INSTRUCTIONS FOR USE

Product Name: Rodent Cryoplateable Hepatocytes

Product No.	Description	Size
F00005-P	Female Sprague-Dawley Rat	5 million cells
M00005-P	Male Sprague-Dawley Rat	5 million cells
F00025-P	Female Wistar Rat	5 million cells
M00025-P	Male Wistar Rat	5 million cells
F00065-P	Female Han Wistar Rat	5 million cells
M00065-P	Male Han Wistar Rat	5 million cells
F005052-P	Female ICR/CD-1 Mouse	5 million cells
M005052-P	Male ICR/CD-1 Mouse	5 million cells
F005152-P	Male C57BL/6 Mouse	5 million cells
M005152-P	Male C57BL/6 Mouse	5 million cells

Product Description

Hepatocytes are freshly isolated and cryopreserved on the same day. Cryoplateable hepatocytes are used for induction, metabolism and toxicity studies. Rodent cryoplateable hepatocytes will attach to a collagen coated plate for 5 days. Our Rodent hepatocytes perform the best when used with BioIVT INVITROGRO™ CP Rodent Hepatocyte Medium (Z990028) and TORPEDO™ Rodent Antibiotic Mix (Z99027).

Storage

Stable for 5 years at ≤ –150°C

Handling/Caution Statement

Caution: *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.*

Materials

Product No.	Description	Size
Z990028	INVITROGRO™ CP Rodent Medium	250 mL
Z990027	TORPEDO ™Rodent Antibiotic Mix	5.5 mL

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Protocol

Medium preparation

1. Prepare **complete** INVITROGRO CP Rodent Medium (Z990028)
   - Place the TORPEDO Rodent Antibiotic Mix (Z990027) in a 37° C water bath until thawed, then remove from water bath.
   - Add 1.0 mL TORPEDO Rodent Antibiotic Mix per 45 mL INVITROGRO CP Rodent Medium.

   Note: Following the addition of TORPEDO Rodent 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 Rodent Medium to 37° C.
2. Transfer 5 mL of warm INVITROGRO CP Rodent 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 Rodent 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. For **Rat** hepatocytes dilute the **Rat** cells to 0.70 X 10_6 viable cells/mL with INVITROGRO CP Rodent Medium. For **Mouse** hepatocytes dilute the **Mouse** cells to 0.35 X 106 viable cells/mL with INVITROGRO CP Rodent Medium.

Thawing multiple vials

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

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

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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 Rodent 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).

8. For **Rat** hepatocytes dilute the **Rat** cells to 0.70 X 10_6 viable cells/mL with INVITROGRO CP Rodent Medium. For **Mouse** hepatocytes dilute the **Mouse** cells to 0.35 X 106 viable cells/mL with INVITROGRO CP Rodent 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.

Wash Plated Cells

1. **After 2-4hrs wash plate with completed** CP Rodent Medium (Z990025) from media preparation above.

Related Products

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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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