Takara Bio Europe AB

Cellartis® Enhanced hiPS-HEP v2 Kits User Manual

Cat. Nos. Y10133, Y10134 & Y10135
(012720)

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

United States/Canada	Asia Pacific	Europe	Japan
800.662.2566	+1.650.919.7300	+33.(0)1.3904.6880	+81.(0)77.565.6999

Table of Contents

Table of Figures

Table of Tables

I. Introduction

Cellartis enhanced hiPS-HEP v2 kits contain an optimized, complete system for the long-term maintenance of Cellartis enhanced hiPS-HEP cells. These cells, included in the kits, are cryopreserved human hepatocytes derived from Cellartis human induced pluripotent stem cell lines. The iPS cells were differentiated into hepatocytes *in vitro*, dissociated into a cell suspension, and frozen in vials. Cellartis enhanced hiPS-HEP v2 kits also contain a kit for thawing and plating of the enhanced hiPS-HEP cells and a long-term maintenance medium.

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 cell performance. Takara Bio Europe AB cannot guarantee correct technical feedback on customer cultures unless the subsequent culture instructions have been followed.

II. List of Components

- **Cellartis Enhanced hiPS-HEP v2 (from ChiPSC12) Kit (Cat. No. Y10133)**
  - Cellartis Enhanced hiPS-HEP (from ChiPSC12) (Cat. No. Y10059; not sold separately)
  - Cellartis Enhanced hiPS-HEP Thawing and Plating Kit (Cat. No. Y10132; not sold separately)
  - Cellartis Enhanced hiPS-HEP Long-Term Maintenance Medium (Cat. No. Y30052; not sold separately)
- **Cellartis Enhanced hiPS-HEP v2 (from ChiPSC18) Kit (Cat. No. Y10134)**
  - Cellartis Enhanced hiPS-HEP (from ChiPSC18) (Cat. No. Y10051; not sold separately)
  - Cellartis Enhanced hiPS-HEP Thawing and Plating Kit (Cat. No. Y10132; not sold separately)
  - Cellartis Enhanced hiPS-HEP Long-Term Maintenance Medium (Cat. No. Y30052; not sold separately)
- **Cellartis Enhanced hiPS-HEP v2 (from ChiPSC22) Kit (Cat. No. Y10135)**
  - Cellartis Enhanced hiPS-HEP (from ChiPSC22) (Cat. No. Y10057; not sold separately)
  - Cellartis Enhanced hiPS-HEP Thawing and Plating Kit (Cat. No. Y10132; not sold separately)
  - Cellartis Enhanced hiPS-HEP Long-Term Maintenance Medium (Cat. No. Y30052; not sold separately)

III. Additional Material Required

- Cell culture vessels with tissue-culture treated polystyrene surfaces
- General cell culture equipment

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.

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

The Cellartis Enhanced hiPS-HEP Thawing and Plating Kit should be stored at –20°C and expires as indicated on the label. Thaw the Cellartis Enhanced hiPS-HEP Thawing and Plating Kit overnight at 2–8°C and keep cold until use. Thawed components should be used within two days.

Cellartis Enhanced hiPS-HEP Long-Term Maintenance Medium (hiPS-HEP LTM medium) should be stored at –20°C and expires as indicated on the label. Before use, thaw the bottle of hiPS-HEP LTM medium overnight at 2–8°C and aliquot; one aliquot should be enough for one media change (see Table I). Aliquots should be stored at –20°C and expire as indicated on the original bottle. Use thawed aliquots of hiPS-HEP LTM medium on the same day they are thawed. Do not refreeze aliquots after thawing. Always discard warmed, leftover hiPS-HEP LTM medium. This medium is light sensitive; therefore, avoid unnecessary exposure to light.

V. Culture of Cellartis Enhanced hiPS-HEP Cells

Cellartis enhanced hiPS-HEP cells are thawed in Hepatocyte Thawing Medium and plated in coated culture vessels using Hepatocyte 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 the medium is replaced with hiPS-HEP LTM medium. Subsequently, the media is changed every second to third day. The cell culture workflow is depicted in Table I.

