Takara Bio Europe AB

Cellartis® Definitive Endoderm Cells (from ChiPSC18) User Manual

Cat. No. Y10040
(111815)

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I. Introduction

Cellartis Definitive Endoderm Cells are composed of human definitive endoderm (DE) cells derived from human induced pluripotent stem (iPS) cell lines. The cells have been differentiated to definitive endoderm *in vitro* to generate a population of >90% SOX-17 protein expressing cells, dissociated to a cell suspension, and frozen in vials. These definitive endoderm cells are suitable for further differentiation into hepatocytes as well as differentiation into pancreatic endoderm cells.

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.

II. List of Components

- Cellartis Definitive Endoderm Cells (from ChiPSC18) (Cat. No. Y10040)

III. Additional Material Required

The following materials are required but not supplied:

- Y-27632
- Cell culture vessels with tissue culture-treated polystyrene surface
- General cell culture equipment used in cell culture laboratory

The following coatings are recommended when seeding DE cells, but not supplied:

- Cellartis HEP Coat (Takara Clontech, Cat. No. Y10052)
- Cellartis DEF-CS™ 500 COAT-1 (Takara Clontech, Cat. No. Y30012)
- iMatrix-511 (Takara Clontech, Cat. No. T300)

The following material are recommended for hepatocyte differentiation, but not supplied:

- Cellartis Hepatocyte Differentiation Kit (Takara Clontech, Cat. No. Y30050)

IV. General Considerations

A. Storage and Handling

Cellartis Definitive Endoderm Cells (from ChiPSC18) should be stored at ≤ –150°C. Under recommended storage conditions, the cells can be stored for up to one year from the date of receipt.

Culture thawed Cellartis Definitive Endoderm Cells (from ChiPSC18) at 37°C ± 1°C, 5% CO_2 and >90% humidity.

B. Culture of Cellartis Definitive Endoderm Cells (from ChiPSC18)

Cellartis Definitive Endoderm Cells can be thawed and plated into any format, e.g. 96-well plates, 24-well plates, or T25 cell culture flasks. One vial contains 6 x 10^6 viable DE cells.

Cellartis Definitive Endoderm Cells (from ChiPSC18) can preferably be thawed and differentiated into hepatocytes by using the Cellartis Hepatocyte Differentiation Kit. Methods for thawing and further differentiating the DE cells can be found in the Cellartis Hepatocyte Differentiation Kit User Manual.

For other applications, DE cells can be seeded on Cellartis HEP Coat, Cellartis DEF-CS COAT-1, iMatrix-511, or other substrates. Thawing on other coatings or in other media may require optimization, depending on the chosen application.

NOTE: Always work under aseptic conditions.

V. Thawing Cellartis Definitive Endoderm Cells (from ChiPSC18)

NOTE: Thawed Cellartis Definitive Endoderm Cells (from ChiPSC18) are fragile. Avoid using a pipette for mixing the cell suspension, if possible. Only use the pipette to seed the cells.

A. Preparations

- Prepare the appropriate thawing and/or plating medium and warm to 37°C ± 1°C.
- Coat the cell culture vessels with an appropriate coating solution; see section V.C for suggestions.
- Add 10 µM of Y27632 (ROCK inhibitor) to the seeding media.

B. Thawing Cells

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.

- One vial of thawed Cellartis Definitive Endoderm Cells contains 6.0 x 10^6 viable cells.
- Suggested seeding density is 1.25 x 10^3 viable cells/cm^2. Seeding density may be optimized for certain applications.
- The thawing procedure and media for thawing and/or plating of Cellartis Definitive Endoderm Cells are dependent on the application. For differentiation to hepatocytes using the Cellartis Hepatocyte Differentiation Kit, please refer to Cellartis Hepatocyte Differentiation Kit User Manual.
- Optional: Count the viable cells upon thawing.

C. Procedure for Recommended Coatings

Coating of Cell Culture Vessels with Cellartis HEP Coat

1. Thaw the frozen Cellartis HEP Coat overnight at 4°C. Keep thawed Cellartis HEP Coat at 2–8°C until use.
2. Add the Cellartis HEP Coat to the cell culture vessels (0.15 ml/cm^2). Make sure the entire surface of each vessel is covered.
3. Incubate at room temperature (RT, 15–25°C) for 30–60 min.
4. Aspirate excess Cellartis HEP Coat from the cell culture vessels just before seeding.

