Takara Bio Europe AB

Cellartis® DEF-CS™ Xeno-Free Culture Media User Manual

Cat. Nos. Y30045
(030917)

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I. Introduction

Cellartis DEF-CS Xeno-Free Culture Media are pre-clinical grade, chemically defined culture media that are free from human- and animal-derived components and are used for efficient expansion of undifferentiated human induced pluripotent stem (iPS) cells.

All procedures described in the manual are optimised for Cellartis human iPS cell lines. If you wish to use Cellartis DEF-CS Xeno-Free Culture Media for other human induced pluripotent stem cells, please be aware that procedures and protocols may have to be adjusted.

This product should only be handled by persons who have been trained in laboratory techniques 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. 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 DEF-CS Xeno-Free Culture Medium without Antibiotics (Cat. No. Y30045)

- **500 ml Cellartis DEF-CS 500 Xeno-Free Basal Medium without Antibiotics (Cat. No Y30046; not sold separately)**
- **Cellartis DEF-CS 500 Xeno-Free Additives (Cat. No. Y30042)**
    - 500 µl DEF-CS Xeno-Free Additive 1 (1000X)
    - 200 µl DEF-CS Xeno-Free Additive 2 (1000X)

III. Additional Materials Required

The following materials are required but not supplied:

- Culture substrate:
    - Corning Synthemax II-SC Substrate (Corning, Cat. No. 3535) or
    - iMatrix-511 (Takara Clontech, Cat. No. T303)
- PBS Dulbecco's with Ca^{2+} & Mg^{2+} (D-PBS +/+)
- PBS Dulbecco's w/o Ca^{2+} & Mg^{2+} (D-PBS –/–)
- Versene Solution (Life Technologies, Cat. No. 15040)
- Cell culture vessels, tissue culture treated polystyrene surface
- General cell culture equipment used in cell culture laboratory

If applicable

- Gentamicin (Life Technologies, Cat. No. 15750)

IV. General Considerations

A. Storage and Handling

Cellartis DEF-CS Xeno-Free Basal Medium without Antibiotics (Cat. No. Y30046) should be stored at 4°C and expires according to the label.

Cellartis DEF-CS Xeno-Free Additives 1 and 2 should be stored at –20°C; shelf life specified on product label. At first use, thaw provided vials and aliquot each component separately into appropriate volumes (mix gently before aliquoting). Store at –20°C according to expiry date on original vial. Thawed vials may be stored at 4˚C for up to one week. Do not subject the additives to more than a single refreeze and thaw cycle.

B. Antibiotics

If you elect to use an antibiotic in your medium, we recommend using Gentamicin. Add 0.5 ml to 500 ml of Cellartis DEF-CS Xeno-Free Basal Medium without Antibiotics for a final concentration of 50 mg/l.

V. Culture of Human iPS Cells in Cellartis DEF-CS Xeno-Free Culture Media

A schematic picture of the thawing, maintenance and cryopreservation of hiPS cell lines in Cellartis DEF-CS Xeno-Free Culture Media is shown in Figure 1. The cell expansion capability for 500 ml of Cellartis DEF-CS Xeno-Free Culture Media is: 20x T25 or 8x T75 or 4x T150 flasks.