The hepatocytes are ready to use after four days in culture. The cells are functional until Day 19 after thawing if handled according to the provided protocol.

For applications that require high CYP activity, use cells from Day 6 after thawing. For optimal results in CYP activity assays, change the media the day before starting the assay.

Table I. Recommended culture schedule for Cellartis enhanced hiPS-HEP cells. Corresponding sections of this user manual are referenced.

Day	Preparation	Section
–1	Thaw the Cellartis Enhanced hiPS-HEP Thawing and Plating Kit and Cellartis Enhanced hiPS-HEP Long-Term Maintenance Medium (hiPS-HEP LTM medium) overnight at 2–8°C.	**VI.A**
0	Aliquot hiPS-HEP LTM medium. Store the aliquots at –20°.	**VI.B**

Day	Cell culture	Volume of medium per well	Recommended aliquot volume of hiPS-HEP LTM medium	Section	Usage window
0	Thaw the enhanced hiPS-HEP cells.			**VII.A–D**	
1	Media change Day 1	225 µl (96-well plate) 1.5 ml (24-well plate)	25 ml	**VIII.A**	
2					
3					
4	Media change	150 µl (96-well plate) 1.0 ml (24-well plate)	17 ml	**VIII.B**	
5					
6	Media change	150 µl (96-well plate) 1.0 ml (24-well plate)	17 ml	**VIII.B**	
7					
8	Media change	225 µl (96-well plate) 1.5 ml (24-well plate)	25 ml	**VIII.B**	
9					
10					
11	Media change	150 µl (96-well plate) 1.0 ml (24-well plate)	17 ml	**VIII.B**	
12					
13	Media change	150 µl (96-well plate) 1.0 ml (24-well plate)	17 ml	**VIII.B**	
14					
15	Media change	225 µl (96-well plate) 1.5 ml (24-well plate)	25 ml	**VIII.B**	
16					
17					
18	Media change	150 µl (96-well plate) 1.0 ml (24-well plate)	17 ml	**VIII.B**	
19					

NOTE: Always work under aseptic conditions.

VI. Preparing Media

A. Day –1

Thaw the Cellartis Enhanced hiPS-HEP Thawing and Plating Kit and hiPS-HEP LTM medium overnight at 2–8°C and keep cold until use. Use within two days.

B. Day 0

Aliquot Cellartis Enhanced hiPS-HEP Long-Term Maintenance Medium. One aliquot should be enough for one media change (see Table I). Store aliquots at –20°C. For optimal results in CYP activity assays, it is recommended to add an extra medium change the day before starting the assay, which might increase the number of aliquots needed.

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

VII. Thawing of Cellartis Enhanced hiPS-HEP Cells (Day 0)

One vial of Cellartis enhanced hiPS-HEP cells contains ≥12.3 x 10^6 viable cells and requires a growth area of 30 cm^2, corresponding to 100 wells in 96-well plates or 15 wells in a 24-well plate. Cells from one vial are to be resuspended in 15 ml Hepatocyte Plating Medium and plated using 150 µl of cell suspension/well of a 96-well plate or 1.0 ml/well of a 24-well plate (corresponding to a density of 4.0 x 10^5 viable cells/cm^2).

A. Coating

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

B. Hepatocyte Thawing Medium and Hepatocyte Plating Medium

Warm Hepatocyte Thawing Medium and Hepatocyte Plating Medium to 37°C ± 1°C. Once this temperature is reached, use the preheated medium within one hour.

C. 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. Do not submerge the cap.
2. After approximately 1 min, carefully invert the vial to check if the cell suspension has thawed enough to be poured from the vial. The cell suspension should be slushy, but not completely fluid.
3. Decontaminate the external surface with an appropriate disinfectant, place the vial into the biological safety cabinet, and pour the cell suspension into 19 ml of 37°C ± 1°C Hepatocyte Thawing Medium. Wash the vial with 1 ml of 37°C ± 1°C thawing medium and add this to the cells in thawing medium.
4. Mix by carefully inverting the closed tube containing the cell suspension approximately 10 times. Do not mix by pipetting.

