Human Hepatic Kupffer Cell Manual

INSTRUCTION MANUAL ZBM0099.02

SHIPPING CONDITIONS

Human Hepatic Kupffer Cells.
All US and Canada orders are shipped via Federal Express Priority service and are usually received the next day. International orders are shipped via FedEx or DHL service using dry ice or a dry vapor shipper if transit time will exceed 3 days. Primary human hepatic cells are very sensitive to extended times (> 3 days) transported using dry ice. Please inquire for dry vapor shipper availability if your transit time will exceed 3 days. Cells should always be stored in liquid nitrogen vapor phase immediately upon arrival.

Must be processed upon shipment receipt.

STORAGE CONDITIONS

Media: Store as indicated IMMEDIATELY UPON ARRIVAL

Cells: Human Hepatic Kupffer Cells are to be stored in vapor phase nitrogen (-150°C to -190°C) IMMEDIATELY UPON RECEIPT.

All Zen-Bio Inc. products are for research use only. Not approved for use in humans. Not approved for human or veterinary therapeutic, diagnostic or clinical procedures.

Ordering Information and Technical Services

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THIS MANUAL IS SUITABLE FOR USE WITH THE FOLLOWING PRODUCT(S):

HP-KC-F	CRYOPRESERVED HUMAN HEPATIC KUPFFER CELLS, 1 MILLION CELLS/VIAL /

LIMITED PRODUCT WARRANTY

This warranty limits our liability to replacement of this product. No other warranties of any kind, expressed or implied, including without limitation implied warranties of merchantability or fitness for a particular purpose, are provided by Zen-Bio, Inc. Zen-Bio, Inc. shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

Zen-Bio, Inc. warrants its cells only if Zen-Bio media are used and the recommended storage conditions, media and protocols are followed without amendment or substitution.

Contact ZenBio, Inc. within no more than 24 hours after receipt of products for all claims regarding shipment damage, incorrect ordering or other delivery issues. Delivery claims received after 7 days of receipt of products are not subject to replacement or refund.

PRECAUTIONS

This product is for research use only. *It is not intended for human, veterinary, or in vitro diagnostic use*. Proper precautions and biological containment should be taken when handling cells of human origin, due to their potential biohazardous nature. **Always wear gloves and work behind a protective screen when handling primary human cells.** All media, supplements, and tissue culture ware used in this protocol should be sterile.

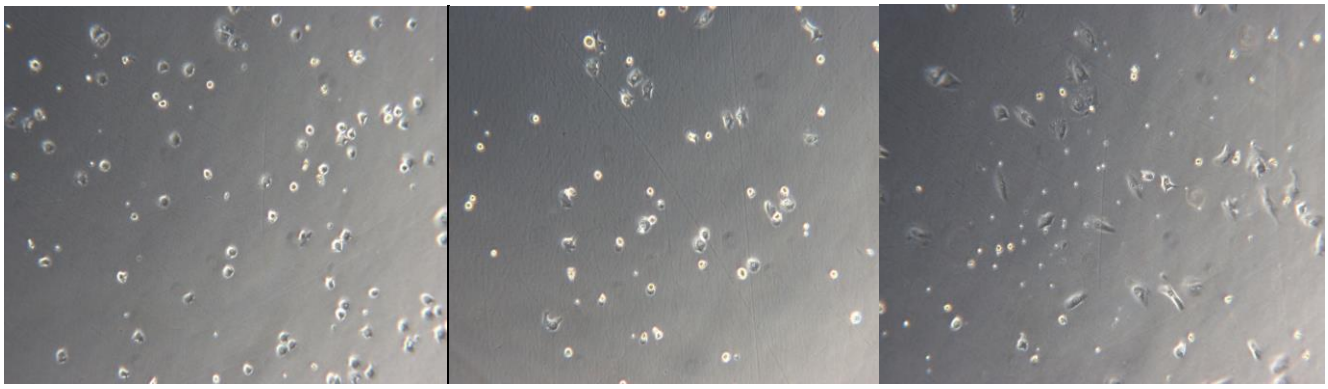
Human Hepatic Kupffer Cell viability depends greatly on the use of the recommended storage temperature, protocols, suitable media, reagents, and sterile plastic wear. If these parameters are not carefully observed this may result in poor plating and viability of the cells.

