Celo.Cardiomyocytes User Guide

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Contents

Getting Started

Celo.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 various electrophysiological behavior assays.
- ✓ 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. We recommend carefully observing the edges of the wells when using different ECM sources.

Media for Celo.Cardiomyocytes

- 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 Storage Condition

Type	Volume	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 contents and recommended storage conditions

2. Safety Precaution & User Notice

All components including Celo.Cardiomyocytes should be handled according to 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	Matrigel, Basal membrane	356235	Corning
	Fibronectin, Human	F0895	Sigma
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		
Consumables	Centrifuge tubes		
	Cell culture plates		
	Pipette tips		
	Trypan blue		
	Phosphate buffered saline (PBS)		

Table 2. Overview of required consumables and equipment

4. Preparation Protocols

4.1 Media

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.

   ✓ 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,

Prepared media referred as	Components mixed
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.

4.2 Cell Culture Surfaces & Coating

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

Coating volume for each plate 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 agent	Working concentration
Fibronectin	**50 µg/ml (1:20 dilution)**
Matrigel	**1:100 dilution**

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

- ✓ Fibronectin > Laminin > Matrigel
- ✓ Fibronectin consistently gives the best results for Celo.Cardiomyocytes culture. For long-term cultures, we highly recommend using Fibronectin.

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. Prepare the coating agent to a working concentration immediately before use as calculated.
4. Pipette the adequate amount of working solution to each well (see Table 4 for volume).
5. Gently rock the plate back and forth to cover the entire grown area with the solution.
6. Incubate at 37°C for 24 hours.

- ✓ Aspirate residual 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 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 4.1 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.

5.2 Seeding

Celogics recommends seeding Celo.Cardiomyocytes at a density of ~1.5 x 10^5 cells/cm^2 for most standard applications. Application-specific protocols are available upon request.

1. Using the number of live cells per mL counted previously from 5.1 Thawing step 9, calculate the volume of suspension (with the desired number of cells) needed to obtain the desired number of cells for seeding. Refer to Table 7 and the formula provided below for calculation.

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
Number of cells needed for each well	1,440,000	570,000	285,000	150,000	50,000

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

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

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

2. Calculate the total volume of Plating Media required to match the seeding volume suggested in Table 7 (number of wells x seeding volume per well).

✓ Calculation example;

To seed 3 wells from a 6-well plate, and C = 5 x 10^6;

Step 1. T= 1.44 x 10^6, W=3, C= 5 x 10^6. V= (1.44 x 10^6/5 x 10^6) x 3 = (0.288) x 3 = 0.864 mL = 864 µL

864 µL of the cell suspension to a new tube.

Step 2. The total volume of cell suspension should be 6 mL (2 mL x 3).

5.136 mL of Plating Media is required to bring the total volume of the cell suspension to 6 mL.

2 ml of cell suspension from step 3 to each well

3. Aliquot the volume of cell suspension calculated (V value) from step 1 to a new tube.

4. Add room temperature Plating Media to the aliquoted cell suspension and bring the total volume to the value calculated in Step 2.

5. Aspirate the residual coating solution from the cell culture plate pre-coated in 4.2 Cell culture surface & coating.

6. Gently mix the solution from step 4 by pipetting and evenly distribute the appropriate volumes to the cell culture plate.

7. Agitate the plate forward and backward and left to right 2 to 3 times each way to evenly distribute the cells.

   ✓ Make sure to stop for 5 seconds before changing the direction for better distribution.

8. Incubate the plate seeded with Celo.Cardiomyocytes in a cell culture incubator at 37°C, 5% CO_2 for 24 hours.

9. 24 hours after seeding, warm the recommended volume of Advanced Media at 37°C.

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

10. Aspirate 90% of the media from each well and replace it with the recommended volume of Advanced Media.

    ✓ To protect the seeded cells from oxidation and ECM from drying out, avoid tilting or lifting the plate while changing the media. Leaving a small amount of media in each well would make sure the cells are immersed in the media.

5.3 Maintenance

After changing the media for the first time (5.2 Seeding Step 10), 90% of 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.

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

Table 8: Recommended volume of media per well for each plate type.

1. Calculate the desired volume of Advanced Media required using Table 8 (volume suggested x number of wells).

   ✓ Consider the full volume suggested in the table instead of 90% for each well. An additional 10% is added to compensate for the volume that may evaporate within the incubator. For example, to change media for a 6-well plate, aspirate 1.8 mL and add 2 mL for each well.

2. 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.

3. Aspirate 90% of the media using a pipettor from each well and add the appropriate volume of pre-warmed Advance Media.

   ✓ Gently release the medium against the wall to minimize disrupting the cells.

4. Incubate the plate with Celo.Cardiomyocytes in a cell culture incubator at 37°C, 5% CO_2.

5. Replace Advanced Media every 48 hours.

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