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

5. Incubate the cell suspension in thawing medium at RT for 15–20 min. Longer incubation may negatively impact cell viability.
6. Centrifuge at 100*g* at RT for 2 min, with the slowest deceleration possible.
7. Remove the Hepatocyte Coating from the cell culture wells immediately before seeding the cells.
8. 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 Hepatocyte Plating Medium. We recommend using exactly 15 ml of plating medium per vial. Mix the vial by carefully inverting the closed tube containing the cell suspension once. Repeat after seeding every four wells. If seeding using a multichannel pipette for 96-well plates, mix after seeding into one column by placing the trough onto a sturdy horizontal surface and gently moving it back and forth once or twice. Do not mix by pipetting.
9. Carefully seed the cells into the coated cell culture vessels, using 150 µl/well for 96-well plates or 1.0 ml/well for 24-well plates.
10. When seeding into a 24-well plate, after the cell plating is complete, gently move the plate onto a sturdy horizontal surface and move in a crisscross or square pattern to evenly distribute the cells.
11. Place the culture vessels in an incubator at 37°C ± 1°C, 5% CO_2, and ≥90% humidity. Ideally, plates should be spread out in the incubator (avoid stacking plates) to allow them to warm quickly, and plates should not be moved until the next day, at the earliest.

D. Thawing Multiple Vials

Several vials can be thawed at the same time using the protocol in Section VII.C. Consider the following when thawing multiple vials:

1. Scale up the volume of Hepatocyte Thawing Medium according to the number of vials (e.g., 80 ml for four vials).
2. Pour the thawed cell suspensions from all vials into the total volume of thawing medium and evenly distribute the cell suspension into 50-ml centrifuge tubes, adding no more than 40 ml per tube.
3. After centrifugation, the cell suspension can be pooled from different tubes before seeding by combining them into one bottle or tube. Add Hepatocyte Plating Medium according to the number of vials used initially (e.g., 60 ml for four vials).

VIII. Maintenance of Cellartis Enhanced hiPS-HEP Cells (Day 1 onward)

The media should be changed every second to third day, with three media changes a week. For every second day media change, use 150 µl/well of a 96-well plate or 1.0 ml/well of a 24-well plate. **For every third day media change (typically over the weekend), add 50% more medium** (225 µl/well of a 96-well plate or 1.5 ml/well of a 24-well plate). In this case, it is best to change the media in the late afternoon (typically Friday afternoon) and early morning (typically Monday morning).

Maintaining a confluent and undisturbed cell layer is important for the functionality of the enhanced hiPS-HEP cells. Therefore, we recommend being very careful when removing and adding media during media changes.

- **Manual pipetting** is recommended for media changes instead of using a vacuum pump. If using a pipette controller, decrease the speed to minimum. For 96-well plates, use a multichannel pipette.
- To make sure that the cells are not left without media for longer than a few seconds, change the media in at most four wells at a time, or one column at the time if using a multichannel pipette for 96-well

plates, and leave approximately 10% of the media in the wells during each media change. **Do not let the cells dry out.**

A. Day 1

Wash the hepatocytes twice with Hepatocyte Washing Medium and add hiPS-HEP LTM medium.

1. Preparation

Warm Hepatocyte Washing Medium to 37°C ± 1°C. Thaw one aliquot of hiPS-HEP LTM medium and warm to 37°C ± 1°C.

2. Media Change

1. To remove unattached cells, very gently wash the cells twice with prewarmed Hepatocyte Washing Medium using 200 µl per well of a 96-well plate or 1.0 ml per well of a 24-well plate.
2. After the second wash step, very carefully add warm hiPS-HEP LTM medium to the cell culture plate using 150 (or 225) µl per well of a 96-well plate or 1.0 (or 1.5) ml per well of a 24-well plate.
3. Place the cell culture vessels in an incubator at 37°C ± 1°C, 5% CO_2, and ≥90% humidity.
4. Discard any leftover warm hiPS-HEP LTM medium.

