Takara Bio Europe AB

Cellartis® Human ES Cell Lines User Manual

Cat. No. Y00025, Y00065, Y00105, Y00145
(102318)

Takara Bio Europe AB
A Takara Bio Company
Arvid Wallgrens backe 20, SE-413 46 Göteborg, Sweden
Europe Technical Support: techEU@takarabio.com

Table of Contents

Table of Figures

Table of Tables

I. Introduction

Cellartis Human Embryonic Stem (ES) 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 ES 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 pluripotent stem 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 ES Cell Lines

- Cellartis human ES cell line 121 (SA121) (Cat. No. Y00020, not sold separately; sold as a part of Cat. No. Y00025)
- Cellartis human ES cell line 167 (SA167) (Cat. No. Y00060, not sold separately; sold as a part of Cat. No. Y00065)
- Cellartis human ES cell line 181 (SA181) (Cat. No. Y00100, not sold separately; sold as a part of Cat. No. Y00105)
- Cellartis human ES cell line 461 (SA461) (Cat. No. Y00140, not sold separately; sold as a part of Cat. No. Y00145)

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. Y00025, Y00065, Y00105, and Y00145)
    - 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)

A larger 500 ml version of this product is sold as Cat. No. Y30010.

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+} & 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 ES Cell Lines

Cellartis Human ES 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 ES 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 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 Human ES cells. Only Additives GF-1 and GF-2 are needed when changing medium on Human ES cells.

V. Culturing Cellartis Human ES Cell Lines

Cellartis Human ES 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 >1.5 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 do not recommend more than a 10 passage expansion 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 Cellartis Human ES cell lines in Cellartis DEF-CS Culture System is shown in Figure 1.

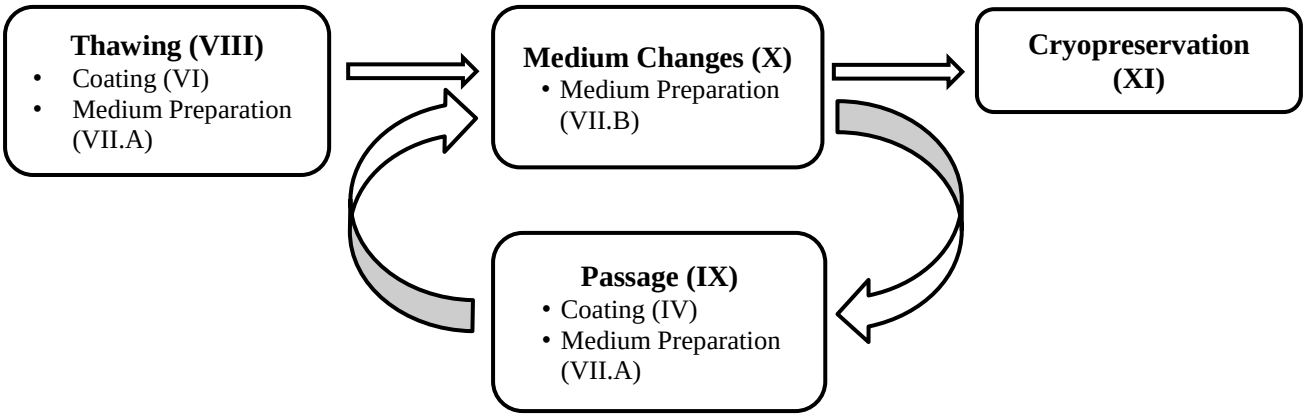


Figure 1. Schematic presentation of the Cellartis Human ES cell line work flow. Corresponding sections of this user manual are referenced in brackets.

Cellartis Human ES 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.

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 min. in an incubator at 37°C ± 1°C, 5% CO2, and >90% humidity or 0.5–3 hrs. 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

VII. Preparing Cellartis DEF-CS Medium

A. Medium for Thawing or Passaging Human ES 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 thawing or 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 Human ES 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 ml	T225 Flask	60.0

VIII. Thawing Cellartis Human ES Cell Lines

Thaw one vial of your Cellartis Human ES cell line in one 12.5 cm^2 cell culture flask, in 3 ml of Cellartis DEF-CS medium for thawing or passaging.

