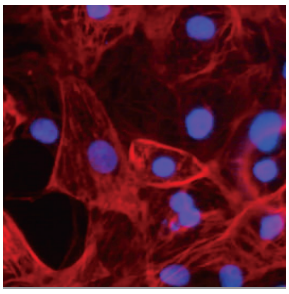

Stem Cell Application Protocol

Application of Cellartis® Cardiomyocytes in Axion BioSystems' Maestro Multi-Electrode Array system for EFP recordings

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I. Introduction

Cellartis Cardiomyocytes are derived from human induced pluripotent stem cells and provide a promising physiologically-relevant, human model for pre-clinical testing and drug screening. Axion BioSystems' Maestro multi-electrode array (MEA) platform allows for non-invasive detection of extracellular field potential (EFP) recordings in high throughput format. Cellartis Cardiomyocytes used in combination with Axion BioSystems' MEA technology demonstrate the potential to accurately predict cardiotoxic responses and to screen compound efficacy.

II. Materials Required

- Cellartis Cardiomyocytes (from ChiPSC22) Kit (Takara Bio, Cat. No Y10075)
  - Cellartis Cardiomyocytes (from ChiPSC22)
  - Cellartis CM Thawing Base
  - Cellartis CM Culture Base
- Fetal Bovine Serum (FBS) (Life Technologies, Cat. No. 16140)
- Y-27632
- Fibronectin (Sigma-Aldrich, Cat. No. F0895)
- Maestro MEA 48-well plate (Axion BioSystems, M768-KAP-48)
- PBS Dulbecco's with Ca2+ & Mg2+ (D-PBS +/+)
- Fibronectin (Sigma-Aldrich, Cat. No. F0895)
- The Maestro MEA System (Axion BioSystems)
- General cell culture equipment used in cell culture laboratory

III. Protocol

NOTE: Avoid contact with the electrodes in all of the following procedures as they are extremely fragile. These procedures should be performed under aseptic conditions as much as possible.

A. Coating of the Maestro 48-well Plate

1. Dilute the required volume of Fibronectin in D-PBS +/+ to a final concentration of 10 µg/ml.
2. Add the diluted Fibronectin solution to the center of each well (on top of the electrodes). Use 8 µl/well. Be careful not to touch the electrodes.

NOTE: Rapid plating is preferred to avoid drying of the coating.

3. Place the plate in the incubator (37°C ± 1°C, 5% CO2, and >90% humidity) for 1–2 hrs.

B. Medium Preparation

Preparing Cellartis CM Thawing Medium

1. Thaw Cellartis CM Thawing Base.
2. Decontaminate the external surface of all bottles with an appropriate disinfectant and place into the biological safety cabinet.
3. Add 8 ml FBS per 32 ml Cellartis CM Thawing Base to achieve Cellartis CM Thawing Medium.
4. Cellartis CM Thawing Medium should be stored at 4°C and expires one month after the date of preparation.
5. Always discard any leftover warmed Cellartis CM Thawing Medium.
6. Preparing Cellartis CM Thawing Medium with Y-27632
7. On the day of use, prepare Cellartis CM Thawing Medium with Y-27632 by adding Y-27632 to a final concentration of 10 µM to Cellartis CM Thawing Medium.
8. Cellartis CM Thawing Medium with Y-27632 should be used on the day of preparation.

Preparing Cellartis CM Culture Medium

1. Thaw Cellartis CM Culture Base.
2. Decontaminate the external surface of supplement and medium bottle with appropriate disinfectant and place into the biological safety cabinet.
3. Add 10 ml FBS per 90 ml Cellartis CM Culture Base to achieve Cellartis CM Culture Medium.
4. Cellartis CM Culture Medium should be stored at 4°C and expires one month after the date of preparation.
5. Always discard any leftover warmed Cellartis CM Culture Medium.

Preparing Cellartis CM Culture Medium with Y-27632

1. On the day of use, prepare Cellartis CM Culture Medium with Y-27632 by adding Y-27632 to a final concentration of 10 µM to Cellartis CM Culture Medium.
2. Cellartis CM Thawing Medium with Y-27632 should be used on the day of preparation.

