Takara Bio Europe AB

Cellartis® DEF-CS™ Culture System User Manual

Cat. No. Y30010 and Y30020
(111518)

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I. Introduction

Cellartis DEF-CS 500 Culture System is a complete system for efficient expansion and scale-up manufacturing of human pluripotent stem (hPS) cells in a feeder-free and defined environment.

All procedures described in the manual are optimized for Cellartis hPS cell lines. If you wish to use Cellartis DEF-CS Culture Medium 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 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

A. Cellartis DEF-CS 500 Culture System (Cat. No. Y30010)

- 500 ml Cellartis DEF-CS 500 Basal Medium (Cat. No. Y30011; not sold separately)
- 4 ml Cellartis DEF-CS 500 COAT-1 (Cat. No. Y30012)
- Cellartis DEF-CS 500 Additives (Cat. No. Y30016; not sold separately)
    - 2 x 750 µl DEF-CS GF-1
    - 500 µl DEF-CS GF-2
    - 200 µl DEF-CS GF-3

III. Additional Materials Required

The following materials are required but not supplied:

- PBS Dulbecco's with Ca^{2+} & Mg^{2+} (D-PBS +/+)
- PBS Dulbecco's w/o Ca^{2+} & Mg^{2+} (D-PBS –/–)
- TrypLE Select Enzyme (1X), no phenol red
- Cell culture vessels, Tissue culture treated polystyrene surface
- General cell culture equipment used in cell culture laboratory

IV. Recommended Materials

The following materials are recommended but not supplied:

- 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)

V. General Considerations

A. Storage and Handling

Cellartis DEF-CS Basal Medium and Cellartis DEF-CS COAT-1 should be stored at 2–8°C; shelf life specified on product label. The Cellartis DEF-CS Basal Medium formulation contains Penicillin and Streptomycin.

Cellartis DEF-CS Additives (GF-1, GF-2, and GF-3) 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 2–8°C for up to one week. Do not re-freeze aliquots after thawing.

NOTE: All three Cellartis DEF-CS Additives (GF-1, GF-2 and GF-3) are used when thawing and passaging hPS cells. Only Additives DEF-CS GF-1 and DEF-CS GF-2 are needed when changing medium on hPS cells.

VI. Culturing of hPS Cells in Cellartis DEF-CS Culture System

A schematic picture of thawing, maintenance (medium changes and passage), and cryopreservation of hPS cell lines in Cellartis DEF-CS Culture System is shown in Figure 1. The cell expansion capability for 500 ml of Cellartis DEF-CS Culture Medium is: 20x T25 or 8x T75 or 4x T150 flasks.

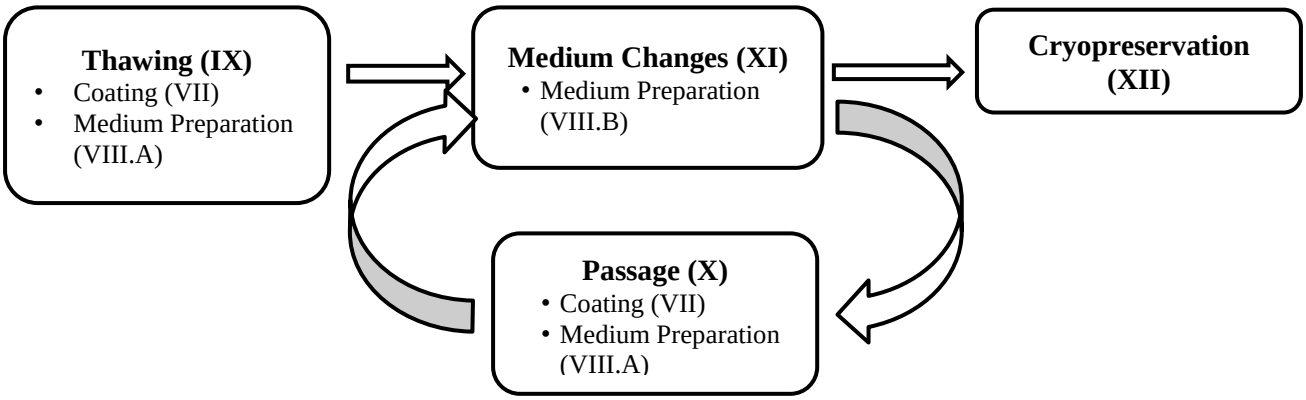

Figure 1. Schematic presentation of the Cellartis hPS cell line work flow. Corresponding sections of this user manual are referenced in brackets.

