Takara Bio Europe AB

Cellartis® DEF-CS™ 500 Xeno-Free GMP Grade Basal Medium User Manual

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(010820)

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I. Introduction

Cellartis DEF-CS 500 Xeno-Free GMP Grade Basal Medium is a chemically defined basal media that is free from human- and animal-derived components and is used for the efficient expansion of undifferentiated human pluripotent stem (hPS) cells. Cellartis DEF-CS 500 Xeno-Free GMP Grade Basal Medium is manufactured as a quality-assured product, according to guidelines for Good Manufacturing Practice (GMP) for Medicinal Products.

The procedures described in the manual relate to non-colony type monolayer culture and have been optimised for use with Cellartis hPS cell lines. If you wish to use Cellartis DEF-CS 500 Xeno-Free GMP Grade Basal Medium for other human induced pluripotent stem cells, please be aware that procedures and protocols may have to be adjusted.

Cellartis DEF-CS 500 Xeno-Free GMP Grade Basal Medium can also be used for dynamic suspension culture of human induced pluripotent stem cells as 3D spheroids. A separate protocol for 3D spheroid suspension culture is available upon request.

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. 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 500 Xeno-Free GMP Grade Basal Medium (Cat. No. Y30071)

III. Additional Materials Required

The following materials are required but not supplied:

- Culture substrate: Corning Synthemax II-SC Substrate (Corning, Cat. No. 3535)
- bFGF (Recombinant Human FGF basic GMP; Peprotech, Cat. No. 233-GMP)
- Y-27632, MF (Wako Pure Chemical Industries, Ltd. Cat. No. 257-00613)
- Versene Solution (Thermo Fisher, Cat. No. 15040)
- Albix (Recombinant human albumin solution) 10 % (w/v) (Novozymes, Cat. No. 205-005)
- PBS Dulbecco's with Ca^{2+} & Mg^{2+} (D-PBS +/+)
- PBS Dulbecco's w/o Ca^{2+} & Mg^{2+} (D-PBS –/–)
- Sterile water
- Cell culture vessels, tissue culture treated polystyrene surface
- General cell culture equipment used in cell culture laboratory

Compatible research-grade components:

- bFGF (Recombinant Human FGF basic GMP; Peprotech, GMP100-18B)
- Y-27632 from (Sigma Aldrich, Cat. No. Y0503)
- Culture substrate: iMatrix-511 (Cat. No. T303)
- Cellartis Human ES Cell Line 121 (SA121) Kit (Cat No. Y00025)
- Cellartis Human ES Cell Line 167 (SA167) Kit (Cat. No. Y00065)
- Cellartis Human ES Cell Line 181 (SA181) Kit (Cat. No. Y00105)
- Cellartis Human ES Cell Line 461 (SA461) Kit (Cat. No. Y00145)
- Cellartis Human iPS Cell Line 7 (ChiPSC7) Kit (Cat. No. Y00275)
- Cellartis Human iPS Cell Line 12 (ChiPSC12) Kit (Cat. No. Y00285)
- Cellartis Human iPS Cell Line 18 (ChiPSC18) Kit (Cat. No. Y00305)
- Cellartis Human iPS Cell Line 22 (ChiPSC22) Kit (Cat. No. Y00325)

IV. General Considerations

A. Storage and Handling

Cellartis DEF-CS 500 Xeno-Free GMP Grade Basal Medium (Cat. No. Y30071) should be stored at 2–8°C and expires according to the label.

V. Culture of hPS Cells in Cellartis DEF-CS 500 Xeno-Free GMP Grade Medium

A schematic picture of the thawing, maintenance and cryopreservation of hPS cell lines in Cellartis DEF-CS 500 xenofree GMP grade medium is shown in Figure 1. The cell expansion capability for 500 ml of medium is: 20x T25 or 8x T75 or 4x T150 flasks or equivalent.

