Takara Bio Europe AB

Cellartis® Enhanced hiPS-HEP User Manual

Cat. Nos. Y10050, Y10056, Y10058
(100615)

Takara Bio Europe AB
A Takara Bio Company
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I. Introduction

Cellartis Enhanced hiPS-HEP are human hepatocyte-like cells derived from human induced pluripotent stem (iPS) cell lines. The cells have been differentiated to hepatocyte-like cells *in vitro*, dissociated into cell suspensions, and frozen in vials. Cellartis Enhanced hiPS-HEP cells are provided with coating substrate and maintenance medium supplement.

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 for optimal performance of the cells. 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 Enhanced hiPS-HEP (from ChiPSC18) Kit (Cat. No. Y10050)**
  - Cellartis Enhanced hiPS-HEP (from ChiPSC18) (Cat. No. Y10051; not sold separately)
  - 2 tubes Cellartis HEP Coat (Cat. No Y10052) (3 ml)
  - 2 tubes Cellartis HEP Supplement (Cat. No. Y10053) (1 ml)
- **Cellartis Enhanced hiPS-HEP (from ChiPSC22) Kit (Cat. No. Y10056)**
  - Cellartis Enhanced hiPS-HEP (from ChiPSC22) (Cat. No. Y10057; not sold separately)
  - 2 tubes Cellartis HEP Coat (Cat. No Y10052) (3 ml)
  - 2 tubes Cellartis HEP Supplement (Cat. No. Y10053) (1 ml)
- **Cellartis Enhanced hiPS-HEP (from ChiPSC12) Kit (Cat. No. Y10058)**
  - Cellartis Enhanced hiPS-HEP (from ChiPSC12) (Cat. No. Y10059; not sold separately)
  - 2 tubes Cellartis HEP Coat (Cat. No Y10052) (3 ml)
  - 2 tubes Cellartis HEP Supplement (Cat. No. Y10053) (1 ml)

III. Additional Material Required

The following materials are required but not supplied:

- DMSO
- I*nVitro*GRO CP medium (BioreclamationIVT, Cat. No. Z99029)
- I*nVitro*GRO HT medium (BioreclamationIVT, Cat. No. Z99019)
- Penicillin/Streptomycin (PEST) (10,000 units/ml of penicillin and 10,000 µg/ml of streptomycin)
- Williams Medium E (WME) (Life Technologies, Cat. No. 32551)
- Y-27632
- Cell culture vessels, tissue culture treated polystyrene surface
- General cell culture equipment used in cell culture laboratory

IV. General Considerations:

A. Storage and Handling

Cellartis Enhanced hiPS-HEP cells should be stored at ≤–150°C. Under the recommended storage conditions, the cells can be stored for up to one year from the date of receipt.

Cellartis Enhanced hiPS-HEP cells should be maintained in an incubator at 37°C ± 1°C, 5% CO_2, and >90% humidity.

Cellartis HEP Supplement should be stored at –20°C and expires as indicated on the label. Use Cellartis HEP Supplement to prepare Cellartis Enhanced hiPS-HEP Maintenance Medium directly when thawed. Cellartis Enhanced hiPS-HEP Maintenance Medium should be used fresh or aliquoted and frozen the same day. Always discard warmed leftover Cellartis Enhanced hiPS-HEP Maintenance Medium. Both Cellartis HEP Supplement and Enhanced hiPS-HEP Maintenance Medium are light sensitive; therefore avoid unnecessary exposure to light.

Cellartis HEP Coat should be stored at –20°C and expires as indicated on the label. Thawed Cellartis HEP Coat should be kept cold until handling and should be used directly.

V. Culture of Cellartis Enhanced hiPS-HEP

Cellartis Enhanced hiPS-HEP cells are thawed in Thawing Medium and plated in coated culture vessels using Plating Medium. It is recommended that the cells are seeded in 24- or 96-well plates. The day after thawing, the cultures are washed to remove non-attached cells and medium is replaced with Cellartis Enhanced hiPS-HEP Maintenance Medium. Subsequently, medium is changed every second to third day. The workflow is depicted in Table I.

The hepatocytes are ready to use after five days in culture. The cells can be maintained at least until day 11 after thawing if handled according to the provided protocol. For applications that require high CYP activity, use cells between days 6 and day 11 after thawing. For optimal results in CYP activity assays, change the medium the day before starting the assay.