All hPS cell lines that are maintained in Cellartis DEF-CS should be passaged every three to four days, with daily medium changes. When the cell density is sparse, you can change the medium every other day; however, it is 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 confluence of 1.5–3.0 x 10^5 cells/cm^2. A suggested weekly schedule is depicted in Table I.

Table I. Weekly schedule for medium changes and passaging.

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

NOTE: Always work under aseptic conditions.

A. Transfer of hPS Cells to Cellartis DEF-CS Culture System

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

When initially transferring hPS cells to this system, some cell characteristics might be different from previously used culture systems. First, the Cellartis DEF-CS system utilizes single-cell passaging, and therefore the morphology of cells cultured in the Cellartis DEF-CS system differs from that of cells cultured in systems using aggregate passaging methods. Second, newly passaged cells tend to spread out. However, when proliferating, the cells get denser, and the typical undifferentiated stem cell morphology (i.e., high nucleus to cytoplasm ratio, defined borders, and prominent nucleoli) appears.

VII. Coating Cell Culture Vessels

1. Dilute the required volume of Cellartis DEF-CS COAT-1 in D-PBS +/+ before use. Make a 1:20 dilution.
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), make sure the entire surface is covered.
4. Place the cell culture flasks for a minimum of 20 min in an incubator at 37°C ± 1°C, 5% CO_2, and >90% humidity or 0.5–3 hr at room temperature (RT, 15–25°C).
5. Aspirate Cellartis DEF-CS COAT-1 solution from cell culture flasks just before seeding of the cells.

Table II. Recommended volumes of COAT-1 for different cell culture vessels

Format	COAT-1 solution (1:20 dilution) (ml)	Format	COAT-1 solution (1:20 dilution) (ml)
6-well	1.5	T75 flask	7.5
T12.5 flask	1.25	T150 flask	15.0
T25 flask	2.5	T225 Flask	22.5

VIII. Preparing Cellartis DEF-CS Medium

A. Medium for Thawing or Passaging hPS Cells

1. Decontaminate the external surface of all additives and the medium bottle with an appropriate disinfectant and place in the biological safety cabinet.
2. Prepare the appropriate volume of “Cellartis DEF-CS medium for thawing or passaging” by adding DEF-CS GF-1 (dilute 1:333), GF-2 (dilute 1:1000), and GF-3 (dilute 1:1000) to Cellartis DEF-CS Basal Medium.
3. Prepare fresh medium on the day of intended use.

Table III. Recommended volumes for seeding of the cell suspension at passage, for different cell culture vessels

Format	DEF-CS medium (ml)	Format	DEF-CS medium (ml)
6-well	2.0	T75 flask	15.0
T12.5 flask	3.0	T150 flask	25.0
T25 flask	4.0	T225 Flask	35.0

B. Medium for Maintenance of hPS Cells

1. Decontaminate the external surface of all additives and the medium bottle with an appropriate disinfectant and place into the biological safety cabinet.
2. Prepare the appropriate volume of "Cellartis DEF-CS medium for maintenance" by adding DEF-CS GF-1 (dilute 1:333) and GF-2 (dilute 1:1000) to Cellartis DEF-CS Basal Medium.
   NOTE: Do not add DEF-CS GF-3 to the maintenance medium.
3. Prepare fresh medium on the day of intended use.

Table IV. Recommended volumes of DEF-CS medium at medium change, for different cell culture vessels

Format	DEF-CS medium (ml)	Format	DEF-CS medium (ml)
6-well	4.0	T75 flask	20.0
T12.5 flask	4.0	T150 flask	40.0
T25 flask	7.0	T225 Flask	60.0

IX. Thawing hPS Cell Lines

When thawing hPS cells in Cellartis DEF-CS Culture System, approximately 1.5–2.5 x 10^5 cells/cm^2 should be seeded in 0.3–0.4 ml medium/cm^2.