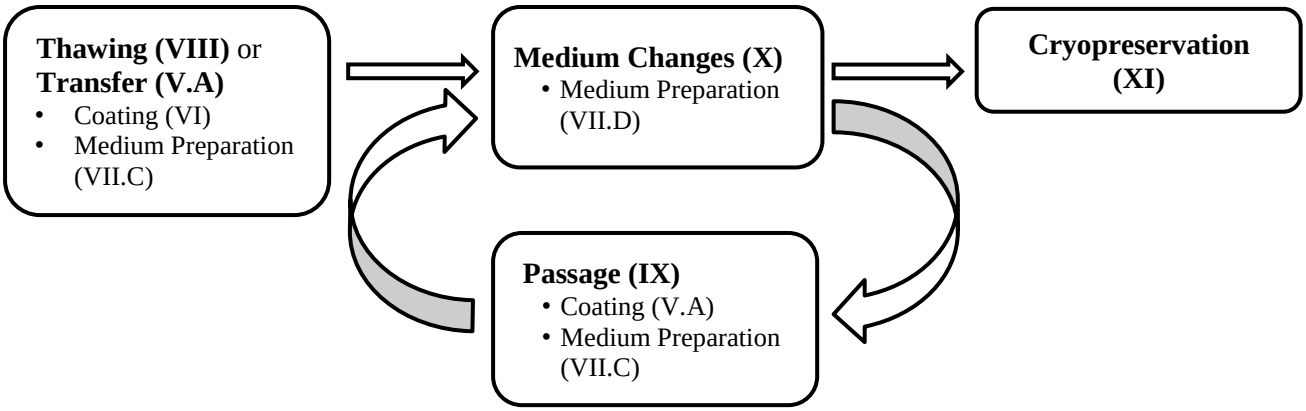

Figure 1. Schematic presentation of the Cellartis DEF-CS xeno-free GMP grade medium work flow. Corresponding sections of this user manual are referenced in brackets.

All hPS cell lines that are maintained using this workflow 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 a weekly schedule is 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.

A. Transfer of hPS Cells to Cellartis DEF-CS DEF-CS Xeno-Free Free GMP Grade Medium

Undifferentiated hPS cells maintained in other culture systems can be readily transferred to Cellartis DEF-CS xeno-free GMP grade medium. Fresh cultures can be transferred at passage, (Section IX.C) and cryopreserved cultures can be thawed using Cellartis DEF-CS xeno-free GMP grade medium (Section VIII.C). It takes between 2 and 5 passages to adapt a cell line to the Cellartis DEF-CS xeno-free GMP grade medium.

VI. Coating Cell Culture Vessels

Coat the appropriate cell culture vessels with Synthemax II-SC Substrate according to manufacturers' instructions. The recommended concentrations may need to be optimized for certain cell lines.

VII. Preparing Cellartis DEF-CS 500 Xeno-Free GMP Grade Medium

A. bFGF Stock Solution

1. Decontaminate the external surfaces of all reagents with an appropriate disinfectant and place into the biological safety cabinet.
2. Prepare bFGF stock solution by dissolving the bFGF in 0.1% Albix in D-PBS to a final concentration of 0.1 mg/ml.
3. Aliquot the stock solution and store at –20°C. Aliquots can be stored at –20°C for 12 months after the date of preparation. Thawed vials may be stored at 2–8˚C for up to one week. Do not subject the aliquots to more than a single thaw and refreeze cycle.

B. Y-27632 Stock Solution

1. Decontaminate the external surfaces of all reagents with an appropriate disinfectant and place into the biological safety cabinet.
2. Prepare the Y-27632 stock solution by diluting Y-27632 in sterile water to a final concentration of 5 mM.
3. Aliquot the stock solution and store at –20°C. Aliquots can be stored at –20°C for 12 months after the date of preparation. Thawed vials may be stored at 2–8˚C for up to one week. Do not subject the aliquots to more than a single thaw and refreeze cycle.

C. Medium for Thawing or Passaging of hPS Cells

1. Decontaminate the external surfaces of reagents and the medium bottle with an appropriate disinfectant and place into the biological safety cabinet.
2. Prepare the appropriate volume of "Cellartis DEF-CS xeno-free GMP grade medium for thawing or passaging" by adding bFGF (dilute stock solution 1:1,000 to a final concentration of 100 ng/ml) and Y-27632 (dilute stock solution 1:1,000 to a final concentration of 5 µM) to Cellartis DEF-CS 500 Xeno-Free GMP-Grade Basal Medium.
3. Medium should be freshly prepared on the day of use. Discard any leftover warm medium.

D. Medium for Maintenance of hPS Cells

1. Decontaminate the external surfaces of all reagents and the medium bottle with an appropriate disinfectant and place into the biological safety cabinet.
2. Prepare the appropriate volume of "Cellartis DEF-CS xeno-free GMP grade medium for maintenance" by adding bFGF (dilute stock solution 1:1,000 to a final concentration of 100 ng/ml) to Cellartis DEF-CS 500 Xeno-Free GMP Grade Basal Medium. Do not add Y-27632 to maintenance medium.
3. Medium should be freshly prepared on the day of use. Discard any leftover warm medium.

VIII. Thawing hPS Cell Lines

When thawing hPS cells for use in this workflow, 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 with Synthemax II-SC Substrate according to manufacturers' instructions; the recommended concentrations may need to be optimized for certain cell lines. Cellartis DEF-CS xeno-free GMP grade medium for thawing or passaging should be prepared as described in Section VII.C 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 cryovial may explode due to rapid temperature changes.

