Takara Bio Europe AB

Cellartis® hiPS Beta Cells Kits User Manual

Cat. Nos. Y10100 & Y10106
(020218)

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I. Introduction

The Cellartis hiPS Beta Cells Kits provide cryopreserved beta cells derived from Cellartis human induced pluripotent stem (hiPS) cell lines. These hiPS cells have been differentiated *in vitro*, dissociated into a single cell suspension, and frozen in vials. The kits are frozen and contain beta cells, a coating substrate, two types of basal media, and supplement.

The beta cell kits are available with beta cells derived from two hiPS different cell lines; ChiPSC12 and ChiPSC22. The beta cells have been derived using an identical manufacturing protocol thus sharing a lot of features and functionalities.

This product should only be handled by persons who have been trained in laboratory techniques and should only be used 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 technical performance unless the subsequent culture instructions have been followed.

II. List of Components

- **Cellartis hiPS Beta Cells (from ChiPSC12) Kit (Cat. No. Y10100)**
    - Cellartis hiPS Beta Cells (from ChiPSC12), 1 vial (≥ 4.8 x 10^6 viable cells/vial) (Cat. No. Y10101; not sold separately)
    - Cellartis Beta Cell Supplement, 11 x 360 µl (Cat. No. Y10102; not sold separately)
    - Cellartis Beta Cell Coating, 4 ml (Cat. No. Y10103; not sold separately)
    - Cellartis Beta Cell Basal Medium, 160 ml (Cat. No. Y10104; not sold separately)
    - Cellartis Beta Cell Basal Medium 2, 30 ml (Cat. No. Y10105; not sold separately)
- **Cellartis hiPS Beta Cells (from ChiPSC22) Kit (Cat. No. Y10106)**
    - Cellartis hiPS Beta Cells (from ChiPSC22), 1 vial (≥ 4.8 x 10^6 viable cells/vial) (Cat. No. Y10107; not sold separately)
    - Cellartis Beta Cell Supplement, 11 x 360 µl (Cat. No. Y10102; not sold separately)
    - Cellartis Beta Cell Coating, 4 ml (Cat. No. Y10103; not sold separately)
    - Cellartis Beta Cell Basal Medium, 160 ml (Cat. No. Y10104; not sold separately)
    - Cellartis Beta Cell Basal Medium 2, 30 ml (Cat. No. Y10105; not sold separately)

III. Additional Material Required

The following materials are required but not supplied:

- 24-well plate (cell culture vessel), tissue-culture-treated polystyrene surface
- General cell culture equipment used in cell culture laboratory

IV. General Considerations

A. Storage and Handling

Component	Storage	Expiration/Stability	Handling
Cellartis hiPS Beta Cells	Liquid nitrogen (–196°C) or –150°C	Under recommended storage conditions, the beta cells can be stored for up to one year from the date of receipt.	Incubate at 37°C ± 1°C, 5% CO2, and ≥90% humidity.
Cellartis Beta Cell Basal Medium & Cellartis Beta Cell Basal Medium 2	–20°C	Shelf life specified on product label. Thawed medium should be stored at 2–8°C and used within 1 month.	Thaw overnight at 2–8°C.
Cellartis Beta Cell Supplement	–20°C	Shelf life specified on product label.	Light sensitive; avoid exposure to light. Thaw immediately before use.
Cellartis Beta Cell Coating	–20°C	Shelf life specified on product label.	Thaw immediately before use.

V. Culture of Cellartis hiPS Beta Cells

Cellartis hiPS beta cells are thawed, resuspended in Cellartis Beta Cell Maintenance Medium and plated in culture vessels coated with Cellartis Beta Cell Coating. It is recommended that the cells are seeded in 24-well plates; one kit is sufficient for 12 wells.

Cellartis hiPS beta cells express some insulin when cryopreserved. When the beta cells are cultured in the Cellartis Beta Cell Maintenance Medium, an enrichment of beta cells will occur during the first days, as evidenced by the detachment of non-beta cells. The recommended assay window is between days 8 and 16 post-thaw.

VI. Coating of Cell Culture Vessels

1. Thaw the frozen Cellartis Beta Cell Coating at Room Temperature (RT, 15–25°C).

NOTE: In case of any visible precipitates upon thawing of Beta Cell Coating we recommend mixing the solution properly when the solution has reached RT. Mix by inverting the vial multiple times.