Table I. Recommended Culture Schedule for Cellartis Enhanced hiPS-HEP. Corresponding sections of this user manual are referenced in parentheses.

Day	Coating (VI)	Thawing & Plating (VIII)	Wash (IX.A)	Maintenance Medium (IX.A & IX.B)	Usage Window	High & Stable CYP Activity
0	0.15 ml/cm^2	4.0 x 10^5 cells/cm^2 0.5 ml/cm^2				
1			2 x 0.5 ml/cm^2	0.5 ml/cm^2		
2						
3				0.47 ml/cm^2		
4						
5				0.47 ml/cm^2		
6						
7				0.47 ml/cm^2		
8						
9				0.47 ml/cm^2		
10						
11						

NOTE: Always work under aseptic conditions

VI. Coating of Cell Culture Vessels

1. Thaw the frozen Cellartis HEP Coat overnight at 4°C. Keep thawed Cellartis HEP Coat cold until use.
2. Add Cellartis HEP Coat to the cell culture vessels (0.15 ml/cm^2). Make sure the entire surface is covered. The recommended volume for coating is 50 µl/well for 96-well plates or 300 µl/well for 24-well plates.
3. Incubate at room temperature (RT, 15–25°C) for 30–60 min.
4. Remove excess Cellartis HEP Coat from the wells just before seeding.

VII. Medium Preparation

A. Y-27632 Stock Solution

Prepare a 5-mM stock solution of Y-27632 by dissolving 10 mg Y-27632 in 5.92 ml sterile distilled water. Aliquot in appropriate volumes and store at –20°C for up to 12 months. Thawed aliquots can be stored at 4°C and should be used within one week.

B. Thawing Medium

1. Decontaminate the external surfaces of all bottles with an appropriate disinfectant and place into the biological safety cabinet.
2. Add PEST (final concentration 0.5%) to the whole bottle InVitroGRO HT (250 ml). This medium can be used for up to one month when stored at 4°C.
3. Add Y-27632 (final concentration 5 µM) to the required amount of medium to prepare complete Thawing Medium. This medium should be freshly prepared immediately before use.

C. Plating Medium

1. Decontaminate the external surfaces of all bottles with an appropriate disinfectant and place into the biological safety cabinet.
2. Add PEST (final concentration 0.5%) to the whole bottle InVitroGRO CP (250 ml). This medium can be used for up to one month when stored at 4°C.
3. Add Y-27632 (final concentration 5 µM) to the required amount of medium to prepare complete Plating Medium. This medium should be freshly prepared immediately before use.

D. Base Maintenance Medium

1. Decontaminate the external surfaces of all bottles with an appropriate disinfectant and place into the biological safety cabinet.
2. To prepare 500 ml Base Maintenance Medium, add 0.1% PEST to 500 ml WME. Base Maintenance Medium can be stored at 4°C for up to one month.

E. Cellartis Enhanced hiPS-HEP Maintenance Medium

NOTE: Cellartis Enhanced hiPS-HEP Maintenance Medium contains DMSO. Therefore, use nitrile gloves when preparing and changing medium and discard old medium in a closed container as hazardous waste.

1. Decontaminate the external surface of all bottles with an appropriate disinfectant and place into the biological safety cabinet.
2. To prepare Cellartis Enhanced hiPS-HEP Maintenance Medium, add 250 µl DMSO and 1 ml Cellartis HEP Supplement per 49 ml of Base Maintenance Medium.
3. Mix the complete medium carefully, aliquot appropriate amounts for medium changes (day 1, 3, 5, etc.) and store the aliquots at –20°C.
4. The complete medium can be stored at –20°C for up to one month. Once thawed, use the same day and discard any remaining media.

VIII. Thawing of Cellartis Enhanced hiPS-HEP

One vial of Cellartis Enhanced hiPS-HEP contains ≥1.23 x 10^7 viable cells. It is recommended that cells are resuspended in 15 ml Plating Medium and plated at a density of 4.0 x 10^5 viable cells/cm^2 (150 µl/well of a 96-well plate or 1,000 µl/well of a 24-well plate). To count the number of viable cells, refer to step 7 in section VIII.B. However, it is not recommended to use more than 15 ml Plating Medium per vial.

