Cellartis Cardiomyocytes — derived from Human Induced Pluripotent Stem Cells

PRODUCTS

製品コード	製品名	容量	価格(税別)
Y30075	Cellartis Cardiomyocytes (from ChiPSC22) Kit	1 Kit (>3M viable cells/vial)	¥180,000
Y30062	Cellartis CM Thawing Base	32 ml	¥9,000
Y30063	Cellartis CM Culture Base	90 ml	¥21,000

Clontech TaKaRa cellartis

About us

With more than 15 years of experience in stem cell generation and differentiation, spanning the development of this field, Takara Bio Europe AB has developed the dedicated portfolio of Cellartis stem cell products. The company was first in the world to bring to the market human embryonic stem cell-derived cardiomyocytes, which were launched in 2008.

Human cardiomyocytes derived from induced pluripotent stem cells

An effective tool for *in vitro* evaluation of cardiotoxicity, drug discovery and basic research

Cellartis Cardiomyocytes are spontaneously beating cardiomyocytes derived from human induced pluripotent stem cells. The cells express the major cardiac markers and ion channels, are functionally similar to adult human cardiomyocytes, and exhibit the expected responses to cardiac stimuli, making them excellent *in vitro* tools for studies of human cardiomyocyte function and for cardiac safety pharmacology assays. The cardiomyocytes have been dissociated into a single-cell suspension and frozen in vials for convenient use in downstream applications, providing accurate and reproducible data acquisition.

Features

- Expression of major cardiac markers and ion channels
- Physiological response to cardiac stimuli
- Generated using a standardized protocol that mimics human cardiac developmental pathways
    - No genetic engineering or purification/selection procedure
    - High yield (>80% cardiomyocytes)
    - About 20% supporting cells contributing to more physiological relevance
- Produced in consistent, quality controlled batches
- 3D compatible
- Large proportion of the cells express calcium and sodium currents

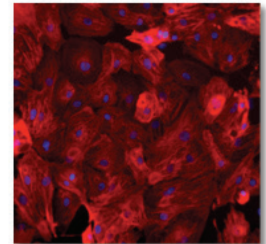


Figure 1. ICC staining of Cellartis Cardiomyocytes (from ChiPSC22).

Red = cTroponin, Blue = DAPI (20x magnification).

Cardiac markers and ion channels

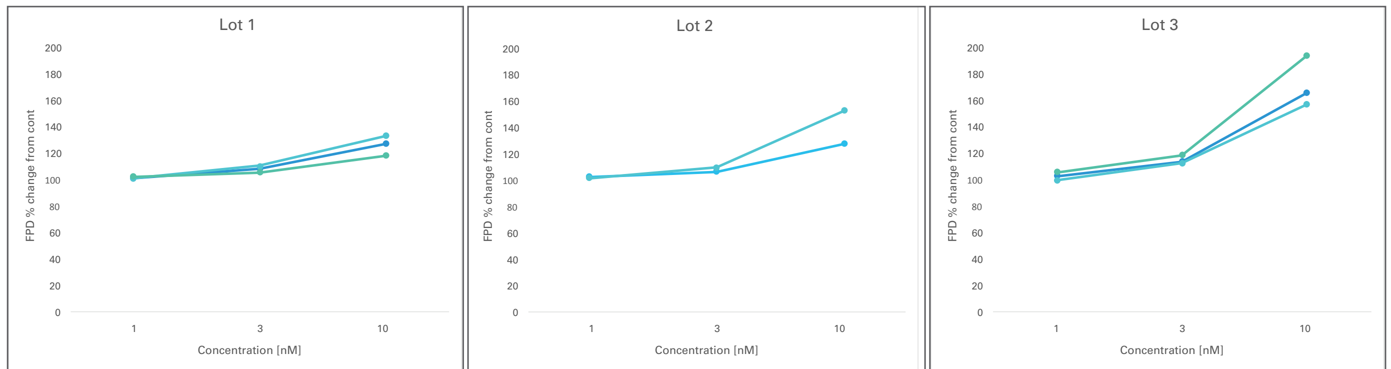
Cellartis Cardiomyocytes express major cardiac markers and ion channels.

Characteristic	Assay	Analyte
Protein markers	ICC	e.g. c-Troponin, Actin, Myomesin
Gene Expression	qPCR	e.g. MLC2V, ACTC1, TNNT2, MYH6, Connexin 43, HCN4, SERCA2, KCNa5, SCN5A, KCNIP2, KCNE2