2. Add 300 µl/well of Cellartis Beta Cell Coating to 12 wells of a 24-well plate.
3. Incubate for 3 hr at RT or 1 hr at 37°C.
4. Aspirate Cellartis Beta Cell Coating from the wells immediately prior to seeding.

VII. Medium Preparation

Combine the Cellartis Beta Cell Supplement with the Cellartis Beta Cell Basal Medium to prepare the Cellartis Beta Cell Maintenance Medium or with Cellartis Beta Cell Basal Medium 2 to prepare the Cellartis Beta Cell Medium 2. Thaw Cellartis Beta Cell Basal Medium and Cellartis Beta Cell Basal Medium 2 overnight at 2–8°C. Once the basal medium has been thawed, it can be store in 2–8°C for 1 month. Each vial of Cellartis Beta Cell Supplement should be thawed at RT immediately before use.

A. Cellartis Beta Cell Maintenance Medium

1. Warm 12.9 ml of Cellartis Beta Cell Basal Medium to 37°C ± 1°C.
2. Thaw one vial of Beta Cell Supplement (360 µl) and add it to the 12.9 ml of pre-warmed Cellartis Beta Cell Basal Medium. **This is the Cellartis Beta Cell Maintenance Medium.**
3. Prepare the Cellartis Beta Cell Maintenance Medium immediately prior to use. Discard any excess warmed medium.

B. Cellartis Beta Cell Medium 2

1. Warm 12.9 ml of Cellartis Beta Cell Basal Medium 2 to 37°C ± 1°C.
2. Thaw one vial of Beta Cell Supplement (360 µl) and add it to the 12.9 ml of pre-warmed Cellartis Beta Cell Basal Medium 2. **This is the Cellartis Beta Cell Medium 2.**
3. Prepare the Cellartis Beta Cell Medium 2 immediately prior to use. Discard any excess warmed medium.

VIII. Thawing of Cellartis hiPS Beta Cells

Cellartis hiPS beta cells is resuspended and seeded in Cellartis Beta Cell Maintenance Medium. Seed the cells from one vial into 12 coated wells of a 24-well plate, generating a cell density of at least 2.0 x 10^5 viable cells/cm^2. Use 0.5 ml/well in a 24-well plate.

A. Preparation

- Coat the appropriate number of cell culture vessels with Cellartis Beta Cell Coating according to Section VI. The cell suspension is sufficient for 12 wells in a 24-well plate.
- Prepare the appropriate volume of Cellartis Beta Cell Maintenance Medium according to Section VII.A.

NOTE: For your protection, wear a protective face mask and protective gloves. Use forceps when handling frozen vials. Never hold the vial in your hand as the cryovial may explode due to rapid temperature changes.

B. Thawing Cells

NOTE: Recently thawed Cellartis hiPS beta cells are fragile, so please follow the thawing instructions closely.

1. Transfer the vial directly from liquid nitrogen or –150°C freezer to a 37°C ± 1°C water bath using forceps.
2. The cell suspension should be thawed until the ice has almost fully melted.
3. Decontaminate the external surface of the vial with an appropriate disinfectant and place into the biological safety cabinet.
4. Pour the thawed cell suspension into 4 ml of 37°C ± 1°C Cellartis Beta Cell Maintenance Medium. Wash the vial with 1 ml of 37°C ± 1°C Cellartis Beta Cell Maintenance Medium and add to the cells.
5. Centrifuge at 300*g* at RT for 2 min.
6. Using a pipette, remove the supernatant. Loosen the cell pellet by flicking the tube and re-suspend the cell pellet very carefully by slowly adding 6.5 ml of 37°C ± 1°C Cellartis Beta Cell Maintenance Medium.
7. Remove the Cellartis Beta Cell Coating from the wells immediately prior to seeding the cells.
8. Carefully mix the cell suspension and seed 500 µl/well of cell suspension into the coated 24-well plate.
9. Incubate the culture vessel at 37°C ± 1°C, 5% CO_2, and ≥90% humidity and leave the cells undisturbed until the next day.

C. Thawing Multiple Vials

Several vials can be thawed at the same time using the protocol in Section VIII.B. Consider the following points when thawing multiple vials:

- Scale up the volume of Cellartis Beta Cell Maintenance Medium in Step 4.
- Pour the thawed cell suspensions from all vials into the total volume of Cellartis Beta Cell Maintenance Medium.