NOTE: Newly thawed Cellartis Enhanced hiPS-HEP cells are very fragile. Do not use a pipette for mixing the cell suspension. Only use pipette when seeding the cells.

A. Preparation

- Coat the appropriate number of cell culture vessels with Cellartis HEP Coat according to section VI.
- Prepare and warm the appropriate volume of Thawing Medium and Plating Medium to 37°C ± 1°C.

B. Thawing Cells

NOTE—FOR YOUR PROTECTION: Wear a protective face mask and protective gloves. Use forceps when handling frozen vials. Never hold the vial in your hand as the cryovial may explode due to rapid temperature changes.

1. Transfer the vial directly from liquid nitrogen to a 37°C ± 1°C water bath using forceps.
2. The cell suspension should be thawed just until it is possible to pour it from the vial, including any frozen parts.
3. After approximately 1 minute, check if the cell suspension is sufficiently thawed by carefully turning the tube upside down. Decontaminate the external surface with an appropriate disinfectant and place into the biological safety cabinet.
4. Once thawed, pour the cell suspension into 19 ml of 37°C ± 1°C Thawing Medium. Wash the vial with 1 ml of 37°C ± 1°C Thawing Medium and add to the cells in Thawing Medium.
5. Mix by carefully inverting the tube with cell suspension approximately 10 times. Do not mix by pipetting.
6. Incubate the cell suspension in Thawing Medium at RT for 15-20 minutes. Longer incubation may negatively impact cell viability.
7. Optional: Count the viable cells using a hemocytometer. Take 50 µl cell suspension, add 5 µl trypan blue, count viable cells, and multiply the number of viable cells by 1.1 to obtain the concentration of viable cells. The cell suspension contains both single cells and small cell clusters. Make sure to count the viable cells in the clusters as well.
8. Centrifuge at 100 x g at RT for 2 min, with the slowest deceleration possible.
9. Remove the Thawing Medium with a pipette without disturbing the cell pellet. Loosen the cell pellet by flicking the tube and resuspend the cell pellet very carefully by slowly adding 15 ml of 37°C ± 1°C Plating Medium. It is not recommended to use more than 15 ml Plating Medium per vial.
10. Remove the Cellartis HEP Coat from the cell culture wells just prior seeding the cells.
11. Carefully seed the cells into the coated cell culture units, using 150 µl/well for 96-well plates or 1,000 µl/well for 24-well plates.
12. Place the culture vessels in an incubator at 37°C ± 1°C, 5% CO_2, ≥90% humidity. Ideally, plates should be spread out in the incubator (avoid stacking of several plates) to allow them to warm quickly and should not be moved the next day at the earliest.

C. Thawing Multiple Vials

Several vials can be thawed at the same time using the protocol in section VIII.B. Consider the following points when thawing multiple vials:

- Scale up the volume of Thawing Medium according to the number of vials (e.g., 80 ml for four vials).
- Pour the thawed cell suspensions from all vials into the total volume of Thawing Medium and distribute the cell suspension in 50 ml centrifuge tubes, adding no more than 40 ml per tube.
- Add Plating Medium according to the number of vials (e.g., 30 ml for two vials) equally distributed to the centrifugation tubes.
- Cell suspension in different tubes can be pooled before seeding by combining into one bottle or tube.

IX. Maintenance of Cellartis Enhanced hiPS-HEP

It is recommended that manual pipetting is used for medium changes instead of a vacuum pump (use a multichannel pipette for 96-well plates). To make sure that the cells are not left without medium for longer than a few seconds, change medium only in four to eight wells at a time.

NOTE: Do not remove the entire Cellartis Enhanced hiPS-HEP Maintenance Medium volume. Leave approximately 10% of the medium during each medium change, making sure that the cells are covered by a thin layer of medium at **all times.** Do not let the cells dry completely.

A. Day 1

Wash the hepatocytes twice (Base Maintenance Medium) and change medium (Cellartis Enhanced hiPS-HEP Maintenance Medium, 0.5 ml/cm^2).

1. Preparation

- Prepare and warm an appropriate volume of Base Maintenance Medium to 37°C ± 1°C as described in section VII.D.
- Prepare and warm complete Cellartis Enhanced hiPS-HEP Maintenance Medium to 37°C ± 1°C as described in section VII.E.