The cell culture vessels are now coated and can be used for seeding of Cellartis Definitive Endoderm Cells.

Coating of Cell Culture Vessels with Cellartis DEF-CS COAT-1

1. Dilute the required volume of Cellartis DEF-CS COAT-1 1:20 in D-PBS (+/+) before use.
2. Mix the diluted Cellartis DEF-CS COAT-1 solution gently and thoroughly by pipetting up and down.
3. Add the appropriate volume of diluted Cellartis DEF-CS COAT-1 solution to the cell culture flasks (use 0.1 ml/cm^2), making sure that the entire surface is covered.
4. Place the cell culture vessels in the incubator at 37°C ±1°C for a minimum of 20 minutes or at RT for 0.5–3 hours.
5. Aspirate the Cellartis DEF-CS COAT-1 solution from the cell culture vessels just before use.

The cell culture vessels are now coated and can be used for seeding of Cellartis Definitive Endoderm Cells.

VI. RNA Profile of Cellartis Definitive Endoderm Cells (from ChiPSC18)

Figure 1 shows the RNA profile of hiPS cell line ChiPSC18 differentiated to Cellartis Definitive Endoderm Cells (from ChiPSC18). The mRNA levels were analyzed using RT-qPCR.

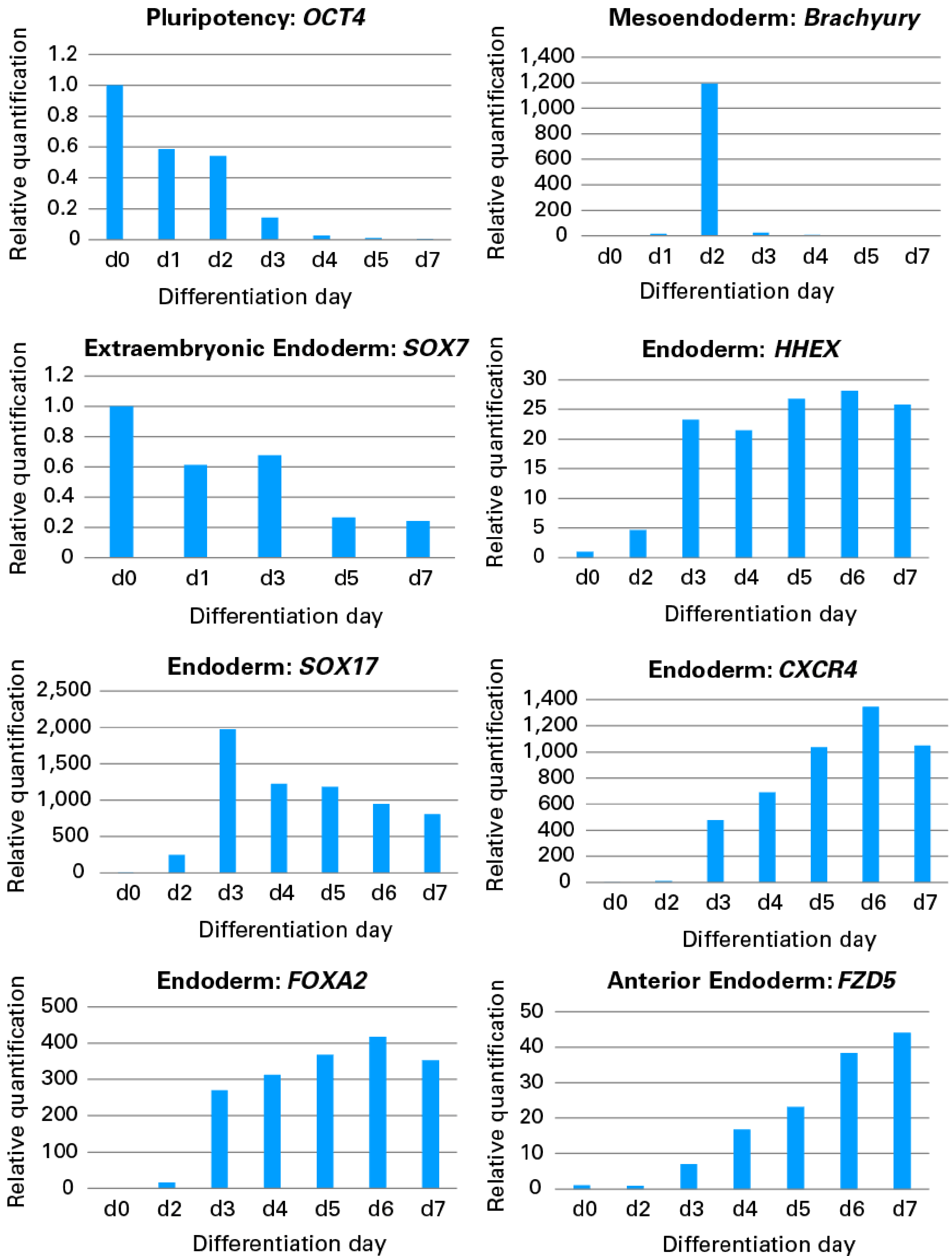