TaKaRa

Produced in consistent, quality-controlled batches

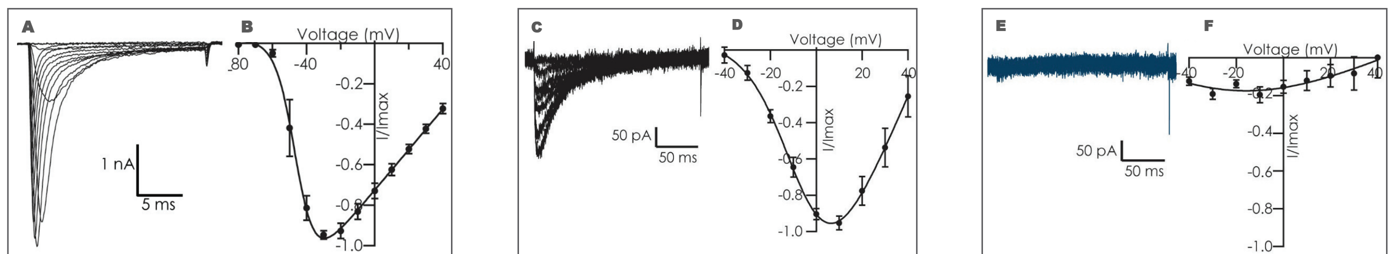
Reproducible behavior of three lots of Cellartis Cardiomyocytes in response to E-4031 (MED64 system; Alpha MED Scientific)



Field potential duration (FPD) was increased in a dose-dependent manner with E-4031 administration. EADs, typically, preceding TdP, were observed at an E-4031 concentration of 30 nM and higher. These results were reproducible across all lots tested. Each color indicates a measurement from a different channel of the MEA probe.

Physiologically relevant NaIV and CaIV

Evaluation of NaIV and CaIV in Cellartis Cardiomyocytes (Patchliner, Nanion)



NaIV (A) and IV plot (B) in control, n = 7. The NaIV was fit using a Boltzmann equation which revealed a Vhalf of activation of -46 mV. This is in excellent agreement with the cardiac NaV channel, NaV1.5 (also known as h1), expressed in tsA-201 cells (see Catterall *et al*, 2005, Pharmacological Reviews, Vol. 57, No.4 pp. 397-409; Li *et al*, 2002, Molecular Pharmacology, Vol. 61, No. 1, pp. 136-141).

CaIV (C) and IV plot (D) in control, n = 18. The CaIV was also fit using a Boltzmann equation which revealed a Vhalf of activation of -5.8 mV. This value agrees well with e.g. Benitah *et al*, 1992, Pflügers Arch, Vol. 421, pp. 176-187 (-7.8 mV) recording human ventricular cells and Li *et al*, 1999, Am. J. Physiol. Heart. Circ. Physiol. Vol 276:H98-H106 (-4.8 mV) from human ventricular myocytes.

CaIV (E) and IV plot (F) in 10 µM nifedipine, n = 5. Complete block.

Large proportion of the cells express calcium and sodium currents

Statistics of NaV and CaV distribution in Cellartis Cardiomyocytes (Patchliner, Nanion)

Table 1	Capture rate (%)	% cells with NaV (> -50 pA)	% cells with CaV (> -50 pA)
	58 (28/48)	71 (20/28)	68 (19/28)

Table 2	RSeal (MOhm)	Cm (pF)	Rs (MOhm)	INa at -30 mV (nA)	ICa at 10 mV (pA)
	976 ± 144 (28)	37 ± 6 (28)	6.0 ± 0.9 (28)	-5.4 ± 1.5 (7)	-157 ± 24 (18)

Table 1: Success rates for cell capture and cells expressing NaV and CaV currents. 6 experiments were performed using a total of 3 chips, therefore 48 potential sites on the chip were available and 28 cells were captured (with seal resistance > 150 MOhm) resulting in a success rate for 58% for capture. Of the cells captured, 71% showed NaV current >-50 pA and 68% showed CaV current > -50 pA.

Table 2: Average values for seal resistance (RSeal), cell capacitance (Cm) and series resistance (Rs) captured with seal resistance > 150 MOhm. NaV current at -30 mV and CaV current at 10 mV is also shown. Number of cells shown in brackets.

Physiologically relevant responses to compounds in various systems

Multiwell MEA System (Multi Channel System)

Cellartis Cardiomyocytes display expected response to the L-type Ca^{2+} channel blocker Nifedipine.