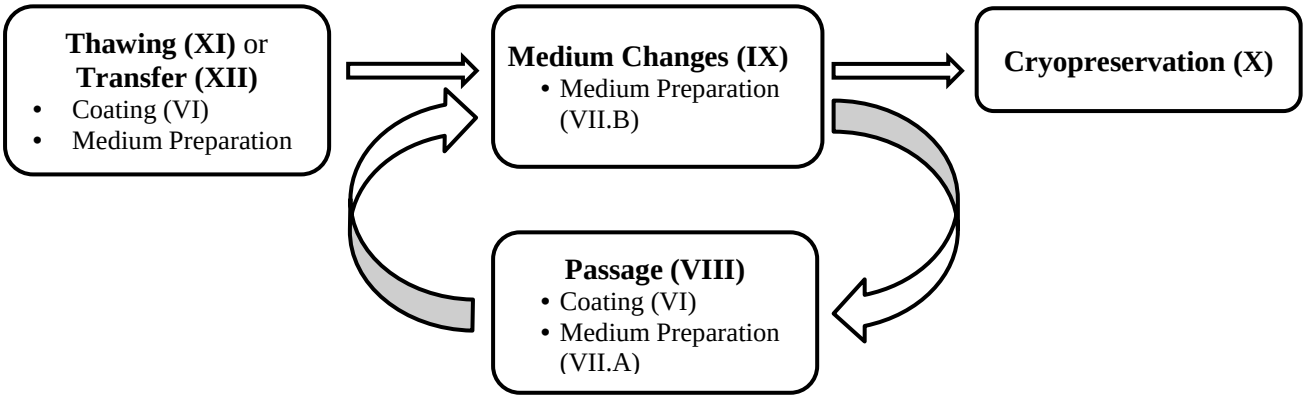

Figure 1. Schematic presentation of the Cellartis DEF-CS Xeno-Free Culture Medium work flow. Corresponding sections of this user manual are referenced in brackets.

Human iPS cell lines that are maintained in Cellartis DEF-CS Xeno-Free Culture Media should be passaged every 3–4 days with daily medium changes. When the cell density is sparse, you can change the medium every other day, however it is always important to change medium the day after passage or thawing, and the day before passage or freezing. It is recommended that the cells are grown to a maximum confluence of 1.5–3.0 x 10^5 cells/cm^2. A suggestion for weekly schedule are depicted in Table I.

Table I. Weekly Schedule

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Passage	Medium Change	Medium Change	Passage	Medium Change	-	Medium Change

NOTE: Always work under aseptic conditions.

VI. Coating of Cell Culture Vessels

Coat the appropriate cell culture vessel with Synthemax or iMatrix according to manufacturers' instructions. The recommended concentrations may need to be optimized for certain cell lines.

VII. Cellartis DEF-CS Xeno-Free Medium Preparation

A. Medium for Thawing or Passage of Human iPS Cells

1. Decontaminate the external surfaces of all additives and the medium bottle with an appropriate disinfectant and place into the biological safety cabinet.
2. The appropriate volume of supplemented Cellartis DEF-CS Xeno-Free Medium is prepared by adding Cellartis DEF-CS Xeno-Free Additive 1 (dilute 1:1000) and Additive 2 (dilute 1:1000) to Cellartis DEF-CS Xeno-Free Basal Medium.
3. Medium should be freshly prepared on the day of use. Discard any left-over warm medium.

B. Medium for Maintenance of Human iPS Cells

1. Decontaminate the external surfaces of all additives and the medium bottle with an appropriate disinfectant and place into the biological safety cabinet.
2. The appropriate volume of supplemented Cellartis DEF-CS Xeno-Free Medium is prepared by adding Cellartis DEF-CS Xeno-Free Additive 1 (dilute 1:1000) to Cellartis DEF-CS Xeno-Free Basal Medium. Do not add Cellartis DEF-CS Xeno-Free Additive 2 to maintenance medium.
3. Medium should be freshly prepared on the day of use. Discard any left-over warm medium.

VIII. Passage of Human iPS Cells

As a general rule, cells should be seeded at a density of 3–4 x 10^4 cells/cm^2 (use 4 x 10^4 cells/cm^2 if leaving the cells three days and 3 x 10^4 cells/cm^2 if leaving the cells four days in between passages). This can be adjusted to suit the cell line as appropriate.

When passaging the cells it is highly recommended that the cells are grown to a confluence of 1.5–3 x 10^5 cells/cm^2; see Figure 3 and Figure 5 for corresponding images of human iPS cells in culture. The cultures may not look so dense, but for consistent cell growth it is important to keep the cell density between 1.5–3 x 10^5 cells/cm^2 at day of passage. If cells are allowed to grow to a higher density, it will have an impact for the next passage, and some cell lines might also be at increased risk of unwanted differentiation. If cultures should appear suboptimal after a few passages, it is recommended to decrease or increase the seeding density. The passage interval may have to be adjusted accordingly.

A. Preparations

Cell culture flasks should be coated as described above. The appropriate volume of supplemented Cellartis DEF-CS Xeno-Free Medium for Thawing or Passage should be prepared as described in Section VII.A and warmed to 37°C ± 1°C before use. Warm all other reagents to RT before use.

