Cat. # Y50015

For Research Use

TaKaRa

MiraCell™ Cardiomyocytes (from ChiPSC12) Kit

Product Manual

v201610

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I. Description

Primary human cardiomyocytes can be useful tools testing cardiotoxicity as well as exploratory testing of drug efficacy. However, acquiring a stable supply of these cells is challenging because they are difficult to collect compared to adipocytes, skin fibroblasts, etc. Additionally, adult cardiomyocytes do not have the proliferative capacity of stem cells. Because of this, cardiomyocytes whose differentiation is induced from human iPS (Induced Pluripotent Stem) cells have attracted attention as a new substitute for primary human cardiomyocytes in toxicity studies and drug discovery research.

MiraCell Cardiomyocytes (from ChiPSC12) are highly pure cardiomyocytes induced from human iPS cells, and are available for analysis of characteristics and functions of cardiomyocytes. Not only can they be used for analysis of the physiological function of cardiomyocytes, they can also be widely used for cardiotoxicity testing with various drugs using an MEA (Multi-electrode array) system, etc.

This product was jointly developed by Takara Bio Inc. and iHeart Japan Corporation, after Takara Bio Inc. introduced a production technique for human cardiomyocytes developed by professor Jun Yamashita of the Center for iPS Cell Research and Application in Kyoto University, from iHeart Japan Corporation in June 2014. By using this cardiomyocyte production technique, highly pure cardiomyocytes are prepared without performing drug selection (e.g., puromycin, etc.) using a cardiomyocyte -specific promoter (α -MHC etc.). Therefore, the reduction in purity that normally occurs with long-term culture is not an issue with this system. It has been reported that a reduction in purity was not observed after a 90-day culture period (under paper submission).
The iPS cell line ChiPSC12 (Cat. #Y00285)* cultured under feeder-free conditions using the Cellartis® DEF-CS™ 500 Culture System (Cat. #Y30010) is used in the manufacturing process for this product.

* http://catalog.takara-bio.co.jp/product/basic_info.php?unitid=U100009146

Product features

- More than 95% purity (cTnT positive rate)
- Purified without drug selection
- Spontaneously beat *in vitro*
- Electrophysiological responsiveness to a wide range of ion channel inhibitors (E-4031 and Chromanol 293B, etc.)

II. Components

MiraCell Cardiomyocytes (from ChiPSC12)	Frozen vial, 1 tube	$> 3 \times 10^6$ cells
MiraCell CM Thawing Medium	1 bottle	20 ml
MiraCell CM Culture Medium	1 bottle	100 ml

Necessary reagents and equipment not supplied in this kit

- 37℃, 5% CO_2 incubator
- Clean bench or safety cabinet
- Refrigerated centrifuge
- Pipet controller and plastic pipet
- Micropipette and sterilized tip (with filter)
- 50-ml centrifuge tube
- Dulbecco's PBS with Ca^{++} & Mg^{++} (D-PBS (+/+))
- Dulbecco's PBS without Ca^{++} & Mg^{++} (D-PBS (-/-))
- 0.25% Trypsin-EDTA solution
- Cell culture vessel (6 well tissue culture plates etc.)
- 1 mg/ml fibronectin solution (fibronectin from human plasma, Sigma Cat. #F0895 or equivalent)
- Trypan blue solution (0.4% trypan blue solution, Thermo Fisher Scientific Cat. #15250-061 or equivalent)
- Hemocytometer

III. Storage

- After receiving this product, immediately store frozen MiraCell Cardiomyocytes (from ChiPSC12), in liquid nitrogen.

- Store MiraCell CM Thawing Medium at -20℃ or colder until use. Before use, thaw the medium by leaving it overnight at 4℃. After thawing, store at 4℃ and use within 1 week (Do not re-freeze and thaw).

- Store MiraCell CM Culture Medium at -20℃ or colder until use. Before use, thaw the medium by leaving it overnight at 4 ℃. After thawing, store at 4℃ and use within 1 month. (Do not re-freeze and thaw.)