A. Preparations

Coat cell culture vessels as described above (Section VII). Prepare Cellartis DEF-CS medium for thawing or passaging as described above (Section VIII.A) and warm it to the appropriate temperature. See below for recommended volumes.

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 4 ml of Cellartis DEF-CS medium for thawing or passaging to a sterile centrifuge tube and warm to RT.
2. Using forceps, transfer the vial directly from liquid nitrogen into a container of 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. (It is okay if the suspension has a slushy consistency, as long as it can be poured out.)
4. Decontaminate the vial in an appropriate disinfectant.
5. Pour the entire contents of the vial into the sterile tube containing 4 ml Cellartis DEF-CS medium for thawing or passaging (RT).
6. Rinse the vial with 1 ml Cellartis DEF-CS medium for thawing or passaging, warmed to RT. Add to the cell suspension.
7. Centrifuge at 300 x g for 1 minute.
8. After centrifugation, aspirate the supernatant and gently resuspend the pellet in Cellartis DEF-CS medium for thawing or passaging (37°C ±1°C).
9. Count the cells in a haemocytometer or in a cell counter (optimized for the cell type).
10. Dilute the cell suspension to achieve a seeding density of 1.5–2.5 x 10^5 cells/cm^2 in 0.3–0.4 ml medium/cm^2.
11. Aspirate the COAT-1 solution.
12. Pipet the cell suspension into the cell culture vessel.
13. Ensure that the cells and medium are evenly distributed across the surface of the cell culture vessel and place the cell culture vessel in an incubator at 37°C ± 1°C, 5% CO_2, and >90% humidity.

C. Thawing Cells from Other Culture Systems

Cryopreserved cells can be thawed directly into the DEF-CS Culture System. 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 Cellartis DEF-CS COAT-1. Use a dilution of 1:5 at thawing and the first few passages to provide extra support during the adaptation period.
- 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 are ready for passage when they have acquired the morphology displayed in Figure 3 and Figure 4. If the cells are sparse after seven days in culture, a passage is still recommended.

X. Passaging hPS Cell Lines

As a general rule, cells should be seeded at a density of 4.0–5.0 x 10^4 cells/cm^2 (use 4.0 x 10^4 cells/cm^2 if leaving the cells four days between passages and 5.0 x 10^4 cells/cm^2 if leaving three days between passages). Adjust the density to suit your particular cell line as appropriate. When passaging the cells, we strongly recommend growing them to a confluence of 1.5–3.0 x 10^5 cells/cm^2 (see Figures 2–4 for images of a variety of Cellartis hPS cell lines in culture).

A. Preparations

Coat cell culture flasks as described above (Section VII). Prepare the appropriate volume of Cellartis DEF-CS medium for thawing or passaging as described above (Section VIII.A) and warm it to 37°C ± 1°C before use. Warm all other reagents to RT before use. Discard any leftover warmed medium.

B. Passaging

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 20 µl/cm^2 of TrypLE Select to the cell culture flasks and incubate in an incubator at 37°C ± 1°C for 5 minutes or until the cell layer has detached. Detachment can be aided by tapping the side of the cell culture flask firmly but gently. It is not recommended to tilt or swirl the cell culture flask.
4. Resuspend the cells in Cellartis DEF-CS medium for thawing or passaging and pipet up and down several times to ensure a single cell suspension. (The cells will aggregate if left too long in TrypLE Select).

Table V. Recommended volumes of TrypLE Select (1X) and DEF-CS medium for resuspension for different cell culture vessels

Format	TrypLE Select (1X) (ml)	DEF-CS medium for resuspension (ml)	Format	TrypLE Select (1X) (ml)	DEF-CS medium for resuspension (ml)
6-well	0.3	1.7	T75 flask	1.5	8.5
T12.5 flask	0.3	1.7	T150 flask	3	17
T25 flask	0.5	2	T225 Flask	4.5	25.5

5. **OPTIONAL:** (To remove TrypLE Select.) Centrifuge the cells at 200 x g for 2–5 minutes. There is no need to centrifuge the cell suspension after dissociation if the TrypLE Select will be diluted at least 1:10 after the adjustment of the medium volume to 0.15–0.25 ml/cm^2.
6. Count the cells in a haemocytometer or in a cell counter (optimized for the cell type).
7. Aspirate the COAT-1 solution.
8. Add the appropriate volume of cell suspension to the newly coated cell culture flasks to obtain the selected density. Adjust the medium volume of Cellartis DEF-CS medium for thawing or passaging to 0.15–0.25 ml/cm^2.
9. Tilt the flask backwards and forwards gently to ensure that the cell suspension is dispersed evenly over the surface, then place in an incubator at 37°C ± 1°C, 5% CO_2, and >90% humidity.

