Human Tenocyte Care Manual

INSTRUCTION MANUAL ZBM0075.04

SHIPPING CONDITIONS

Human Tenocytes

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 using dry ice or a dry vapor shipper (if transit time for the cells will exceed 3 days). Primary human cells are very sensitive to extended times (> 3 days) transported using dry ice (-80°C). 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 immediately upon shipment receipt.**

STORAGE CONDITIONS

Media: +4°C: 30 days after ship date -20°C: 6 months after ship date

Cryopreserved cells: Vials of cells are to be stored in vapor phase nitrogen (-150°C to -190°C).

All Zen-Bio Inc products are for research use only. ZenBio, Inc products are 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:

TEN-F	CRYOPRESERVED HUMAN TENOCYTES, 1 MILLION CELLS/VIAL

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 cultureware used in this protocol should be sterile.

Human tenocyte viability depends greatly on the use of suitable media, reagents, and sterile collagen-1 coated plastic wear. If these parameters are not carefully observed, limited differentiation may occur and cell growth may be slow.

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 shipping recommendations and protocols are followed. Cryopreserved cells are assured to be viable when thawed according to Zen-Bio protocols using ZenBio media.

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.

INTRODUCTION

Cryopreserved human tenocytes are obtained via the gift of organ donation of human tendon tissues. Each donor has confirmed documentation consent on file allowing for research use of non-transplantable organs or tissues. The tenocytes are isolated from tendon tissue using enzymatic treatment and stored in vapor phase liquid nitrogen. Tenocytes are shipped on dry ice (or a dry vapor shipper if transit > 3 days) and should be stored in vapor phase liquid nitrogen immediately upon arrival. This instruction manual describes procedures to culture the human tenocytes.

QUALITY CONTROL

The tenocytes are isolated from tendon tissue using enzymatic treatment. The tenocytes are assessed via qRT-PCR to confirm expression of collagen (Collagen 1A, Collagen 3A1) thrombospondin 4, a glycoprotein that mediates cell-to-cell and cell-to-matrix interactions, and the tenomodulin activator scleraxis. The cells are assessed for viability and confirmed positive for cell surface markers CD44 and CD90 and negative for CD45, CD31.

MATERIALS PROVIDED FOR EACH CATALOG ITEM

- **Cryopreserved Human Tenocytes**
  - Cat # TEN-F
  - Frozen vial containing 1.0 million viable human tenocytes
    (Store in vapor phase of liquid nitrogen immediately upon receipt)

MEDIUM COMPOSTION

Tenocyte Growth Medium Cat# TEN-1, 500ml

DMEM
Fetal Bovine Serum (FBS)
Penicillin
Streptomycin
Amphotericin B

TEN-1 contains 4.5 g/L (25 mmol/L) D-glucose.
The medium is also available as without serum and/or phenol red.
Please inquire for custom media requests.

MEDIUM EXPIRATION DATES:

- 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 at 1% when you are ready to use. The media will now expire 30 days after the thaw date

PLEASE INQUIRE FOR CUSTOM MEDIA REQUESTS

PLATING AND EXPANSION PROCEDURES

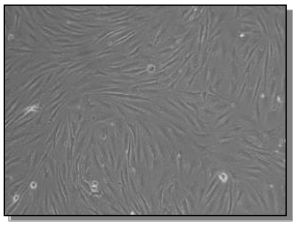
Primary human cells can be sensitive to brands of cultureware. ZenBio scientists recommend the use Corning BD Biocoat or ZenBio, Inc collagen I coated cultureware.

Cryopreserved Tenocytes

1. Remove cells from liquid nitrogen and place immediately into a 37°C water bath with agitation. Be careful not to submerge the cap of the vial into water. Do not leave the vials in water bath after most of the content has thawed. Rinse the vials with 70% ethanol before taking them to the culture hood.
2. Upon the thawing, add the cells to a sterile conical bottom centrifuge tube, containing 9 ml of Growth Medium (TEN-1).