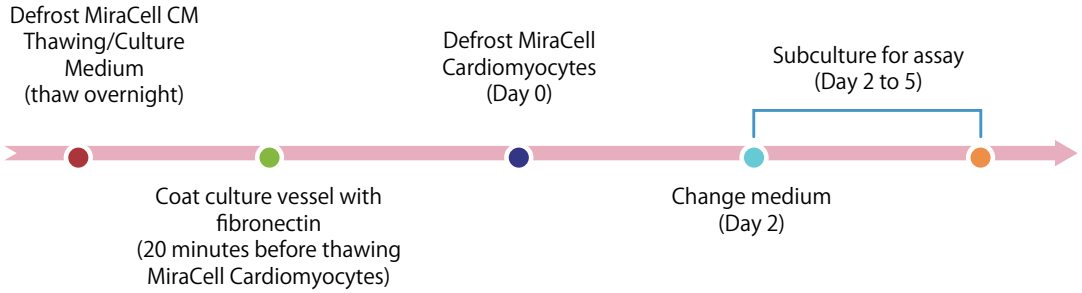
IV. Preparation before use

1. Cardiomyocytes are susceptible to physical damage. When pipetting, please do so slowly (taking more than 3 seconds for 1 ml) and take care to minimize the number of pipetting steps.

2. Before use, always pre-heat the medium up to between room temperature and 37℃.

V. Protocol

V-1. Cell culture timeline



V-2. Coating culture vessels with fibronectin

[Prepare 20 minutes before thawing cardiomyocytes]

1. As shown below, dilute 1 mg/ml fibronectin solution 20X with D-PBS (+/+) to prepare a final solution at a concentration of 50 μg/ml.

D-PBS (+/+)	1.9 ml
1 mg/ml fibronectin solution	0.1 ml

2. Mix by pipetting, taking care to not produce bubbles. *1

3. Add 50 μg/ml fibronectin solution (0.1 ml/cm^2) to a culture vessel *2 and spread the solution such that it covers the entire surface of the vessel.

Multiwell culture vessels	50 μg/ml fibronectin
48-well	0.1 ml/well
24-well	0.2 ml/well
12-well	0.4 ml/well
6-well (35-mm dish)	1.0 ml/well

4. Let the culture vessel sit for 20 to 60 minutes at 37℃ (or for 30 minutes to 3 hours at room temperature).

*1 Avoid intense mixing, such as vortexing, etc., as it affects fibronectin activity.

*2 Cardiomyocytes can be spread into at least 1 well of a 6 -well plate, 3 wells of a 12-well plate, or 6 wells of a 24-well plate.

V-3. Thawing and culturing cardiomyocytes

- Prepare a 37℃ water bath before thawing the MiraCell Cardiomyocytes vial.
- Dispense 20 ml of MiraCell CM Thawing Medium into a 50-ml tube and warm to 37℃.
- Transfer a frozen vial of cardiomyocytes from liquid nitrogen storage to a container with liquid nitrogen or dry ice.

[Day 0]

1. Place the MiraCell Cardiomyocytes cryo vial in a float and thaw in a 37℃ water bath for 2 minutes. *1
2. Sterilize the vial with 70% ethanol, remove any extra ethanol with a kimwipe (or equivalent).
3. Slowly transfer the cells in the vial into a new 50-ml tube using a 1-ml pipette. *2
4. Add 1 ml of MiraCell CM Thawing Medium (pre-warmed to 37℃) to the vial. Take the remaining cells using a 1-ml micropipette and slowly transfer it to the 50-ml tube from step 3. *3
5. Gradually add 1 ml of MiraCell CM Thawing Medium into the cell suspension solution using a 1-ml micropipette. *4
6. Gradually add a total of 2 ml of MiraCell CM Thawing Medium to the cell suspension using a 1-ml micropipette. *5
7. Use a 5-ml pipette to transfer 5 ml of MiraCell CM Thawing Medium to the cell suspension from Step 5 at a rate of one drop per second.
8. Centrifuge the 10-ml cell suspension at 200*g* at 20℃ for 5 minutes. *6
9. Remove the supernatant using an aspirator. *7
10. Loosen the cell pellet by tapping 10 to 20 times.
11. Add 3 ml of MiraCell CM Thawing Medium using a 5-ml pipette*8, and mix by pipetting once. *8

*1 Do not shake the vial while it is thawing. (This affects cell viability after thawing.)