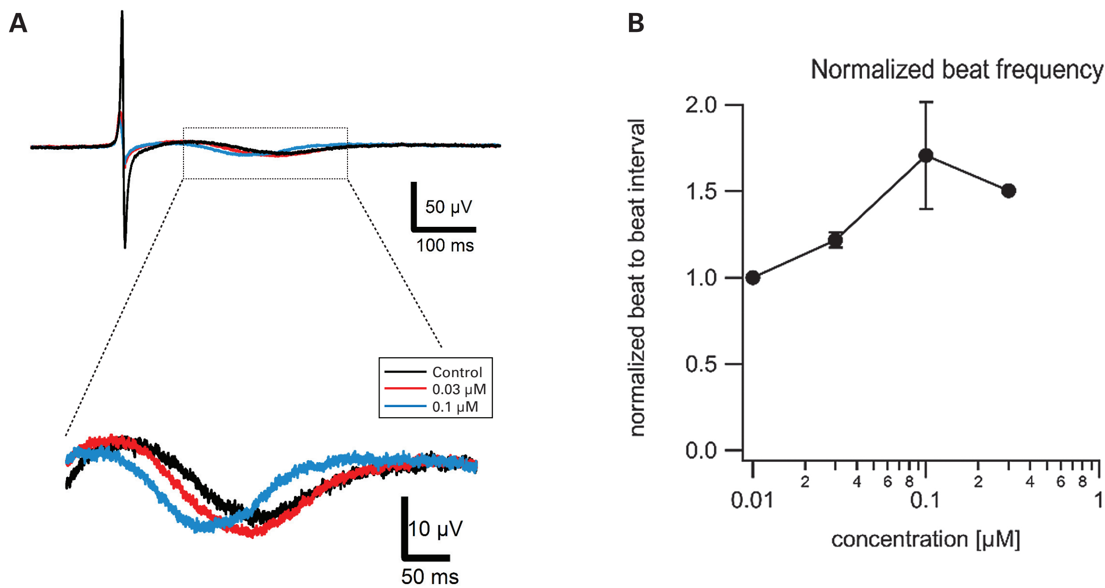
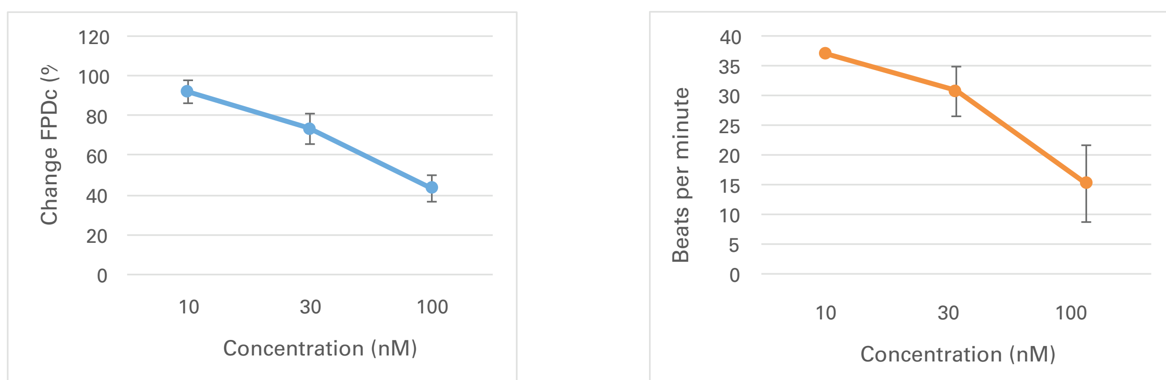


Fig A. Superposition of averaged fAP traces at different concentrations of Nifedipine with magnification of the repolarization component at the end of the fAP. Note the concentration-dependent left shift of the repolarization resulting in a shortening of the fAP.

Fig B. Normalized beat frequency. The beat to beat interval was prolonged with increasing concentrations of Nifedipine resulting in an expected decreased beat frequency. Each data point represents the average of n=8 data points. Error bars indicate SEM.

MED64 system (Alpha MED Scientific)

Physiological FPD shortening and beat rate decreases with Verapamil.



FPD (left) and beat rate (right) at different concentrations of Verapamil. Verapamil inhibits hERG and L-type Ca^{2+} channels at overlapping concentrations and is known to shorten FPD and decrease beat rate *in vivo*. These expected effects of Verapamil were reproduced *in vitro* in dose-dependent manner, suggesting high physiological relevance of the cells. n=3 Error bars indicate SEM.

