Celo.Cardiomyocytes MEA User Guide

celogics

Contents

Getting Started

Celo.Cardiomyocytes Cardiomyocytes

Cell Type	Human iPSC-derived Cardiomyocytes
Volume	Approximately 1 mL (≥ 5 x 10^6 cells per vial)
Product Format	Cryopreserved cells in the optimized cryopreservation medium
Source	Differentiated from a human iPSC line (fibroblast, Caucasian male donor)
Expiration Date	Printed on individual vials (≤2 years from manufacturing)
Quality Control	Please refer to the CoA for lot-specific information. Virus clearance & STR analysis data are available upon request.

Celogics strives to provide fully functional human cardiomyocytes applicable for different types of experiments in cardiovascular research. Celo.Cardiomyocytes are derived from induced Pluripotent Stem Cells (iPSCs) using proprietary protocols to optimize user experience by prioritizing purity, reproducibility, and electrophysiology. Our proprietary Advanced Media is designed to enhance the electrophysiological profiles of Celo.Cardiomyocytes by promoting maturation as well as excitation-contraction coupling. Together with Advanced Media, Celo.Cardiomyocytes have been validated on multiple electrophysiological platforms, showing synchronous beating with physiologically relevant field potential duration (FPD), high calcium influx, and strong contraction. Celo.Cardiomyocytes are a reliable source of human iPSC-derived cardiomyocytes making them an excellent choice in the advanced science in tissue-specific research, toxicity screening, efficacy testing, and drug discovery.

- ✓ This User Guide will help you seed Celo.Cardiomyocytes at the appropriate densities to create synchronous layers of cardiomyocytes for **48-well Microelectrode array (MEA)** applications and possibly for similar platforms.
- ✓ We recommend performing assays with Celo.Cardiomyocytes from **Day 7 onwards**.
- ✓ We highly recommend using **fibronectin** as the source of **extracellular matrices (ECM)** for **long-term culture** of over 14 days on cell culture plates. Celo.Cardiomyocytes start beating from day 2-3 and stretch/pull ECM from the plate surface. By far, fibronectin has shown to have the best endurance over such mechanical force applied.

Advanced Media & Supplements

- Thaw Advanced or Plating Supplement and combine with the entire vial of its complement medium. Aliquot and store the prepared medium at **4°C for up to 1 month**.
- **DO NOT FREEZE** the supplements.
- Plating and Advanced Media are **serum-free**. For additional information on the composition, please contact us for assistance.

All media and supplements are free of antibiotics and antimycotics. For the best possible result, we do not recommend adding such agents unless aseptic cell culture conditions are not possible.

Technical Support

Our technical support team is ready to help you with your inquiries. Please visit our website for general information, frequently asked questions, and product documentation. For all other questions and support, please contact us at celogics@celogics.com.

1. Handling & Storage

1.1 Unpacking & Handling

- Upon receiving the shipment, immediately transfer each of the components to the appropriate storage conditions.
- Check the catalog number, lot number, and expiry date.
  - ✓ The expiry date of the Basal medium is usually the shortest (indicated on the label) so experiments should be planned accordingly.
- Celo.Cardiomyocytes should be handled by technically qualified individuals complying with good laboratory practices, applicable laboratory regulations, and the MSDS. Following the User Guide herein is recommended for the best results.
- Celo.Cardiomyocytes are for *in vitro* research use only, not intended for human or animal *in vivo* applications.

1.2 Package Description

Full Kit	Components	CAT#	Storage
Celo.Cardiomyocytes	Cryopreserved, >5 million cells	C50	Liquid nitrogen
Basal medium	200 mL	CM200	4°C
Advanced Supplement	2 mL	C50-MS	Frozen, -20°C
Plating Medium	45 mL	C50-PM	4°C
Plating Supplement	0.9 mL	C50-PS	Frozen, -20°C

Table 1. Product package description and appropriate storage condition

2. Safety Precaution & User Notice

All components including Celo.Cardiomyocytes should be handled according to the Biosafety Level 1 or equivalent local directives.

For *in vitro* research use only, not intended for human or animal *in vivo* applications. Appropriate safety procedures should always be used. Please refer to the MSDS for detailed instructions.