1. Transfer 9 ml of Cellartis DEF-CS xeno-free GMP grade medium for thawing or passaging 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 contents of the vial into the sterile tube containing 9 ml Cellartis DEF-CS xeno-free GMP grade medium for thawing or passaging at RT.
6. Use 1 ml Cellartis DEF-CS xeno-free GMP grade medium for thawing or passaging, warmed to RT, to rinse the vial. Add to the cell suspension.
7. Centrifuge at 300*g* for 1 minute.
8. After centrifugation, aspirate the supernatant and gently resuspend the pellet in a volume corresponding to 0.3–0.4 ml Cellartis DEF-CS xeno-free GMP grade medium for thawing or passaging per 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.

C. Thawing Cells from Other Culture Systems

Cryopreserved cells can be thawed directly into Cellartis DEF-CS xeno-free GMP grade medium for thawing or passaging. The standard thawing protocol should be followed, although some modifications may increase the success of transfer:

- The cells may benefit from a higher concentration of coating.
- The cells might initially grow at a slightly slower rate. A suitable passage interval might therefore be between three and seven days for the first few passages. The cells should adapt to the morphology as displayed in Figure 3 and Figure 5 prior to passage. If the cells are sparse after seven days in culture, a passage is still recommended.

IX. Passaging hPS Cell Lines

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 density of 1.5–3 x 10^5 cells/cm^2; see Figure 2 for corresponding images of hPS cells in culture. Please note that the cells will cover the surface of the culture vessel (be confluent) at a cell density of approximately 0.8–1 x 10^5 cells/cm^2. Do not passage the cells for at least another day, to achieve the recommended density at passage. If cells are passaged too soon, it might have a negative impact on the growth rate during the next passage, and some cell lines might also be at increased risk of unwanted differentiation. If cells are allowed to grow to density of > 3 x 10^5 cells/cm^2, it might have a negative impact on the growth rate during the next passage. 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 vessels should be coated as described above. The appropriate volume of Cellartis DEF-CS xeno-free GMP grade medium for thawing or passaging should be prepared as described in Section VII.C and warmed to 37°C ± 1°C before use. Warm all other reagents to RT before use.

B. Passaging

1. Check cells under microscope; photo document as necessary.
2. Aspirate medium from the cell culture vessel and wash the cell layer once with D-PBS (–/–).
3. Add 0.1 ml/cm^2 of Versene to the cell culture vessel and incubate for 20 minutes or until the cells round up. Tap the side of the cell culture vessel firmly against the bench 3-5 times to detach cells. Detachment should be aided by beating the side of the cell culture vessel firmly or by hitting the short side of the culture vessel against the bench 3–5 times. Avoid flushing the cell layer with Versene to detach cells.
4. Dilute the cells in Cellartis DEF-CS xeno-free GMP grade medium for thawing or passaging (1:1 dilution) and pipette up and down several times to achieve a single cell suspension.
5. Centrifuge the cells at 300*g* for 2–5 minutes.
6. Resuspend the cells in the Cellartis DEF-CS xeno-free GMP grade medium for thawing or passaging.
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 vessel to obtain the selected density. The seeding volume of Cellartis DEF-CS xeno-free GMP grade medium for thawing or passaging should be 0.3 ml/cm^2.
9. Tilt the vessel backwards and forwards gently to ensure the cell suspension is dispersed evenly over the surface and place in the incubator.

C. Transfer from Other Culture Systems at Passage

Fresh cultures can be transferred to Cellartis DEF-CS xeno-free GMP grade medium at passage. The cells should be dissociated according to the protocol of the previous system, seeded as single cells or aggregates using a 1:1 split ratio based on culture area. Some modifications may increase the success of transfer:

- The cells may benefit from a higher concentration of coating.
- Newly transferred cells might initially grow at a slightly slower rate. A suitable passage interval might therefore be between 3 and 7 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 sparse after seven days in culture, a passage is still recommended.

X. Changing Medium for hPS Cell Lines

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

A. Preparation

The appropriate volume of Cellartis DEF-CS xeno-free GMP grade medium for maintenance should be prepared as described in section VII.D 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 vessel. Avoid flushing medium directly on the cell layer.
3. Place the cell culture vessel in the incubator.

XI. Cryopreserving hPS Cell Lines

Cellartis hPS cells cultured using this workflow can be cryopreserved by using common slow freezing protocols for cell suspensions using STEM-CELLBANKER® GMP Grade (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.