2. Medium Change

1. Wash the cells twice very gently with warm Base Maintenance Medium (0.5 ml/cm^2) to remove unattached cells. Do not use a vacuum pump; pipette manually (use a multichannel pipette for 96-well plates).
2. After the second wash step, carefully add warm Cellartis Enhanced hiPS-HEP Maintenance Medium to the cell culture plate (0.5 ml/cm^2).
3. Place the Cellartis Enhanced hiPS-HEP in an incubator at 37°C ± 1°C, 5% CO_2, ≥90% humidity.
4. Discard any leftover warm Cellartis Enhanced hiPS-HEP Maintenance Medium.

B. Day 3 Onward

The medium should be changed every second to third day. If leaving the Cellartis Enhanced hiPS-HEP without a medium change over the weekend, add 50% more medium on Friday (i.e., 1.5 ml/well of a 24-well plate and 230 µl/well of a 96-well plate). It is best to change the medium late on Friday and early on Monday.

1. Preparation

- Thaw and warm complete Cellartis Enhanced hiPS-HEP Maintenance Medium to 37°C ± 1°C as described in section VII.E.

2. Medium Change

1. Very gently remove approximately 90% of the Cellartis Enhanced hiPS-HEP Maintenance Medium from the cell culture plate and discard (i.e., a maximum of 150 µl per 96 well or 0.94 ml per 24 well). Do not use a vacuum pump; pipette manually (use a multichannel pipette for 96 well plates).
2. Very carefully add warm Cellartis Enhanced hiPS-HEP Maintenance Medium to the cell culture plate, using 0.47 ml/cm^2 (i.e., 150 µl per 96 well and 0.94 ml per 24 well).
3. Place the Cellartis Enhanced hiPS-HEP in an incubator at 37°C ± 1°C, 5% CO_2, ≥90% humidity.
4. Discard any leftover warm Cellartis Enhanced hiPS-HEP Maintenance Medium.

