Ncardia
Stem cell experts

Pluricyte® Cardiomyocytes

Manual

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Getting Started

Please make sure to read this entire Pluricyte® Cardiomyocyte Manual carefully before you start to thaw the Pluricyte® Cardiomyocytes.
Pluricyte® Cardiomyocytes are for *in vitro* life science research use only. See **Appendix A** for general terms and conditions of sale.
A Material Safety Data Sheet (MSDS) for Pluricyte® Cardiomyocytes is available online at ncardia.com.

Technical support and training

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

Important Recommendations

- Carefully follow the thawing and seeding instructions, this step is essential for optimal cell survival and attachment (**Section 3.2**).
- This manual describes the thawing and culture procedures of Pluricyte® Cardiomyocytes on regular tissue culture plates. For plating Pluricyte® Cardiomyocytes on specific MEA-plates, including E-plates® (ACEA Biosciences), 48-well MEA plates (Axion Biosystems), or 6-/1-well MEAs (Multi Channel Systems), we kindly refer you to our assay-specific application notes available online.
- We strongly recommend to use fibronectin as coating substrate for MEA plates and to use Matrigel™ or fibronectin coating substrate for standard tissue culture plates. Other types of coating substrates may impact the condition of the cells.
- Always refresh the Pluricyte® Cardiomyocyte Medium (PCM) of the cells the day after seeding the cells (**Section 3.3**). Subsequently, refresh the PCM of the cells every 2 days, or 3 days when refreshing at Friday and Monday to prevent weekend work.
- First contractions of Pluricyte® Cardiomyocytes appear between 24-48 hours post-thaw. 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-thaw. The optimal time window to perform electrophysiology-based assays with Pluricyte® Cardiomyocytes is between 8-12 days after plating the cardiomyocytes.

1. Introduction

Pluricyte® Cardiomyocytes are fully functional human induced pluripotent stem cell (hiPSC) derived ventricular cardiomyocytes that are particularly suitable for predictive electrophysiology- and contractility assays for safety pharmacology, toxicity testing and efficacy screening in early drug discovery. Pluricyte® Cardiomyocytes are cultured in Pluricyte® Cardiomyocyte Medium (PCM), which is a serum-free and chemically defined medium designed to promote cardiomyocyte maturation and cell function.[1] Pluricyte® Cardiomyocytes can be maintained in culture for at least two weeks post-thaw.

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 contains well-pronounced depolarization and repolarization peaks, enabling easy detection of field potential durations in MEA assays

This manual describes the thawing and culture procedures of Pluricyte® Cardiomyocytes on regular tissue culture plates (different formats).

2. Equipment, Materials and Reagents

Equipment	Manufacturer
Flow cabinet	Various
Incubator at 37°C, with 5% CO_2 and humidified air	Various
Centrifuge	Various
P10 pipette	Various
P20 pipette	Various
P1000 pipette	Various
Multichannel pipette 30-300µl (when plating on 96-well plates)	Various
Pipette controller	Various
Optional: Dry-ice in foam box	Various
Optional: Foam float for in water bath	Various
Optional: water bath at 37°C	Various
Materials	
sterile disposable 5 ml pipettes	Various
sterile 50 ml conical tubes	Various
sterile filter tips for p10 pipette	Various
sterile filter tips for p20 pipette	Various
sterile filter tips for p1000 pipette	Various
sterile filter tips multichannel (when plating on 96-well plates)	Various
sterile tissue culture plates, flat bottom, clear plates, TC treated	Various
sterile multichannel reservoirs (when plating on 96-well plates)	Various
Parafilm™	Various
Reagents	**Cat no**
Pluricyte® Cardiomyocyte Kit, including: Pluricyte® Cardiomyocytes Pluricyte® Cardiomyocyte Medium	PCK-1.5
1x DPBS + Ca^{2+} + Mg^{2+} *	Gibco Cat#14040
Fibronectin (1 mg/ml) *	Sigma F1141
DMEM/F12*	Gibco Cat#31331
Matrigel™ hESC-qualified Matrix*	Corning Cat#354277

Table 1. Equipment, materials and reagents

**We strongly recommend to use fibronectin as coating substrate for MEA plates and to use Matrigel™ or fibronectin coating substrate for standard tissue culture plates. Other types of coating substrates may impact the condition of the cells.*

3. Methods

3.1 Coating of tissue culture plates

For cardiomyocyte adhesion, plastic ware needs to be coated before plating the cells. We strongly recommend to use fibronectin as coating substrate for MEA plates and to use Matrigel™ or fibronectin coating substrate for standard tissue culture plates. Other types of coating substrates may impact the condition of the cells.

