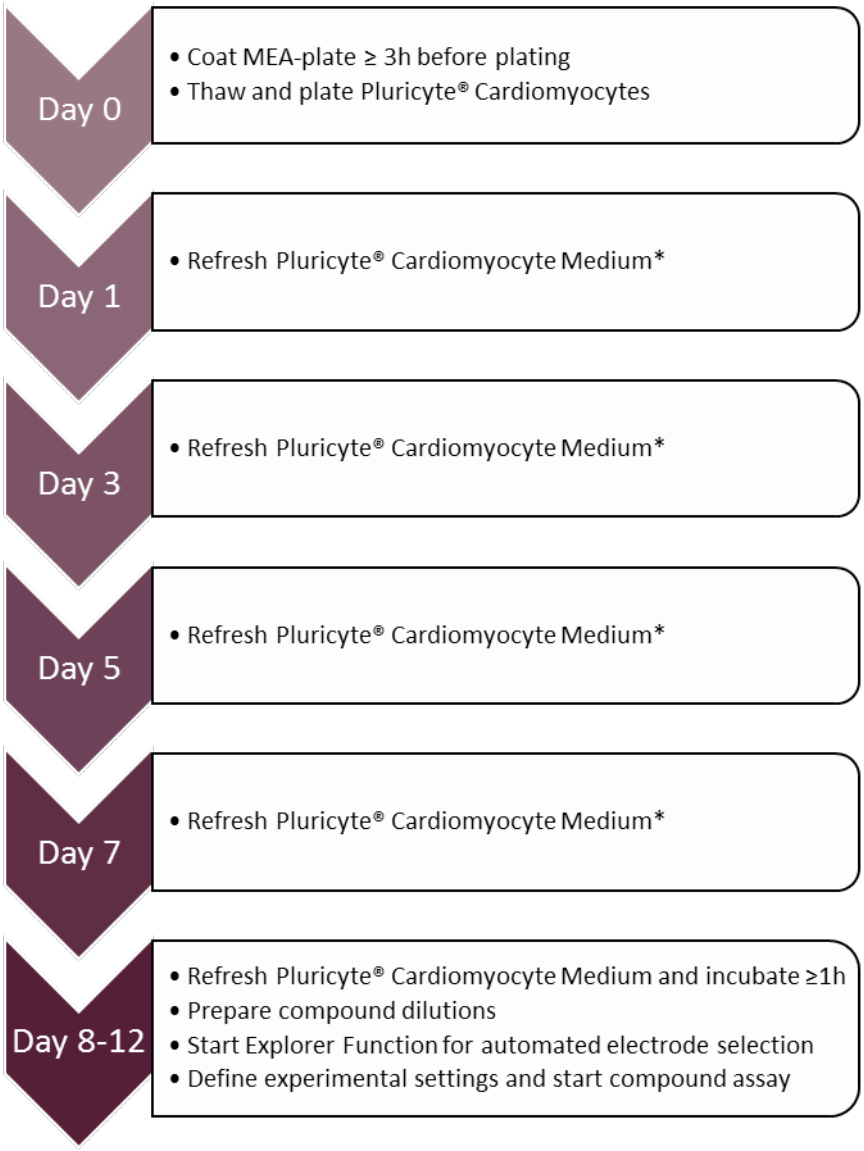
Pluricyte® Cardiomyocytes - strengths and characteristics

Pluricyte® Cardiomyocytes exhibit a relatively high level of maturity, when compared to other human stem cell-derived cardiomyocytes and present the following unique characteristics:

- High purity of ventricular cardiomyocytes
- Low resting membrane potentials (~-78 mV)
- Fast upstroke velocities and action potential amplitudes
- Organized sarcomeric structures
- Monolayer field potential has well-pronounced depolarization and repolarization peaks, enabling easy detection of field potential durations in MEA assays

This application note describes our recommendations for the use of Pluricyte® Cardiomyocytes in the Multiwell-MEA-System from Multi Channel Systems. In addition, a case study describing the assessment of the effects of a set of pro-arrhythmic compounds in Pluricyte® Cardiomyocytes, showing the expected pharmacological responses, can be downloaded at www.ncardia.com. Pluricyte® Cardiomyocytes, cultured in Pluricyte® Cardiomyocyte Medium, in combination with the Multiwell-MEA-System from Multi Channel Systems provide a highly relevant in vitro assay platform to study the cardiac safety profile of compounds during drug development.

2. Workflow



* Optional: in order to monitor the condition of the Pluricyte® Cardiomyocyte monolayer it is advised to perform daily measurements (≥ 1h after refreshment).

