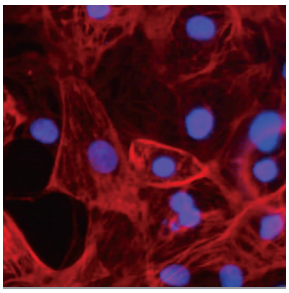

Stem Cell Application Protocol

Application of Cellartis® Cardiomyocytes in Multi Channel System's 96-well Multiwell-MEA-System for EFP Recordings

that's GOOD science!

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. Multi Channel System's Multiwell-MEA-System allows for detection of extracellular field potential (EFP) recordings in high throughput format. Cellartis Cardiomyocytes used in combination with Multi Channel System's Multiwell-MEA-System 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
- Multiwell plate (72- or 96-well, Multi Channel Systems, #72W500/100F and 96W700/100F, respectively) + lid to cover
- Fibronectin (Corning #354008)
- Sterile water
- Storage box
- Multiwell-MEA-System (Multi Channel System)
- General cell culture equipment used in cell culture laboratory

III. Protocol

A. Coating of the Multiwell plate

1. Prepare a storage box by placing a moisturized paper tissue at the bottom of the box (see Figure 1).

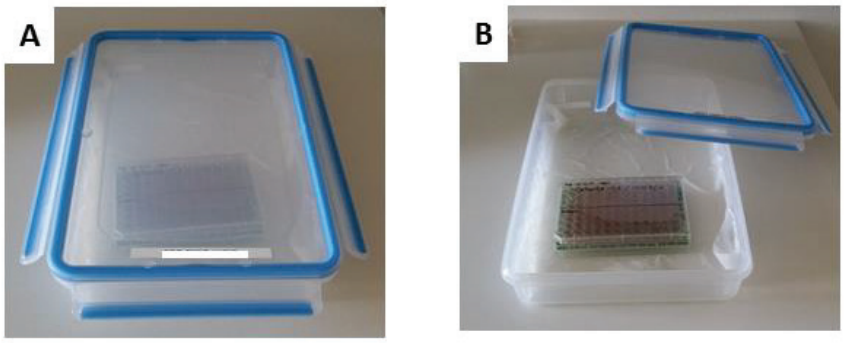

Fig. 1 Storage box for moisturized environment.
A: Box with covering lid. B: opened box.
Note the moisturized paper towel at the bottom of the box.

2. Dilute the required volume of Fibronectin in sterile water to a final concentration of 1 mg/ml).

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.

3. Add the diluted Fibronectin solution directly on the electrode area into each well to be used. Use 5 µl/well, avoiding to touch the electrode field.

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

4. Keep the plate in the storage box in the incubator (37°C ± 1°C, 5% CO_2, and >90% humidity) for a minimum of 1 hr. Tight sealing of the box is not necessary.
5. Aspirate the Fibronectin solution from the wells 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 one time.

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 200g for 5 min at RT and remove the supernatant.
10. Carefully resuspend the cell pellet with Cellartis CM Thawing Medium with Y-27632, using 400 µl of medium per thawed vial.
11. Count the cells and measure viability.
12. Adjust the number of viable cells to 6 x 10^6 cells/ml with Cellartis CM Thawing Medium with Y-27632.

NOTE: Aspirate the Fibronectin solution just before adding the cell suspension. Prepare max. 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 2–4 wells.
14. Carefully mix your cell suspension to ensure that a single cell suspension is achieved and plate 5 µl of suspension per well onto the electrode field (3 x 10^4 cells/well). Use the lid of the plate to cover the wells *immediately* after the cell suspension has been added, in order to avoid evaporation.
15. Proceed rapidly with the remaining wells, repeat bullet 13–15 until the cell suspension has been added to all the wells.
16. Put the plate in the storage container and keep in incubator for 3 hours to allow the cells to settle.
17. After 3 hrs., very carefully add additionally 200 µl Cellartis CM Culture Medium with Y-27632 per well, a few wells at a time. Use the lid of the plate to cover the wells in which the volume has not yet been increased by the addition of teh 200 µl Cellartis CM Culture Medium with Y-27632.
18. Place the plate in the storage box, in the incubator (37°C ± 1°C, 5% CO_2, and >90% humidity) and leave undisturbed for 24 hrs.

D. Medium change

It is recommended to do the first medium change 24 ± 2 hrs. after thawing and plating, and further every other day.

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

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

1. Replace 90% of the medium (180 ml) with fresh Cellartis CM Culture Medium.
2. Place the plate in the incubator (37°C ± 1°C, 5% CO2, and >90% humidity).

NOTE: Cells are optimally recorded between day 6 and 7 post-thaw.