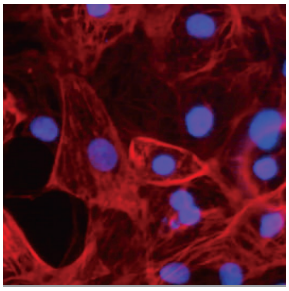

Stem Cell Application Protocol

Application of Cellartis® Cardiomyocytes in 384-well plate format for FLIPR 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 safety evaluation and drug screening. The FLIPR Tetra High-Throughput Cellular Screening System is a real-time kinetic cellular assay screening system that allows for detection of intracellular Ca^{2+} flux and cardiac beating in high-throughput scale. Cellartis Cardiomyocytes used in combination with this 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)
- 384-well plate (Corning Costar, Cat. No. 3712)
- MicroClime lid (Labcyte, Cat. No. LLS-0310)
- PBS Dulbecco's with Ca2+ & Mg2+ (D-PBS +/+)
- FLIPR Calcium 5 Assay Kit (Molecular Devices, Cat No. R8185, R8186, R8187)
- FLIPR Tetra High-Throughput Cellular Screening System (Molecular Devices)
- General cell culture equipment used in cell culture laboratory

III. Protocol

NOTE: These procedures should be performed under aseptic conditions as much as possible.

A. Coating of the 384-well plate

1. Dilute the required volume of Fibronectin in D-PBS +/+ to a final concentration of 50 µg/ml.
2. Add the diluted Fibronectin solution into each well to be used. Use 18 µl/well.

NOTE: To minimize edge effects, seed cells in the central 308 wells only. Fill the outside

wells with the PBS and cover the plates with a MicroClime lid filled with PBS for optimal recordings.

3. Incubate at 37°C for a minimum of 2.5 hrs.
4. Aspirate the Fibronectin solution from the cell culture plate just before use.

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 cabine.
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.

Preparing Cellartis CM Thawing Medium with Y-27632

1. 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.
2. 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.

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 6 ml medium per thawed vial.
11. Count the cells and measure viability.
12. Adjust the number of viable cells to 2.5 x 10^5 cells/ml with Cellartis CM Thawing Medium with Y-27632.

NOTE: Aspirate the Fibronectin solution just before adding the cell suspension. Prepare one column 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 column.
14. Carefully mix your cell suspension and pipet 36 µl per well (corresponding to 9 x 10^3 cells/well).
15. Optional: If foaming of the medium occurs, spin the plate for 1 minute at 200*g* at room temperature. This will remove the majority of bubbles seen in the media. Spin another minute if bubbles still remain.
16. Place the plate in the incubator (37°C ± 1°C, 5% CO2, and >90% humidity).

D. Medium change day 1

It is recommended to wash the cells with fresh medium the day after thawing and plating

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

Medium preparation

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

Medium change including washing the cells once

1. Aspirate most of the medium per well column-by-column with a multichannel aspirator taking care not to touch the bottom of the wells.
2. Add 36 ul of fresh Cellartis CM Culture Medium.
3. Aspirate the newly added medium as described in bullet 1 above.
4. Add 50 µl of fresh Cellartis CM Culture Medium.
5. Place the plate in the incubator (37°C ± 1°C, 5% CO2, and >90% humidity).

E. Medium change day 3 and onwards

It is recommended to perform medium changes every 2–3 day until analysis. Also perform a medium change in the morning of the day of recording.

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

Medium preparation

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

Medium change

1. Aspirate most of the medium per well column-by-column with a multichannel aspirator taking care not to touch the bottom of the well.
2. Add 50 µl of fresh Cellartis CM Culture Medium.
3. Place the plate in the incubator (37°C ± 1°C, 5% CO2, and >90% humidity).

NOTE: Cells are optimally recorded on day 10 post-thaw (in the afternoon to let cells recover after the morning medium change).

PRODUCTS

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

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We are committed to developing and providing optimal tools that are tailored to your projects. To ensure your success, our Cellartis iPS Cell Services team delivers professional expertise through close collaboration.

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