



Human Stellate Cell Care Manual

INSTRUCTION MANUAL ZBM0069.07

SHIPPING CONDITIONS

Human Stellate Cells, cryopreserved

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 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: Store in vapor phase nitrogen (-150°C to -190°C) IMMEDIATELY UPON RECEIPT.
Use a dry vapor shipper if total transit time will be longer than 3 days.

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

ORDERING INFORMATION AND TECHNICAL SERVICES

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THIS MANUAL IS SUITABLE FOR USE WITH THE FOLLOWING PRODUCTS:

HP-F-S	HUMAN HEPATIC STELLATE CELLS, 100,000 VIABLE CELLS/VIAL
P-F-S	HUMAN PANCREATIC STELLATE CELLS, 100,000 VIABLE 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 protocols are followed without amendment or substitution. Human stellate cell viability depends greatly on the use of suitable media, reagents, and appropriate sterile plastic wear. If these parameters are not carefully observed cell responsiveness in assays may be lower than expected.

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.

QUALITY CONTROL

Human stellate cells from Zen-Bio are isolated from human liver or pancreas obtained via the gift of organ donation from donor tissue that is not suitable for organ transplantation. Each donor has confirmed documentation on file allowing for research use of any non-transplantable organs or tissues in compliance with ethical policies. All samples are collected in the United States.

Each vial of hepatic or pancreatic stellate cells contains at least 100,000 viable cells/vial.

Each lot of primary cells is tested found non-reactive to HIV-1, HIV-2, Hepatitis B, Hepatitis C, and syphilis using US Food and Drug Administration (FDA) licensed tests and procedures. However, no known test can offer complete assurance that these viruses are not present. Since we cannot test all pathogens, always treat the culture as a potentially 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 or higher.

Human Stellate cells are assessed for viability and characterized for cell surface markers using flow cytometry for population distributions. The cells are characterized by a panel of markers to verify cell type. Stellate cells are positive for vimentin, smooth muscle alpha actin, and vinculin.

INTRODUCTION

Hepatic stellate cells (HSCs; also called as vitamin A-storing cells, lipocytes, interstitial cells, fat-storing cells, Ito cells) exist in the space between parenchymal cells and sinusoidal endothelial cells of the hepatic lobule. Stellate cells store 80% of vitamin A in the whole body as retinyl palmitate in lipid droplets in the cytoplasm and are responsible for Vitamin A homeostasis. Stellate cells are present in extra hepatic organs such as the pancreas, lung, kidney, intestine, spleen, adrenal gland, ductus deferens and vocal cords. Stellate cells of liver and pancreas and have been described in all vertebrates ranging from lampreys (primitive fish) to humans, demonstrating their major importance.

Although stellate cells, and in particular hepatic stellate cells (HSC), were described more than a century ago, their real physiological and pathophysiological relevance has emerged only in the last 20 years. Hepatic and extra hepatic stellate cells form what has been defined as “the stellate cell system”. Besides clear morphological similarities, two main features are peculiar to this type of cell in the liver as well as in other organs: (1) a perivascular location and a distribution typical of a pericyte, particularly when associated with a sinusoidal type of endothelium; and (2) the capability of storing retinoids. Stellate cells are normally quiescent and have a star-like appearance. Once activation occurs, they undergo cellular proliferation and differentiate into a contractile cell type (myofibroblast). Activation and transformation of stellate cells into myofibroblast-like cells is an essential step in fibrosis. In fibrosis, activated stellate cells display a loss of stored vitamin A, and increased collagen synthesis along with other extracellular matrix components. This increased ECM deposition contributes to the wound healing process before the establishment and progression of hepatic and pancreatic fibrosis. The fibrogenic role of stellates has grown exponentially and the elucidation of the cellular and molecular basis of fibrogenesis has brought important advances in the knowledge of chronic fibrogenic disorders of the liver and pancreas.

Another important property of HSCs and pancreatic stellate cells (PaSCs), is that they possess signaling pathways important for maintenance of stemness. Stellate cells display a differentiation potential as investigated *in vitro* and *in vivo*. These properties commonly found in stem/progenitor cells do support the concept that stellate cells are undifferentiated cells, which might play an important role in liver regeneration.