*2 Slowly pipette the cell suspension, taking more than 3 seconds per ml.

*3 Tilt the 50-ml tube about 45 degrees, add one drop along the wall of the tube and mix the cell suspension 3 to 5 times by gently moving the tube back and forth. Wait for 5 seconds, add the next drop, and mix again. Repeat until the entire 1 ml of Medium has been added.

*4 After adding one drop, mix the solution 3 to 5 times by gently moving the tube back and forth. Wait for 3 seconds and then add the next one drop. Repeat until the entire 1 ml of Medium has been added.

*5 Add the Medium one drop per second while mixing the cell suspension 3 to 5 times by light tapping.

*6 Strictly maintain the centrifugation strength, as it affects cell recovery and viability.

*7 When removing the supernatant, leave about 0.2 ml of supernatant in order to decrease the risk of cell loss and/or loss in cell viability.

*8 Pipette the cell suspension only once (taking 2 to 3 seconds per ml). Pipetting multiple times may decrease cell number and cell viability.

12. Take 20 μl of the cell suspension and count the number of cells as follows. *9

<Example of cell counting method>

1) Dilute a 20-μl aliquot of the cell suspension 2X by adding 20 μl of trypan blue solution, and count the number of unstained cells using a hemocytometer.

2) After counting the number of cells in four areas (see Figure 1), calculate the cell concentration (cells/ml) and the total number of living cells as shown below.

(The total number of cells x 10^4 cells) / 4 x 2 = Cell concentration (cells/ml)
Cell concentration x 3 ml = Total number of living cells (cells)

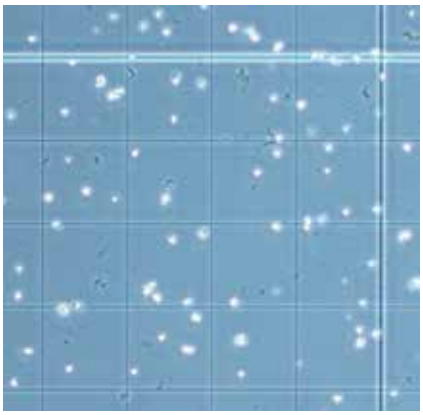


Figure 1. Cardiomyocytes after thawing (one area of a hemocytometer)

13. Add an appropriate amount of MiraCell CM Thawing Medium (prewarmed to 37℃) to adjust to a cell concentration of 8 x 10^5 cells/ml.

14. After aspirating off the fibronectin solution from the plate prepared in V-2. 4, immediately add 2.5 to 3.4 ml of cell suspension to 1 well of a 6-well plate, for a cell density of 2.0 - 3.0 x 10^5 cells/cm^2. (See table below for other culture vessels.)

Culture vessels	The volume of cell suspension (8 x 10^5 cells/ml)	Cell number/well
48-well	0.25 - 0.31 ml	2.0 - 2.5 x 10^5
24-well	0.5 - 0.63 ml	4.0 - 5.0 x 10^5
12-well	1.0 -1.25 ml	8.0 - 10.0 x 10^5
6-well (35-mm dish)	2.5 - 3.4 ml	20 - 27 x 10^5

15. After evenly dispersing the cells by gently shaking the plate left and right, culture the cells in a 5% CO_2 incubator at 37℃. *10

*9 Cell aggregation may occur, but you can proceed with the protocol.

*10 Do not move the culture vessel for at least 24 hours after placing it in the incubator. Spontaneous beating of cardiomyocytes is observed from the day after thawing.