INTRODUCTION

Kupffer Cells, also known as Browicz-Kupffer cells or stellate macrophages, are specialized macrophages located in the liver lining the walls of the sinusoids. These cells are constantly exposed to gut-derived bacteria, microbial debris and bacterial endotoxins, known to activate macrophages. Upon activation Kupffer cells release various products, including cytokines, prostanoides, nitric oxide and reactive oxygen species. These factors regulate the phenotype of Kupffer cells themselves, and the phenotypes of neighboring cells, such as hepatocytes, stellate cells, endothelial cells and other immune cells that traffic through the liver. Therefore, Kupffer cells are intimately involved in the liver's response to infection, toxins, ischemia, resection and other stresses. **Figure 1** shows the attachment of Zen Bio Kupffer Cells after 6 hr. (A), 24 hr (B), and 48 hr. (C). Note the morphology change over time.

Figure 1.

(A) (B) (C)



QUALITY CONTROL

Human Hepatic Kupffer Cells from Zen-Bio are obtained via the gift of organ donation from livers not suitable for organ transplant. Each donor has confirmed documentation on file allowing for non-clinical research use of any non-transplantable organs or tissues in compliance with ethical regulations. The procedures for processing the samples are Standard Operating Procedure (SOP) managed GLP protocols. All samples are collected and processed in the United States.

Each vial contains 1 million cells viable cells. Cells are assessed for viability and cell surface marker analysis using a panel of markers to verify cell type. Each lot is tested and found non-reactive to viral DNA from HIV, hepatitis B and hepatitis C. Kupffer Cells are characterized using flow cytometry for population distributions, and are positive for CD11b, CD14, and CD68 (Seiki *et al*., 2014).

MATERIALS PROVIDED FOR EACH CATALOG ITEM

- **Cryopreserved Human Hepatic Kupffer Cells**
  - Cat # HP-KC-F
  - Frozen vial containing 1 million viable Human Hepatic Kupffer cells.
    (Store cells in vapor phase nitrogen (-150°C to -190°C) immediately upon receipt)

MEDIA COMPOSITION

Human Hepatic Kupffer Cell Plating Medium Cat# KC-1 (250ml)	**Storage and Expiration Date**
• DMEM- high glucose (4.5g/L) • Insulin, human recombinant • Transferrin, human halo • Selenium • L-alanyl-L-glutamine • Glycine • L-Alanine • L-Asparagine • L-Aspartic • L-Glutamic Acid • L-Proline • L-Serine • HEPES • Fetal Bovine Serum (FBS) • Penicillin • Streptomycin • Amphotericin B	• Store at 4°C • The expiration date is 30 days from the ship date. • Medium is provided ready to use and prepared fresh prior to shipment.

Human Hepatic Kupffer Cell Maintenance Medium Cat# KC-2 (250ml)	**Storage and Expiration Date**
• RPMI 1640 • L-alanyl-L-glutamine • HEPES • Fetal Bovine Serum (FBS) • Penicillin • Streptomycin • Amphotericin B	• If placed at 4°C upon arrival, the media is stable until the expiration date on the bottle label. • If stored at -20°C upon arrival, the media is stable for 6 months. Add fresh antibiotics when you are ready to use. The media will expire 30 days after the thaw date.

THAWING AND PLATING CRYOPRESERVED KUPFFER CELLS

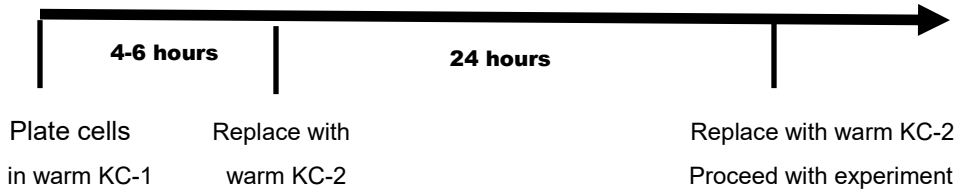
NOTE: Collagen I coated cultureware is required. Read the FAQ section for details on recommended brands.

DO NOT pre-warm medium to thaw cells. READ instructions listed below.

1. Place vial in a 37°C water bath, hold and rotate vial gently until the contents are completely thawed. Remove the vial from the water bath immediately, wipe dry, rinse the vial with 70% ethanol and transfer to a sterile field. Remove cap, being careful not to touch the interior threads with fingers.
1. Using a pipette, gently transfer contents of vial to a 15 ml conical tube containing 9 mL of **COLD** (4°C) Kupffer Cell Plating Medium (KC-1) and place the tube on ice.
    a. **Note:** Kupffer cells easily attach to the walls of the conical tube at 37°C. Therefore, use of pre-warmed media is **not** recommended at this step.
2. Centrifuge tube at 500xg for 5 minutes. After centrifugation, aspirate medium and re-suspend cell pellet in 1mL **COLD** Kupffer Cell Plating Medium KC-1.
    a. **Note:** the pellet will be very small. Resuspend using a P1000 micropipette, as resuspension using a serological pipette may lead to clumping of the cells.
3. Count the cells using the trypan blue exclusion assay.
4. Dilute the cells in **WARM** Kupffer Cell Plating Medium (KC-1) to 400,000 cells/ml.
5. Plate 100,000 cells/cm^2 on cultureware coated with collagen type I (see FAQ on page 6)
    a. The table below lists surface area information for ZenBio collagen coated cultureware.
    b. Please confirm the surface area if you are using a different brand of collagen I coated cultureware and calculate the number of cells you will require using the amount of 100,000 cells/cm^2. There are differences in the surface area of cultureware from different vendors.

