Takara Bio Europe AB

Cellartis® Human iPS Cell Lines User Manual

Cat. Nos. Y00185, Y00225, Y00265, Y00275, Y00285, Y00305, Y00315, Y00325 (061215)

Takara Bio Europe AB
A Takara Bio Company
Arvid Wallgrens backe 20, SE-413 46 Göteborg, Sweden
Europe Technical Support: tech-cellartis@takara-clontech.eu

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I. Introduction

Cellartis human induced pluripotent stem (iPS) cell lines are available from several donors and are supplied from fully characterised cell banks. Please refer to the certificate of analysis for cell line-specific information, such as donor information and karyotype.

Cellartis human iPS cell lines are delivered with the Cellartis DEF-CS™ 100 Culture System (not sold separately), which is a complete system for efficient expansion and scale-up manufacturing of human iPS cells in a feeder-free and defined environment. The Cellartis DEF-CS Culture System is sold in a 500 ml size as Cat. No. Y30010.

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 Human iPS Cell Lines

- Cellartis human iPS cell line P11025 (Cat. No. Y00180, not sold separately; sold as a part of Cat. No. Y00185)
- Cellartis human iPS cell line P11032 (Cat. No. Y00220, not sold separately; sold as a part of Cat. No. Y00225)
- Cellartis human iPS cell line 4 (ChiPSC4) (Cat. No. Y00260, not sold separately; sold as a part of Cat. No. Y00265)
- Cellartis human iPS cell line 7 (ChiPSC7) (Cat. No. Y00270, not sold separately; sold as a part of Cat. No. Y00275)
- Cellartis human iPS cell line 12 (ChiPSC12) (Cat. No. Y00280, not sold separately; sold as a part of Cat. No. Y00285)
- Cellartis human iPS cell line 18 (ChiPSC18) (Cat. No. Y00300, not sold separately; sold as a part of Cat. No. Y00305)
- Cellartis human iPS cell line 21 (ChiPSC21) (Cat. No. Y00310, not sold separately; sold as a part of Cat. No. Y00315)
- Cellartis human iPS cell line 22 (ChiPSC22) (Cat. No. Y00320, not sold separately; sold as a part of Cat. No. Y00325)

B. Cellartis DEF-CS 100 Culture System

- Cellartis DEF-CS 100 Culture System (Cat. No. Y30020, not sold separately; sold as a part of Cat. Nos. Y00185, Y00225, Y00265, Y00275, Y00285, Y00305, Y00305, and Y00315. A larger 500 ml version of this product is sold as Cat. No. Y00310.)
    - DEF-CS Basal Medium (100 ml)
    - DEF-CS COAT-1 (for 100 ml) (800 µl)
    - DEF-CS GF-1 (for 100 ml) (300 µl)
    - DEF-CS GF-2 (for 100 ml) (100 µl)
    - DEF-CS GF-3 (for 100 ml) (40 µl)

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 without Ca^{2+} or 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. General Considerations

A. Storage and Handling

1. Cellartis Human iPS Cell Lines

Cellartis human iPS cell lines should be stored at ≤ –150°C. The cells can be stored for one year from date of receipt under proper storage conditions.

Cellartis human iPS cell lines should be maintained in an incubator at 37°C ± 1°C, 5% CO_2, and >90% humidity.

NOTE: When transferring the cells from the transport vessel to long term storage, *immediate* transfer is essential since variations in temperature may have an adverse effect on cell survival and quality.

2. Cellartis DEF-CS 100 Culture System

Cellartis DEF-CS Basal Medium and Cellartis DEF-CS COAT-1 should be stored at 4°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, mix gently and aliquot into appropriate volumes. 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 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 human iPS cells. Only Additives GF-1 and GF-2 are needed when changing medium on human iPS cells.

V. Culturing Cellartis Human iPS Cell Lines

Cellartis human iPS cell lines are cultured in DEF-CS Medium, which is sold as part of the Cellartis DEF-CS Culture System, and subsequently frozen as a single cell suspension, with approximately 3 x 10^6 cells per vial. After thawing, cells need to be passaged at least once in Cellartis DEF-CS Culture System for recovery. After this period, the cells can be transferred to other media although we recommend to continue culturing them in Cellartis DEF-CS Culture System. We recommend up to 10 passage expansions in Cellartis DEF-CS Culture System from each vial, in order to ensure consistent phenotype and genotype. A schematic picture of thawing, maintenance (medium changes and passage), and cryopreservation of hiPS cell lines in Cellartis DEF-CS Culture System is shown in Figure 1.