C. Transfer from Other Culture Systems at Passage

Fresh cultures can be transferred to Cellartis DEF-CS Culture System at passage. The cells should be dissociated according to the protocol of the previous system, seeded as single cells or aggregates seeded at a density according to the Cellartis DEF-CS Culture System protocol or 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 Cellartis DEF-CS COAT-1. Use a dilution of 1:5 during the first few passages to provide extra support during the adaptation process.
- 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 are ready for passage when they have acquired the morphology displayed in Figure 3 and Figure 4. If the cells are sparse after seven days in culture, a passage is still recommended.

XI. Changing Medium for hPS Cell Lines

Medium change is recommended daily (except day of passage). Use 0.25–0.4 ml/cm^2 of medium. If the medium turns yellow due to high metabolic activity, increase the medium volume.

A. Preparation

Prepare the appropriate volume of Cellartis DEF-CS medium for maintenance as described above (Section VIII.B) and warm it to 37°C ± 1°C before use. Do not add Cellartis DEF-CS GF-3 at medium change. Discard any leftover warm medium.

B. Medium Change

1. Check cells under microscope; photo document as necessary.
2. Carefully aspirate the medium and pipet newly warmed medium into the cell culture flask. Avoid flushing medium directly onto the cell layer.
3. Place the cell culture flask in an incubator at 37°C ± 1°C, 5% CO_2, and >90% humidity.

XII. Cryopreserving hPS Cell Lines

All hPS cells cultured in Cellartis DEF-CS Culture System can be cryopreserved using common slow freezing protocols for cell suspensions with STEM-CELLBANKER (Zenoaq Resource Co.Ltd. Cat. No. ZR636) or DMSO and FBS. 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.

