CRYOPRESERVED HEPATOCYTES

Product No.	Description	Size
M00995	Male Human, individual	5 million cells
F00995	Female Human, individual	5 million cells
M00005	Male Sprague–Dawley rat, pooled	5 million cells
F00005	Female Sprague–Dawley rat, pooled	5 million cells
M00025	Male Wistar rat, pooled	5 million cells
F00025	Female Wistar rat, pooled	5 million cells
M00065	Male Han Wistar rat, pooled	5 million cells
F00065	Female Han Wistar rat, pooled	5 million cells
M00105	Male Dunkin–Hartley guinea pig, pooled	5 million cells
F00105	Female Dunkin–Hartley guinea pig, pooled	5 million cells
M00205	Male beagle dog, pooled	5 million cells
F00205	Female beagle dog, pooled	5 million cells
M00305	Male cynomolgus monkey, pooled	5 million cells
F00305	Female cynomolgus monkey, pooled	5 million cells
M00315	Male rhesus monkey, pooled	5 million cells
F00315	Female rhesus monkey, pooled	5 million cells
M00325	Male Marmoset monkey, pooled	5 million cells
F00325	Female Marmoset monkey, pooled	5 million cells
M00405	Male New Zealand white rabbit, pooled	5 million cells
F00405	Female New Zealand white rabbit, pooled	5 million cells
M00505	Male ICR/CD–1 mouse, pooled	1 million cells
F00505	Female ICR/CD–1 mouse, pooled	1 million cells
M005052	Male ICR/CD-1 mouse, pooled	5 million cells
F005052	Female ICR/CD-1 mouse, pooled	5 million cells
M005152	Male C57BL/6 mouse, pooled	5 million cells
F005152	Female C57BL/6 mouse, pooled	5 million cells
M00615	Male Gottingen minipig, pooled	5 million cells
F00615	Female Gottingen minipig, pooled	5 million cells
X00005	Mixed Gender Sprague–Dawley rat, pooled	5 million cells
X00065	Mixed Gender Han Wistar rat, pooled	5 million cells
X00205	Mixed Gender beagle dog, pooled	5 million cells
X00305	Mixed Gender cynomolgus monkey, pooled	5 million cells
X005052	Mixed Gender ICR/CD-1 mouse, pooled	5 million cells

Product Description:

Hepatocytes are freshly isolated and cryopreserved on the same day. Cryopreserved hepatocytes in suspension are typically used to study phase I and phase II metabolism[1-4] in short-term studies ≤ 4 hours. Our hepatocytes perform the best when used with BioIVT INVITROGRO™ hepatocyte media.

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Stability: Stable for 5 years at ≤ –150°C
Storage: ≤–150 °C

Materials:

Item	Manufacturer	Product Number
INVITROGRO™ HT Medium	BioIVT	Z99019
INVITROGRO™ KHB	BioIVT	Z99074
Trypan Blue solution	Sigma	T8154

Procedure:

Thawing a single vial

1. Pre-warm INVITROGRO HT Medium to 37° C.
2. Transfer 48 mL of warm INVITROGRO HT Medium to a sterile 50 mL conical tube.
3. Carefully remove the vial from the shipping container or freezer. If the vial was stored in the liquid phase, carefully remove the cap and pour off any liquid nitrogen. Close the cap firmly before placing the vial into the water bath.
4. Immediately immerse the vial into a 37° C water bath. Shake gently until the ice is entirely melted, but no longer than it takes to completely thaw the vial. It may be helpful to remove the label from the vial so it is easier to view the vial contents.
5. Empty the contents of the vial into the pre-warmed INVITROGRO HT Medium.
6. Add 1.0 mL of pre-warmed INVITROGRO HT Medium to each vial to resuspend any remaining cells. Decant or pipette the contents into the hepatocyte suspension.
7. Resuspend the hepatocytes by gently inverting the tube several times (3 times is sufficient).
8. Centrifuge the cell suspension at 50 x g in a room temperature centrifuge for 5 minutes.
9. Discard the supernatant by either pouring in one motion (do not pour partially and re-invert centrifuge tube), or aspirating using a vacuum pump.
10. Loosen the cell pellet by gently swirling the centrifuge tube.
11. Add 2 mL of INVITROGRO KHB (or other appropriate) buffer. Invert the tube gently to resuspend the hepatocytes.
12. Determine the total cell count and the number of viable cells using the Trypan Blue exclusion method.

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Trypan Blue Cell Count Worksheet:

Remove a cell suspension aliquot and perform the following:

- Dilute cells for a Trypan Blue Exclusion cell count.

Example for a 10X dilution:

700 µL Medium or Buffer + 200 µL Trypan Blue + 100 µL diluted cells

- Mix and incubate for 1 minute
- Apply 10µL aliquot to one side of hemacytometer
- Count cells under 10X magnification
- Calculate total viable cells and percent viability

Cell Count:

Dilution Factor: _____X

Total Viable Cells: _____

Number of squares counted: _____

Total Nonviable Cells: _____

Total Cell Count: _____

% Viability = Total Viable Cells/Total Cell Count x 100 = _____

Dilution of Cell Suspension

$$\text{Cell Concentration (\# Viable Cells/mL)} = \frac{\text{Total Viable Cells}}{\text{\# squares counted}} \times 10{,}000 \times \text{Dilution Factor} \_\_\_\_ = \_\_\_\_ \text{cells/mL}$$

Cell Concentration x _____ mL Total Cell Suspension Volume = _____ Total Yield (cells)

$$\text{Total Resuspension Volume} = \frac{\text{Total Yield (cells)}}{\text{Target Cell Concentration (cells/mL)}} = \_\_\_\_\_ \text{ mL}$$

Resuspension Volume to be added = Total Resupension Volume – Original Suspension Volume =_____mL

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References:

1. Li, A. P. (**1997**). Primary hepatocyte cultures as an in vitro experimental model for the evaluation of pharmacokinetic drug-drug interactions. *Adv. Pharmacol. Series* 43, 103–130.

2. Loretz, L. J.; Li, A. P.; Flye, M. W.; Wilson, A. G. Optimization of cryopreservation procedures for rat and human hepatocytes. *Xenobiotica* **1989**, *19*(5), 489–498.

3. Ruegg, C. E.; Silber, P. M.; Mughal, R. A.; Ismail, J.; Lu, C.; Bode, D. C.; Li, A. P. Cytochrome-P450 induction and conjugated metabolism in primary human hepatocytes after cryopreservation. *In Vitro Toxicology* **1997**, *10*(2), 217–222.

4. Li, A. P.; Lu, C.; Brent, J. A.; Pham, C.; Fackett, A.; Ruegg, C. E.; and Silber, P. M. Cryopreserved human hepatocytes: characterization of drug-metabolizing enzyme activities and applications in higher throughput screening assays for hepatotoxicity, metabolic stability, and drug-drug interaction potential. *Chem. Biol. Interact*. **1999**, *121*, 17–35.

Caution: Treat all products containing human- and monkey-derived materials as potentially infectious since no known test methods can offer assurance that products derived from human or monkey tissues will not transmit infectious agents.

All products are for research use only. Do not use in animals or humans. These products have not been approved for any diagnostic or clinical procedures.

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