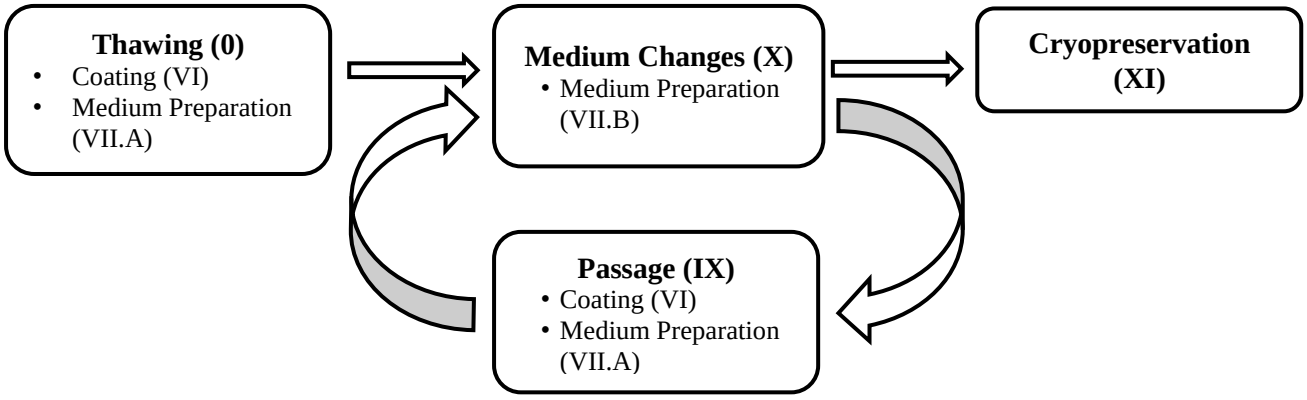

Figure 1. Schematic presentation of the Cellartis human iPS cell line work flow. Corresponding sections of this user manual are referenced in brackets.

Human iPS 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 maximum 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.

VI. 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 minutes in the incubator at 37°C ±1°C 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 use.

VII. Preparing Cellartis DEF-CS Medium

A. Medium for Thawing or Passaging Human iPS 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 supplemented Cellartis DEF-CS medium 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. Discard any left-over warm medium.

B. Medium for Maintenance of Human iPS 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 supplemented Cellartis DEF-CS medium by adding DEF-CS GF-1 (dilute 1:333) and GF-2 (dilute 1:1000) to Cellartis DEF-CS Basal Medium. Do not add Cellartis DEF-CS GF-3 to maintenance medium.
3. Prepare fresh medium on the day of intended use. Discard any left-over warm medium.

VIII. Thawing Cellartis Human iPS Cell Lines

Thaw one vial of your Cellartis human iPS cell line in one 12.5 cm^2 cell culture flask, in 4 ml of supplemented Cellartis DEF-CS medium.

A. Preparations

Coat cell culture units as described above (Section VI). Prepare supplemented Cellartis DEF-CS medium as described above (Section VII.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 supplemented Cellartis DEF-CS medium 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 supplemented Cellartis DEF-CS medium (RT).
6. Rinse the vial with 1 ml supplemented Cellartis DEF-CS medium, 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 4 ml supplemented Cellartis DEF-CS medium (37°C ±1°C). It is not necessary to count the cells at this time.
9. Pipet the cell suspension into the cell culture unit.
10. Ensure that the cells and medium are evenly distributed across the surface of the cell culture unit, and place the cell culture unit in the incubator.

IX. Passaging Cellartis Human iPS 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).

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 human iPS cell lines in culture). If cultures should appear suboptimal after a few passages, we recommend to vary seeding density and passage interval.

A. Preparations

Coat cell culture flasks as described above (Section VI). Prepare the appropriate volume of supplemented Cellartis DEF-CS maintenance medium as described above (Section VII.B) and warm it 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 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 for 5 minutes or until the cell layer has detached. Detachment can be aided by swirling the cell culture flask or by tapping the side of the cell culture flask firmly but gently.
4. Resuspend the cells in the supplemented Cellartis DEF-CS medium and pipet up and down several times to ensure a single cell suspension. (The cells will aggregate if left too long in TrypLE Select).
5. **OPTIONAL:** (To remove TrypLE Select if present at greater than 1:10 ratio.) 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 was diluted at least 1:10.
6. Count the cells in a haemocytometer or in a cell counter (optimized for the cell type).
7. 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 medium should be 0.15–0.25 ml/cm^2.
8. Tilt the flask backwards and forwards gently to ensure that the cell suspension is dispersed evenly over the surface, then place in the incubator.