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MATERIALS PROVIDED FOR EACH CATALOG ITEM _____

Note: Store in vapor phase nitrogen (-150°C to -190°C) IMMEDIATELY UPON RECEIPT

- Cryopreserved human hepatic stellate cells, $\geq 100,000$ cells/vial, cat# HP-F-S
- Cryopreserved human pancreatic stellate cells, $\geq 100,000$ cells/vial, cat# P-F-S

MEDIA COMPOSTIONS _____

Human Stellate Growth Medium	Human Stellate Growth Medium without Serum
Cat# HSGM-500 500ml	Cat# HSGM-500-SF 500ml
Cat# HSGM-250 250ml	Cat# HSGM-250-SF 250ml
DMEM / Ham's F-12 (1:1, v/v Fetal Bovine Serum (FBS; US origin) HEPES Buffer pH 7.4 Penicillin Streptomycin Amphotericin B	DMEM / Ham's F-12 (1:1, v/v HEPES Buffer pH 7.4 Penicillin Streptomycin Amphotericin B

All human stellate growth media contain 3.15g/L D-glucose)

MEDIA EXPIRATION DATES:

- If placed at 4°C upon arrival, the media is stable 1 month from the ship date
 - the +4°C expiration date listed on the bottle label.
- If stored at -20°C upon arrival, it is stable 6 months after the ship date
 - the -20°C expiration date listed on the bottle.
 - Upon thawing, add fresh antibiotics at 1% volume when you are ready to use.
 - The media will expire 30 days after the thawing date.

THAWING AND PLATING CRYOPRESERVED HUMAN STELLATE CELLS

NOTE: Handle gently and quickly to maintain viability.

Use collagen I coated culture ware only.

We recommend Corning Biocoat or ZenBio, collagen I coated cultureware. See FAQ for more details

Instructions for seeding stellate cells

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 work area. Remove cap, being careful not to touch the interior threads with fingers.
2. Using a pipette, gently transfer contents of vial to a sterile 15 ml conical tube.
3. Wash vial with 5 ml HSGM medium and add this wash to conical tube.
4. Centrifuge the tube at 250xg for 5 minutes. After centrifugation, aspirate medium and re-suspend the contents in fresh HSGM medium.
5. Perform a cell count.
6. For expansion, seed the cells at a density of 4,000 cells/cm² on collagen I coated plates.
7. For best results, do not disturb the culture for at least 12 hours after seeding. Change growth medium the next day to remove any residual DMSO or unattached cells, then every other day thereafter.

Instructions for sub-culturing stellate cells

1. Subculture cells when they have reached 90% confluency.
2. Warm medium, 0.25% trypsin solution, and Dulbecco's Phosphate Buffered Saline, without Calcium & Magnesium (DPBS) to room temperature.
3. Aspirate medium, then rinse cells with DPBS. Add trypsin solution into flask and incubate in a 37°C incubator for 3-5 minutes, or until the cells detach.
4. At the end of trypsinization, wash cells off flask with an appropriate amount of medium.
5. Transfer to centrifuge tube and centrifuge at 250xg for 5 minutes.
6. After centrifugation, aspirate the medium, re-suspend in 1-2 ml fresh medium and count cells for seeding.
7. Seed the cells at a density of 4,000 cells/cm² on collagen I coated plates.

FREQUENTLY ASKED QUESTIONS

1	How high can I expand the cells?	Human primary stellate cells have no confirmed limit for expansion. Cells are cryopreserved after establishing viable cultures.
2	Should antibiotics be included in the medium	Yes. Antibiotics and anti-fungal agents are always recommended since the cells are primary cells.
3	Do you test for pathogens? Which ones?	Yes. Samples from each donor are tested via PCR to confirm non-reactivity for HIV-1, HIV-2, hepatitis B, hepatitis C and syphilis. However, since we cannot test all pathogens, please treat the culture as a potentially infectious agent.
4	What is the concentration of ingredients in your media?	We do not disclose the concentrations of the components of our media. We are happy to prepare custom media to your specifications.
5	What formats are available for the ZenBio collagen coated cultureware?	See the chart below.

ZenBio Collagen I Coated Cultureware

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