TaKaRa

Electrical activity of Cellartis Cardiomyocytes exposed to hERG blocker E-4031

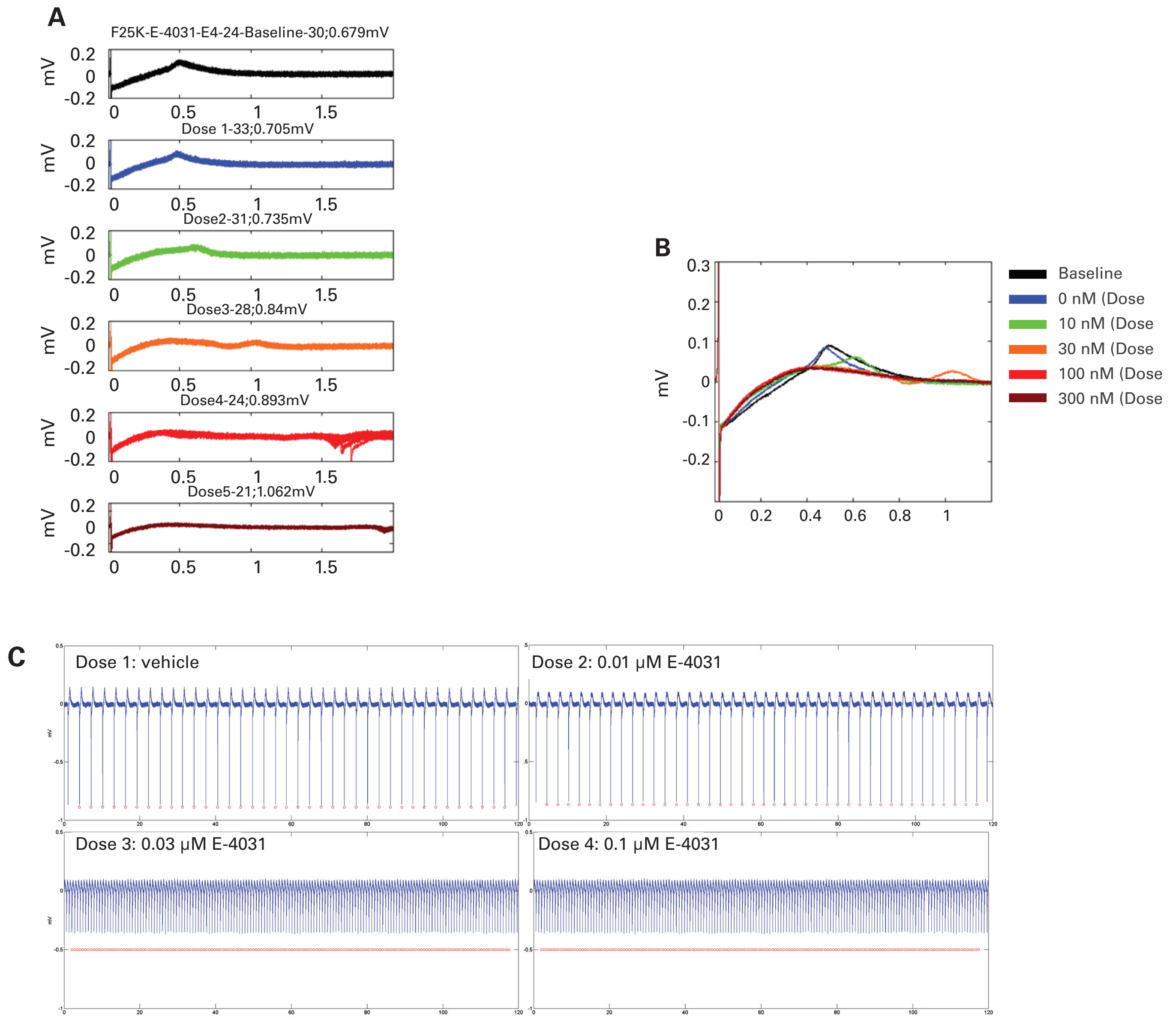


Fig A. Sodium spikes. Overlay of all detected sodium spikes during a two minute recording from a single electrode. Sodium spikes after were temporally aligned at time zero to emphasize the subsequent electrical activity. Asterisk indicate triggered activity defined as sodium spike activity of low amplitude that follows the repolarizing T wave.

Fig B. Average of the overlaid records. Average of the overlaid records shown in A (same color code) to emphasize the changes on the field potential duration.

Fig C. Tachycardic response. Example of high frequency tachycardic response in Matlab generated figures. Red dots indicate detection events.

xCELLigence RTCA CardioECR instrument (ACEA)

Impedance and Field Potential recordings of Cellartis Cardiomyocytes exposed to Moxifloxacin