3.1.1 Coating of plastic ware with fibronectin

Fibronectin is a biological matrix preparation used to coat plastic ware in order to enable cardiomyocytes adhere to the surface.

1. Dilute fibronectin 1:100 in D-PBS (incl. Ca^{2+}/Mg^{2+}) to get a 10 µg/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. Plate the coating solution immediately onto plates (see **Table 2** for recommended coating solution volumes).
   Incubate the fibronectin-coated plate in a cell culture incubator at 37°C, with 5% CO_2 for 3 hours.

Note: longer incubation times are acceptable, however, the fibronectin coating solution should not dry out; this causes irreversible loss of extracellular matrix properties.

3. Aspirate excess fibronectin coating solution right before plating the cells (see **section 3.2** for thawing and plating the Pluricyte® Cardiomyocytes).

Plate format	Volume to plate per well
96-well	0.05 ml
48-well	0.25 ml
24-well	0.50 ml
12-well	1.00 ml
6-well	2.00 ml
T25 flask	5.00 ml

Table 2. Recommended fibronectin coating solution volumes per well.

3.1.2 Coating of plastic ware with Matrigel™

Matrigel™ is a biological matrix preparation used to coat plastic ware in order to enable cardiomyocytes adhere to the surface.
Note: Matrigel™ polymerizes above 10°C. Keep all reagents and final coating solution at 4°C until use.

1. Thaw an aliquot of Matrigel™ 1:100 in cold DMEM/F12 on ice into a 50 ml conical tube following Manufacturer's protocol.
2. Mix the diluted Matrigel™ coating solution carefully.
3. Plate the Matrigel™ coating solution immediately onto the plates (see **Table 3** for recommended coating solution volumes) and allow for polymerization for at least 45 min at room temperature.

Note: never let the Matrigel™ coating solution dry out as this causes irreversible loss of extracellular matrix properties.

4. Aspirate excess Matrigel™ coating solution right before plating the cells (see **section 3.2** for thawing and plating the Pluricyte® Cardiomyocytes).

Plate format	Volume to plate per well
96-well	0.10 ml
48-well	0.25 ml
24-well	0.50 ml
12-well	1.00 ml
6-well	2.00 ml
T25 flask	5.00 ml

Table 3. Recommended Matrigel™ coating solution volumes per well.

3.2 Thawing Pluricyte® Cardiomyocytes

This part of the protocol describes the thawing of Pluricyte® Cardiomyocytes (stored in vapor phase of liquid nitrogen). Complete the following steps of the thawing procedure in a time-efficient manner to facilitate optimal viability and performance.

Note: the volumes used below are calculated for 1 Pluricyte® Cardiomyocyte vial. If more cells are needed, combine the contents of the vials in the 50 ml conical tube (see step 4) and adjust the volumes of Pluricyte® Cardiomyocyte Medium (PCM) to add accordingly. We recommend to thaw maximum 3 vials per operator at a time.

1. Coat the tissue culture plate(s) with fibronectin or Matrigel™ coating solution as described in **section 3.1**.
2. Warm 6 ml PCM to room temperature (RT).

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

3. Take 1 vial of Pluricyte® Cardiomyocytes from LN_2 storage (optional: transport the vial on dry ice) and place the vial in a 37°C incubator for exactly 4 minutes.
4. Gently transfer the contents of the vial to a 50 ml tube using a p1000 pipet. Avoid pipetting up and down.
5. Rinse the empty vial with 1 ml PCM (RT) and add the 1 ml PCM drop-wise to the 50 ml tube containing the cells: add 1 drop every 5 seconds using a p1000 pipette while gently swirling the cells after each drop.
   This step is crucial for the recovery of the cardiomyocytes. We recommend to use a timer.
6. Add 4.7 ml of PCM drop-wise to the 50 ml tube, 1 drop every 2 seconds using a 5 ml pipette.

Note: the total volume of the cell suspension is now 6 ml.