B. Day 3 Onward

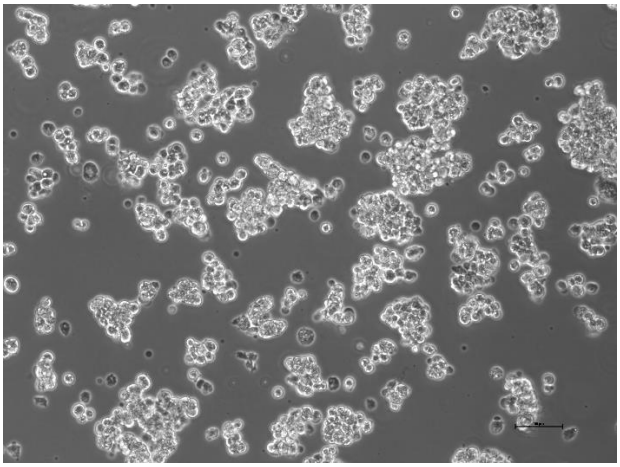
1. Preparation

Thaw one aliquot of hiPS-HEP LTM medium and warm to 37°C ± 1°C.

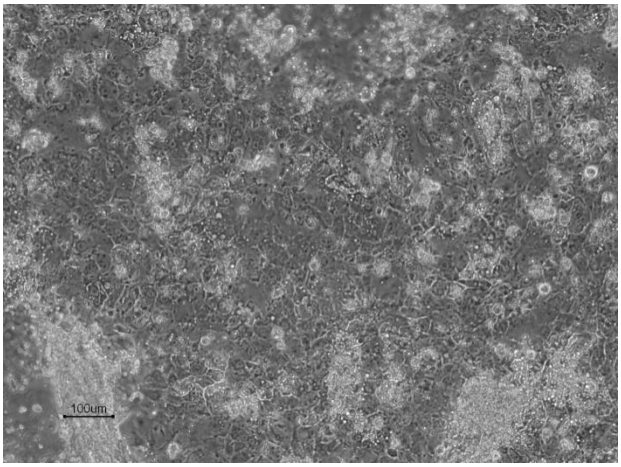
2. Media Change

1. Very gently remove approximately 90% of the hiPS-HEP LTM medium from the cell culture vessels and discard. Leave approximately 15 µl per well of a 96-well plate and 100 µl per well of a 24-well plate.
2. Very carefully, add warm hiPS-HEP LTM medium to the cell culture plate, using 150 (or 225) µl per well of a 96-well plate and 1.0 (or 1.5 ml) per well of a 24-well plate.
3. Place the vessels in an incubator at 37°C ± 1°C, 5% CO_2, and ≥90% humidity.
4. Discard any leftover warm hiPS-HEP LTM medium.
5. Repeat medium change according to schedule in Table I.

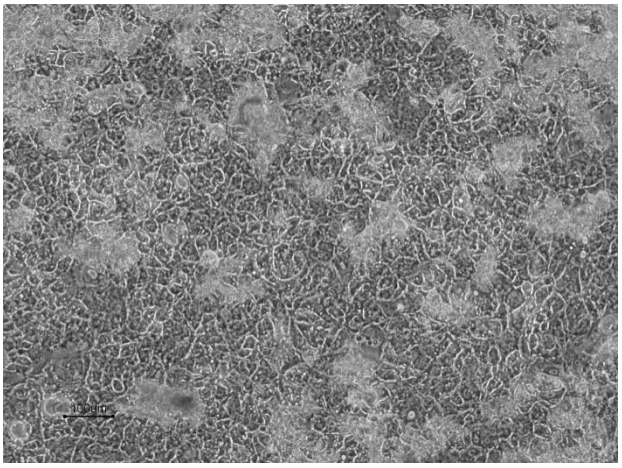
IX. Images of Thawed Cellartis Enhanced hiPS-HEP Cells