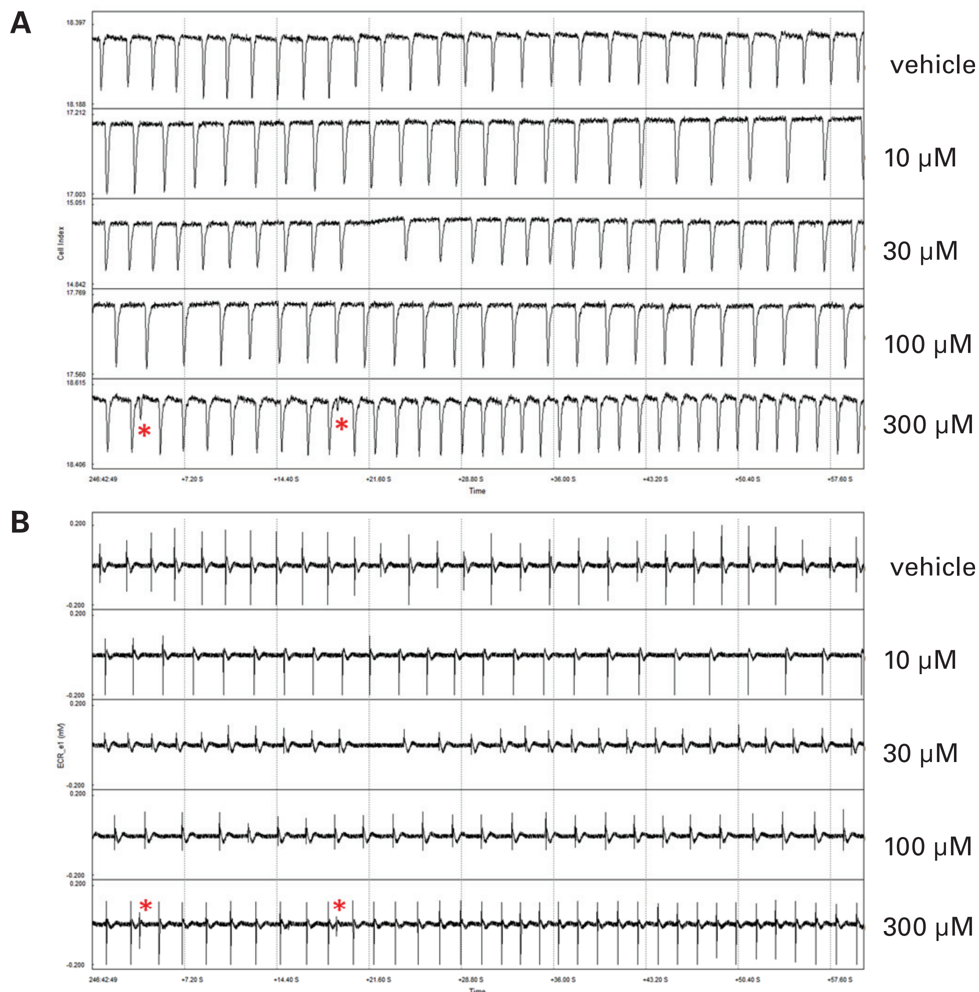


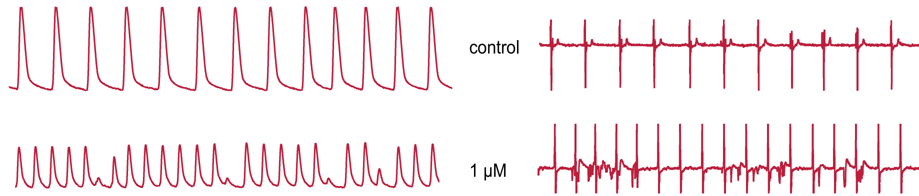
Table 1:

Moxifloxacin treatment	Time of exposure			
	Baseline	2 hours	5 hours	24 hours
10 µM	0 (0/4)	0 (0/4)	0 (0/4)	0 (0/4)
30 µM	0 (0/4)	0 (0/4)	0 (0/4)	0 (0/4)
100 µM	0 (0/4)	0 (0/4)	0 (0/4)	0 (0/4)
300 µM	0 (0/4)	0 (0/4)	1.3 (1/4)	1.3 (1/4)

Impedance (A), Field Potential (B) and Averaged Irregular Beat Rate (Table 1) at different concentrations of Moxifloxacin. Impedance and Field Potential recordings after 5 hours' exposure to vehicle and increased moxifloxacin concentrations. Asterisks indicate ectopic beats due to triggered activity. Averaged Irregular Beat rate was calculated as [Number of irregular beats/(Number of irregular beats + number or regular beats) x 100]. Number in parenthesis indicate the number of wells with proarrhythmic activity to the total number of wells in that condition. Cellartis cardiomyocytes respond to moxifloxacin with prolongation of the FPD and proarrhythmic markers only at the highest concentration tested consistent with the clinical observations.

CardioExcyte 96 system (Nanion)

Detection of EADs after Sotalol administration



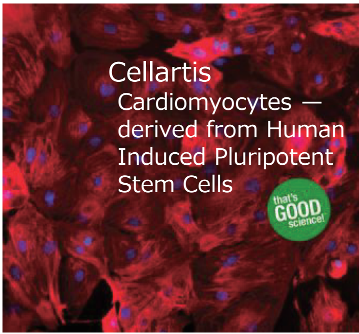
Representative Impedance (left) and Electrical Field Potential (right) traces after treatment of Cellartis Cardiomyocytes with hERG blocker Sotalol. The cells showed sensitivity to Sotalol and produced EADs at 1 µM.

Takara and the CiPA initiative

The objective of the Comprehensive *in vitro* Proarrhythmic Assay (CiPA) initiative is to facilitate the adoption of a new paradigm for assessment of clinical potential of TdP that is not measured exclusively by potency of hERG block and not at all by QT prolongation. The new CIPA paradigm will be driven by a suite of mechanistically based *in vitro* assays coupled to in silico reconstructions of cellular cardiac electrophysiologic activity, with verification of completeness through comparison of predicted and observed responses in human-derived cardiac myocytes.

Takara is actively supporting the CiPA initiative to improve the current regulatory guidance for preclinical cardiac safety assessment. Takara is participating in the CiPA Myocyte Subteam and has played an important role in establishing the minimum criteria to which stem cell cardiomyocyte providers and cardiomyocytes must adhere in order to be considered for use in the CIPA validation study.

Takara is a reliable provider of clinically relevant human iPS derived cardiomyocytes and Cellartis cardiomyocytes are currently supplied to participating sites globally for use in the CiPA validation study.