C. Thawing and Plating of Cellartis Cardiomyocytes

NOTE: It is recommended that not more than two to three vials are thawed at once.

NOTE: For your protection, wear a protective face mask and protective gloves. Use forceps when handling a frozen vial. Never hold the vial in your hand as it may explode due to rapid temperature changes.

1. Prepare the appropriate volume of Cellartis CM Thawing Medium with Y-27632 (see Section B) and warm to room temperature (RT, 15–25°C).
2. Transfer, as quickly as possible, the frozen vial from liquid nitrogen to a 37°C ± 1°C water bath using forceps.
3. Thaw the cells by gently pushing the vial under the surface of the water, without swirling the vial. Do not submerge the cap of the vial in the water bath as this could contaminate the cells.
4. Take the vial out of the water bath as soon as the thawing is completed (approximately 3 min.; the vial should still be cold on the outside).
5. Wipe the vial with an appropriate disinfectant and place into the biological safety cabinet.
6. As soon as possible, gently transfer the cell suspension into a sterile 50 ml tube by using a pipette.
7. Rinse the vial with 1 ml of Cellartis CM Thawing Medium with Y-27632 and carefully add it to the cell suspension dropwise.

8. Add 8 ml of Cellartis CM Thawing Medium with Y-27632 dropwise. Gently swirl the tube a few times in between.
9. Centrifuge the tube at 200*g* for 5 min at RT and remove the supernatant.
10. Carefully re-suspend the cell pellet with Cellartis CM Thawing Medium with Y-27632, using 700 µl medium per thawed vial.
11. Count the cells and measure viability.
12. Adjust the number of viable cells to 3,125,000 cells/ml with Cellartis CM Thawing Medium with Y-27632.

NOTE: Aspirate the Fibronectin solution just before adding the cell suspension. Prepare 2–4 wells at a time, since drying of the surface might result in crystallization of the Fibronectin and subsequent damaging of the cells.

13. Aspirate the Fibronectin solution from the first 2–4 wells.
14. Proceed rapidly with the remaining wells.
15. Carefully mix your cell suspension and pipet 8 µl of the cell suspension on top of the electrodes in each well (2,5 x 10^4 cells/well).
16. Proceed rapidly with the remaining wells.
17. Place the plate in the incubator (37°C ± 1°C, 5% CO2, and >90% humidity) to allow the cells to settle.
18. After 1–2 hours, carefully add additionally 390 µl Cellartis CM Thawing Medium with Y-27632 to each well to reach a final volume of 400 µl.
19. Place the plate in the incubator (37°C ± 1°C, 5% CO2, and >90% humidity).

D. Medium Change

It is recommended to do the first medium change 48 ± 5 hrs. after thawing and plating, and further every 2–3 days (e.g. Monday, Wednesday, Friday).

Medium preparation

Prepare the appropriate volume of Cellartis CM Culture Medium with Y27632 as described in Section B and warm to 37°C ± 1°C before use.

Medium change

NOTE: Work very gently in order not to detach the cells.

1. Replace the medium with 400 µl of fresh Cellartis CM Culture Medium with Y-27632.
2. Place the plate in the incubator (37°C ± 1°C, 5% CO2, and >90% humidity).

NOTE: Pharmacological experiments are best conducted on day 7-9 post-thaw, or when the T-waves are well defined.

3. On the day of recording, perform a complete medium change as described above, but reduce the volume to 300 µl of fresh Cellartis CM Culture Medium with Y-27632. Cells norml

PRODUCTS

Cat. #	Product	Size
Y10075	Cellartis Cardiomyocytes (from ChiPSC22)	1 kit

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Takara Bio USA, Inc.
United States/Canada: +1.800.662.2566 • Asia Pacific: +1.650.919.7300 • Europe: +33.(0)1.3904.6880 • Japan: +81.(0)77.565.6999