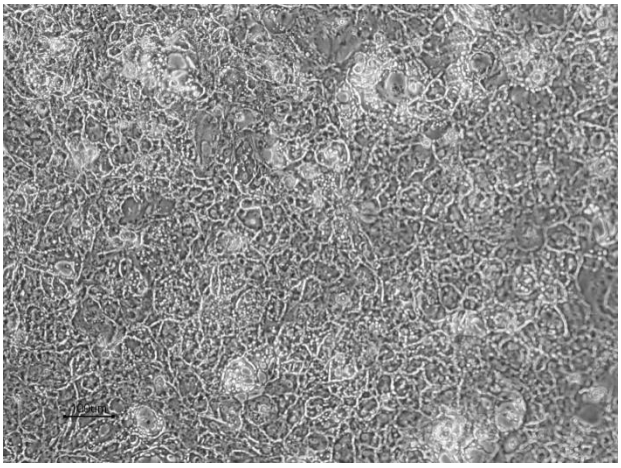
A



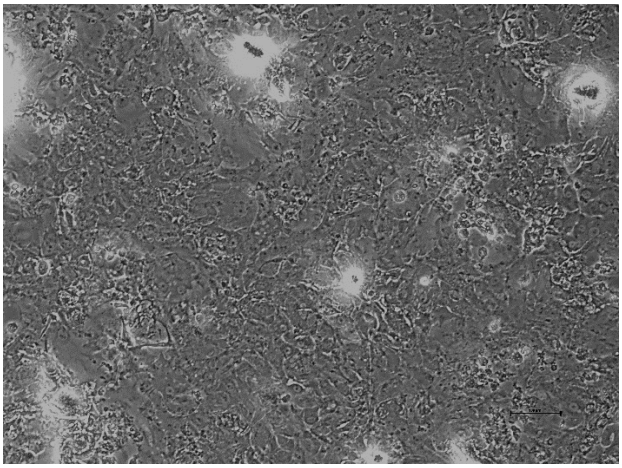
B



C



D



E

Figure 1. Cellartis enhanced hiPS-HEP cells in culture. Panel A. Cells after thawing (Day 0). The cultures are a mixture of single cells and cell clusters of various sizes. Cells were cultured as described and images were taken 3 **(Panel B)**, 7 **(Panel C)**, 11 **(Panel D)** and 19 **(Panel E)** days after thawing. For all images, the scale bar is 100 microns.

Appendix A. CYP Activity Assay

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

A. Additional Materials Required

1. CYP activity assay basal medium
    - Penicillin/Streptomycin (PEST) (10,000 units/ml of penicillin and 10,000 µg/ml of streptomycin)
    - Williams Medium E (WME) w/o phenol red (Sigma-Aldrich, Cat. No. W1878)
    - HEPES Solution, 1 M
    - L-Glutamine Solution, 200 mM
2. CYP substrate cocktail:
    - Phenacetin (Sigma-Aldrich, Cat. No. 77440)
    - Bupropion (Sigma-Aldrich, Cat. No. B102)
    - Mephenytoin (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)
3. Pierce BCA Protein Assay Kit (Life Technologies, Cat. No. 23225)

B. Preparation

1. For optimal results in CYP activity assays, perform media changes according to Table I, and always the day before starting the assay.
2. Prepare a stock solution of the CYP substrate cocktail. In the assay, use the final assay concentrations listed in Table II.
3. Prepare CYP activity assay basal medium: Mix WME, 0.1% PEST, 25 mM HEPES, and 2 mM L-Glutamine, then warm to 37°C ± 1°C.
4. Prepare the supplemented CYP activity assay medium by adding the CYP substrate cocktail to CYP activity assay basal medium just prior to use.

Table II. CYP substrate cocktail ingredients.

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. Very gently, wash Cellartis enhanced hiPS-HEP cells twice with 0.5 ml/cm^2 warm CYP activity assay basal medium.
2. Add warm, supplemented CYP activity assay medium to the cells. Use 100 µl/well in a 96-well plate or 440 µl/well in 24-well plate.
3. Incubate for 2 hr at 37°C ± 1°C, 5% CO_2, and ≥90% humidity.
4. Collect 100 µl of 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).

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.