X. Changing Medium for Cellartis Human iPS 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 supplemented Cellartis DEF-CS medium as described above (Section VII.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 the incubator.

XI. Cryopreserving Cellartis Human iPS Cell Lines

Cellartis human iPS cells cultured in Cellartis DEF-CS Culture System can be cryopreserved using common slow freezing protocols for cell suspensions with STEM-CELLBANKER (Takara Clontech, Cat. No. CB041) 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.

XII. Transferring to Other Culture Media

We recommend thawing and maintaining Cellartis human iPS cell lines in the Cellartis DEF-CS Culture System. After thawing and recovery (at least one regular passage) the iPS cells can be transferred to other culture systems if desired, following the instructions of the preferred culture system.

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

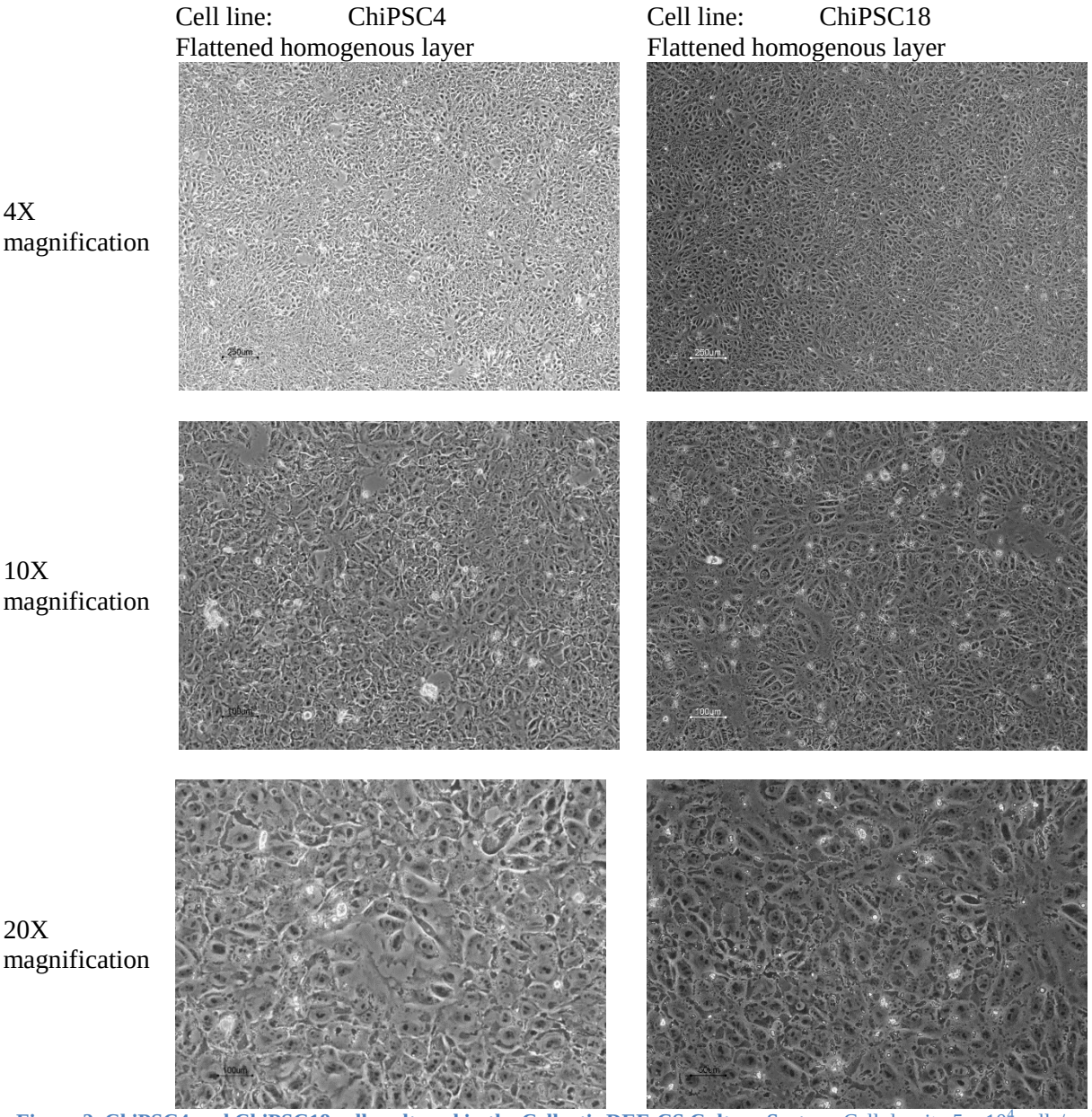

Figure 2. ChiPSC4 and ChiPSC18 cells cultured in the Cellartis DEF-CS Culture System. Cell density 5 x 10^4 cells/cm^2.