- For seeding, scale up the volume of Cellartis Beta Cell Maintenance Medium in Step 6.
- Ideally, plates should be spread out in the incubator (avoid stacking several plates) to allow them to warm quickly.

IX. Maintenance of Cellartis hiPS Beta Cells

The day after thawing, the medium is replaced with freshly prepared Cellartis Beta Cell Maintenance Medium. Subsequently, the media is changed every day or every second day. 1–3 days prior to performing your assay, you should switch to using Cellartis Beta Cell Medium 2. A suggested workflow is depicted in Table 1.

A. Day 1

Replace Cellartis Beta Cell Maintenance Medium. Use 1 ml per well of the 24-well plate. Use manual pipetting for media changes. **Do not use a suction pump.** During media changes, minimize the time cells are left without medium.

1. Preparation

Prepare and warm an appropriate volume of Cellartis Beta Cell Maintenance Medium to 37°C ± 1°C, as described in Section VII.A.

2. Media Change

1. Carefully remove media from the cells by tilting the plate and pipetting from the edge of the well to avoid harming the cells.
2. Add 1 ml of warm Cellartis Beta Cell Maintenance Medium to each well by gently pipetting medium at the edge of the well.
3. Incubate the cells at 37°C ± 1°C, 5% CO_2, and ≥90% humidity.
4. Discard any unused, warmed Cellartis Beta Cell Maintenance Medium.

B. Day 2 or 3 and Onward

The Cellartis Beta Cell Maintenance Medium should be changed every day or every second day. Do not maintain the beta cells without medium change for longer than 48 hr. The media change should be performed as described in Section IX.A.

NOTE: When the Cellartis hiPS beta cells are cultured in the Cellartis Beta Cell Maintenance Medium, an enrichment of beta cells will occur during the first days, as evidenced by the detachment of non-beta cells. This pronounced cell loss is normal and to be expected. If the Cellartis Beta Cell Maintenance Medium turns yellow or if you observe extensive cell loss, perform a daily media change.

C. Preparing for Assay

1–3 days prior to performing your assay, you should switch to using Cellartis Beta Cell Medium 2. A media change (using Cellartis Beta Cell Medium 2) should be performed the day before the assay.

1. Preparation

Prepare and warm an appropriate volume of Cellartis Beta Cell Medium 2 to 37°C ± 1°C as described in Section VII.B.

2. Media Change

1. Carefully remove media from the cells by tilting the plate and pipetting from the edge of the well to avoid harming the cells.
2. Add 1 ml of warm Cellartis Beta Cell Medium 2 to each well by gently pipetting medium at the edge of the well.

3. Incubate the cells at 37°C ± 1°C, 5% CO_2, and ≥90% humidity.
4. Discard any unused, warmed Cellartis Beta Cell Medium 2.

Table 1. Suggested culture schedule for Cellartis hiPS beta cells.

Day	Suggested Weekday	Media Change (Sections IX.A & IX.B)	Notes
0	Wednesday		Thawing and Plating
1	Thursday	Cellartis Beta Cell Maintenance Medium	
2	Friday	Cellartis Beta Cell Maintenance Medium	Insulin positive cells
3	Saturday	–	
4	Sunday	Cellartis Beta Cell Maintenance Medium	
5	Monday	Cellartis Beta Cell Maintenance Medium	
6	Tuesday	–	
7	Wednesday	Cellartis Beta Cell Maintenance Medium	
8	Thursday	–	Suggested assay window.
9	Friday	Cellartis Beta Cell Maintenance Medium	
10	Saturday	–	
11	Sunday	Cellartis Beta Cell Maintenance Medium	
12	Monday	Cellartis Beta Cell Medium 2	
13	Tuesday	–	
14	Wednesday	Cellartis Beta Cell Medium 2	
15	Thursday	Assay	
16	Friday		

NOTE: 1–3 days prior to performing your assay, switch to using Cellartis Beta Cell Medium 2. A media change (using Cellartis Beta Cell Medium 2) should be performed the day right before the assay. In this example, the assay is performed on day 15, and thus Cellartis Beta Cell Medium 2 is used on Days 12 and 14.

