Takara Bio Europe AB

Cellartis® Cardiomyocytes User Manual

Cat. No. Y10075
(102318)

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I. Introduction

Cellartis Cardiomyocytes are human cardiomyocytes derived from a human induced pluripotent stem (iPS) cell line. The iPS cells have been differentiated into spontaneously beating cardiomyocytes *in vitro*. The cells have subsequently been dissociated into a single cell suspension and frozen in vials. Cellartis Cardiomyocytes are provided with basal media for thawing and maintenance; FBS needs to be added to the media before use.

This product should be handled only by persons who have been trained in laboratory techniques and in accordance with the principles of good cell culture practice. Takara Bio Europe AB recommends the use of media and reagents according to this manual for optimal performance of the cells. Takara Bio Europe AB cannot guarantee correct technical feedback on customer cultures unless the below culture instructions have been followed.

II. List of Components

- **Cellartis Cardiomyocytes (from ChiPSC22) Kit (Cat. No Y10075)**
  - Cellartis Cardiomyocytes (from ChiPSC22) (Cat. No. Y10076; not sold separately)
  - Cellartis CM Thawing Base (Cat. No. Y10062)
  - Cellartis CM Culture Base (Cat. No. Y10063)

III. Additional Material Required

The following materials are required but not supplied:

- Fetal Bovine Serum (FBS) (Life Technologies, Cat. No. 16140)
- Fibronectin (Sigma-Aldrich, Cat. No. F0895)
- PBS Dulbecco's with Ca^{2+} & Mg^{2+} (D-PBS +/+)
- PBS Dulbecco's w/o Ca^{2+} & Mg^{2+} (D-PBS –/–)*
- Trypsin-EDTA (0.25%), phenol red*
- Y-27632
- Cell culture vessels, tissue culture treated polystyrene surface
- General cell culture equipment used in cell culture laboratory

* Only needed if dissociating Cellartis Cardiomyocytes, see section X.

IV. General considerations

A. Storage and Handling

Cellartis Cardiomyocytes should be stored at ≤–150°C. Under recommended storage conditions the cells can be stored for up to one year from date of receipt.

NOTE: When transferring the cells from the transport vessel to long term storage, *immediate* transfer is essential since variations in temperature may have an adverse effect on cell survival and quality.

Cellartis Cardiomyocytes should be maintained in an incubator at 37°C ± 1°C, 5% CO_2, and >90% humidity.

Cellartis CM Thawing Base and Cellartis CM Culture Base should be stored at –20°C. The expiration date is indicated on the label. Complete Cellartis CM Thawing Medium and Cellartis CM Culture Medium should be prepared as described in section VII.

V. Culture of Cellartis Cardiomyocytes

Cellartis Cardiomyocytes are thawed and plated in Cellartis CM Thawing Medium. The day after thawing, the cultures are washed to remove non-attached cells and the medium is changed to Cellartis Culture Medium.

The cardiomyocytes can be used starting at day three after thawing (two days after the first medium change). Medium should be changed every second to third day. The cardiomyocytes can be maintained in culture for at least 14 days after thawing under the recommended conditions. The workflow is depicted in Figure 1.

For applications in which non-standard culture formats are used, it is recommended that the cells are thawed and plated in 6- or 12-well tissue culture-treated plates coated according to section VI for optimal recovery. When the cells have recovered (day three after thawing), the cells can be dissociated and moved to the preferred culture/assay format (see dissociation protocol in section X).

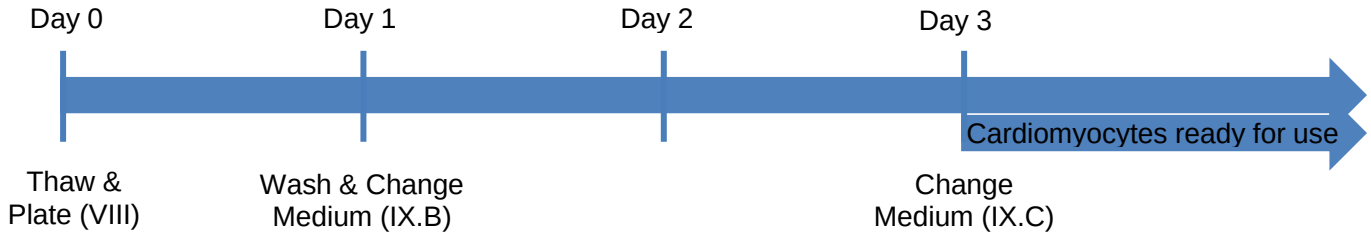

Figure 1. The Cellartis Cardiomyocyte workflow. Corresponding sections of this user manual are referenced in parentheses.