7. Take a 20µl sample of the homogenous cell suspension and add to a micro centrifuge tube.
8. Spin down the cell suspension for 3 minutes at 250xg.
9. Aspirate the medium and gently resuspend the cells in 1 ml Pluricyte® Cardiomyocyte Medium.
10. Determine the total cell number and cell viability as follows:
    *We highly recommend to perform the cell counting manually using a hemocytometer. For in stance, by using the Fuchs Rosenthal Counting Chamber (**Figure 1**):*
    a. Add 20µl Trypan blue solution to the 20µl cell sample (collected in step 7), 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**.*
11. Calculate the dilution factor to reach the desired concentration (see **Table 4**) and add PCM to the cell suspension accordingly.
12. Add the cell suspension to a multichannel reservoir using a 5ml pipette.
13. Transfer the coated plate(s) to the flow cabinet and aspirate the excess of the coating solution.
14. Plate cells according to **Table 4.** Make sure to transfer the cells extremely gently.

Note: avoid air bubbles and gently resuspend cells in the multichannel reservoir in between pipetting steps to evenly distribute the cells.

Place the plate(s) in the incubator at 37 °C and 5% CO_2.

Equation 1 Cell counting.

Count 4 #2 squares according to **Figure 1**

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

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

____/4 x 2 x 5000 =_____cells/ml

[#vc]

_____=_____(= cells in total)

[# of cells/ml] [total volume after step 6]

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

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

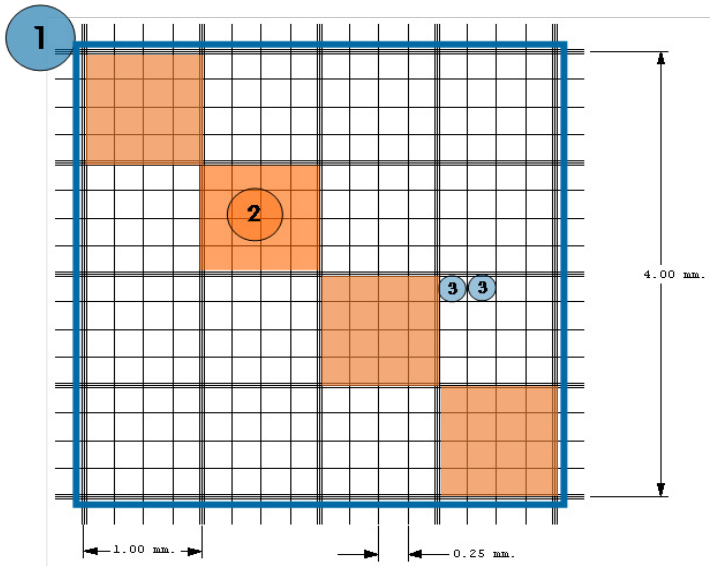

Figure 1. Lay-out of a Fuchs Rosenthal Counting chamber

Table 4. Recommended volumes and cell densities per well per plate format

Plate format	Volume to plate per well	Number of cells per well
96-well	0.10 ml	25,000-40,000
48-well	0.25 ml	50,000-100,000
24-well	0.50 ml	100,000-200,000
12-well	1.00 ml	200,000-400,000
6-well	2.00 ml	500,000-800,000
T25	5.00 ml	1,300,000-1,500,000

3.3 Maintenance of Pluricyte® Cardiomyocytes

It is crucial to always refresh the Pluricyte® Cardiomyocyte Medium (PCM) of the cells one day after seeding the cells (day 1), and subsequently every 2 days (or every 3 days in order to avoid weekend work).

1. Add the required PCM to a 15- or 50 ml tube and warm the PCM to 37°C for 20-30 min. Refer to **Table 4** for appropriate volumes.
2. Immediately before use, transfer the warm PCM into a multichannel reservoir and transfer the plate(s) from the incubator to the flow cabinet.
3. Aspirate the PCM from each well.

Note: avoid touching the bottom of the wells with the pipette tips to not disturb the cardiomyocyte monolayer.

4. Add the appropriate volumes pre-warmed PCM per well (**Table 4**).
5. Transfer the plate(s) back to the incubator.

For further characterization or application of Pluricyte® Cardiomyocytes in different assay platforms, please refer to our user guides that can be found in our application browser for different electrophysiology-based assays. For these assays the recommended assay window is between day 8 and day 12 post-thaw.

4. References

1. Ribeiro, M. C. et al. Functional maturation of human pluripotent stem cell derived cardiomyocytes in vitro Correlation between contraction force and electrophysiology. Biomaterials 51, 138–150 (2015).

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