B. Passage

1. Check cells under microscope; photo document as necessary.
2. Aspirate medium from cell culture flasks and wash the cell layer once with D-PBS (–/–).
3. Add 0.1 ml/cm^2 of Versene to the cell culture flasks and incubate for 20 minutes or until the cells round up. Detachment should be aided by beating the side of the cell culture flask firmly or by hitting the short side of the culture flask against the bench 3–5 times. Avoid flushing the cell layer for detachment if possible.
4. Dilute the cells in supplemented Cellartis DEF-CS Xeno-Free Medium (1:1 dilution) and pipette up and down several times to ensure a single cell suspension is achieved.
5. Centrifuge the cells at 300 x g for 2–5 minutes.
6. Resuspend the cells in the supplemented Cellartis DEF-CS Xeno-Free Medium.
7. Count the cells in a haemocytometer or in a cell counter (optimized for the cell type).
8. Add the appropriate volume of cell suspension and medium to the newly coated cell culture flasks to obtain the selected density. The seeding volume of supplemented Cellartis DEF-CS Xeno-Free Medium should be 0.3 ml/cm^2.
9. Tilt the flask backwards and forwards gently to ensure the cell suspension is dispersed evenly over the surface and place in the incubator.

IX. Medium Change for Human iPS Cells

Medium change is recommended daily (except day of passage). Use 0.3–0.4 ml/cm^2. If the medium colour turns yellow due to high metabolic activity, the medium volume should be increased.

A. Preparation

The appropriate volume of supplemented Cellartis DEF-CS Xeno-Free Medium for Maintenance should be prepared as described in Section VII.B and warmed to 37°C ±1°C before use. Discard any leftover warm medium.

B. Medium Change

1. Check cells under microscope; photo document as necessary.
2. Carefully aspirate the medium and pipette newly warmed medium into the cell culture flask. Avoid flushing medium directly on the cell layer.
3. Place the cell culture flask in the incubator.

X. Cryopreservation of Human iPS Cells

Human iPS cells cultured in Cellartis DEF-CS Xeno-Free Culture System can be cryopreserved by using common slow freezing protocols for cell suspensions using STEM-CELLBANKER (Zenoaq Resource Co.Ltd., Cat. No. ZR636). As a general guide, 2.5–3.5 x 10^6 cells in 1 ml freezing medium should be frozen in a 2 ml cryovial.

XI. Thawing of Human iPS Cells

When thawing human iPS cells in Cellartis DEF-CS Xeno-Free Culture Medium, approximately 1.5–2.0 x 10^5 cells/cm^2 should be seeded in 0.3–0.4 ml medium/cm^2.

A. Preparations

Cell culture vessels should be coated as described above. Supplemented Cellartis DEF-CS Xeno-Free Medium for Thawing or Passage should be prepared as described in Section VII.A and warmed to the appropriate temperature, see below for the recommended volumes and temperatures.

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 cryo vial may explode due to rapid temperature changes.

1. Transfer 9 ml of supplemented Cellartis DEF-CS Xeno-Free Medium to a sterile centrifuge tube and warm to RT.
2. Using forceps, transfer the vial directly into a container with 37°C ± 1°C water. Thaw the vial by gently pushing it under the surface of the water. Do not submerge the cap of the vial in the water bath as this could contaminate the cells.
3. Allow the vial to thaw until the cell suspension can be poured out of the vial, with frozen parts of cell suspension still left in the vial.
4. Decontaminate the vial in appropriate disinfectant.
5. Pour the content of the vial into the sterile tube containing 9 ml supplemented Cellartis DEF-CS Xeno-Free Medium (RT).
6. Use 1 ml supplemented Cellartis DEF-CS Xeno-Free Medium, warmed to RT, to rinse the vial. Add to the cell suspension.
7. Centrifuge at 300g for 1 minute.

8. After centrifugation, aspirate the supernatant and gently resuspend the pellet in a volume corresponding to 0.3–0.4 ml supplemented Cellartis DEF-CS Xeno-Free Medium/cm^2 (37°C ± 1°C), resulting in approximately 1.5–2.0 x 10^5 cells/cm^2.
9. Pipette the cell suspension into the cell culture unit.
10. Ensure the cells and medium are evenly distributed across the surface of the cell culture unit and place the cell culture unit in the incubator.