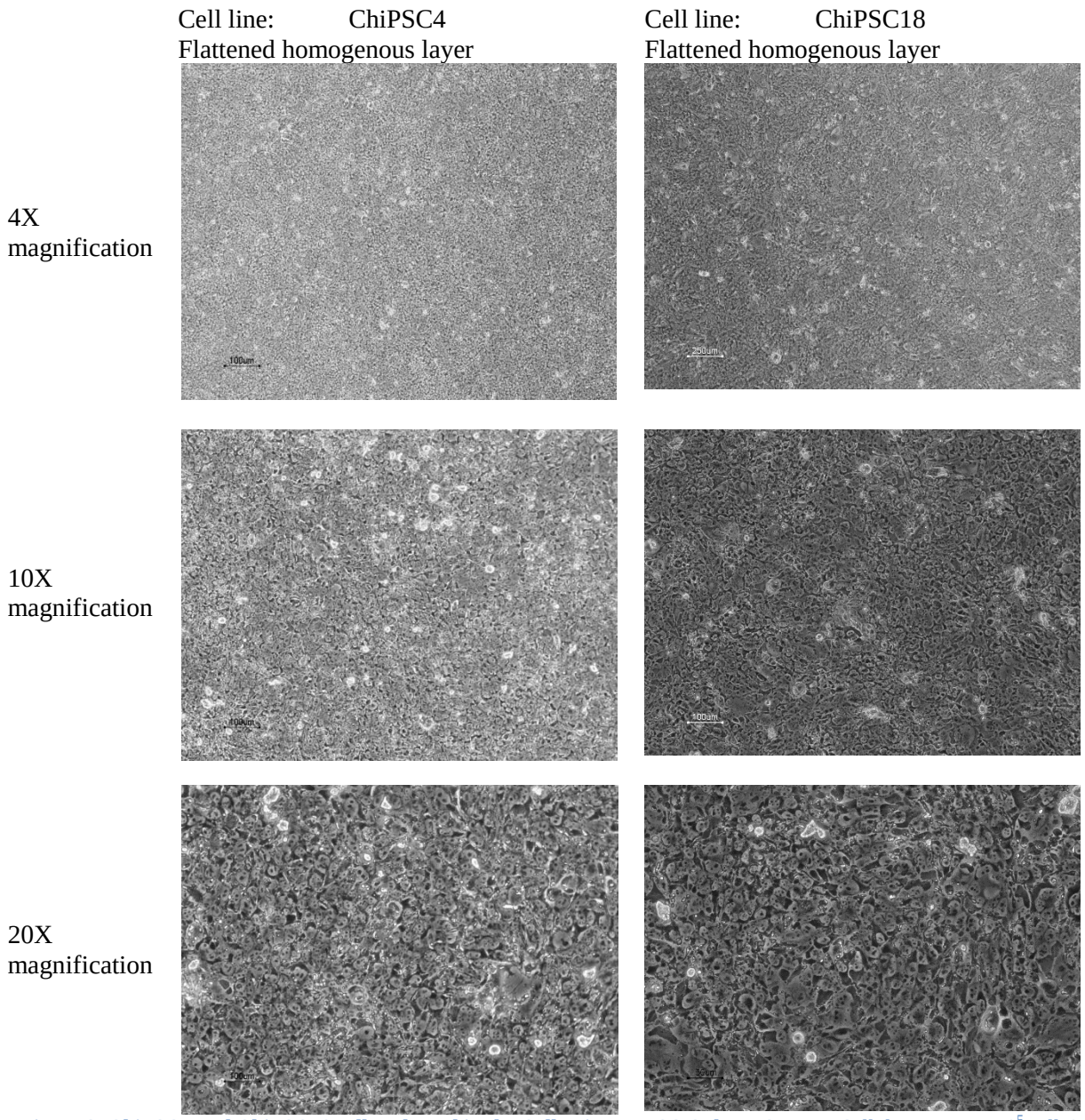

Figure 3. ChiPSC4 and ChiPSC18 cells cultured in the Cellartis DEF-CS Culture System. Cell density 1.5 x 10^5 cells/cm^2.