XIII. Images of Cellartis hPS Cell Lines Maintained in the Cellartis DEF-CS Culture System

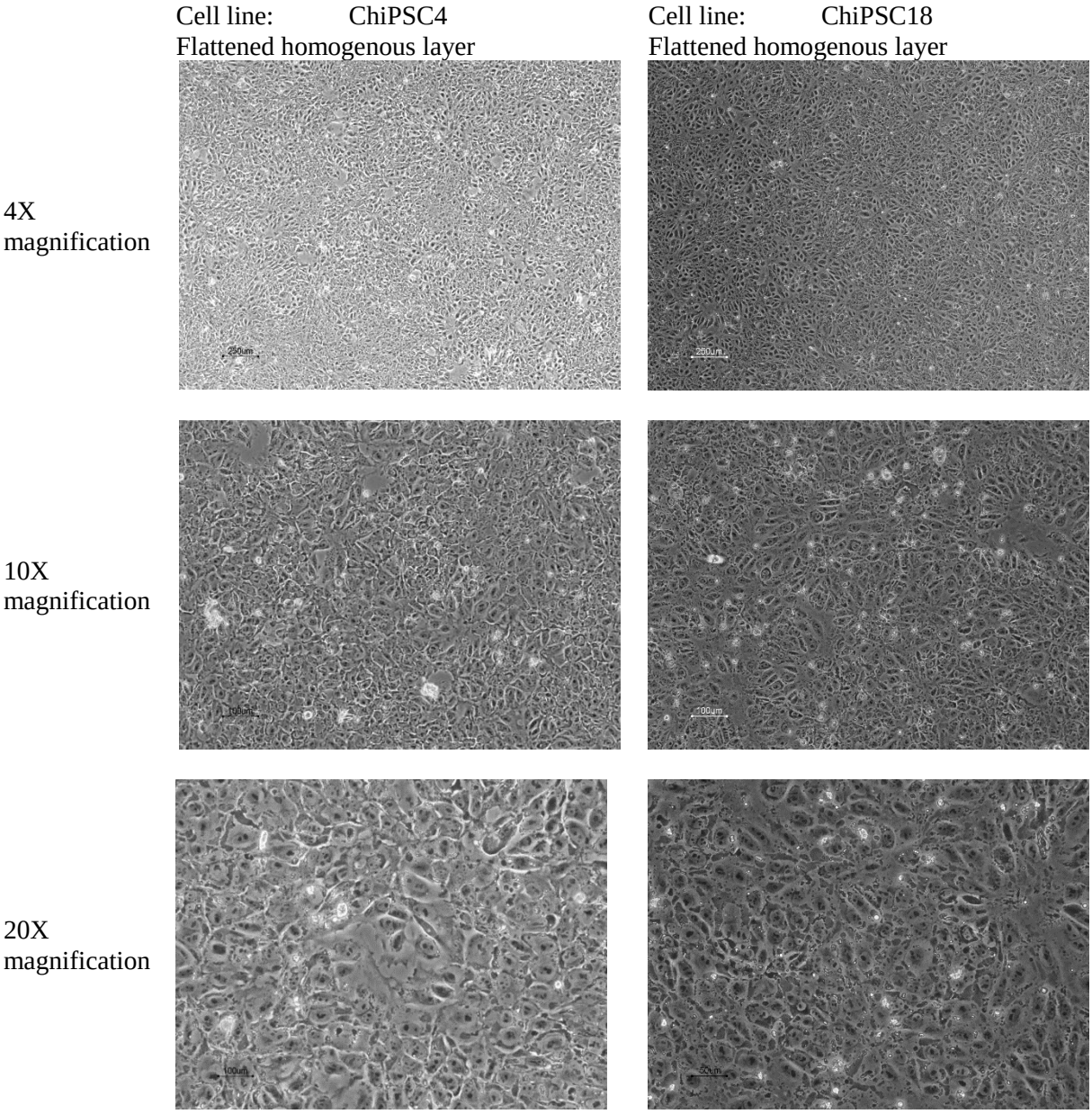

Figure 2. ChiPSC4 and ChiPSC18 cells cultured in the Cellartis DEF-CS Culture System one day after seeding. Cell density 5 x 10^4 cells/cm^2.