User Notice & Restrictions:

- Users may use the Product for internal research including but not limited to screening potential drug compounds for efficacy and safety, and the provision of such services to third parties. No other right is granted to the User whether expressly, by implication, by estoppel, or otherwise. In particular, the purchase of the Product does not include nor carry any right or license to use, develop or otherwise exploit the Product commercially, and no rights are conveyed to the User to use the Product for any other purpose.
- User agrees to use the Product in compliance with all applicable statutes and regulations, but not to use the Product for any administration or application to humans. Moreover, the User agrees not to use the Product in human subjects for human clinical use for therapeutic, diagnostic, or prophylactic purposes, or in vivo application on animals for veterinary use for therapeutic, diagnostic, or prophylactic purposes, including but not limited to clinical applications, cell therapy, transplantation, and/or regenerative medicine without an appropriate license.
- In the case a user transfers the Product to a third party, the user shall convey the User Restrictions set forth herein to such third party.

3. Required Materials

Type	Item	CAT#	Vendor
Coating Material	Fibronectin, Human	F0895	Sigma
Typical Cell Culture Equipment	Liquid nitrogen storage tank		
	37°C water/beads bath		
	Tabletop centrifuge		
	Biological safety cabinet with UV lamp		
	Hemocytometer or automated cell counter		
	Phase contrast microscope		
	Pipettes		
	Cell culture incubator		
Typical Cell Culture Consumables	Centrifuge tubes		
	48-well MEA plates		
	Pipette tips		
	Trypan blue		
	Phosphate Buffered Saline (PBS)		

Table 2. Overview of required consumable materials and equipment

4. Media Preparation

1. Thaw Plating and/or Advanced Supplement by placing it at 4°C for 24 hours before use.
2. In a biosafety cabinet, add thawed Supplement to its complementary medium and mix thoroughly.

   A. Once the supplement is thawed and added to the medium, store at 4°C for up to 1 month. DO NOT FREEZE Plating or Basal medium.

   ✓ Aliquot the media in small volumes (enough for 2-3 days) to avoid oxidation of the medium from repeated warming and opening,

Media Type	Components
Advanced Media	Basal Medium Advanced Supplement
Plating Media	Plating Medium Plating Supplement

Table 3. Celo.Cardiomyocytes medium components. To obtain the best results, each medium should be reconstituted according to the User Guide.

5. Microelectrode Array Assay Protocol

5.1 Coating MEA plates

1. Calculate the amount of coating agent required (5 µl is required for each well).

    ✓ To coat a whole 48-well plate, prepare a total of 250 µl (add an excess 10 µl to account for pipetting error).

2. Cell culture-ware coating should be done according to manufacturer protocols. A general protocol applicable to most coating agents can be found below.

3. Dilute the Fibronectin product in D-PBS to reach the working concentration (50 µg/ml) immediately before use.

Coating Material	Working Concentration
Fibronectin	**50 µg/ml (1:20 dilution)**

Table 4: Recommended working concentration and dilution for each coating reagent

4. Hold the plate at an angle to see the electrode grid in each well.

5. Carefully pipette 5 µl of fibronectin solution to each well by placing the droplet at the center of the wells to cover the measurement electrodes.

    ✓ Make sure the droplet covers measuring electrodes only not touching reference electrodes.

    ✓ This step determines the seeding placement of the cells.

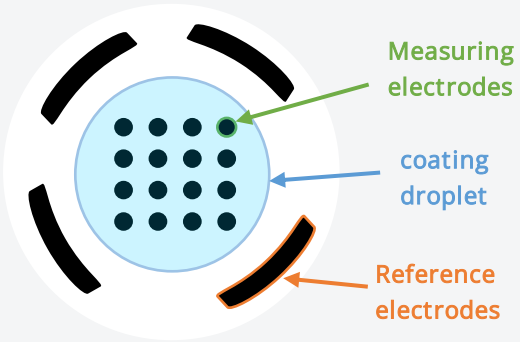

Figure 2. "Electrode Spotting" Method
Most MEA plates are shaped similar with small measuring electrodes at the center and rod-shaped reference electrodes around the edges. A 5 µl droplet will cover the measurement area appropriately as shown.

