FRESH HEPATOCYTE SUSPENSION

Product No.	Description
M00986	Male human
F00986	Female human
M00286	Male beagle dog
M00386	Male cynomolgus monkey
M00586	Male ICR/CD-1 mouse
M00786	Male Sprague-Dawley rat

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.

Stability: Unpack and use immediately upon receipt

Item	Manufacturer	Product Number
INVITROGRO™ HI Medium	BioIVT	Z99009
INVITROGRO™ KHB	BioIVT	Z99074
Trypan Blue solution	Sigma	T8154

Media Preparation: Use INVITROGRO HI Medium or INVITROGRO KHB if running suspension assays. TORPEDO™ Antibiotic Mix not required for short-term assays.

Unpacking Hepatocyte Suspensions:

1. Pre-warm 50 mL INVITROGRO HI for suspension assays to 37° C.
2. Remove the conical tube(s) of cells from the shipment box.
3. Gently resuspend the cells by inverting the tube multiple times. Continue inverting until an even suspension is created.
4. Spin the cells at 50 × g for 5 minutes in a refrigerated centrifuge set to 4° C.
5. Pour the supernatant from the conical tube and add 50 mL of pre-warmed INVITROGRO HI to the cell pellet.
6. Invert the tube 3 times to resuspend the cell pellet.
7. Centrifuge the cell suspension at 50 × g in a room temperature centrifuge for 5 minutes.

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8. Discard the supernatant by either pouring in one motion (do not pour partially and re-invert centrifuge tube), or aspirating using a vacuum pump.
9. Loosen the cell pellet by gently swirling the centrifuge tube.
10. Add 2 mL of INVITROGRO KHB (or other appropriate) buffer per 10 million cells ordered. Invert the tube gently to resuspend the hepatocytes. Determine the total cell count and the number of viable cells using the Trypan Blue exclusion method (see Trypan blue cell count worksheet). Dilute to desired concentration for suspension assay, like 2 million viable cells per mL.

References:

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

Caution: Treat all products containing human and monkey-derived materials as potentially infectious, as 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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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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