Dog Cryoplateable Hepatocytes

Product No.	Description	Size
M002055-P	Male Beagle Dog	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 studies. Dog cryoplateable hepatocytes will attach to a collagen coated plate for 5 days. Our dog hepatocytes perform the best when used with BioreclamationIVT *InVitro*GRO™ CP rat hepatocyte medium (S01494) and *Torpedo*™ Antibiotic Mix (Z99000).

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

Thawing Procedure:

Thawing a single vial

1. Pre-warm *InVitro*GRO CP Rat Medium to 37° C. **Do not add the *Torpedo* Antibiotic Mix to the Media at this step. The thawing is done without the *Torpedo* antibiotic mix.**

2. Transfer 5 mL of warm *InVitro*GRO CP Rat 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 *InVitro*GRO CP Rat medium. 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(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 **dog** cells to 0.70 X 10^6 viable cells/mL with *InVitro*GRO CP Rat Medium.

Thawing multiple vials

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

1. Pre-warm *InVitro*GRO CP Rat Medium to 37° C. Ensure that there is enough medium for 5 mL of pre-warmed *InVitro*GRO CP Rat 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 Rat 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 **dog** cells to 0.70 X 10^6 viable cells/mL with *InVitro*GRO CP Rat 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.

Medium preparation

1. Prepare **complete** *InVitro*GRO CP Rat Medium (S01494)
   - 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 *InVitro*GRO CP Rat 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.

Wash Plated Cells

1. After 2-4hrs wash plate with completed CP Rat Medium (S01494) from media preparation above.

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
S01494	*InVitro*GRO™ CP Rat medium, custom formulation	250 mL
Z99000	*Torpedo* ™ Antibiotic Mix	5.5 mL

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.