Takara Bio Europe AB

Cellartis® Power™ Primary HEP Medium User Manual

Cat. No. Y20020
(122118)

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

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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

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I. Introduction

Cellartis Power Primary HEP Medium (Power HEP medium) is a medium for long-term maintenance of cryopreserved plateable human primary hepatocytes. It should be used for the entire culture period, with the first media change into Power HEP medium four hours after thawing and plating. Power HEP medium has an advanced formulation, optimized to prolong the life span and prevent dedifferentiation of primary hepatocytes in conventional 2D cultures. This is achieved with a weekend-free feeding schedule and without the need of an overlay or sandwich culture.

Using Power HEP medium, primary hepatocytes remain viable and functional for four weeks post-thawing. It has been observed that some functions vary during the first week of culture, likely due to a recovery phase following the freezing/thawing procedure. This recovery phase varies with different primary hepatocyte donors; therefore, Takara Bio Europe AB recommends users to evaluate the functions and donors of interest over time to determine the optimal time point for each specific assay.

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 this medium according to this manual for optimal performance of the cells. Takara Bio Europe AB cannot guarantee correct technical feedback on customer cultures unless the subsequent culture instructions have been followed.

II. List of Components

- 250 ml Cellartis Power Primary HEP Medium (Cat. No. Y20020)

III. Additional Material Required

The following materials are required but not supplied:

- General cell culture equipment
- Human cryoplateable primary hepatocytes with the providers recommended thawing and plating medium
- Collagen I from rat tail (e.g., Corning Cat. No. 354236) or pre-coated Collagen I plates (e.g., Corning BioCoat™ Cat. No. 354407 or 354408)
- PBS Dulbecco's with Ca^{2+} & Mg^{2+} (D-PBS +/+)

If applicable:

- Penicillin-Streptomycin (PEST) for your thawing and plating medium (see section V.A.1)

IV. General Considerations

A. Storage and Handling

Power HEP Medium should be stored at –20°C and expires as indicated on the label. At first use, thaw overnight at 2–8°C and aliquot into appropriate volumes. Aliquots can be stored at –20°C until the expiration date indicated on the original bottle. Thaw aliquots at 2–8°C. Thawed aliquots may be stored at 2–8°C for up to one week. Do not refreeze aliquots after thawing. Always discard warmed, leftover Power HEP medium. The medium is light sensitive; therefore, avoid unnecessary exposure to light.

NOTE: Power HEP medium contains DMSO. Therefore, use nitrile gloves when preparing and changing the medium and discard old medium in a closed container as hazardous waste.

B. Primary Hepatocytes

To be able to culture your primary hepatocytes for four weeks, we recommend using high quality cryoplateable human primary hepatocytes with high viability and attachment efficiency. More specifically, we recommend using a seeding density that renders at least 80–90% confluency.

V. Culture of Primary Hepatocytes

Power HEP medium is an optimized medium for prolonged maintenance of human cryopreserved plateable primary hepatocytes. The recommended culture schedule for maintenance up to four weeks is described in Table I below.

Table I. Recommended culture schedule for primary hepatocytes cultured in Cellartis Power Primary HEP Medium. Corresponding sections of this user manual are referenced.

WEEK	Weekday	Cell culture	Volume of medium per well	Section
1	**Monday**	• Thawing and plating	According to protocol from the vendor of the primary hepatocytes*	
		• Media change to Power HEP medium 4 hr after plating	450–500 µl/cm^2 150 µl (96-well plate) 1.0 ml (24-well plate)	**V.B**
	Tuesday			
	Wednesday	• Media change	450–500 µl/cm^2 150 µl (96-well plate) 1.0 ml (24-well plate)	**V.C**
	Thursday			
	Friday	• Media change	675–750 µl/cm$_2$ 225 µl (96-well plate) 1.5 ml (24-well plate)	**V.C**
	Saturday and Sunday			
2 – 4	**Monday**	• Media change	450–500 µl/cm^2 150 µl (96-well plate) 1.0 ml (24-well plate)	**V.C**
	Tuesday			
	Wednesday	• Media change	450–500 µl/cm^2 150 µl (96-well plate) 1.0 ml (24-well plate)	**V.C**
	Thursday			
	Friday	• Media change	675–750 µl/cm^2 225 µl (96-well plate) 1.5 ml (24-well plate)	**V.C**
	Saturday and Sunday			

*Note: When culturing your primary hepatocytes in Power HEP medium, there is no need for an overlay.

IMPORTANT:

- Always work under aseptic conditions.
- It is recommended that manual pipetting is used for media changes instead of a vacuum pump. For 96-well plates, use a multichannel pipette.

A. Thawing and Plating of Primary Hepatocytes

1. Thawing and Plating

Thaw and plate the cells according to the user manual of the vendor of your primary hepatocytes, using the thawing and plating media and seeding density recommended by the vendor to achieve at least 80–90% confluency.

NOTE: Primary hepatocytes are not guaranteed to be sterile. For prolonged culture of up to four weeks, it is crucial that the thawing and plating media contain sufficient antibiotics in order to prevent contamination in your cultures. If the thawing and plating media supplied by the vendor of your choice does not contain antibiotics, we recommend adding 1% PEST before use.

B. Switching to Cellartis Power Primary HEP Medium, 4 hr after Plating

1. Preparation

Warm the appropriate volume of Power HEP medium to 37°C ± 1°C before use.

2. Media Change

1. Very gently remove the plating medium from the cell culture vessels and discard. Do not use a vacuum pump; rather, pipette manually (use a multichannel pipette for 96-well plates).
2. Very carefully, add warm Power HEP medium to the cell culture plate, using 450–500 µl/cm^2 (or, if thawing on a Friday using 675–750 µl/cm^2).
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 Power HEP medium.