XII. Transfer of Human iPS Cells to Cellartis DEF-CS Xeno-Free Culture Medium from Other Culture Media

Human pluripotent stem cells maintained in other culture systems can readily be transferred to Cellartis DEF-CS Xeno-Free Culture Medium. Fresh cultures can be transferred and cryopreserved cultures can be thawed directly into the Cellartis DEF-CS Xeno-Free Culture Medium. The cells might need up to five passages to adjust to the new culture medium.

The normal Cellartis DEF-CS Xeno-Free Culture Medium protocol should be followed although some considerations might need to be taken into account.

A. Passage Interval

When seeding human pluripotent stem cells previously cultured in a different culture system, the cells might initially grow differently than in the former system. Depending on the confluence of the cell monolayer, the suitable interval might be between three to seven days for the first passages. The cells should adapt to the morphology as displayed in Figure 3 and Figure 5 prior to passage. If the cells are still sparse after seven days in culture, a passage is still recommended.

XIII. Images of Human iPS Cells Maintained in Cellartis DEF-CS Xeno-Free Culture Medium

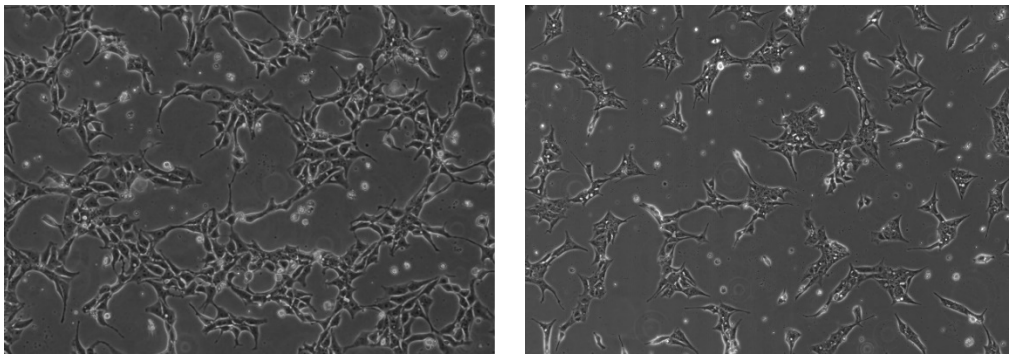

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Figure 2. Human iPS cells in Cellartis DEF-CS Xeno-Free Culture Medium using Synthemax. Day after passage.