Application

Stem Cell Application Protocols of Cellartis® Cardiomyocytes

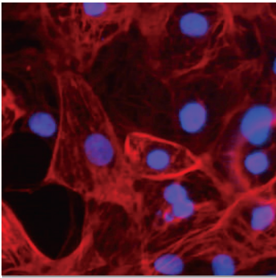
in Maestro Multi-Electrode Array system for EFP

in CardioExcyte® 96 for Impedance and EFP

in 96-well Multiwell-MEA-System for EFP

in xCELLigence RTCA CardioECR Platform for Impedance and EFP

in 384-well plate format for FLIPR



Stem Cell Application Protocol

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

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% CO_2, 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.

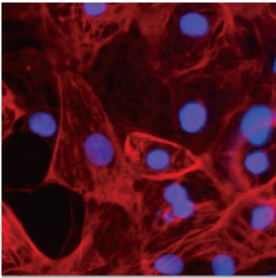
Medium change

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



Stem Cell Application Protocol

Application of Cellartis® Cardiomyocytes in CardioExcyte® 96 for Impedance and EFP recordings

I. Introduction

Cellartis Cardiomyocytes (from ChiPSC22) 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 hybrid instrument CardioExcyte 96 allows for both impedance readout and extracellular field potential (EFP) recordings in high throughput format. Cellartis Cardiomyocytes used in combination with this technique form an excellent platform 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)
- CardioExcyte 96 Sensor Plate (Cat. No. 20 1001, Nanion Technologies)
- PBS Dulbecco's with Ca^{2+} & Mg^{2+} (D-PBS +/+)
- CardioExcyte 96 instrument (Nanion Technologies)
- 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 fragile. These procedures should be performed under aseptic conditions as much as possible.

A. Coating of the CardioExcyte 96 Sensor 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 into each well to be used. Use 50 µl/well.
3. Incubate at 37°C for a minimum of 1.5 hours.

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 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.

. 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 1.5–2.0 x 10^5 cells/ml with Cellartis CM Thawing Medium with Y-27632.

NOTE: It is recommended to remove the coating solution and add the cell suspension column by column. Preparing more wells at a time might result in a drying of the surface, resulting in crystallization of the fibronectin and damaging of the cells afterwards.

13. Aspirate the Fibronectin solution from the first column.
14. Carefully mix your cell suspension and pipet 200 µl into each well (corresponding to 3–4 x 10^4 cells/well).
15. Proceed rapidly with the remaining columns.
16. 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 24 ± 2 hrs. after thawing and plating, and further on day 2, 3 and 4. From day 4 and onwards, it is sufficient to do a medium change every other day.

. Medium preparation

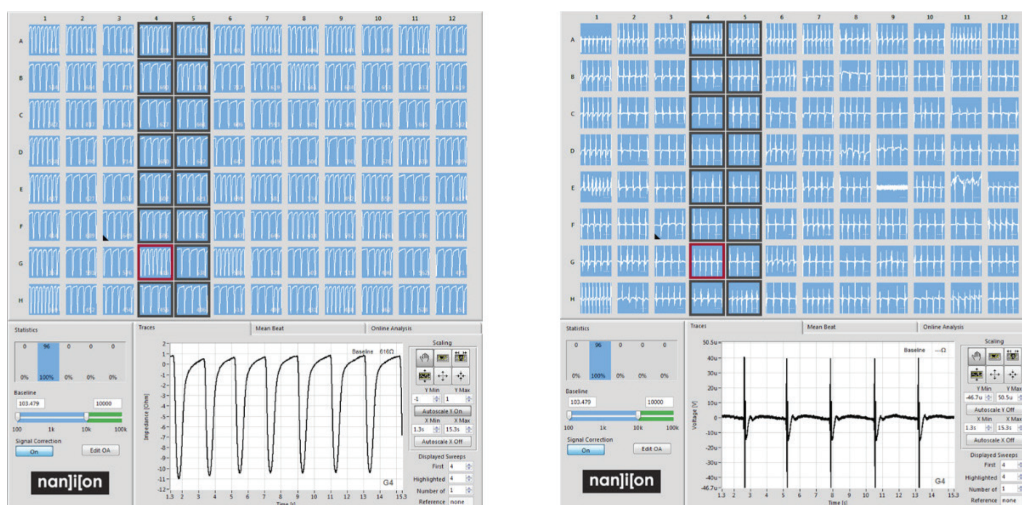
1. Prepare the appropriate volume of Cellartis CM Culture Medium as described in section B and warm to 37°C ± 1°C before use.

. Medium change

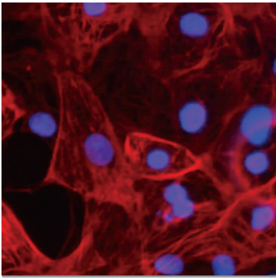
NOTE: Work very gently in order not to detach the cells.

1. Aspirate 100 µl medium per well column-by-column and add 100 µl of fresh medium. Be careful not to touch the electrodes on the bottom of the well.
2. Repeat bullet 1 one time for each well (column-by-column).
3. Place the plate in the incubator (37°C ± 1°C, 5% CO2, and >90% humidity).