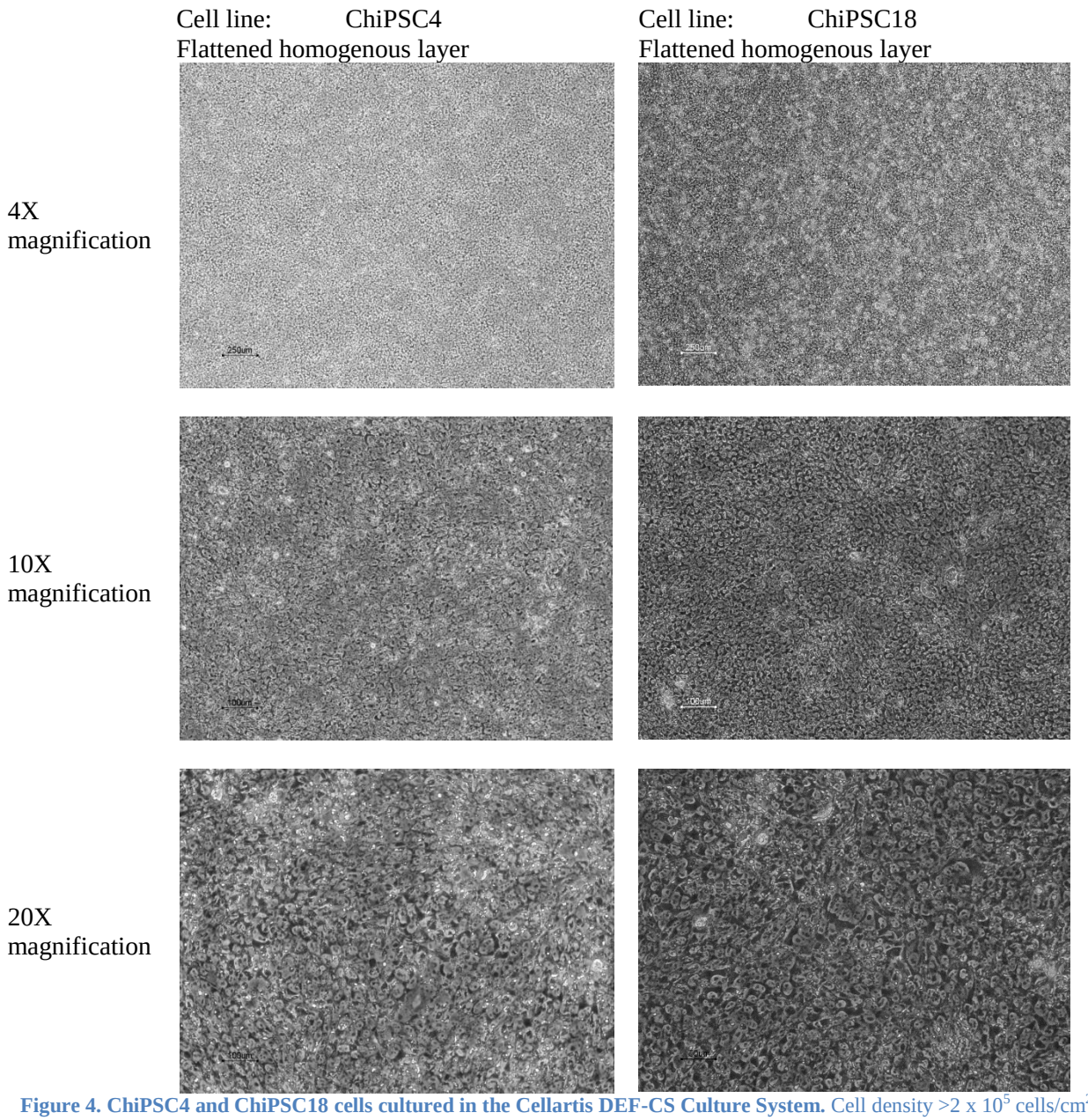

Figure 4. ChiPSC4 and ChiPSC18 cells cultured in the Cellartis DEF-CS Culture System. Cell density >2 x 10^5 cells/cm^2.

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Customer Service/Ordering	**Technical Support**
tel: +46.(0)31.758.0900	tel: +46.(0)31.758.0900
fax: +46.(0)31.758.0910	fax: +46.(0)31.758.0910
web: www.clontech.com	web: www.clontech.com
e-mail: info-cellartis@takara-clontech.eu	e-mail: tech-cellartis@takara-clontech.eu

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