

Cryoplateable Hepatocytes

Product No.	Description	Size
M00995-P	Male Human	5 million viable cells*
F00995-P	Female Human	5 million viable cells*
M003055-P	Male Cynomolgus Monkey	5 million viable cells*
M003155-P	Male Rhesus Monkey	5 million viable cells*
M00615-P	Male Göttingen Minipig	5 million viable cells*

*Visit www.bioreclamationivt.com/tables for lot availability

Product Description:

Hepatocytes are freshly isolated and cryopreserved on the same day. All cryoplateable hepatocyte characterization information can be found by viewing the characterization tables at www.bioreclamationivt.com/tables. Cryoplateable hepatocytes are used for induction and toxicity studies¹. Our hepatocytes perform the best when used with BioreclamationIVT *InVitroGRO*[™] hepatocyte media

Stability: Stable for approximately 5 years at $\leq -150^{\circ}\text{C}$

Storage: $\leq -150^{\circ}\text{C}$

Thawing Procedure:

Medium preparation

1. Prepare complete *InVitroGRO* 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 *InVitroGRO* 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 *InVitroGRO* CP Medium to 37°C .
2. Transfer 5 mL of complete warm *InVitroGRO* 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 *InVitroGRO* 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 0.70×10^6 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 complete 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.
4. Dilute the cells to 0.70×10^6 viable cells/mL with *InVitroGRO* CP Medium, for all species except mouse. For mouse, dilute the cells to 0.35×10^6 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/cm² 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% CO₂, 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 *InVitroGRO* 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 μ L Medium or Buffer + 200 μ L Trypan Blue + 100 μ L diluted cells

- Mix and incubate for 1 minute
- Apply 10 μ L 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

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

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

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

Resuspension Volume to be added = Total Resuspension Volume – Original Suspension Volume = _____ mL

Related Products:

Product No.	Description	Size
Z99029	<i>InVitroGRO</i> TM CP (plating) medium	250 mL
Z990003	<i>InVitroGRO</i> TM CP (plating) medium	500 mL
Z990004	<i>InVitroGRO</i> TM CP (plating) medium	1 L
Z99000	<i>Torpedo</i> TM Antibiotic Mix	5.5 mL
Z990007	<i>Torpedo</i> TM Antibiotic Mix	11 ml
Z990008	<i>Torpedo</i> TM 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.