X. Morphology of Cellartis hiPS Beta Cells

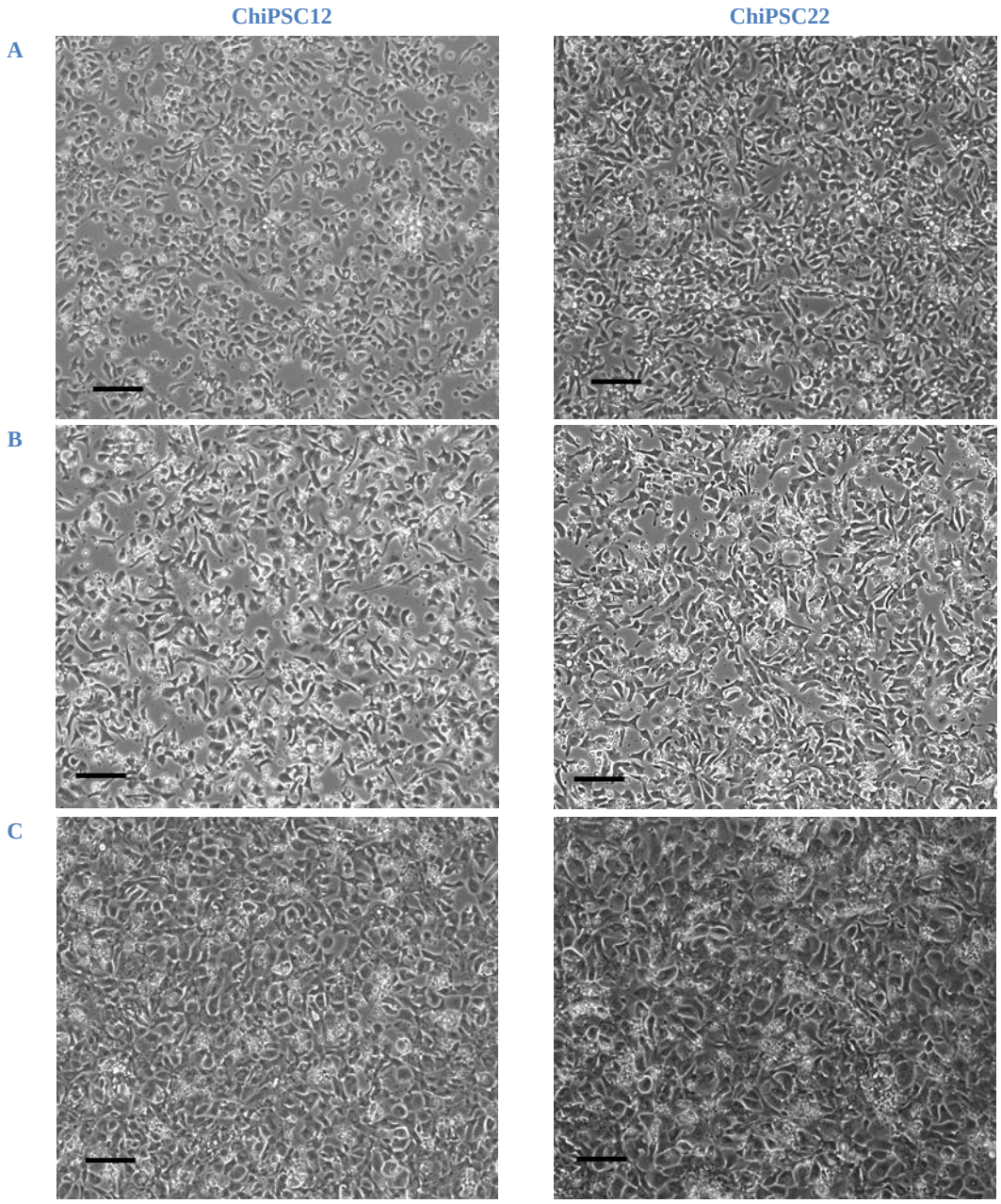

Figure 1. Morphological analysis of Cellartis hiPS beta cells from ChiPSC12 and ChiPSC22. Cells were thawed and cultured according to the protocol, and images were taken on Day 1 (**Panels A**), Day 5 (**Panel B),** and Day 14 (**Panel C**) post-thaw**.** For all images, the scale bar is 100 µm.

Notice to Purchaser

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.

This product may not be resold or transferred, modified for resale or transfer, or used to manufacture commercial products without written approval from Takara Bio Europe AB.

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This document has been reviewed and approved by the Takara Bio Europe AB Quality Assurance Department.