3. Important Recommendations

- Carefully follow the thawing and seeding instructions. This step is essential for optimal cell survival and attachment (Section 5.2). After thawing, Pluricyte® Cardiomyocytes are directly seeded onto the Multiwell MEA-plates.
- We strongly recommend to use fibronectin as coating substrate for the MEA-plates. Other types of coatings may reduce the signal and/or impact the condition of the cells.
- Always refresh the Pluricyte® Cardiomyocyte Medium of the cells the day after seeding the cells (Section 5.3). Subsequently, refresh the Pluricyte® Cardiomoycyte Medium of the cells every 2 days. Pluricyte® Cardiomyocyte Medium could be refreshed on Friday afternoon and Monday morning to prevent weekend-work.
- First contractions of Pluricyte® Cardiomyocytes appear between 24-48 hours post-thawing. It will take 3-4 days before the cells have formed an electrically coupled monolayer. Stable beating monolayers can be observed 7-8 days post-thawing. The optimal time window to perform electrophysiology-based assays with Pluricyte® Cardiomyocytes is between 8-12 days after plating the cardiomyocytes.

4. Equipment, Materials and Reagents

Equipment, materials and reagents are described in Tables 4.1, 4.2, 4.3 and 4.4., respectively.

Equipment	Manufacturer
Multiwell-MEA-system (Interface board, Multiwell-MEA-Headstage, Multiwell-Screen acquisition software)	Multi Channel Systems (MCS)
Data acquisition computer (minimum Windows 8 Software)	Various
Multiwell-Analyzer software (Available online)	Multi Channel Systems
Class 2 laminar flow cabinet	Various
Incubator at 37˚C, with 5% CO_2 and humidified air	Various
P20, P200 and P1000 pipettes	Various
12-channel Multichannel pipette (or adjustable pipette)	Various
Hemocytometer	Various

Table 4.1: Equipment

Materials	Manufacturer	Catalog number
96-well MEA plate	Multi Channel Systems	96w700/100F
Sterile disposable 5ml pipettes	Various	
Sterile disposable 10ml pipettes	Various	
Sterile disposable 25ml pipettes	Various	
Sterile Eppendorf tubes	Various	
Sterile 50ml conical tubes	Various	
Sterile 20µl Filter pipette tips	Various	
Sterile 200µl Filter pipette tips	Various	
Sterile 1000µl Filter pipette tips	Various	
Sterile multichannel reservoirs	Various	

Table 4.2: Materials

Reagents	Manufacturer	Catalog number
Fibronectin (1mg/ml)	Sigma	F1141
1x DPBS $+Ca^{2+}+Mg^{2+}$	e.g. Life technologies	Gibco 14040
Pluricyte® Cardiomyocyte Kit*	Pluriomics	PCK-1.5
• 1.5M Pluricyte® Cardiomyocytes		
• 100 ml Pluricyte® Cardiomyocyte Medium		

Table 4.3: Reagents

* For a full 96-well plate, two Pluricyte® Cardiomyocyte Kits are needed.

5. Methods

5.1 Coating the 96-Well MEA plate

The MEA plate is coated with fibronectin on the day of plating the Pluricyte® Cardiomyocytes (≥ 3h before thawing of the cells).

Note: The volumes used below are calculated for one 96-well MEA plate. For plating more than one 96-well MEA plates, multiply the volumes used by the number of 96-well MEA plates needed.

1. Dilute 25µl of the fibronectin solution in 475µl sterile D-PBS (incl. Ca^{2+} and Mg^{2+}) in an Eppendorf tube to get a 50µg/ml fibronectin coating solution. Mix the solution carefully.

Note: Fibronectin is susceptible to shear stress, do not vortex or spin the solution, and avoid harsh pipetting.

2. Carefully pipette a droplet of 4µl of the 50µg/ml fibronectin coating solution to the center of each well of the MEA plate covering the electrodes. See Figure 5.1 for correct droplet placement.

Note: Avoid touching the bottom of the plate with the pipette tips.

3. Incubate the MEA plate at 37°C, 5% CO_2 for 3 hours.

Note: Avoid drying out of the fibronectin coating, this will cause irreversible loss of matrix properties.

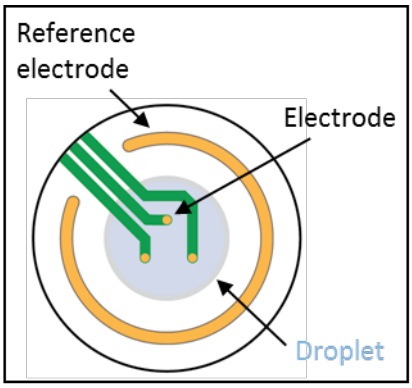


Figure 5.1. Droplet placement. Graphical representation of a well on a 96-well MCS MEA plate. The coating or cell droplet (grey area) should be placed in the center of the well covering the electrodes, while avoiding the reference electrodes.