X. Images of Thawed Cellartis Enhanced hiPS-HEP

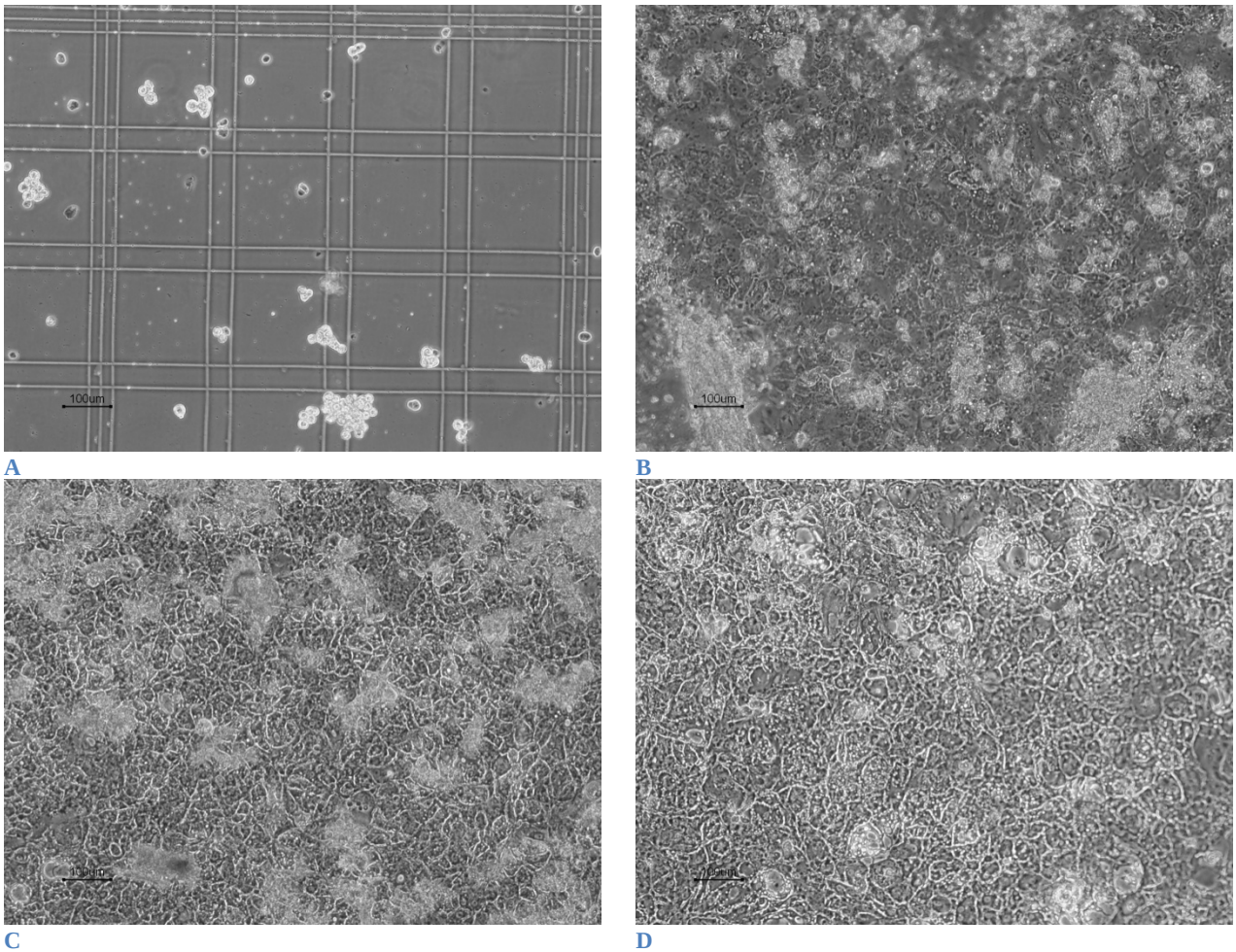

Figure 1. Cellartis Enhanced hiPS-HEP cells in culture. Panel A. Cells in a Bürker chamber after thawing (day 0). Cells were cultured as described and images were taken three **(Panel B**), seven **(Panel C)**, and 11 days **(Panel D)** after thawing. For all images the scale bar is 100 microns.

Appendix A. CYP Activity Assay

After 6 days in culture, Cellartis Enhanced hiPS-HEP cells can be used for Cytochrome P450 (CYP) activity assays. Samples can be analysed using LC/MS to measure the formation of the following specific metabolites: acetaminophen (CYP1A), 4-OH-Diclofenac (CYP2C9), 4-OH-Mephenytoin (CYP2C19), OH-Bufuralol (CYP2D6), and 1-OH-Midazolam (CYP3A).

A. Additional Material Required

- HEPES Solution, 1 M
- L-Glutamine Solution, 200 mM
- CYP Substrate Cocktail:
  - Phenacetin (Sigma-Aldrich, Cat. No. 77440)
  - Bupropion (Sigma-Aldrich, Cat. No B102)
  - Mephenyotin (Santa Cruz, Cat. No. sc-200975A)
  - Diclofenac (Sigma-Aldrich, Cat. No. D6899)
  - Bufuralol (Becton Dickinson, Cat. No. 451034)
  - Midazolam (Loradan, Cat. No. MID-111-HC)
- Pierce BCA Protein Assay Kit (Life Technologies Cat. No. 23225)

B. Preparation

- Prepare a stock solution for the CYP substrate cocktail. In the assay, use the final assay concentrations listed in Table II.
- Warm the appropriate volume of Base Maintenance Medium to 37°C ± 1°C.
- Prepare and warm CYP activity assay medium: Basal Maintenance Medium, 25 mM HEPES, 2 mM L-Glutamine. Add the CYP substrate cocktail just prior to use.

Table II. CYP Substrate Cocktail

CYP	Substrate	Final Assay Concentration
1A	Phenacetin	10 µM
2B6	Bupropion	10 µM
2C19	Mephenytoin	50 µM
2C9	Diclofenac	10 µM
2D6	Bufuralol	10 µM
3A	Midazolam	5 µM

C. Activity Assay

1. Wash Cellartis Enhanced hiPS-HEP cells twice very gently with 0.5 ml/cm^2 warm Base Maintenance Medium.
2. Add 110 µl/cm^2 warm CYP activity assay medium to the cells.
3. Incubate for 2 hours at 37°C ± 1°C, 5% CO_2, ≥90% humidity.
4. Collect 100 µl supernatant and store at –80°C until LC/MS analysis.
5. Determine the amount of protein per well using the Pierce BCA Protein Assay Kit.
6. Normalize the metabolite concentrations measured by LC/MS to the amount of protein per well and the assay duration (120 min).

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.

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This document has been reviewed and approved by the Takara Bio Europe AB Quality Assurance Department.