[Day 2]

16. Aspirate off the medium from the culture vessel 2 days after culturing.
17. Add MiraCell CM Culture prewarmed to 37℃ (see table below).

Culture vessels	Volume of medium to be added
48-well	0.3 ml
24-well	0.6 ml
12-well	1.2 ml
6-well (35-mm dish)	3.0 ml

18. Culture for 2 more days in a 5% CO_2 incubator at 37℃. *11
Perform a medium change every other day, aspirating off all the medium, and the adding the appropriate amount of pre-warmed MiraCell CM Culture Medium as indicated in the table above.

*11 Beating of cardiomyocytes sometimes temporarily stops after a medium change.

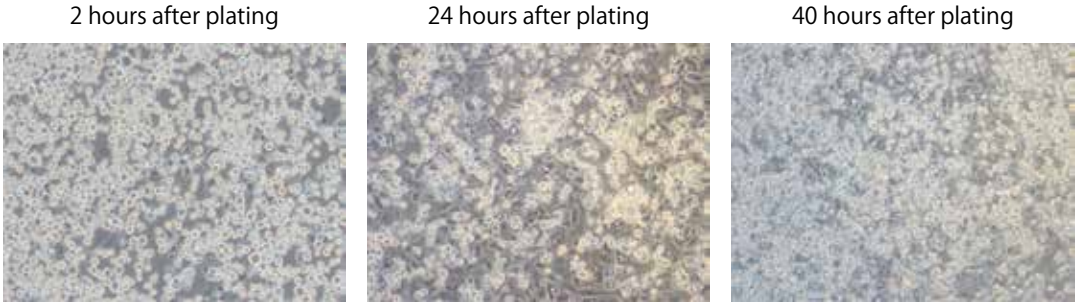


Figure 2. Cell morphology of cardiomyocytes

V-4. Subculturing cardiomyocytes

At 2 to 5 days after culturing, cardiomyocytes can be subcultured for assays. A method for subculturing is shown below.

- Allow the required reagents (D-PBS (-/-), MiraCell CM Culture Medium, and 0.25% trypsin-EDTA solution) to come to room temperature beforehand.

[Day 2 to 5]

1. Remove the medium from the well using an aspirator.
2. Add 2 ml of D-PBS (-/-) to the well.
3. Remove the D-PBS (-/-) using an aspirator, and add another 2 ml of D-PBS (-/-) to the well.
4. Remove the D-PBS (-/-), add 1 ml of 0.25% trypsin-EDTA solution, and incubate at 37℃ for 4 minutes. *1
5. Strongly tap the plate from the side in order to detach the cardiomyocytes as much as possible. *2
6. Add 1 ml of MiraCell CM Culture Medium and transfer the cell suspension from the well to a 50-ml tube using a 1-ml pipette. *3
7. Add 1 ml of MiraCell CM Culture Medium to the well, and detach any remaining cells by pipetting gently and completely.
8. Transfer the Medium to the 50-ml tube from Step 6.
9. After gently pipetting the cell suspension once using a 5-ml pipette, take 20 μl and count the number of cells.
10. Subculture the required number of cells in the appropriate culture vessels for downstream assays.

*1 Keep close track of the time, as a longer trypsin treatment will decrease the viability of the cardiomyocytes.

*2 If the cells are not detached, incubate at 37℃ for 1 more minute and tap the plate again.

*3 Pipetting is not required for this step. In addition, any pipetting of the cell suspension should be done slowly, taking more than 3 seconds for 1 ml for each uptake and ejection step.

*Note Electrophysiological analysis of cardiomyocytes with an MEA (Multi-electrode array) system is shown at the site below:
http://catalog.takara-bio.co.jp/product/basic_info.php?unitid=U100009229

VI. Related Products

Cellartis® human iPS cell line 12 (ChiPSC12) Kit (Cat. #Y00285)
MiraCell™ CM Culture Medium (Cat. #Y50013)

NOTE : This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals. Also, do not use this product as food, cosmetic, or household item, etc.

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