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 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 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. All cryoplateable hepatocyte characterization information can be found by viewing the characterization tables at **www.bioreclamationivt.com/tables**. Our hepatocytes perform the best when used with BioreclamationIVT *InVitro*GRO™ hepatocyte media.

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

Thawing Procedure:

Medium preparation

1. Prepare complete *InVitro*GRO CP Medium
   - Place the *Torpedo* Antibiotic Mix in a 37° C water bath until thawed, then remove from water bath.
   - Add 1.0 mL *Torpedo* Antibiotic Mix per 45 mL *InVitro*GRO CP medium.

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

Thawing a single vial

1. Pre-warm *InVitro*GRO CP Medium to 37° C.

2. Transfer 5 mL of complete warm *InVitro*GRO CP Medium to a sterile 50 mL conical tube.

3. Carefully remove the vial from the shipping container or cryostorage unit. 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.

   Immediately immerse 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 *InVitro*GRO CP medium in biological safety cabinet. This step can take 90-120 seconds.

4. Add 1.0 ml of hepatocyte suspension to the vial to wash any remaining cells from the vial.

5. Cell suspension may be stored at ambient temperature for remaining steps. Do not store on ice.

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

7. Determine the total cell count and the number of viable cells using the Trypan Blue exclusion method.

8. Dilute cells to 0.70 X 10^6 viable cells/mL with *InVitro*GRO CP Medium.

Thawing multiple vials

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

1. Pre-warm *InVitro*GRO CP Medium to 37° C. Ensure that there is enough medium for 5 mL of pre-warmed *InVitro*GRO 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 complete pre-warmed *InVitro*GRO 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.

4. Dilute the cells to 0.70 x 10^6 viable cells/mL with *InVitro*GRO CP Medium, for all species except mouse. For mouse, dilute the cells to 0.35 x 10^6 viable cells/mL.

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: 70 µL/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.

4. Human and monkey hepatocytes will attach within 2-4 hours at which time the medium should be aspirated and gently replaced with *InVitro*GRO CP with antibiotics.

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

Number of squares counted:_____

Total Viable Cells:_____

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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Related Products:

Product No.	Description	Size
Z99029	*InVitro*GRO™ CP (plating) medium	250 mL
Z990003	*InVitro*GRO™ CP (plating) medium	500 mL
Z990004	*InVitro*GRO™ 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: Treat all products containing human and monkey-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.