6. Add 6~8 ml of D-PBS around the wells to increase the humidity to prevent the droplet from drying. (This step may or may not be necessary depending on the incubation condition.)
7. Incubate at 37°C for 1 hour.

- ✓ We recommend starting the thawing process of the cells about 40 minutes after starting the incubation of the coating.

5.2 Thawing

Celo.Cardiomyocytes can be thawed using typical cell culture thawing protocols. Here, we present our optimized protocol and recommend our users to follow the instructions below. We strongly recommend thawing 1 vial at a time to minimize cell exposure to liquid DMSO.

1. Equilibrate the Plating Media prepared from 4. Media at room temperature (RT, 25°C) for at least 30 minutes.
2. For each vial to thaw, aliquot 8 mL of Plating Media in a 15 mL centrifuge tube.
3. Retrieve the cryovial(s) from the liquid nitrogen storage tank.
4. Immerse the cryovial(s) from step 3 in a 37°C water/bead bath. The opening of the vial should not touch the water. Once 80% is thawed (after ~3 mins), spray the cryovial(s) with 70% ethanol, wipe, and place it in the biosafety cabinet. Proceed to step 5 immediately.
5. Open the cryovial and gently transfer the contents (~1 mL) using a 1 mL pipettor to the Plating Media aliquoted from step 2 as droplets while gently swirling the tube.
    - ✓ Dropwise pipetting while gently swirling the tube minimizes osmotic shock and maximizes viability. Droplets will remain on the surface for about 1 second and then drop towards the bottom of the tube (visible due to the DMSO).
    - ✓ Simply pipette slowly into the air about 1 cm above the surface of the solution. It should take approximately 1 min per 1 mL.
6. Rinse the emptied vial with 1 mL of Plating Media and transfer the solution using dropwise pipetting to the same tube from step 5.
7. Centrifuge the cell suspension for 3 minutes (speed of 180 x g, room temperature).
8. Discard the supernatant carefully.
9. Gently resuspend the cells using 1 mL of Plating Media and count live (viable), dead, and total cells using a hematocytometer with Trypan blue or an automated cell counter.
    - ✓ Avoid vigorous pipetting of the cell suspension to maximize viability. Single-cell resuspension of Celo.Cardiomyocytes are easily achieved by pipetting gently 3 to 4 times.
10. Immediately move on to the Plating section.

5.3 Seeding

Celogics recommends seeding 50,000 (5 x 10^4) cells on the fibronectin coated area (5.1 Coating MEA plates, step 5).

1. Using the number of live cells per mL counted previously from 5.2 Thawing, step 9, calculate the volume of suspension (with the desired number of cells) needed for seeding. Refer to the formula provided below for calculation.

$V = \frac{50000}{C} xW$	V= volume of suspension needed W= total number of wells to be plated C= number of live cells per mL

2. Calculate the total volume of Plating Media required (number of wells x 5µl).

   ✓ Calculation example;

   To seed 24 wells;

   Step 1. If C = 5 x 10^6; W=24, C= 5 x 10^6;

   V= (50000/5 x 10^6) x 24 = (0.01) x 24 = 0.24 mL = 240 µL

   Step 2. Total volume of Plating Media required = 24 x 5 µL = 120 µL

3. Aliquot the volume of cell suspension calculated (V value) from step 2 to a new 1.5 mL tube.
4. Centrifuge the suspended cells at 180 g for 3 minutes at room temperature.
5. If necessary, remove the D-PBS added around the wells in 5.1, step 6.
6. Resuspend the cells with room temperature Plating Media (4. Media preparation) and bring the total volume to the value calculated in step 2.
7. Discard the coating solution and add 5 µl of the cell suspension.

   ✓ Seed less than 6 wells at a time to prevent drying out of the fibronectin coating. Dried fibronectin may lead to poor attachment.

   ✓ The droplet of cells should remain within the fibronectin coating area.

8. Add 3 ml of D-PBS around the wells to increase humidity and prevent cells from drying out.
9. Incubate at room temperature for 1 hour.
10. Add 300 µl of pre-warmed Plating Media to each well.