NOTE: Impedance signals are maximal on approximately day 8 post-thaw, while optimal EFP T-waves are detected later, usually on day 9-10. For application of compounds, monitor the T-wave development and add compounds when it can be detected.



Impedance traces (left) and extracellular field potentials (right) from Cellartis Cardiomyocytes, showing strong beating and synchronized monolayers as well as high expression levels of cardiac ion channels in all 96 wells.



Stem Cell Application Protocol

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

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).

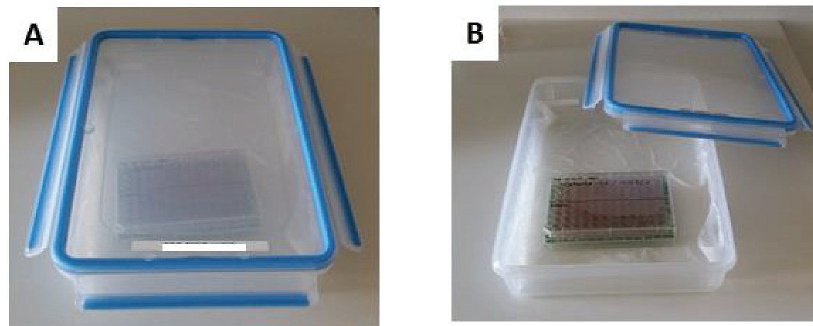


Fig. 1 Storage box for moisturized environment.
A: Box with covering lid. B: opened box.
Note the moisturized paper towel at the bottom of the box.

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% CO2, 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 6×10^6 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 (3×10^4 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

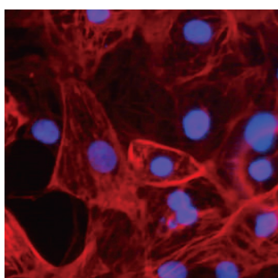
1. Prepare the appropriate volume of Cellartis CM Culture Medium as described in Section B and warm to 37°C ± 1°C before use.

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.



Stem Cell Application Protocol

Application of Cellartis® Cardiomyocytes in ACEA Biosciences' xCELLigence RTCA CardioECR Platform for Impedance and EFP recordings

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 hybrid system xCELLigence RTCA CardioECR allows for both impedance readout and extracellular field potential (EFP) recordings in high throughput format. Cellartis Cardiomyocytes used in combination with this technique form an excellent platform 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
- Electronic 48-well microtiter plate (E-Plate® CardioECR 48, ACEA Biosciences)
- Fibronectin (Sigma-Aldrich, Cat. No. F0895)
- PBS Dulbecco's with Ca^{2+} & Mg^{2+} (D-PBS +/+)
- xCELLigence RTCA CardioECR instrument (ACEA Biosciences)
- 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 fragile. These procedures should be performed under aseptic conditions as much as possible.

A. Coating of the E-Plate® CardioECR 48

1. Dilute the required volume of Fibronectin in D-PBS +/+ to a final concentration of 10 µg/ml.
2. Add the diluted Fibronectin solution into each well to be used. Use 50 µl/well.
3. Incubate at 37°C for 3 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.

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 Culture 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 of 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 4 ml medium per thawed vial.
11. Count the cells and measure viability.
12. Adjust the number of viable cells to 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 just before adding the cell suspension, making sure the wells do not run dry.
14. Carefully mix your cell suspension and pipet 50 µl into each well (corresponding to 2.5 x 10^4 cells/well).
15. Proceed rapidly with the remaining columns.
16. Place the plate in the incubator (37°C ± 1°C, 5% CO2, and >90% humidity).
17. After 3 hrs, carefully add 130 µl Cellartis CM Thawing Medium with Y-27632 to each well to reach a final volume of 180 µl.

D. Medium change

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

Medium preparation

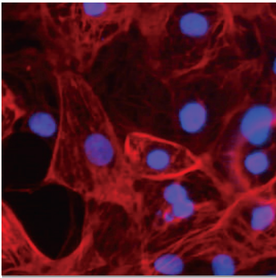
1. Prepare the appropriate volume of Cellartis CM Culture Medium with 10 µM Y-27632 as described in section B and warm to 37°C ± 1°C before use.

Medium change

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

1. Replace the medium with 180 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: Impedance studies are best conducted after 7 days of post-thaw culture, depending on the stabilization of the signals.



Application of Cellartis® Cardiomyocytes in 384-well plate format for FLIPR recordings

Stem Cell Application Protocol

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

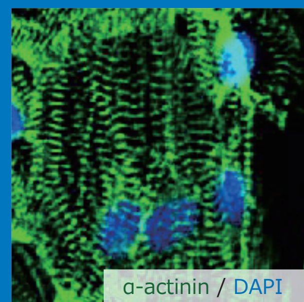
1. Aspirate most of the medium per well column-by-column with a multichannel aspirator taking care not to touch the bottom of the well.
2. Add 50 µl of fresh Cellartis CM Culture Medium.
3. Place the plate in the incubator (37°C ± 1°C, 5% CO2, and >90% humidity).

