3H Biomedical™
More Innovation, Less Preparation

BIOPREDIC INTERNATIONAL

PRODUCT DATA SHEET

Frozen Human Hepatocytes – CYP-controlled

Product:	Frozen Human Hepatocytes – CYP-controlled, for use in monolayer
Cat no:	BHEP187-I
Source:	Human liver
Unit Size:	1 million cells per vial
Storage:	<-135°C

Cell Source

Frozen human hepatocytes – CYP-controlled offered by 3H Biomedical are isolated from human liver by Biopredic International according to the method of Guguen-Guillouzo C and Guillouzo A (In "*Isolated and cultured hepatocytes*", Guillouzo A. and Guguen-Guillouzo C, Eds. INSERM Paris & John Libbey Eurotext London, 1986, pp.1-12.).

Cell Characterization

Frozen human hepatocytes – CYP-controlled are negative for HIV 1-2, HBV, HCV, mycoplasma, bacteria, yeast and fungi, and controlled for CYP-inducibility. The cells are genotyped for CYP2C9, CYP2C19, CYP2D6 and CYP3A5, and are characterized using the following functional controls:

- Phenacetin O-deethylase activity (CYP1A2)
- Tolbutamide hydroxylase activity (CYP2C9)
- Dextromethorphan O-demethylase activity (CYP2D6)
- Testosterone 6β-hydroxylase activity (CYP3A4/5)
- Paracetamol glucuronidation activity
- Paracetamol sulfation activity

Cell Culture Condition

The cells are cryopreserved immediately after purification and are transported at -135°C. If storage of the cells is desired, liquid nitrogen is recommended. Culture the frozen human hepatocytes in a monolayer at the conditions provided in the Instruction for use, and use the cells 24 hours after seeding.

Recommended Cell Culture Medium

- Hepatocyte thawing medium without glucose (100 ml, Cat. no. BMIL261)
- Williams medium for hepatocyte seeding (100 ml, Cat. no. BMIL212)
- Williams medium for hepatocyte incubation (100 ml, Cat. no. BMIL214-100ML; or 500 ml, Cat. no. BMIL214-500ML)
- Minimum Essential Medium (MEM) Earle – without phenol red (100 ml, Cat. no. BMIL236)

Cell Culture Reagents

- Percoll solution (100 ml, Cat. no. BPER212)
- Trypan blue solution (0.4% w/v, 100 ml, Cat. no. SR1007) – dilute to 0.05% in PBS before use

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This product is for research use only. Not approved for human use.

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INSTRUCTION FOR USE

Frozen Human Hepatocytes – CYP-controlled

IMPORTANT: Cryopreserved cells are very delicate. Thaw the vial in a 37°C waterbath and return them to culture as quickly as possible with minimal handling.

Equipment and Consumables

The following equipment and consumables are needed for culture of the cryopreserved hepatocytes:

- Laminar hood
- Small liquid nitrogen container
- Waterbath (37°C)
- Centrifuge
- Light microscope + materials for cell counting
- 37°C incubator with 5% CO_2/95% air and 100% RH
- Pipet-aid and micropipettes
- 2, 5, 10, and 30 ml sterile pipettes
- 5, 10, and 40 ml sterile tubes with round bottoms. **Do not use tubes with cone-shaped bottoms!**
- Plates or culture flasks coated with collagen I

Thawing Procedure

1. Work in sterile conditions. For one vial of cryopreserved hepatocytes, prepare one 40 ml tube with 30 ml of thawing medium. Equilibrate the tube to 37°C in a waterbath. Wipe it dry and rinse the tube with 70% ethanol, and transfer the tube to a sterile field.
2. Thaw the frozen vial in the waterbath until the ice detaches from the interior vial wall.
3. Remove the vial from the waterbath immediately, wipe it dry, rinse the vial with 70% ethanol and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads with fingers.
4. Pour the content of the vial (mainly ice and only a small volume of liquid) directly into the warm thawing medium. Rinse the vial with some thawing medium and gently transfer the cell-containing rinsing medium back to the tube with thawing medium.
5. Replace the cap of the tube containing cells and thawing medium and gently swirl the tube by hand until the ice has melted.
6. Centrifuge the tube for 1 minute at 1000 rpm.
7. Remove the supernatant and resuspend the cell pellet in 2 ml of seeding medium.

Percoll Purification

(Only if specified in the Certificate of analysis for the cell batch.)

1. Prepare one 10 ml tube with 4 ml of Percoll solution (28%).
2. Very slowly, dispense the cell suspension onto the Percoll solution. Tilt the tube 30°, and keep the pipette tip in contact with the inferior inner tube wall. The cell suspension must stay on the surface of the Percoll solution.
3. Centrifuge the tube for 5 minutes at 1000 rpm.
4. Remove the supernatant and resuspend the cell pellet in 2 ml of seeding medium.
5. Keep the cells at 4°C until seeding.

Cell Counting and Viability Estimation

1. Prepare a 5 ml tube with 950 µl trypan blue solution (0.05%).
2. Gently homogenize the cell suspension, and add 50 µl of the suspension to the trypan blue solution. Gently mix the obtained cell suspension.
3. Proceed to count the cells under the microscope. Only dead cells absorb the dye and will appear blue while the living cells remain unstained. Count the living and dead cells and calculate the cell concentration and viability.

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INSTRUCTION FOR USE

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Set Up and Use of Culture

1. Seed the cells according to Table 1 below. Check the cell density in the microscope, and if necessary, adjust the cell concentration. If you do not use 96-well plates, gently rock the culture vessel to distribute the cells evenly. Visually check the homogeneity of the cell suspension and place the cells in the incubator.
2. After the time in culture specified in the Certificate of analysis (3-16 hours), observe the cell morphology, adherence, density and spreading. Cells with visible nuclei must form a monolayer and be 70%, or more, confluent.
3. Preferably, use the Williams medium for hepatocyte incubation during your study. Change the medium after the adherence period (specified in the Certificate of analysis) and then every 24 hours.

Other media, such as MEM Earle without phenol red, can be used when the incubation period does not exceed 24 hours and the incubation is performed with CO_2.

Table 1. Seeding of human hepatocytes

Culture vessel	Number of cells/well or flask	Volume/well or flask	Cell concentration
96-well plate	50 000	100 µl	500 000/ml
24-well plate	400 000	500 µl	800 000/ml
12.5 cm^2 flask	2 300 000	2.5 ml	900 000/ml
25 cm^2 flask	4 700 000	5 ml	900 000/ml
75 cm^2 flask	14 000 000	15 ml	900 000/ml

Caution: Handling human-derived products is potentially biohazardous. Although each cell type has tested negative for HIV, HBV and HCV using serology, diagnostic tests are not necessarily 100% accurate. Therefore, proper precautions must be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working with these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].

[1]. Grizzle, W. E., and Polt, S. S. (1988) Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues. J Tissue Culture Methods. 11(4).

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