ZenBio Cat#	Format	Surface area per well (cm^2)	cells/cm^2	Cells per well	Total cells per plate
CC-96	96	0.29	100,000	29,000	2.78x10^6
CC-48	48	0.64	100,000	64,000	3.07 x10^6
CC-24	24	1.82	100,000	182,000	4.37 x 10^6
CC-12	12	3.65	100,000	365,000	4.38x10^6
CC-6	6	8.87	100,000	887,000	5.32 x10^6
CC-25	T-25	25	100,000		2.5x10^6
CC-75	T-75	75	100,000		7.5x10^6

6. Place the cells in a humidified 37°C/5% CO_2 incubator and allow them to attach for 4–6 hours.
7. After attachment, replace the medium with fresh WARM Kupffer Cell Maintenance Medium (KC-2).
8. After 24 hours, replace the medium with warmed Kupffer Cell Maintenance Medium (KC-2) and proceed with your experiment.



FREQUENTLY ASKED QUESTIONS

1. Can I expand these cells?
    a. We do not recommend expanding these cells. Any other use negates the warranty.
    b. These primary cells are designed to be thaw and use one time only.
    c. We do not recommend thawing a portion of the vial to re-freeze for later use. Use the entire vial contents for one-time use.

2. Is there a specific type of culture ware that should be used?
    a. Yes.
    b. Only Corning/BD Biocoat or ZenBio brand Collagen I Coated Cultureware should be used. Read the chart below for ZenBio collagen coated cultureware information.

ZenBio Collagen I Coated Cultureware

Cat#	ZenBio Collagen I Coated Cultureware Description
CC-96	Collagen I Coated 96-well Plate, Pack of 5
CC-48	Collagen I Coated 48-well Plate, Pack of 5
CC-24	Collagen I Coated 24-well Plate, Pack of 5
CC-12	Collagen I Coated 12-well Plate, Pack of 5
CC-6	Collagen I Coated 6-well Plate, Pack of 5
CC-25	Collagen I Coated T-25 Flask, Vent Cap, Pack of 5
CC-75	Collagen I Coated T-75 Flask, Vent Cap, Pack of 5
CC-225	Collagen I Coated T-225 Flask, Vent Cap, Pack of 1 (EXCLUSIVE!)

3. Why can I not use pre-warmed medium to thaw the Kupffer cells?
    a. Kupffer cells easily attach to the walls of the conical tube at 37°C. Using cold medium helps minimize loss of cells during the thawing process.

4. What happens if the cells are allowed to attach longer than 6 hours?
    a. The cells will begin to die and your attachment will be significantly lower.

5. Do you test for pathogens? Which ones?

    Yes. Samples from each donor are tested for HIV-1, HIV-2, Hepatitis B, Hepatitis C. Some samples may also be tested for Epstein Barr Virus and Cytomegalovirus (CMV). However, since we cannot test all pathogens, please treat the culture as a potentially infectious agent using Biosafety Lever 1 or higher.

PATHOGEN TESTING

Each lot is tested and found non-reactive to viral DNA from Hepatitis B and viral RNA from HIV 1, HIV-2 and Hepatitis C by US Food and Drug Administration (FDA) licensed tests. Hepatitis B Surface antigen (HBsAg) and HIV antibody (Ab), STS (Syphilis) are also tested via by US Food and Drug Administration (FDA) licensed tests. Some lots may also be tested for Cytomegalovirus (CMV) and Epstein Barr virus (EBV). However, no known test can offer complete assurance these viruses are not present. Since we cannot test all potential pathogens, please treat the culture as a potential infectious reagent. We recommend using the US Centers for Disease Control (CDC) Universal Precautions for prevention of blood-borne pathogens as a minimum guideline for standards of practice at Biosafety Level 1 (BSL-1) or higher.

REFERENCE

Seki S, Ikarashi M, Kinoshita M, Nakashima M and Nakashima H. New Findings about Liver Kupffer Cells/Macrophages, B Cells and their Functions. J Hepat Res. 2014;1(1): 1003.

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