TECHNICAL MANUAL

Maintenance of Cellartis® hiPS-CM and Cellartis® Pure hES-CM

Contents

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General information

Cellartis® hiPS-CM

Catalogue number:	CM-208-VIAL-KIT/ Y10070	1 frozen vial of Cellartis® hiPS-CM (CM-208-VIAL-01/Y10071) 1 frozen bottle à 32 mL of Cellartis® CM Thawing Base (CTB-502-0040/Y10062) 1 frozen bottle à 90 mL of Cellartis® CM Culture Base (CCB-503-0100/Y10063)

Cellartis® hiPS-CM are human cardiomyocytes derived from an induced pluripotent stem cell line reprogrammed using episomal technology. The cells have been differentiated to spontaneously beating cardiomyocytes *in vitro*. The cells have subsequently been dissociated to a single cell suspension and frozen in vials.

Cellartis® Pure hES-CM

Catalogue number:	CM-203-VIAL-KIT/ Y10060	1 frozen vial of Cellartis® Pure hES-CM (CM-203-VIAL-01/ Y10061) 1 frozen bottle à 32 mL of Cellartis® CM Thawing Base (CTB-502-0040/Y10062) 1 frozen bottle à 90 mL of Cellartis® CM Culture Base (CCB-503-0100/Y10063)

Cellartis® Pure hES-CM are human cardiomyocytes derived from a genetically modified human embryonic stem cell line. The cells have been differentiated to spontaneously beating cardiomyocytes *in vitro* and purified. The cells have subsequently been dissociated to a single cell suspension and frozen in vials.

Cellartis® hiPS-CM and Pure hES-CM are shipped on dry ice or in a dry shipper and should be handled according to “Unpacking and Handling” upon arrival. Cellartis® CM Thawing Base and CM Culture Base are provided frozen and shipped on dry ice.

Thawed and plated cardiomyocytes can be used from day 3 after thawing, the day after the first medium change. The cardiomyocytes can be maintained in culture for at least 14 days after thawing under the recommended conditions. For applications where non-standard culture formats are used, it is recommended to thaw the cells in 6 or 12-well tissue culture-treated plates for optimal recovery. When the cells have recovered, day 3 after thawing, the cells can be dissociated and moved to the preferred culture/assay format, see dissociation protocol on page 5.

We recommend that this product is handled only by persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good cell culture practice.

Additional Material Required

Product	Suggested Manufacturer	Catalogue number
Fetal Bovine Serum (FBS)	Gibco/Life Technologies	16140
Fibronectin	Sigma-Aldrich	F0895
PBS Dulbecco's with Ca^{2+} & Mg^{2+}	Gibco/Life Technologies	14040
PBS Dulbecco's w/o Ca^{2+} & Mg^{2+} *	Gibco/Life Technologies	14190
Trypsin-EDTA (0.25%), phenol red *	Gibco/Life Technologies	25200
Y-27632	Sigma-Aldrich	Y0503

* Only needed if dissociating Cellartis® hiPS-CM or Pure hES-CM.

Other Equipment Needed

General cell culture equipment used in cell culture laboratory.

Product Quality

Takara Bio Europe AB recommends the use of media and reagents according to this manual. Takara Bio Europe AB cannot give technical feedback on customer cultures unless the below culture instructions have been followed.

Methods

NOTE! Always work under aseptic conditions.

Unpacking of Cellartis® hiPS-CM, Pure hES-CM, CM Thawing Base, and CM Culture Base

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 cryo vial may explode due to rapid temperature changes.

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

1. Unpack Cellartis® hiPS-CM and/or Cellartis® Pure hES-CM as soon as possible after arrival.
2. Check all materials for leakage or breakage.
3. Using forceps immediately place the vial in liquid nitrogen at ≤ -150°C.
4. Cellartis® CM Thawing Base and CM Culture Base should be stored at ≤ -15°C upon arrival.

Storage and Handling of Cellartis® hiPS-CM, Pure hES-CM, CM Thawing Base, and CM Culture Base

Cellartis® hiPS-CM and Pure hES-CM should be stored at ≤ -150°C. Under recommended storage conditions the cells can be stored for up to one year from date of receipt.