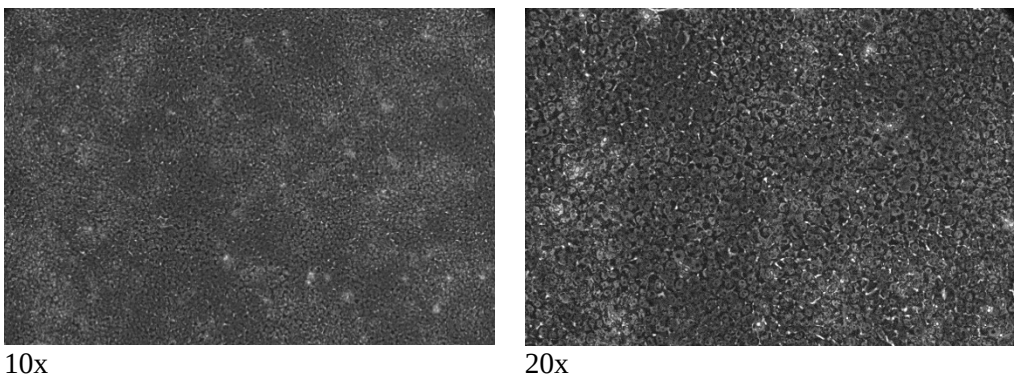

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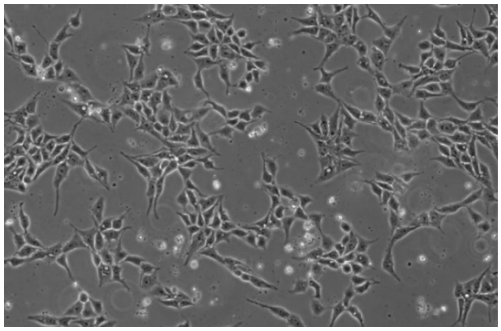
Figure 3. Human iPS cells in Cellartis DEF-CS Xeno-Free Culture Medium using Synthemax. Just prior to passage.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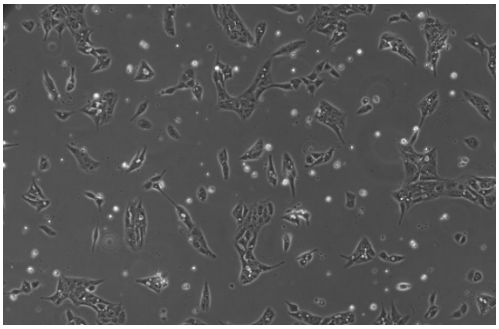
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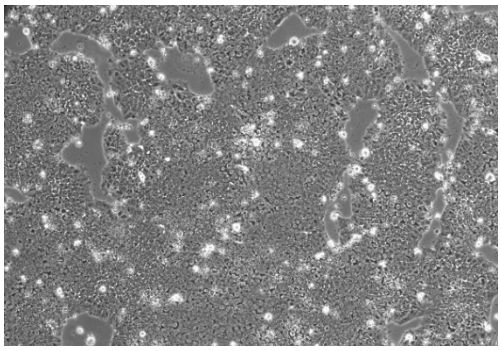
Figure 4. Human iPS cells in Cellartis DEF-CS Xeno-Free Culture Medium using iMatrix. Day after passage.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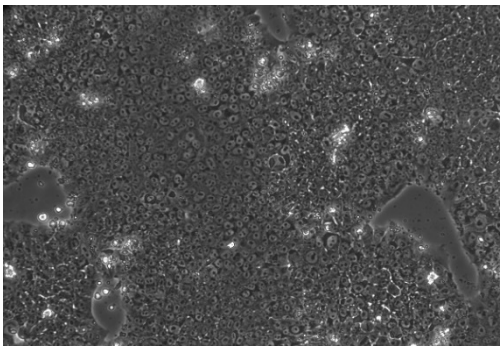
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Figure 5. Human iPS cells in Cellartis DEF-CS Xeno-Free Culture Medium using iMatrix. Just prior to passage.

Appendix A. Troubleshooting Guide

Table II. Troubleshooting Guide

Problem	Possible Explanation	Solution
Cells detach/round up	iMatrix-511 coated surface has dried out.	Add some medium to the surface directly after the coating solution has been removed.
Cells detach	Too low concentration of coating solution, or too short period of coating.	Try other concentrations of coating solution. Coat for a longer period.
Cells do not detach at passage	Too small volume of Versene, too short treatment.	Increase volume to 0.2 ml/cm^2. Use warmed solution. Treat the cells longer in incubator (up to 30 minutes).
Cells do not detach even though Versene is used as described	Different cell lines can react differently to Versene.	Flush off the cells with pipette. Though the cells are quite robust during Versene treatment and flushing, one should account for increased cell death and try to adjust the seeding density accordingly.
The cell density at passage vary considerably	Over-compensated cell seeding at previous passages.	Try to keep passage intervals and seeding densities as persistent as possible, i.e. try to not compensate a slow growth for the next passage, or vice versa.
The cells seem to differentiate	The concentration of coating solution, or the particular coating solution used.	Increase or decrease the coating solution concentration. Try a different coating; some cell lines might not be compatible with Synthemax or iMatrix.
The cells seem to differentiate	Too small media volumes used between passages. Some cell lines have a higher metabolic activity, though they do not necessarily divide faster.	Increase the media volumes used, especially if the medium has turned yellow at higher densities before medium change.
Transferred cells do not adapt to Cellartis DEF-CS Xeno-Free Culture Medium	The cells are not used to the new environment.	The cells could benefit from a higher seeding density for the first few passages, e.g. 8x10^4 cells/cm^2.

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