

## Instruction Manual

Product	Size	Catalog Number
Human Hepatocytes (HHpC)	3-6 x 10 <sup>6</sup> cryopreserved cells	C-12850

### Product Description

The liver, which is mainly formed by highly specialized cells called hepatocytes, is the main metabolic organ of the body performing glycolysis, glycogenesis, amino acid metabolism, lipid metabolism, and ureagenesis. In addition, hepatocytes play a key role in elimination of toxic substances. Therefore, cultured human hepatocytes are perfectly suited for *in vitro* metabolism and detoxification studies. PromoCell Human Hepatocytes (HHpC) are produced at PromoCell's cell culture facility from the normal human liver tissue of single donors.

Immediately after isolation, all PromoCell Human Hepatocytes are cryopreserved as freshly prepared cells using PromoCell's proprietary, serum-free freezing medium, Cryo-SFM.

Each cryo vial contains 3-6 million viable cells with a guaranteed adherence rate of > 50% after thawing.

**Note:** Under standard *in vitro* cell culture conditions, mature human hepatocytes usually do not survive for longer than 10-14 days and do not proliferate.

### Quality Control

Rigid quality control tests are performed for each lot of PromoCell Human Hepatocytes.

They are tested for cell morphology, adherence rate, and cell viability. Furthermore, metabolic tests for phase I and phase II reactions as well as analysis of Albumin synthesis are carried out for each lot.

In addition, all cells have been tested for the absence of HIV-1, HIV-2, HBV, HCV, and microbial contaminants (fungi, bacteria, and mycoplasma).

A detailed certificate of analysis (CoA) for each lot can be downloaded at: [www.promocell.com/coa](http://www.promocell.com/coa)

### Intended Use

PromoCell Human Hepatocytes are for *in vitro* research use only and not for diagnostic or therapeutic procedures.

### Warning

Although tested negative for HIV-1, HIV-2, HBV, and HCV, the cells - like all products of human origin - should be handled as potentially infectious. No test procedure can completely guarantee the absence of infectious agents.

**Follow appropriate safety precautions!**

*After delivery, start immediately with the protocol for cryopreserved cells (see page 2).*

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Use aseptic techniques and a laminar flow bench.*

### Protocol for Cryopreserved Human Hepatocytes (HHpC)

**Straight after arrival, store the cryopreserved cells in liquid nitrogen, or seed them immediately.**

**Note:** Storage at  $-80^{\circ}\text{C}$  is not sufficient for cell preservation and causes irreversible cell damage.

**Note:** For culturing Hepatocytes, Collagen type I coated culture vessels are necessary. Please make sure to have them before starting the protocol.

#### 1. Prepare the medium

Prewarm PromoCell Hepatocyte Growth Medium at  $37^{\circ}\text{C}$  (5 ml Growth Medium for 1 ml cell solution in the cryo vial).



#### 2. Thaw the cells

Remove the cryovial from the liquid nitrogen container and immediately place it on dry ice - even for short transportation. Under a laminar flow bench, briefly twist the cap a quarter turn to relieve pressure, then re-tighten. Immerse the vial into a water bath ( $37^{\circ}\text{C}$ ) just up to the screw cap for 2 minutes. Ensure that no water enters the thread of the screw cap.



#### 3. Disinfect the vial and transfer the cells

Thoroughly rinse the cryovial with 70% ethanol under a laminar flow bench. Then, aspirate the excess ethanol from the thread area of the screw cap. Open the vial and carefully transfer the cells to the tube with the prewarmed medium from step 1. Avoid pipetting the suspension up and down in the cryovial.



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#### 4. Seed and incubate the cells

Agitate the tube carefully and determine the number of viable cells. Dilute the cells to the desired concentration. The recommended seeding density for HHpC on type I Collagen coated culture vessels is 150,000 cells per cm<sup>2</sup>. Place the vessel in an incubator (37°C, 5% CO<sub>2</sub>) for 4 hours.



#### 5. Replace the medium

Viable cells should be attached after 4 hours.

**Note:** Non-attached cells may cause medium turbidity and should not be mistaken for a contamination.

For removal of the non-attached cells aspirate the medium from the vessel. Add new Hepatocyte Growth Medium.



#### 6. Check and incubate the cells

Check the cell density and place the vessel in an incubator (37°C, 5% CO<sub>2</sub>).



## Specifications

Product	Recommended Culture Media*	Plating Density	Passage after Thawing	Marker
Human Hepatocytes (HHpC)	C-25010 C-25020	150,000 cells per cm <sup>2</sup>	P0	Phase I reaction <sup>+</sup> Phase II reaction <sup>+</sup> Albumin <sup>+</sup>

## Related Products

Product	Size	Catalog Number
Hepatocyte Growth Medium (Ready-to-use)	500 ml	C-25010
Hepatocyte Growth Medium Kit	500 ml	C-25110
Hepatocyte Basal Medium	500 ml	C-25210
Hepatocyte Basal Medium, phenol red-free	500 ml	C-25215
Hepatocyte Growth Medium SupplementMix	for 500 ml	C-39642
Hepatocyte Growth Medium SupplementPack	for 500 ml	C-39640
Hepatocyte Maintenance Medium (Ready-to-use)	500 ml	C-25020
Hepatocyte Maintenance Medium Kit	500 ml	C-25120
Hepatocyte Maintenance Medium SupplementMix	for 500 ml	C-39652
Hepatocyte Maintenance Medium SupplementPack	for 500 ml	C-39650
HHpC Pellet	1 million cells per pellet	C-14085
PromoFectin-Hepatocyte	0.1 ml 0.5 ml	PK-CT-2000-HEP-10 PK-CT-2000-HEP-50

\*The catalog numbers in this table are for media in ready-to-use packaging.

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