Keep thawed cells at +37±1ºC, 5% CO_2, and > 90% humidity.

Cellartis® CM Thawing Base and CM Culture Base should be stored at ≤ -15°C and expire according to the label. Complete Cellartis® CM Thawing and Culture Medium should be prepared as described below.

Cellartis® CM Medium Preparation

Cellartis® CM Thawing Medium

1. Thaw Cellartis® CM Thawing Base.
2. Decontaminate the external surface of all supplements and medium bottle with 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 +2-8°C and expires one month after the date of preparation.
5. Before use, add Y-27632 to a final concentration of 10 µM, and warm to RT (+15-25°C). Discard any leftover warm Cellartis® CM Thawing Medium.

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 +2-8°C and expires one month after the date of preparation.
5. Warm to +37±1°C before use. Discard any leftover warm Cellartis® CM Culture Medium.

Coating of Cell Culture Units

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

Thawing of Cardiomyocytes

It is recommended to thaw a maximum of 2-3 vials at one time. The recommended seeding density is 130k viable cells/cm^2.

Preparation

1. Prepare appropriate volume of Cellartis® CM Thawing Medium with Y-27632. Warm to RT.
2. Coat the appropriate number of cell culture units according to “Coating of cell culture units”.

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 cryo vial may explode due to rapid temperature changes.

Thawing Cells

1. Transfer, as quickly as possible, the frozen vials from liquid nitrogen to a +37±1°C water bath.
2. Thaw the cells without swirling the vials. Take the vials out of the water bath as soon as the thawing is completed (approximately 3 minutes, the vials should still be cold on the outside).
3. Wipe the vials with appropriate disinfectant and place into the biological safety cabinet.
4. As soon as possible, transfer the cell suspension into a sterile 50 mL tube.
5. Rinse the vial with 1 mL of Cellartis® CM Thawing Medium and carefully add it to the cell suspension one drop every 2-3 second with gentle swirling in between drops.
6. Add 8 mL of Cellartis® CM Thawing Medium slowly. Add 1 mL with a rate of 2-3 drops at a time gently swirling the tube in between, and the last 7 mL at a rate of 6-8 drops at a time.
7. Centrifuge the tube at 200g at RT, for 5 minutes and remove the supernatant.
8. Carefully resuspend the cell pellet with Cellartis® CM Thawing Medium, using 1.5-4 mL/vial.
9. Count the cells and measure viability.
10. Add Cellartis® CM Thawing Medium to desired cell density and seed the cells, using 0.5-0.6 mL medium/cm^2. Make sure the entire surface is covered with medium.
11. Transfer the cell culture unit to an incubator with +37±1°C, 5% CO_2, and >90% humidity and leave untouched for 48 hours.
12. Perform a medium change (see below), exchanging the entire volume of Cellartis® CM Thawing Medium to Cellartis® CM Culture Medium. Work very gently at this point in order not to dislodge the cells.

NOTE! The cells can be used from day 3 after thawing, the day after the first medium change.

Medium Change of Cardiomyocytes

Change medium every 2nd to 3rd day.

1. Prepare appropriate volume of Cellartis® CM Culture Medium, 0.5-0.6 mL medium/cm^2, and warm to +37±1°C.
2. Gently remove the entire volume of medium from the well or dish and discard.
3. Add warm Cellartis® CM Culture Medium to achieve the final volume.
4. Return the cell culture unit to +37±1°C.

Collecting Cardiomyocytes from the Tissue Culture Plate

Seeding density is dependent on application and may require optimization but a recommended starting point is 130k viable cells/cm^2.

1. Warm an appropriate amount of Cellartis® CM Culture Medium to 37°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 without Ca^{2+}/Mg^{2+}.
4. Add 0.25 % trypsin-EDTA to each well (~100 µL per cm^2) and incubate for 2-4 minutes.
5. Gently detach the cells by dispensing the dissociation solution over the surface using a 1 mL pipette.
6. Add 1 volume (~100 µL per 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 200g for 5 minutes (at room temperature).
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 final concentration.

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