XII. Images of hPS Cells Maintained in Cellartis DEF-CS Xeno-Free GMP Grade Culture Medium

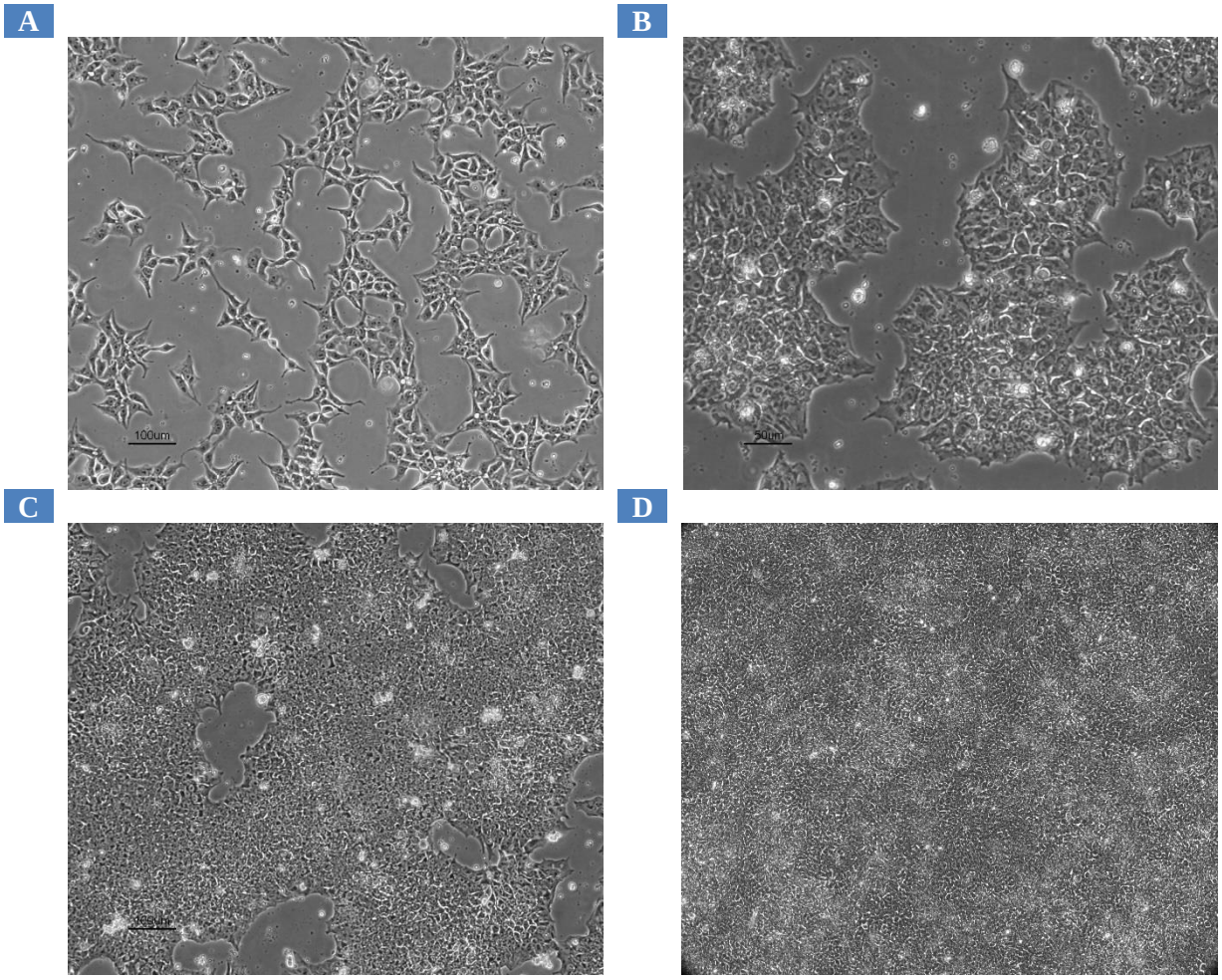

Figure 2. hPS cells in complete Cellartis DEF-CS xeno-free GMP grade culture medium using Synthemax. Panel A. Day 1 after passage (seeded at 3 x 10^4 cells/cm^2) (10X). **Panel B.** Day 2 after passage (approximate density 1 x 10^5 cells/cm^2) (20X). **Panel C.** Day 3 after passage (approximate density 3 x 10^5 cells/cm^2) (10X). **Panel D.** Day 4 after passage (approximate density 4 x 10^5 cells/cm^2) (10X).

Appendix A. Troubleshooting Guide

Table II. Troubleshooting Guide

Problem	Possible Explanation	Solution
Cells detach/round up	Synthemax coated surface has dried out.	Add some medium to the surface directly after the coating solution has been removed.
Cells detach prior to passage	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 varies considerably	Over-compensated cell seeding at previous passages.	Try to keep passage intervals and seeding densities as consistent as possible, i.e. try to not compensate a slow growth for the next passage, or vice versa.
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 GMP grade 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. 6–8x10^4 cells/cm^2.

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