LIVERPOOL® CRYOPLATEABLE HEPATOCYTES

Product No.	Description	Size
X008052-P	5-Donor, Mixed Gender	5 million viable cells
X008001-P	10-Donor, Mixed Gender	5 million viable cells

*The process for producing the LiverPoo;® 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 LIVERPOOL cryoplateable 5- and 10- donor pooled human hepatocytes are produced from non-transplantable liver tissue. Cryoplateable hepatocytes are used for induction and toxicity studies[1]. Each donor pool is characterized for CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, UGT, and ST along with induction for CYP1A2, CYP2B6 and CYP3A4. LIVERPOOL viability is greater than 70% 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

Procedure:

Medium preparation

1. Prepare **complete** INVITROGRO CP Medium (Z99029)
   - Place the TORPEDO Antibiotic Mix (Z99000) in a 37° C water bath until thawed, then remove from water bath.
   - Add 1.0 mL TORPEDO Antibiotic Mix per 45 mL INVITROGRO CP Medium.

   Note: Following the addition of TORPEDO Antibiotic Mix, the shelf life for the complete medium is 7 days.

2. Completed media should be used for all media exchanges following plating.

Thawing a single vial

1. Pre-warm INVITROGRO CP Medium to 37° C.

2. Transfer 5 mL of warm INVITROGRO CP 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 immediately immersing the vial into a 37° C water bath. Shake gently. When the cells pull away from the vial wall, transfer the content of vial into the pre-warmed INVITROGRO CP Medium. This step can take 90-120 seconds.

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4. Add 1.0 mL of hepatocyte suspension to the vial to wash any remaining cells from the vial(s).

5. Resuspend the hepatocytes by gently inverting the tube several times (3 times is sufficient).

6. Determine the total cell count and the number of viable cells using the Trypan Blue exclusion method (reference the "Trypan Blue Cell Count Worksheet" section of this document).

7. Dilute the cells to 0.70 X 10^6 viable cells/mL with INVITROGRO CP Medium.

Thawing multiple vials

Note: All vials should be thawed in the water bath simultaneously.

1. Pre-warm INVITROGRO CP Medium to 37° C. Ensure that there is enough medium for 5 mL of pre-warmed INVITROGRO CP Medium for each vial of cryopreserved hepatocytes. Use a container that will allow for re-suspending the cells.

2. After the cells have pulled away from the vial walls, quickly remove caps from each vial and pour the contents into a sterile tube or beaker that contains at least 5 mL of pre-warmed INVITROGRO CP Medium per vial thawed. For example, use 25 mL for 5 vials in a container that can hold a volume of 50 mL.

3. Determine the total cell count and the number of viable cells using the Trypan Blue exclusion method (reference the "Trypan Blue Cell Count Worksheet" section of this document).

4. Dilute the cells to 0.70 X 10^6 viable cells/mL with INVITROGRO CP Medium.

Procedure for Plating Cryopreserved Hepatocytes:

1. Add an appropriate volume of diluted cells to collagen-coated tissue culture plates as follows:

   6-well plate: 2.5 mL/well (requires a total volume of 15 mL per 6-Well plate)
   12-well plate: 1.0 mL/well (requires a total volume of 12 mL per 12-Well plate)
   24-well plate: 0.5 mL/well (requires a total volume of 12 mL per 24-Well plate)
   48-well plate: 0.2 mL/well (requires a total volume of 10 mL per 48-Well plate)
   96-well plate: 70µL/well (requires a total volume of 10 mL per 96-Well plate)

   For T-flasks, add 0.25 mL/cm^2 to the T-flask.

2. Gently shake the plates in a back-and-forth and side-to-side manner to evenly distribute the cells. Avoid any circular movement, as this will cause the cells to unevenly pool in the center of the plates.

3. Carefully place the plates into a 37° C, 5% CO_2, saturating humidity incubator to allow the cells to attach.

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Wash Plated Cells

1. **After 2-4hrs wash plate with completed** CP Medium (Z99029) from media preparation above.

Related Products:

Product No.	Description	Size
Z99029	INVITROGRO™ CP (plating) medium	250 mL
Z990003	INVITROGRO™ CP (plating) medium	500 mL
Z990004	INVITROGRO™ CP (plating) medium	1 L
Z99000	TORPEDO™ Antibiotic Mix	5.5 mL
Z990007	TORPEDO™ Antibiotic Mix	11 ml
Z990008	TORPEDO™ Antibiotic Mix	22 mL

Reference:

1. Roymans, D.; Van Looveren, C.; Leone, A.; Parker, J. B.; McMillan, M.; Johnson, M. D.; Koganti, A.; Gilissen, R.; Silber, P.; Mannens, G.; Meuldermans, W. Determination of cytochrome P450 1A2 and cytochrome P450 3A4 induction in cryopreserved human hepatocytes. *Biochem. Pharmacol.* **2004**, *67(3),* 427-437.

Caution: *This product was prepared from human tissue. Treat all products containing human-derived materials as potentially infectious, as no known test methods can offer assurance that products derived from human tissues will not transmit infectious agents.*

This product is being sold for research and/or manufacturing purposes only. The biological samples supplied by BioIVT, or any material isolated from the samples, are for in-vitro research use only and are not to be used as a source of material for clinical therapies. Human material may be used in vivo in animals. The user assumes all responsibility for its usage and disposal, in accordance with all regulations.

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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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