LIVERPOOL® 5-, 10-, 20-, 50- AND 200-DONOR HUMAN CRYOPRESERVED HEPATOCYTES

Product No.	Description	Size
X008052	LIVERPOOL 5-Donor Pooled Cryopreserved Human Hepatocytes, Mixed Gender	5 million cells
X008001	LIVERPOOL 10-Donor Pooled Cryopreserved Human Hepatocytes, Mixed Gender	5 million cells
FX008001	LIVERPOOL 10-Donor Pooled Cryopreserved Human Hepatocytes, female pool	5 million cells
MX008001	LIVERPOOL 10-Donor Pooled Cryopreserved Human Hepatocytes, male pool	5 million cells
X008000	LIVERPOOL 20-Donor Pooled Cryopreserved Human Hepatocytes, Mixed Gender	5 million cells
X008005	LIVERPOOL 50-Donor Pooled Cryopreserved Human Hepatocytes, Mixed Gender	5 million cells
X008200	LIVERPOOL 200-Donor Pooled Cryopreserved Human Hepatocytes, Mixed Gender	5 million cells

*The process for producing the LIVERPOOL® pooled human hepatocyte products is covered by one or more U.S. or foreign patents and patent applications, including U.S. Patent No. 7,604,929.

Product Description:

Our patented LIVERPOOL 5- 10-, 20-, 50- and 200-donor pooled cryopreserved human hepatocytes are produced from nontransplantable liver tissue. Each donor pool is characterized for CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, UGT, and ST. LIVERPOOL viability is greater than 80% and the cells exhibit both phase I and II enzyme activities. Our hepatocytes perform the best when used with BioIVT INVITROGRO™ hepatocyte media.

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

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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

Caution: Treat all products containing human-derived materials a 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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