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
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 is available upon request.

Celogics strives to provide fully functional human cardiomyocytes suitable for all types of experiments in the field of cardiomyocytes. Celo.Cardiomyocytes are derived from induced Pluripotent Stem Cells (iPSCs) using proprietary protocols which optimize the user experience by prioritizing purity, reproducibility, and electrophysiology. Advanced Media is designed to enhance the electrophysiological profiles of Celo.Cardiomyocytes, support a robust maturation as well as excitation-contraction coupling. Together with Advanced Media, Celo.Cardiomyocytes have been validated on multiple electrophysiological platforms, resulting in synchronous beating with adequate FPD, higher calcium influx, and stronger contraction. As such, they are the reliable source of human iPSC-derived cardiomyocytes suitable for the advance of 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 appropriate for a variety of applications related to electrophysiological behavior assays.
- ✓ We recommend performing any **planned assays** with Celo.Cardiomyocytes from **Day 7 onwards**.
- ✓ We highly recommend using **fibronectin** as the source of **extracellular matrices (ECM)** for a **long-term culture** over 14 days on cell culture plates. Other ECM may cause detachment of cardiomyocytes from the MEA plate.
- ✓ For the maximum stability of Celo.Cardiomyocytes for **long-term culture** over 14 days, we recommend performing a **half-media change technique**. This minimizes seeded cardiomyocytes and ECM being exposed to the air and prevents their detachment. With a full media change technique, ECM may start to roll up from the edges and detach.

Advanced Media & Supplements

- Advanced Media & Supplements need to be combined before use and stored at 4°C for up to 1 month. DO NOT FREEZE the Advanced or Plating Media, aliquot into smaller quantities for best results.
- Advanced Plating or Advanced Media is serum-free. For additional information on the composition, please contact us for assistance.

All media and supplements are antibiotics and antifungal-free as they are not necessary under appropriate conditions. Celogics does not recommend the use of such agents for accurate results, but they should be used if aseptic cell culture conditions are not possible.

Technical Support

Our Technical Support Scientists are 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, check whether all temperature-sensitive components are correctly frozen. If this is not the case, please contact us immediately.
- Immediately transfer each of the components to the appropriate storage conditions.
- Please check the catalog number, lot number, and expiry date. The expiration date of the Advanced Media is the shortest (date of expiration 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 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
Advanced Media	200 mL	CM200	4°C
Maintenance Supplement	2 mL	C50-MS	Frozen, -20°C
Plating Media	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 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:

- User 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 that User transfers Product to a third party, User shall convey the User Restrictions set forth herein to the 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		
	Appropriate culture plate for your platform		
	Pipette Tips		
	Trypan Blue		
	Phosphate Buffered Saline (PBS)		

Table 2. Overview of required consumable materials and equipment

4. Media Preparation

1. Thaw Advanced Media or Plating Media and its Supplement by placing them at 4°C for 24 hours before use.
2. In a biosafety cabinet, add thawed Plating Supplement (50X) or Maintenance Supplement (100X) to the thawed medium and mix thoroughly. Store at 4°C for up to 1 month after the addition of the supplement. DO NOT FREEZE Advanced Plating or Advanced Media.
   - ✓ To avoid oxidation of the medium from repeated warming/opening, aliquot the medium into quantities enough for 2~3 media changes.

Media Type	Components
Advanced Media	Advanced Media Maintenance Supplement
Plating Media	Plating Media Plating Supplement

Table 3. Each Media type needs its corresponding supplement for the best result

5. Microelectrode Array (MEA) Assay Protocol

5.1 Thawing

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

1. Equilibrate the Plating Media from 4.1 Preparing 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. Submerge the cryovial(s) 2/3 in a 37°C water/bead bath. The mouth of the vial should not come in contact with the water. Constantly check how much has thawed and once ~20% remains (~3 mins), spray the cryovial(s) with 70% Et-OH, wipe and place it in the biosafety cabinet. The cryovial(s) should have completely thawed exactly when you start step 5.

5. Open the cryovial and transfer the contents (~1 ml) using a 1 ml pipette to the aliquoted 8 ml of Plating Media dropwise while gently swirling the tube.

    ✓ Dropwise pipetting while gently swirling the tube minimizes osmotic shock and maximizes mixing, which ensures high viability. Drops will remain on the surface for ~1 second and then drop towards the bottom of the tube (visible due to the DMSO content). For dropwise pipetting, simply pipette slowly into the air ~1 cm above the media surface. It should take approximately 1 min per 1 ml.

6. Use 1 ml of Plating Media to gently rinse the emptied vial and transfer dropwise to the centrifuge tube containing the cells from step V while gently swirling the tube.

7. Centrifuge the suspended cells at the speed of 180 x g for 3 minutes at room temperature.

8. Carefully discard the supernatant.

9. Resuspend the cells gently 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. Immediately move on to the Plating section.

    ✓ Avoid vigorous pipetting of the cell suspension to maximize viability. Single-cell resuspension of Celo.Cardiomyocytes during thawing should easily be achieved by gently pipetting 3~4 times.

5.2 Coating the MEA plate

1. Calculate the amount of coating media to be prepared (5 µl is required for each well). E.g., To coat a whole 48-well plate, prepare a total of 250 µl (add excess 10 µl to account for pipetting error).
2. 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)

✓ It is not recommended to use any other coating material than Fibronectin.

3. Hold the plate at an angle to see the electrode grid in each well. Pipette the correct amount of coating solution (5 µl) to the center of the wells in a manner that forms a drop over the measurement electrodes. This step determines the seeding placement of the cells – covering all the measurement electrodes is best. It is preferable to avoid covering the T-shaped reference electrodes.