A. Preparations

Coat cell culture vessels as described above (Section VI). Prepare Cellartis DEF-CS medium for thawing or passaging as described above (Section VII.A) and warm it to the appropriate temperature. Discard any leftover warm medium. 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 mlCellartis 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 3 ml Cellartis DEF-CS medium for thawing or passaging (37°C ±1°C). It is not necessary to count the cells at this time.
9. Pipet the cell suspension into the cell culture vessel.
10. 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% CO2, and >90% humidity.

IX. Passaging Cellartis Human ES 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 ES cell lines in culture). If cultures should appear suboptimal after a few passages, we recommend varying seeding density and passage interval.

A. Preparations

Coat cell culture flasks as described above (Section VI). Prepare the appropriate volume of Cellartis DEF-CS medium for thawing or passaging as described above (Section VII.A) and warm it to 37°C ±1°C before use. Warm all other reagents to RT before use. Discard any leftover warm 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. 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 Cellartis DEF-CS medium for thawing or passaging should be 0.15–0.25 ml/cm^2, see table III.
8. 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% CO2, and >90% humidity.

X. Changing Medium for Cellartis Human ES 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 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 pipetting medium directly onto the cell layer.
3. Place the cell culture flask in an incubator at 37°C ± 1°C, 5% CO2, and >90% humidity.

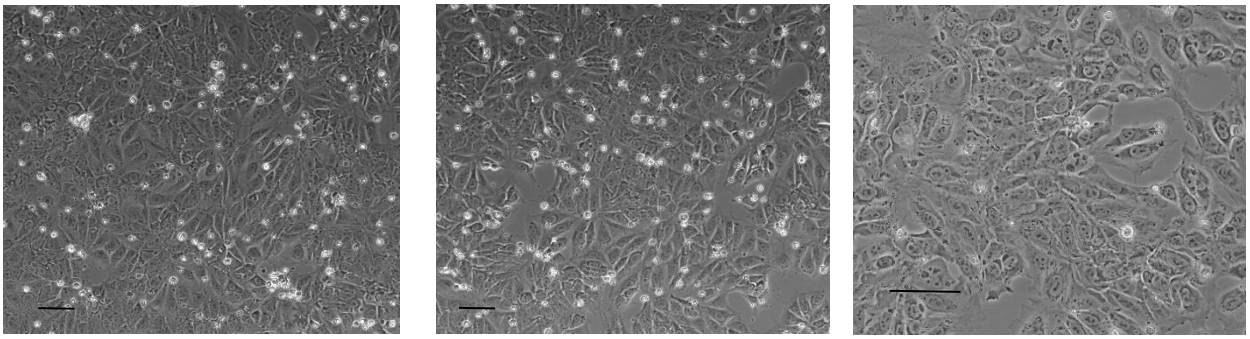
XI. Cryopreserving Cellartis Human ES Cell Lines

Cellartis Human ES 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.