5.2 Thawing Pluricyte® Cardiomyocytes and seeding onto the 96-well MEA plate

This part of the protocol describes the thawing and direct plating of Pluricyte® Cardiomyocytes onto the 96-well MEA plate. Complete the following steps of the thawing procedure in a time-efficient manner to facilitate optimal viability and performance.

4. Pipette 12ml Pluricyte® Cardiomyocyte Medium into a sterile 15ml conical tube and warm the medium to room temperature (RT).

Note: make sure to mix the medium by inverting before use.

5. Take 2 vials of Pluricyte® Cardiomyocytes per 96-well MEA plate from the LN2 storage.

Note: The volumes used below are calculated for two vials of Pluricyte® Cardiomyocytes to be plated on one 96-well MEA plate. We recommend to thaw a maximum of 3 vials per operator at a time.

6. Thaw the vials in an incubator at 37˚C for 4 minutes.
7. Gently transfer the contents of both vials (300 µl/vial) to a 50 ml tube using a P1000 pipette.
8. Rinse both vials with 1ml Pluricyte® Cardiomyocyte Medium (PCM) and add the 1 ml drop-wise to the 50ml tube, 1 drop every 5 seconds using a P1000 pipette.
9. Add another 1ml Pluricyte® Cardiomyocyte Medium drop-wise to the 50ml tube, 1 drop every 5 seconds under gentle continuous swirling.
10. Add 9.4ml Pluricyte® Cardiomyocyte Medium drop-wise to the 50ml tube, 1 drop every 2 seconds using a 5ml pipette.

Note: the total volume of the cell suspension is now 12ml.

11. Take a 20µl sample of the homogenous cell suspension and add to a micro centrifuge tube.
12. Spin down the cell suspension for 3 minutes at 250xg.
13. Aspirate the medium and gently resuspend the cells in 200µl Pluricyte® Cardiomyocyte Medium.
14. Determine the total cell number and cell viability as follows:

    We highly recommend to perform the cell counting manually using a hemocytometer. For instance, by using the Fuchs Rosenthal Counting Chamber (Figure 5.2):

    a. Add 20µl Trypan blue solution to the 20µl cell sample (collected in step 11), mix carefully.
    b. Add 20µl of the Trypan blue/cell suspension mix to the counting chamber.
    c. Calculate the total number of cells according to equation 1.

15. Calculate the dilution factor to reach 20,000 cells/4µl and add Pluricyte® Cardiomyocyte Medium to the cell suspension accordingly.
16. Carefully aspirate the coating solution from the MEA plate.
17. Place a 4µl droplet of the cell suspension (20,000 cells) to the center of each well, covering the electrodes. See Figure 5.1 for correct droplet placement.

18. Incubate the MEA plate with the seeded Pluricyte® Cardiomyocytes at 37°C, 5% CO2 for 1 hour.

19. After 1 hour, gently add 100µl of Pluricyte® Cardiomyocyte Medium to the side of each well.

Note: adding Pluricyte® Cardiomyocyte Medium too quickly will dislodge the adhered cardiomyocytes.

20. Incubate the MEA plate at 37°C, 5% CO_2.

Equation 1 . Cell counting

Count 4 #2 squares according to Figure 5.2

Viable cells: ___ + ___ + ___ + ___ = ____ (#vc)

Non-viable (blue) cells: ___ + ___ + ___ + ___ = ____(#nvc)

_____ / 4 x 2 x 5000 = _____ cells/ml

[#vc]

_____ x _____ = _____ (cells in total)

[# of cells/ml] [volume after step 13]

Viability = ___: (___+ ____) x 100 = _____ %

[#vc] [#vc] [#nvc]

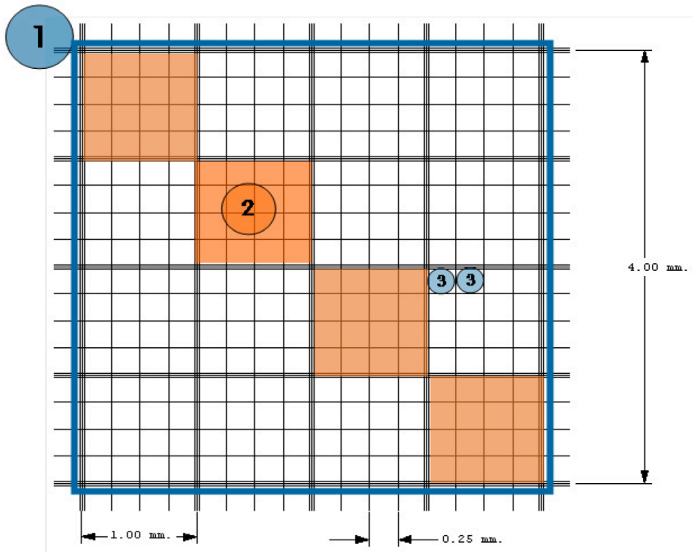


Figure 5.2 Lay-out of a Fuchs Rosenthal Counting chamber.