Figure 1. RNA profile of Cellartis Definitive Endoderm Cells (from ChiPSC18).

VII. Images of Cellartis Definitive Endoderm Cells (from ChiPSC18)

Figure 2 shows an example of typical morphology two days after thawing Cellartis Definitive Endoderm Cells (from ChiPSC18) using the Cellartis Hepatocyte Differentiation Kit. Figure 3 shows immunocytochemistry (ICC) results for Cellartis Definitive Endoderm Cells (from ChiPSC18) cells that were stained two days after thawing.

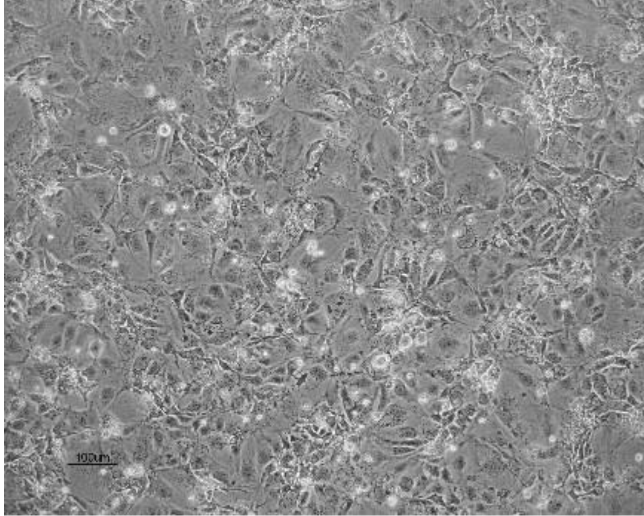
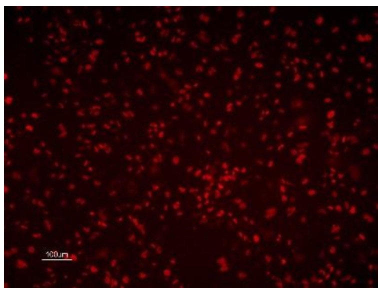


Figure 2. Cellartis Definitive Endoderm Cells (from ChiPSC18). Morphology two days after thawing. Scale bar corresponds to 100 µm.



Sox-17

Oct-4

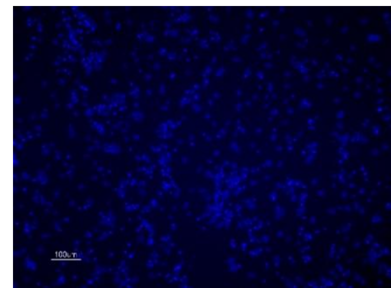


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Figure 3. ICC results for Cellartis Definitive Endoderm Cells (from ChiPSC18). Cellartis Definitive Endoderm Cells was thawed and fixed two days after thawing. The cells were stained against Oct-4 and Sox-17. Scale bar corresponds to 100 µm.

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