XII. Transferring to Other Culture Media

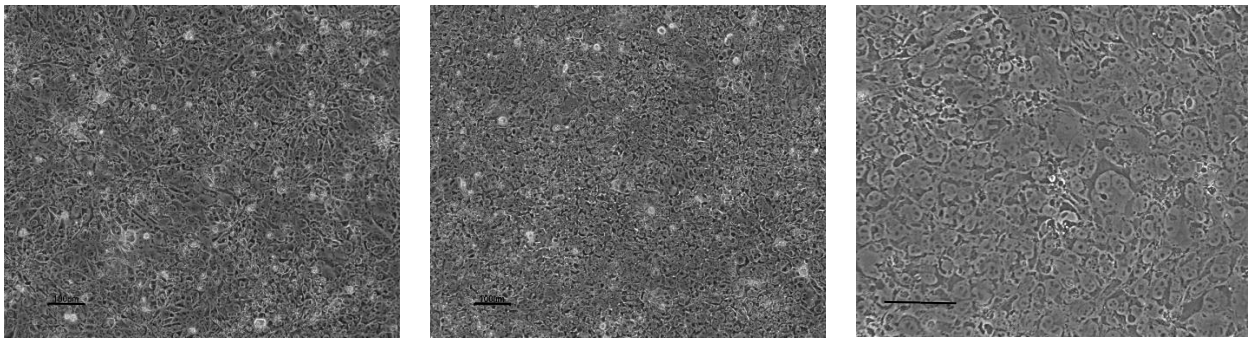
We recommend thawing and maintaining Cellartis Human ES cell lines in the Cellartis DEF-CS Culture System. After thawing and recovery (at least one regular passage) the ES cells can be transferred to other culture systems if desired, following the instructions of the preferred culture system.

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



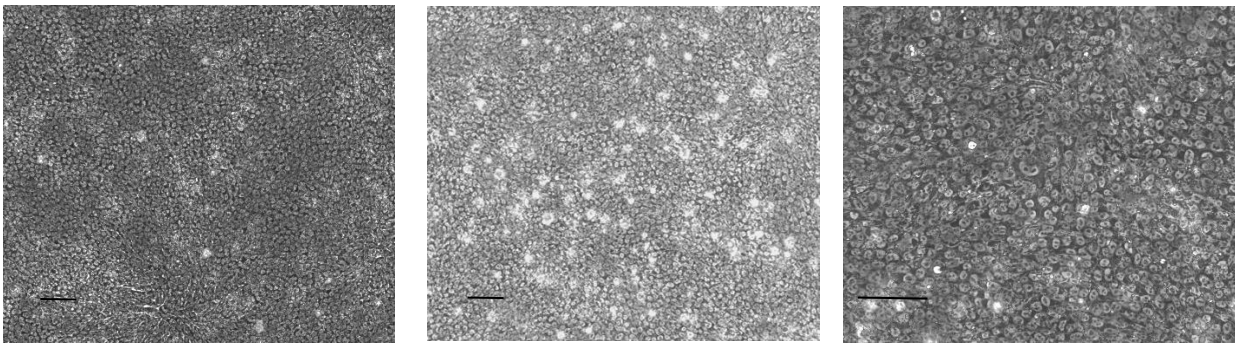
10x magnification | 10x magnification | 20x magnification

Figure 2. Human embryonic stem cells cultured in the Cellartis DEF-CS Culture System one day after seeding.
Cell density approximately 5 x 10^4 cells/cm^2. Scale bar 100 µm.



10x magnification | 10x magnification | 20x magnification

Figure 3. Human embryonic stem cells cultured in the Cellartis DEF-CS Culture System two days after seeding.
Cell density 1–1.5 x 10^5 cells/cm^2. Scale bar 100 µm.



10x magnification | 10x magnification | 20x magnification

Figure 4. Human embryonic stem cells cultured in the Cellartis DEF-CS Culture System three to four days after seeding.
Cell density > 2 x 10^5 cells/cm^2. Scale bar 100 µm.

Contact Us	
Customer Service/Ordering	**Technical Support**
Tel: +33.(0)1.3904.6880	tel: +33.(0)1.3904.6880
fax: +33.(0)1.3904.6870	fax: +33.(0)1.3904.6870
web: www.takarabio.com	web: www.takarabio.com
e-mail: ordersEU@takarabio.com	e-mail: techEU@takarabio.com

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.

If you require licenses for other use, please contact us by phone at +46.(0)31.758.0900.

Your use of this product is also subject to compliance with any applicable licensing requirements as detailed in our catalogues, on our website at http://www.takarabio.com, on the label or other documentation accompanying the goods. It is your responsibility to review, understand and adhere to any restrictions imposed by such statements.