5.3 Maintenance of the Pluricyte® Cardiomyocytes in the 96-well MEA plate

It is crucial to always refresh the Pluricyte® Cardiomyocyte Medium of the cells one day after seeding the cells (day 1), and subsequently every 2 days (see workflow in Section 2).

21. Pipette 11ml Pluricyte® Cardiomyocyte Medium (PCM) into a sterile 15 ml conical tube and incubate the tube at 37°C, 5% CO_2 for at least 20 min.
22. Transfer the MEA plate from the incubator to the flow cabinet.
23. Add the warm Pluricyte® Cardiomyocyte Medium to a multichannel reservoir.
24. Aspirate the medium from each well or remove the medium using a multichannel pipette. Make sure to avoid disturbing the cell monolayer.
25. Add 100µl medium per well using a multichannel pipette. Avoid disturbing the cell monolayer by gently pipetting to the side of each well.
26. Incubate the MEA plate at 37°C, 5% CO_2.
27. Maintain the cardiomyocytes for 8-12 days, refreshing the medium every 2 days. Pluricyte® Cardiomyocyte Medium could be refreshed on Friday afternoon and Monday morning to prevent weekend-work.

5.4 Data acquisition during maintenance

In order to monitor the condition of the Pluricyte® Cardiomyocyte monolayer, we advise to perform daily measurements during the maintenance, starting at day 1. See the Multiwell-Analyzer software Manual for specific instructions on using the software for data acquisition and analysis. First contractions of Pluricyte® Cardiomyocytes appear between 24-48 hours post-thawing. It will take 3-4 days before the cells have formed an electrically coupled monolayer. The amplitudes of the field potential signals increase with prolonged culturing. Stable beating monolayers can be observed 7-8 days post-thawing.

For each measurement:

28. Place the MEA plate in the Multiwell-MEA-Headstage and start a measurement (e.g. 2 minutes).

Note: Wait >1 hour after medium refreshments before measurement to avoid unstable signals caused by medium change.

5.5 Compound assay

The optimal time window to perform electrophysiology-based assays with Pluricyte® Cardiomyocytes is between 8-12 days after plating the cardiomyocytes.

We recommend to prepare 3- or 10-fold serial dilutions of test compounds in Pluricyte® Cardiomyocyte Medium and to add the compound in 3-fold or 10-fold increasing concentration-steps, up to a volume of maximum 10% of the final volume of medium in the well (e.g. 3-7-10µl in a final volume of 100µl). We recommend not to use DMSO concentrations above 0.1%.

27. Replace the Pluricyte® Cardiomyocyte Medium in the MEA-plate ≥1 hour before the compound assay as described in Section 5.3, and place the plate back into the incubator.
28. Prepare the test compounds in 3- or 10-fold serial dilutions in Pluricyte® Cardiomyocyte Medium, in a 96-well plate and place this compound-plate in an incubator at 37˚C, 5% CO_2 for at least 10 minutes.
29. Transfer the MEA plate from the incubator into the Multiwell-Headstage (pre-warmed at 37˚C).
30. Define experimental set-up and plate lay-out in the Multiwell-Screen software (Settings Panel).

Note: Choose between single well-dose or cumulative-dose experiment.

31. Start Explore function (Experiment Control Panel) to perform automated electrode selection.
32. Start experiment and follow pipetting instructions as indicated by the software.
33. For each pipetting step, remove the chosen volume (e.g. 3-7-10µl from a final volume of 100µl) from each well and add the same volume from the 96-well compound plate to the MEA plate.

Note: Mix gently by pipetting at least 3 times. Our case study report (see www.ncardia.com) provides an example of data acquisition for acute studies.

34. Generate a Report Sheet directly after running an experiment to obtain immediate preliminary data regarding compound-induced effects on Pluricyte® Cardiomyocytes.

5.6 Data analysis

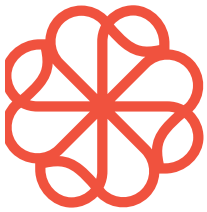
35. Analyze the acquired data using the Multiwell-Analyzer software.

Note: See the Multiwell-MEA-Systems Manual for specific instructions for data analysis

36. Export quantitative results in the desired extension (e.g. .csv files) for further analysis.

Acknowledgement

This project has received funding from the European Union's Horizon 2020 funded Ecsel Joint Undertaking Research and Innovation Programme "InForMed" under grant agreement No. 2014-2-662155.



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