C. Maintenance of Primary Hepatocytes

The media should be changed every second to third day, with three media changes a week. For every second day media change, use 450–500 µl/cm^2 corresponding to 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), increase the medium volume with 50%** to 675–750 µl/cm^2 corresponding to 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 the early morning (typically Monday morning).

NOTE: To make sure that the cells are not left without media for longer than a few seconds, change the media in four wells at a time or one column at the time if using a multichannel pipette for 96-well plates.

1. Preparation

Warm the appropriate volume of Power HEP medium to 37°C ± 1°C before use.

2. Media Change

1. Very gently remove the old Power HEP medium from the cell culture vessels and discard. Do not use a vacuum pump; rather, pipette manually (use a multichannel pipette for 96-well plates).
2. Very carefully, add new warm Power HEP medium to the cell culture plate, using 450–500 µl/cm^2 (or 675–750 µl/cm^2).
3. Place the vessels in an incubator at 37°C ± 1°C, 5% CO_2, and ≥90% humidity.
4. Discard any leftover warm Power HEP medium.
5. Repeat medium change according to schedule in Table I.

VI. Images of Primary Hepatocytes Cultured in Cellartis Power Primary HEP Medium

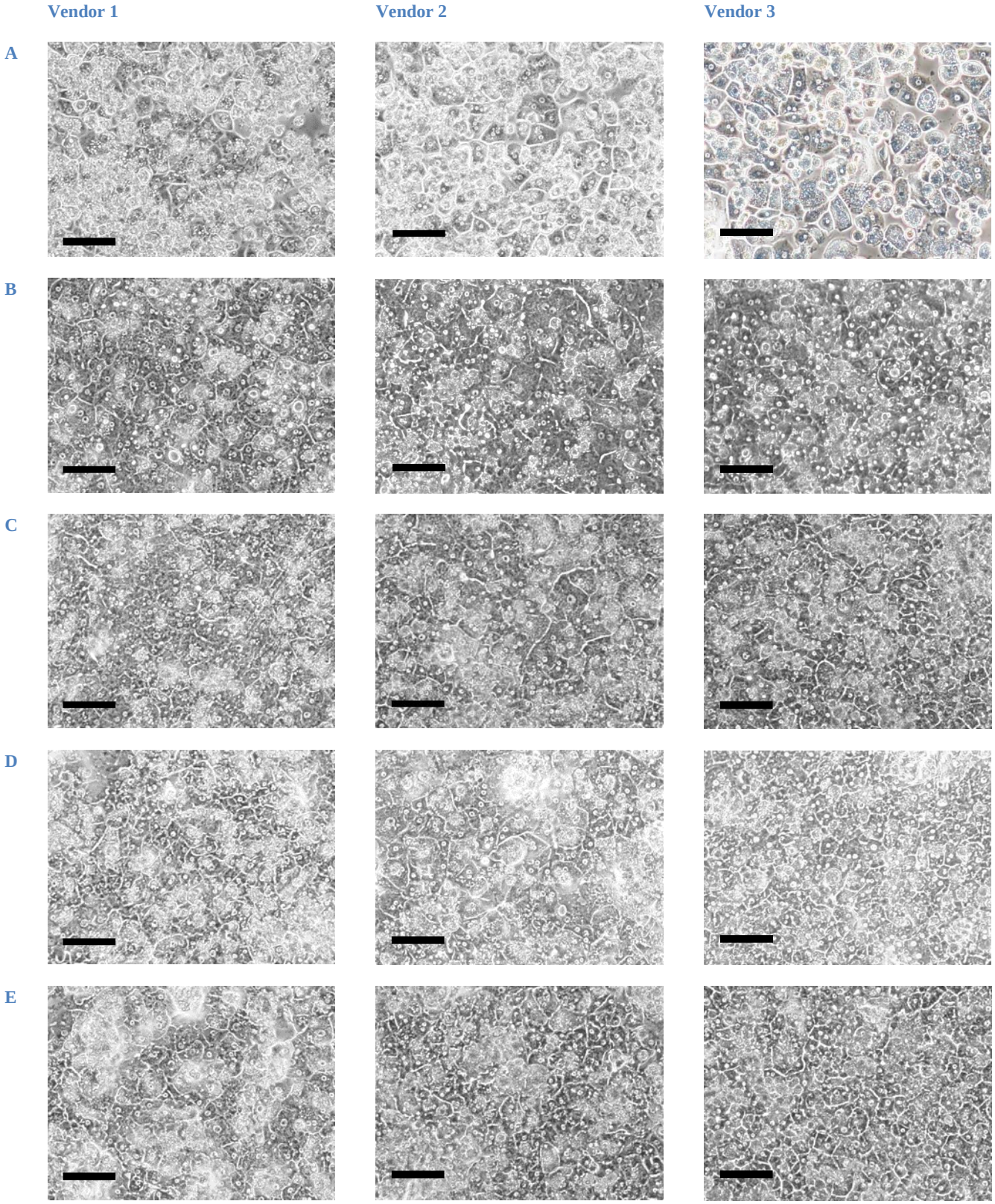

Figure 1. Cryoplateable human primary hepatocytes from three different vendors (1, 2, and 3) cultured in Cellartis Power Primary HEP medium. Cells were cultured as described and images were taken 1 **(Panel A**), 7 **(Panel B)**, 14 **(Panel C),** 21 **(Panel D)** and 28 **(Panel E)** days after thawing. For all images, the scale bar is 100 µm.