VI. Coating of Cell Culture Vessels

1. Dilute the required volume of fibronectin in D-PBS +/+ (final concentration 50 µg/ml).
2. Add the fibronectin solution to the cell culture vessels (0.3 ml/cm^2, corresponding to 15 µg/cm^2). Make sure the entire surface is covered.
3. Incubate at 37°C ± 1°C and 5% CO_2 for >3 hr.
4. Remove the fibronectin solution from the cell culture vessels just before use.

VII. Cellartis CM Medium Preparation

A. 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.

B. 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.

VIII. Thawing of Cellartis Cardiomyocytes

It is recommended that a maximum of two to three vials are thawed at one time. The recommended seeding density is 1.2–1.5 x 10^5 viable cells/cm^2.

A. Preparation

- Prepare the appropriate volume of Cellartis CM Thawing Medium with Y-27632 by adding Y-27632 to a final concentration of 10 µM. Warm to room temperature (RT, 15–25°C).
- Coat the appropriate number of cell culture vessels according to section VI.

B. Thawing Cells

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 the cryovial may explode due to rapid temperature changes.

1. Transfer, as quickly as possible, the frozen vials from liquid nitrogen to a 37°C ± 1°C water bath using forceps.
2. 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.
3. Take the vials out of the water bath as soon as the thawing is completed (approximately 3 min; the vials should still be cold on the outside).
4. Wipe the vials with an appropriate disinfectant and place into the biological safety cabinet.
5. As soon as possible, gently transfer the cell suspension into a sterile 50 ml tube by using a pipette.
6. Rinse the vial with 1 ml of Cellartis CM Thawing Medium (with 10 µM Y-27632) and carefully add it to the cell suspension dropwise.
7. Add 8 ml of Cellartis CM Thawing Medium (with 10 µM Y-27632) dropwise. Gently swirl the tube a few times in between.
8. Centrifuge the tube at 200 x g for 5 min at RT and remove the supernatant.
9. Carefully resuspend the cell pellet with Cellartis CM Thawing Medium (with 10 µM Y-27632), using 6 ml medium per thawed vial.
10. Count the cells and measure viability.
11. Add Cellartis CM Thawing Medium (with 10 µM Y-27632) to the desired cell density and seed the cells, using 0.4–0.6 ml /cm^2. Make sure the entire surface is covered with medium.
12. Transfer the cell culture unit to an incubator at 37°C ± 1°C, 5% CO_2, and >90% humidity and leave untouched for 24 hr.

NOTE: The cells can be used starting at day three after thawing (two days after the first medium change).

IX. Medium Change of Cellartis Cardiomyocytes

At day one after thawing, the non-attached cells are washed away and the medium is changed. After day one, the medium should be changed every second to third day.

A. Preparation

- Prepare Cellartis CM Culture Medium as described in section VII.B. Warm to 37°C ± 1°C.

B. Medium Change Day 1

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

1. Wash the cells carefully by aspirating the Cellartis CM Thawing Medium and gently flush the cells with the medium two to three times in order to remove non-attached cells.
2. Aspirate the old medium and add warm Cellartis CM Culture Medium (0.4–0.6 ml/cm^2).
3. Return the cell culture unit to an incubator with 37°C ± 1°C, 5% CO_2, and >90% humidity.

C. Medium Change Day 3 Onwards

1. Gently aspirate the entire volume of medium from the culture vessel and discard.
2. Add warm Cellartis CM Culture Medium, 0.4–0.6 ml/cm^2.
3. Return the cell culture vessel to the incubator with 37°C ± 1°C, 5% CO_2, and >90% humidity.

X. Re-seeding of Cellartis Cardiomyocytes in Application Formats

Seeding density and coating are dependent on the intended application and may require optimization. A recommended starting point is to seed 1.2–1.5 x 10^5 viable cells/cm^2 on culture vessels coated with 7.3 µg fibronectin/cm^2.

1. Warm an appropriate amount of Cellartis CM Culture Medium to 37°C ± 1°C. Add Y-27632 to a final concentration of 5 µM prior to use.
2. Aspirate the medium from each well containing cardiomyocytes.
3. Rinse the wells with D-PBS –/–, using approximately 0.25 ml/cm^2.
4. Add 0.25 % trypsin-EDTA to each well (~100 µl/cm^2) and incubate for 2–4 min.
5. Gently detach the cells by dispensing the dissociation solution over the surface using a 1 ml pipette.
6. Add one volume (~100 µl/cm^2) of Cellartis CM Culture Medium (with 5 µM Y-27632) to each well to deactivate the trypsin.
7. Transfer the cell suspension into a suitable tube.
8. Count the cardiomyocytes.
9. Centrifuge the cells at 200 x g for 5 min at RT.
10. Aspirate the supernatant and gently resuspend the cell pellet in an appropriate volume of Cellartis CM Culture Medium (with 5 µM Y-27632) to the desired concentration.
11. Seed the cells at the desired density, using 0.4–0.6 ml/cm^2 Cellartis CM Culture Medium (with 5 µM Y-27632).

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