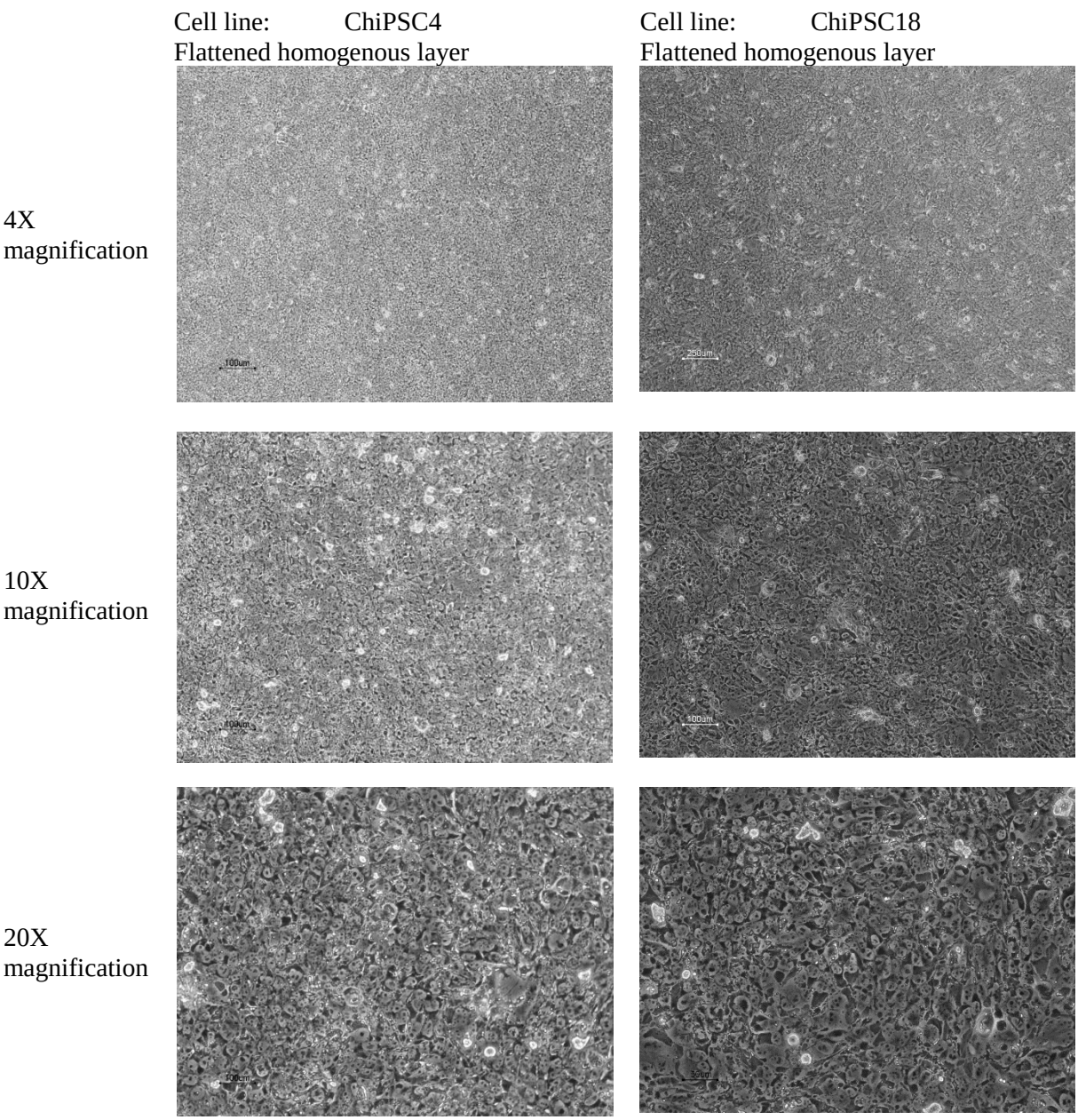

Figure 3. ChiPSC4 and ChiPSC18 cells cultured in the Cellartis DEF-CS Culture System. Cell density 1.5 x 10^5 cells/cm^2.