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

MiraCell™ Cardiomyocytes

◆ 新世代高純度心筋分化誘導技術による優れたロット間再現性

◆ Cellarts心筋と異なるドナー由来のヒトiPS細胞より調製

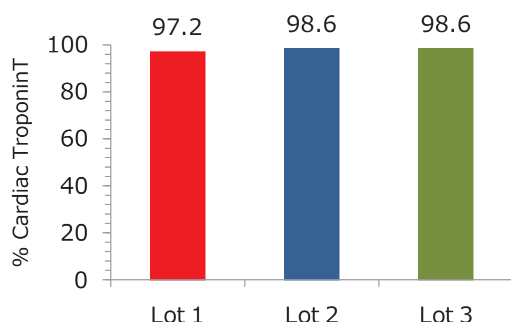


製 品 名	内容	容 量	製品コード	価格(税別)
New MiraCell™ Cardiomyocytes (from ChiPSC12) Kit	MiraCell Cardiomyocytes (from ChiPSC12) ・・・ >3x10^6 cells/vial MiraCell CM Thawing Medium ・・・20 mL MiraCell CM Culture Medium* ・・・100 mL	1 Kit	Y50015	¥200,000

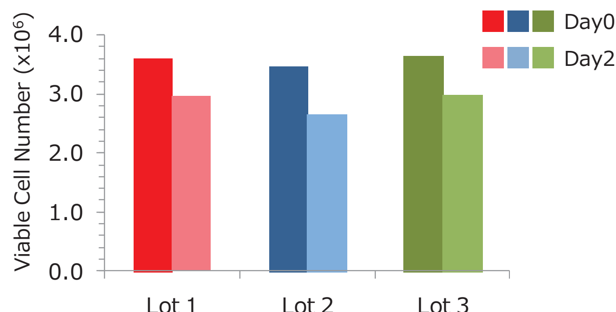
*MiraCell CM Culture Medium(製品コード: Y50013, 価格(税別) ¥21,000)のみも購入可能。

薬剤選択に依存しない高純度化技術による優れたロット間安定性

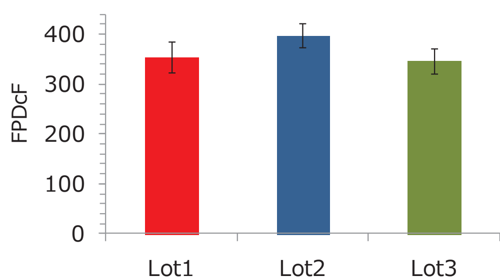
A) 心筋マーカーcTnTの陽性率



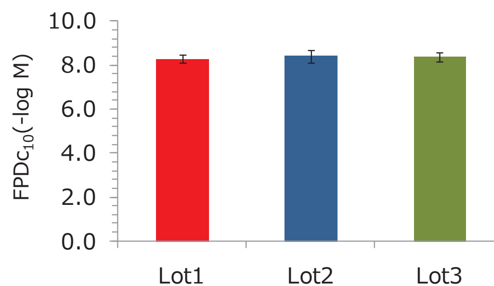
B) 解凍直後(Day0)、及び解凍二日目(Day2)の生細胞数



C) MEDプローブ上でのFPDcF



D) E-4031応答性 (FPDc$_{10}$)



MiraCell Cardiomyocytes (from ChiPSC12)のロット間差(3ロット)の確認

A) 心筋マーカーcTnT(Cardiac TroponinT)陽性率(解凍二日目)比較結果 **B)** 解凍直後及び解凍二日目の生細胞数比較結果 **C)** MEDプローブ上(アルファメッドサイエンティフィック社製)でのFPDcF(Field potential duration corrected by Fridericia's formula)の比較結果。※BPM35以上のプローブを使用。 **D)** 各ロットにおけるI_{Kr}阻害剤(E-4031)によるFPDc$_{10}$(FPDcFが10%延長する際の薬剤濃度)比較結果。いずれの評価方法においても、ロット間差は少ないことが確認された。

- 本製品は、iHeart Japan株式会社よりライセンスを受け、タカラバイオ(株)が製造、販売しています。
- MiraCell™ はiHeart Japan株式会社の商標です。
- 本資料で紹介した製品はすべて研究用として販売しております。ヒト、動物への医療、臨床診断用には使用しないようご注意ください。また、食品、化粧品、家庭用品等として使用しないでください。
- タカラバイオの承認を得ずに製品の再販・譲渡、再販・譲渡のための改変、商用製品の製造に使用することは禁止されています。
- 本チラシ記載の価格は2016年10月1日現在の希望小売価格です。価格に消費税は含まれておりません。

iHeart

2016年10月作成

タカラバイオ株式会社

東京支店 TEL 03-3271-8553 FAX 03-3271-7282
関西支店 TEL 077-565-6969 FAX 077-565-6995
TaKaRaテクニカルサポートライン TEL 077-565-6999 FAX 077-565-6995
Website http://www.takara-bio.co.jp
Facebook http://www.facebook.com/takarabio.jp

取扱店