CRYOPRESERVED KUPFFER CELLS

Product No.	Description	Size
F00903	Female Human High-purity Kupffer Cells	1 million viable
M00903	Male Human High-purity Kupffer Cells	1 million viable
S00352	Female Human Kupffer-enriched NPCs	1 million viable
S00353	Male Human Kupffer-enriched NPCs	1 million viable

Product Description:

Kupffer cells are freshly isolated and cryopreserved on the same day. Cryopreserved Kupffer cells are used for disease modeling, metabolism and drug-induced liver injury (DILI) studies. Cryopreserved Kupffer cells will attach to a collagen coated plate in monoculture, or to pre-plated hepatocytes in co-culture. Our cryopreserved Kupffer cells perform the best when used with BioIVT INVITROGRO™ CP NPC Medium (Z990031) and TORPEDO™ Antibiotic Mix (Z99000).

Our high-purity Kupffer cells have a purity level of > 70% measured by flow cytometry and characterized for cell function. Our Kupffer-enriched NPCs contain a mixture of NPCs with <40% population of Kupffer cells. Both products have a minimum of 1 million viable cells per vial. Select the best product suited for your assay.

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

Preparation:

Medium preparation

1. Prepare **complete** INVITROGRO CP NPC Medium (Z990031)
   - 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 INVITROGRO CP NPC Medium.

   Note: Following the addition of TORPEDO Antibiotic Mix, the shelf life for the complete medium is 7 days and should be stored at 4° C.

2. Completed medium should be used for all medium exchanges following plating.

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Procedure for Plating Kupffer Cells in Monoculture:

Thawing a single vial

1. INVITROGRO CP NPC Medium should be used at 4° C to maximize cell yield.

2. Transfer 10 mL of cold completed INVITROGRO CP NPC Medium to a sterile 50 mL conical tube.

3. Carefully remove the vial of cryopreserved Kupffer Cells 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 INVITROGRO CP NPC Medium. This step can take 90-120 seconds.

4. Add 1.0 mL of Kupffer cell suspension to the vial to wash any remaining cells from the vial(s).

5. Spin at 500 x g for 10 minutes at 4° C to pellet the Kupffer cells

6. Aspirate the supernatant, being careful not to disrupt the pellet.

7. Re-suspend the Kupffer cells in 1.0 mL completed INVITROGRO CP NPC Medium.

8. 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).

9. Dilute the cells to the desired number of viable cells/mL (appropriate cell number is assay dependent- recommended $0.25 \times 10^6 - 0.5 \times 10^6$) with completed INVITROGRO CP NPC Medium.

10. 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)

11. 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.

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12. Carefully place the plates into a 37° C, 5% CO_2, saturating humidity incubator to allow the cells to attach.

Thawing multiple vials

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

1. Use completed INVITROGRO CP NPC Medium at 4° C. Ensure that there is enough medium for 10 mL of INVITROGRO CP NPC Medium for each vial of cryopreserved Kupffer cells. Use one or more centrifuge tubes that will allow for centrifugation and re-suspension of the cells.

2. After the cells have pulled away from the vial walls, quickly remove caps from each vial and pour the contents into the sterile centrifuge tube(s) containing at least 10 mL of cold INVITROGRO CP NPC Medium per vial thawed. For example, use 50 mL for 5 vials in a centrifuge tube that can hold a volume of 50 mL.

3. Spin at 500 x g for 10 minutes at 4° C to pellet the Kupffer cells

4. Aspirate the supernatant, being careful not to disrupt the pellet.

5. Re-suspend the Kupffer cells in 1.0 mL INVITROGRO CP NPC Medium per vial of cells. For example, if 5 vials were thawed into a single 50mL conical tube resuspend the cells in 5 mL INVITROGRO CP NPC Medium after centrifugation.

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 cells to the desired number of viable cells/mL (appropriate cell number is assay dependent) with INVITROGRO CP NPC Medium.

8. 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)

9. 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.

10. Carefully place the plates into a 37° C, 5% CO_2, saturating humidity incubator to allow the cells to attach.

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Procedure for Plating Kupffer Cells in Co-culture with Hepatocytes:

1. 24 hours prior to plating Kupffer cells, plate primary human hepatocytes in one or more collagen-coated cell culture plates.

2. For Kupffer cell thawing and plating use INVITROGRO CP NPC Medium at 4° C. Transfer 10 mL of cold completed INVITROGRO CP NPC Medium per vial thawed a sterile conical tube.

3. 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 INVITROGRO CP NPC Medium.

4. Spin at 500 x g for 10 minutes at 4° C to pellet the Kupffer cells

5. Aspirate the supernatant, being careful not to disrupt the pellet.

6. Re-suspend the Kupffer cells in 1.0 mL INVITROGRO CP NPC Medium per vial of cells.

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 the desired number of viable cells/mL. Common hepatocyte: Kupffer cell ratios include 1:0.1and 1:0.4, which have been suggested to simulate the cell ratios in normal and inflamed livers respectively[1,2]. Different hepatocyte: Kupffer cell ratios may be used depending on application requirements and assay optimization.

    - For a hepatocyte: Kupffer cell ratio of 1:0.1, dilute Kupffer cells to $7.0x10^4$ cells/mL using completed INVITROGRO CP NPC Medium and add to hepatocytes plated at $0.7x10^6$ cells/mL

    - For a hepatocyte: Kupffer cell ratio of 1:0.4, dilute Kupffer cells to $0.28x10^6$ cells/mL using completed INVITROGRO CP NPC Medium and add to hepatocytes plated at $0.7x10^6$ cells/mL

9. Aspirate the media from the previously plated hepatocytes. Add an appropriate volume of diluted cells to each well 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)

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11. 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.

12. Carefully place the plates into a 37° C, 5% CO_2, saturating humidity incubator to allow the cells to attach.

Media Change

1. **After 24hrs exchange the media with pre-warmed completed** INVITROGRO CP NPC Medium (Z990031) from media preparation above.

Related Products:

Product No.	Description	Size
Z990031	INVITROGRO™ CP (plating) NPC medium	250 mL
Z99000	TORPEDO™ Antibiotic Mix	5.5 mL

References:

1. Theresa V. Nguyen, Okechukwu Ukairo, Salman R. Khetani, Michael McVay, Chitra Kanchagar, Wolfgang Seghezzi, Gulesi Ayanoglu, Onyi Irrechukwu and Raymond Evers. Drug Metabolism and Disposition. May 1, 2015, 43 (5) 774-785

2. Jeffrey A. Sunman, Roy L. Hawke, Edward L. LeCluyse and Angela D. M. Kashuba. Drug Metabolism and Disposition. March 1, 2004, 32 (3) 359-363

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 hemocytometer
- 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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