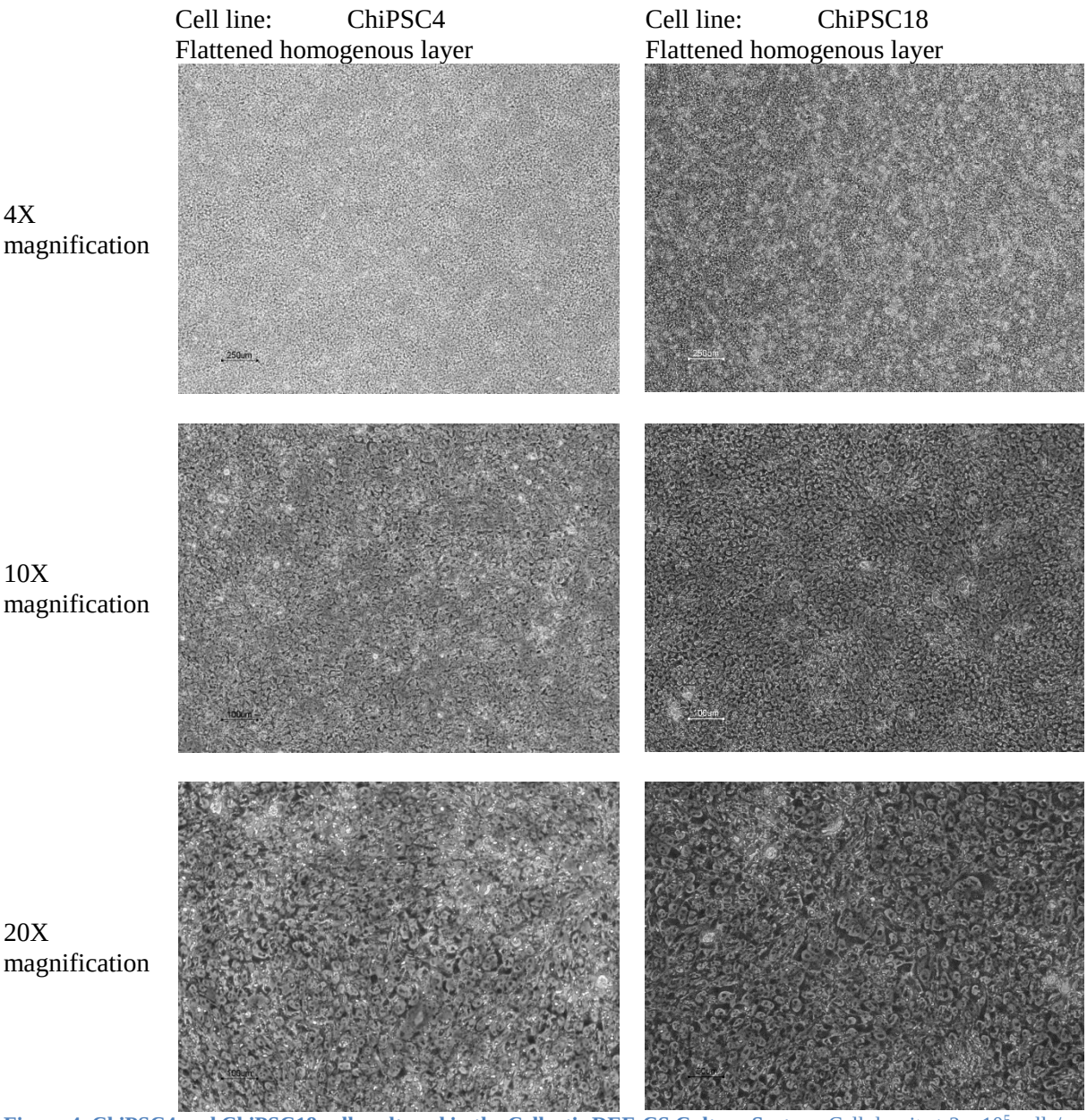

Figure 4. ChiPSC4 and ChiPSC18 cells cultured in the Cellartis DEF-CS Culture System. Cell density >2 x 10^5 cells/cm^2.

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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Appendix A. Troubleshooting Guide

Table VII. Troubleshooting Guide

Problem	Possible Explanation	Solution
Cells do not detach at passage	Too cold TrypLE Select Enzyme.	Make sure TrypLE Select Enzyme is room tempered before use.
Cells do not detach at passage	Cell density too low at passage.	Cells are normally easier to detach at higher densities.
The cell density at passage vary considerably	Recommended seeding densities and passage interval is not optimal for the used cell line.	Seeding densities and passage intervals needs to be optimized.
The cells seem to differentiate	Cells has been seeded too sparse.	Make sure that the seeding density is at least 4.0 x 10^4 cells/cm^2, some cell lines may require higher seeding densities.
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.
Cell density is higher than 3x10^5 cells/cm^2 at passage	Recommended seeding densities and passage interval is not optimal for the used cell line.	If the cells are extremely dense at passage: *increase* the seeding density at passage slightly since cell growth inhibition might cause a reduced generation time for the next passage. When cells have retained their normal generation time, optimize seeding densities and passage interval.
Transferred cells do not adapt to Cellartis DEF-CS Culture System	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.
Transferred cells do not adapt to Cellartis DEF-CS Culture System	The cells are not used to the new environment.	The cells may benefit from a higher concentration of Cellartis DEF-CS COAT-1. Use a dilution of 1:5 during the first few passages to provide extra support during the adaptation process.
Transferred cells do not adapt to Cellartis DEF-CS Culture System	The cells are not used to the new environment.	Newly transferred cells might initially grow at a slightly slower rate. Extend the passage interval up to 7 days for the first passages.
Cells do not adhere at passage or thawing	DEF-CS COAT-1 has been diluted in D-PBS –/–.	Make sure to use D-PBS with Mg^{2+} and Ca^{2+}.
Cells do not adhere at passage or thawing	Too short incubation with DEF-CS COAT-1.	Prolong the incubation time with DEF-CS COAT-1.
Cells are sparse even after seven days in culture	Too few cells attached at passage.	Increase the seeding density.
Cells are sparse even after seven days in culture	Slow growth rate of the used cell line	Increase the volume of GF-1, use maximum a 1:111 dilution.

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