    ✓ We recommend equilibrating the medium in the incubator while allowing the gas flow.

11. 24 hours after seeding, warm the recommended volume of Advanced Media (4. Media preparation) at 37°C.

    ✓ We recommend equilibrating the medium in the incubator for at least 20 minutes to allow the gas flow.

12. Slightly tilt the MEA plate and aspirate the media using a pipette.

    ✓ Leaving a small amount of medium in each well would make sure the cells are immersed in the medium.

13. Gently release 300 µl of pre-warm Advanced Media against the wall to avoid disturbing the cell layer.

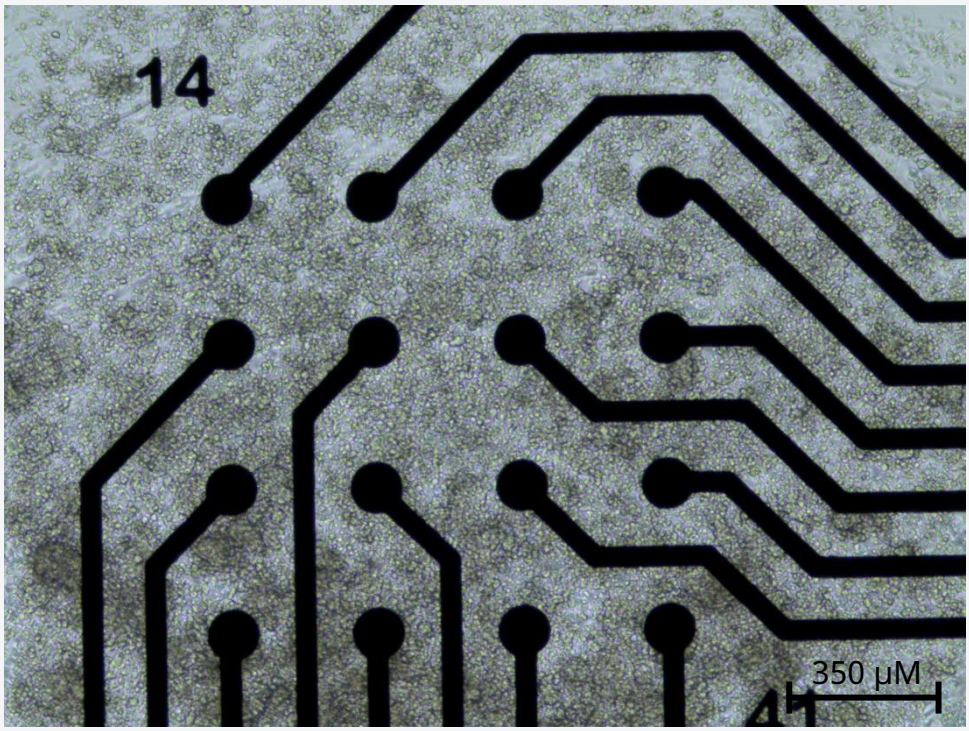

Figure 3. Morphology of Celo.Cardiomyocytes on MEA plate on day 1 after thawing (40X). The recommended density for the MEA plate is considerably higher than what is expected for the general cell culture.

5.4 Maintenance

After changing the media for the first time (5.2 Seeding, Step 12), the medium should be replaced at 48-hour intervals. When performing electrophysiological assays, it is recommended to change the media in the morning and perform the experiment in the afternoon to allow cells to stabilize before the experiment.

1. Warm the desired volume of Advanced Media prepared from 4.1 Media at 37°C in a water bath or a cell culture incubator for at least 20 mins.
2. Slightly tilt the MEA plate and aspirate the media using a pipette.
    - ✓ Leaving a small amount of medium in each well would make sure the cells are immersed in the medium.
3. Gently release 300 µl of pre-warm Advanced Media against the wall to avoid disturbing the cell layer
    - ✓ Avoid changing more than 6 wells at a time to avoid damage due to air contact.
4. Incubate the plate with Celo.Cardiomyocytes in a cell culture incubator at 37°C, 5% CO_2.
5. Replace Advanced Media on each well every 48 hours.
    - ✓ We recommend performing an MEA assay from day 7 post-plating.

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Quality Research