3. Centrifuge at 400 x g, 20°C, 10 minutes. Aspirate the medium and resuspend cells in a volume of TEN-1 appropriate for counting the cells. Count cells using a hemocytometer.
4. Place approximately 370,000-500,000 cells per collagen I coated T-75 culture flask using 20 ml TEN-1. **[We recommend using collagen-I coated plates/flasks].**
5. Incubate cells until they are 70-80% confluent (in about 4-5 days). Cells will need to be fed every other day with TEN-1. Remove 12 ml of medium per T-75 flask and replace with 12 ml fresh TEN-1.
6. Aspirate medium and wash tenocytes 4-5 times using sterile Phosphate Buffered Saline (PBS) to remove all traces of medium. Remove the PBS and release the cells from the flask bottom by adding 2 mL/T-75 flask (or 6 ml/T-225 flask) of 0.25% trypsin/ 2.21mM EDTA solution. Allow cells to trypsinize for 5 minutes at 37°C. Tap the flask gently to loosen the cells.
7. Neutralize the trypsin using an equal volume of 0.5mg/ml soybean trypsin inhibitor or serum containing medium. Check the flask under a microscope to ensure all cells are free of the flask bottom.
8. Count the cells and plate in desired format. Ensure cells are evenly suspended when plating large numbers of plates or flasks. Do not agitate plates and flasks after plating. Place in a humidified incubator at 37°C and 5% CO_2, making sure the surface is level for even cell distribution.

Human Tenocytes



TROUBLESHOOTING GUIDE

Observation	Possible causes	Suggestions
Tenocytes do not grow	1. Cells have been passaged too many times 2. Cells expanded too high 3. Cell stored improperly	1. Use cells of a lower passage number 2. Do not exceed 1:6 expansion ratio 3. Store cells vapor phase liquid nitrogen only
Edge effects	1. Medium in outside wells evaporated	1. Ensure a saturated humidity in the incubator and feed the cells no less than every 3 days. Make sure multiple plates are stacked no more than 3 plates high.

FREQUENTLY ASKED QUESTIONS

Can I pass the cells?	Yes. Tenocytes can be trypsinized and re-plated. The cells are NOT suitable for culture after passage 4. All cells are shipped at passage 2-3 after establishing a primary culture. We do not recommend re-freezing the cells for a stock
How fast do the cells replicate?	The average doubling time is 24-36 hours. However, keep in mind that the replication rate for human tenocytes varies slightly from donor to donor.
Should antibiotics be included in the medium?	Yes. Antibiotics and anti-fungal agents are always recommended since the cells are primary cells.
From what source are the cells obtained?	The tenocytes are isolated from human tendon tissue, including Achilles, patellar and flexor tendons. Cryopreserved human tendon tissue is obtained via the gift of organ donation. Each donor has confirmed documentation consent on file allowing for research use of non-transplantable organs or tissues
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, and Hepatitis C. However, since we cannot test all pathogens, please treat the culture as a potentially infectious agent.
What donor information do I receive?	The donor's age, gender, tissue site, and other information that we receive are provided in the certificate of analysis that accompanies each lot of cells.
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.
Is there a specific type of culture ware that should be used?	Yes. Only Corning/BD Biocoat or ZenBio brand Collagen I Coated Cultureware are recommended.

ZenBio Collagen Coated Culture ware

Catalog#	Item Description	Unit
CC-25	Collagen Coated I T-25 Flask, Vent Cap	PACK/5
CC-75	Collagen Coated I T-75 Flask, Vent Cap	PACK/5
CC-225	Collagen Coated I T-225 Flask (EXCLUSIVE!), Vent Cap	PACK/1
CC-6	Collagen Coated I 6-well Plate	PACK/5
CC-12	Collagen Coated I 12-well Plate	PACK/5
CC-24	Collagen Coated I 24-well Plate	PACK/5
CC-96	Collagen Coated I 96-well Plate	PACK/5

PATHOGEN TESTING

All donor lots are found to be negative for HIV-1, HIV-2, Hepatitis B, and Hepatitis C via US Food and Drug Administration (FDA) licensed tests. However, no known test can offer complete assurance that the viruses that these infectious diseases are not present. Since we cannot test all pathogens, please treat the culture as a potential infectious reagent at Biosafety Level 1 or higher.

Our products are tested and are free from mycoplasma contamination. 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. Always wear gloves and work behind a protective screen when handling primary human cell