SPHEROID CERTIFIED™ HEPATOCYTES

Product No.	Description	Size
M00995-SCERT	Male Human Cryoplateable Hepatocytes, SPHEROID CERTIFIED	5 million
F00995-SCERT	Female Human Cryoplateable Hepatocytes, SPHEROID CERTIFIED	5 million

Product Description:

Spheroid forming hepatocytes are freshly isolated and cryopreserved on the same day. SPHEROID CERTIFIED hepatocytes are used for making 3D spheroids which can be used in metabolism, cytotoxicity, and disease modelling studies. SPHEROID CERTIFIED hepatocytes will form a spheroid within 7 days of plating in a cell repellent U bottom plate. Our cryoplateable spheroid forming hepatocytes perform the best when thawed into BioIVT INVITROGRO™ Spheroid Spin Medium (Z990032) followed by plating in INVITROGRO™ Spheroid Plating Medium (Z990033) and maintained in INVITROGRO™ Spheroid Maintenance Medium (Z990034) along with Spheroid Media Supplement A (Z990035) and TORPEDO™ Antibiotic Mix (Z99000).

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

Procedure:

Medium preparation

1. Day 0:

Prepare **complete** INVITROGRO Spheroid Plating Medium (Z990033). Place one vial of the Spheroid Medium Supplement A (Z990034) and TORPEDO Antibiotic Mix (Z99000) in a 37° C water bath until thawed, then remove from water bath.

- Add 1 ml Spheroid Media Supplement A (Z990035) and 5.5 mL TORPEDO Antibiotic Mix per 250 mL INVITROGRO Spheroid Plating medium (Z990033).

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

2. Day 5:

Prepare **complete** INVITROGRO Spheroid Maintenance Medium (Z990034). Place one vial of the Spheroid Medium Supplement A (Z990034) and TORPEDO Antibiotic Mix (Z99000) in a 37° C water bath until thawed, then remove from water bath.

- Add 1 ml Spheroid Medium Supplement A (Z990035) and 5.5 mL TORPEDO Antibiotic Mix per 250 mL INVITROGRO Spheroid Maintenance medium (Z990034).

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

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- Medium can be completed in smaller batches utilizing the ratio of 1.1ml Antibiotic Mix/50ml media. Avoid freeze thaw in the TORPEDO Antibiotic Mix by aliquoting, as necessary.

Completed INVITROGRO Spheroid Maintenance Medium (Z990034) should be used for all medium exchanges following plating.

Thawing and plating single vial

1. Pre-warm INVITROGRO Spheroid Spin Medium (Z990032) and INVITROGRO Spheroid Plating Medium (Z990033) to 37° C.

2. Transfer 25 mL of warm INVITROGRO Spheroid Spin Medium to a sterile 50 mL conical tube. If thawing more than two vials, 50ml of INVITROGRO Spheroid Spin Medium should be utilized with a maximum of 5 vials per 50ml conical.

3. Carefully remove the vial from the shipping container or liquid nitrogen storage. Immediately immerse the vial into a 37° C water bath. This step can take 90-120 seconds. When the cells pull away from the vial wall, transfer the content of vial into the INVITROGRO Spheroid Spin Medium. The vial may be rinsed by transferring 1ml of medium.

4. Resuspend the hepatocytes in the INVITROGRO Spheroid Spin Medium by gently inverting the tube several times (3 times is sufficient).

5. Spin the tube at 100g for 10 minutes.

6. Aspirate supernatant and resuspend cells in 5 mL of complete INVITROGRO Spheroid Plating Medium (Z990033).

7. 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. Dilute the cells to 1.5 X 104 viable cells/mL with INVITROGRO Spheroid Plating Medium.
NOTE: 96-well plate: 100 µL/well for a 1500 cell spheroid (requires a total volume of 10 mL per 96-Well plate)

9. Gently mix the diluted cells in a tube to ensure a homogenous solution.

10. To each well of a cell repellent U-bottom 96-well plate to receive cells, add 100ul of INVITROGRO Spheroid Plating Medium (Z990033).

11. Using a repeater, add 100 µL of the diluted cells per well of a sterile, 96-well U-bottom Ultra low attachment microplate recommended for Spheroid formation.

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Note: BioIVT utilized Nunclon™ Sphera™ 96-Well, U-Shaped-Bottom Microplates in their testing and recommends for usage, other similar plates may be utilized with incorporation of manufactures recommended protocol.

12. Spin plate at 250g for 2 mins.

13. Check the bottom of the plates for cell aggregation in each well.

14. Place in the incubator set at 37°C, 5% CO_2 for 5 days.

Maintaining spheroids

1. Day 5: Carefully remove 100 µL of medium from each well without disturbing the spheroid and add 100 µL of fresh INVITROGRO Spheroid Maintenance Medium (Z990034).

2. Change media every other day after Day 5 with INVITROGRO Spheroid Maintenance Medium.

Related Products:

Product No.	Description	Size
Z990032	INVITROGRO™ Spheroid Spin Medium	50 mL
Z990033	INVITROGRO™ Spheroid Plating Medium	250 mL
Z990034	INVITROGRO™ Spheroid Maintenance Medium	250 mL
Z990035	INVITROGRO™ Spheroid Media Supplement A	1 mL
Z99000	TORPEDO™ Antibiotic Mix	5.5 mL
Z990036	INVITROGRO™ Spheroid Media Kit	1 Kit

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 Resuspension Volume – Original Suspension Volume =_____mL

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