Celo.Cardiomyocytes User Guide

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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, which supports 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 ECMs may detach from the culture plate due to the applied physical force by beating cardiomyocytes. We recommend carefully observing the edges of the wells when using different ECM sources.

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 Storage

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	Matrigel, Basal Membrane	356235	Corning
	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		
	Cell Culture Plates		
	Pipette Tips		
	Trypan Blue		
	Phosphate Buffered Saline (PBS)		

Table 2. Overview of required consumable materials and equipment

4. Preparation Protocols

4.1 Preparing Media

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

4.2 Preparing Cell Culture Surfaces & Coating

1. Calculate the amount of coating media required (see Tables 4 & 5).

Coating Volume for each Cell Culture Vessel type				
6-well plate (9.6 cm^2)	12-well plate (3.8 cm^2)	24-well plate (1.9 cm^2)	48-well plate (1.0 cm^2)	96-well plate (0.33 cm^2)
1 ml/well	500 µl/well	300 µl/well	100 µl/well	50 µl/well

Table 4: Recommended coating volume per well for each plate type

Coating Material	Working Concentration
Matrigel	**1:100 dilution**
Fibronectin	**50 µg/ml (1:20 dilution)**

Table 5: Suggested working concentration and dilution for each coating reagent

- ✓ Fibronectin > Matrigel > Gelatin
- ✓ Fibronectin consistently gives the best results for Celo.Cardiomyocytes culture. Gelatin 0.1% solution coating is the most economic option at the cost of attachment quality. For long-term cultures, we strongly suggest using Fibronectin for consistent results.

2. Prepare the coating media to working concentrations before use as calculated.
3. Pipette the adequate amount of coating solution to each well (see table 4 for volume).
4. Gently swirl the plate until the solution completely covers the growth area of each well.
5. Incubate at 37°C for at least an hour.
6. Aspirate coating solution right before seeding cardiomyocytes to avoid the surface from drying out.

5. Cell Culture Protocols

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 Seeding

Celogics recommends seeding Celo.Cardiomyocytes at a density of ~1.5 x 10^6 cells/cm^2 for most standard applications. Application-specific protocols are available upon request.

1. Calculate and adjust the cell suspension with Plating Media to reach the desired cell plating density using the number obtained from the cell count (from Thawing step 9). The number of cells and total seeding volume required per each well is calculated below (see table 7).

Seeding Volume per well	6-well (9.6 cm^2)	12-well (3.8 cm^2)	24-well (1.9 cm^2)	48-well (1.0 cm^2)	96-well (0.33 cm^2)
	2 ml	1 ml	500 µl	300 µl	200 µl
Cell Counts	1,440,000	570,000	285,000	150,000	50,000

Table 7: Recommended number of cells to seed per well for each plate type

- ✓ Growth area (cm^2) can vary between different vendors.

2. Aspirate the remaining coating solution from each well.
3. Gently mix the solution from step 1. by pipetting and evenly distribute the appropriate volumes of cells with Plating Media.
4. Incubate the culture plate. (Move the plate gently in 4 directions a few times to evenly distribute the cells)
5. The day after, perform the first media change with Advanced Media (Celo.Cardiomyocytes Maintenance Step 1).

5.3 Maintenance

Starting on the day after plating the cells, the media should be changed at 48-hour intervals. When performing electrophysiological assays on the cells, we recommend changing the media on the morning of the experiment to ensure the cells are stabilized with enough nutrients.

1. Warm the desired volume of Advanced Media at 37°C in a water bath or a cell culture incubator for at least 20 mins. Refer to table 7 for the recommended volume for each culture vessel type.
2. Replace the plating media with the newly warmed media in a biosafety cabinet. Pipette onto the cell culture plate walls to avoid any damage to the cell.
3. Place the plate back in the incubator.

Repeat 1 to 3 every 2 days.

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