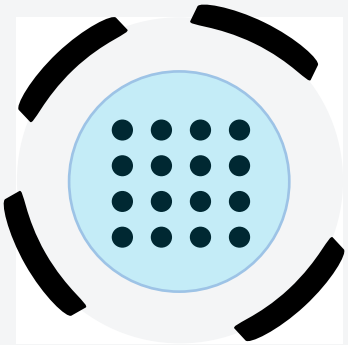

Figure 2. "Electrode Spotting" Method
Independently of the plate type, most MEA plates are shaped similar with smaller round measuring electrodes in the center and reference electrodes on the outside. A 5 µl droplet will cover the measurement area appropriately as shown in red.

4. Handle the plate gently and 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)
5. Incubate at 37°C for exactly 1 hour.

✓ It is important to let the coating incubate for one hour but also to not let it dry. We recommend starting the thawing process of the cells with about 40 minutes after starting the incubation of the coating.

5.3 Seeding

1. Calculate the number of total cells keeping in mind that 50,000 cells are required for each well. E.g., To plate a whole 48-well plate, calculate the volume that corresponds to 2,500,000 cells (excess of 100,000 cells; 50 wells total).
2. Transfer the corresponding volume to a 1.5 ml tube.
3. Centrifuge the suspended cells at 180 g for 3 minutes at room temperature.
4. During the centrifugation, remove the D-PBS which had been added around the wells. This will allow for easier handling of the plate when seeding the cells.
5. Resuspend the cells using Celo.Cardiomyocytes Plating Media to match the plating density (5 µl per 50,000 cells). E.g., For 50 wells, 250 µl is the correct volume.
6. Discard the coating solution and add 5 µl of the cell suspension 6 wells at a time at most. Drying out of the fibronectin can lead to poor cell attachment. The droplet of cells should not be able to spread out of the coating area due to surface tension.
7. Handle the plate gently and add 3~5 ml of D-PBS around the wells to increase the humidity. Should the droplet dry out, cells will not be able to attach properly.
8. Incubate at room temperature for exactly 1 hour.
9. Add 300 µl of pre-warmed Celo.Cardiomyocytes Plating Media to each well.

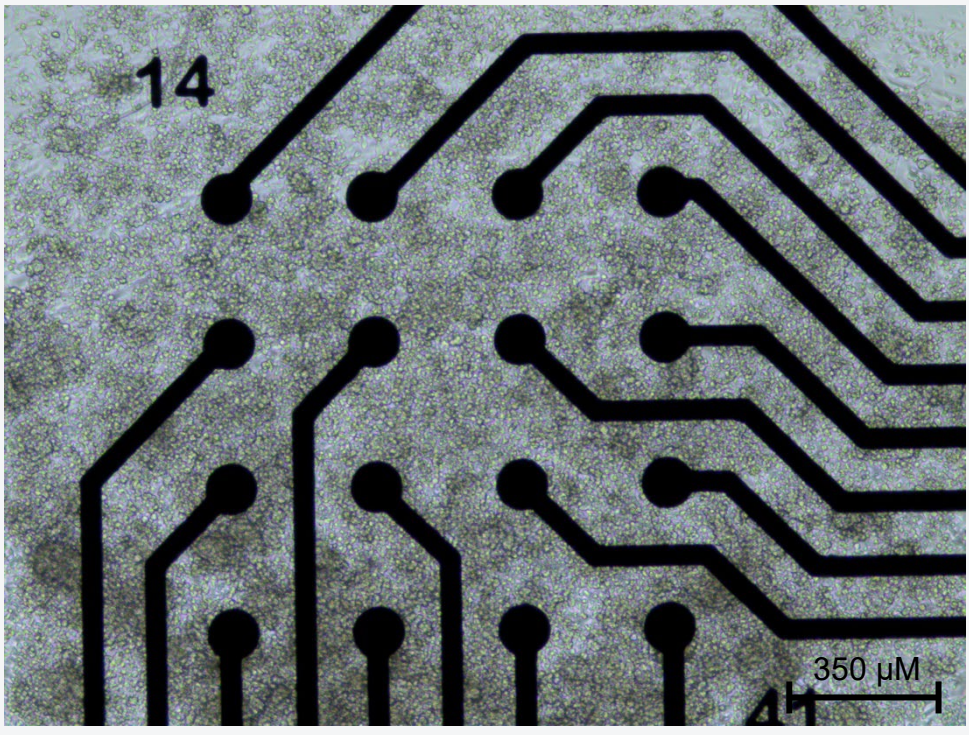

Figure 3. Morphology of Celo.Cardiomyocytes on Axion MEA plate day 1 after thawing (40X). The recommended density for the MEA plate is considerably higher than what is expected for the general cell culture.

5.4 Maintenance

1. Prepare Celo.Cardiomyocytes Advanced Media as described in Celo.Cardiomyocytes User Guide.
2. Immediately before use, equilibrate an aliquot of Advanced Media at room temperature for 30 minutes.
3. One-day post-plating, replace the Plating Media with Advanced Media. To remove the spent media, slightly tilt the MEA plate and aspirate the media using a pipette. Then, gently add 300 µl/well of pre-warm Manteinance Media from the top of the well to avoid disturbing the cardiomyocyte monolayer, and make sure not to touch the electrodes.
    - ✓ Avoid changing more than 6 wells at a time to avoid damage due to air contact.
4. Maintain the cardiomyocyte culture on the MEA plate by replacing 100% of the spent media with 300 µl/well of fresh pre-warm Advanced Media every 48 hours.
5. Continue to culture the cardiomyocytes in a cell culture incubator at 37°C, 5% CO_2.
6. We recommend performing an MEA assay from day 7 post-plating.

Quality Cells
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