Human Adult Mammary Fibroblast Care Manual

INSTRUCTIONAL MANUAL ZBM0062.05

SHIPPING CONDITIONS

Human Adult Mammary Fibroblast Cells

Orders are delivered via Federal Express courier. All US and Canada orders are shipped via Federal Express Priority service and are usually received the next day. International orders are usually received in 2-4 days. Please inquire if alternate couriers are needed.
Must be processed immediately upon shipment receipt.

STORAGE CONDITIONS

- Media: Short Term (30 days from ship date): +4°C 6 months: -20°C
- Cryopreservation Medium: -20°C
- Cryopreserved cells: Vials of frozen mammary fibroblasts are to be stored in vapor phase nitrogen (-150°C to -190°C).

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:

MF-F	HUMAN ADULT MAMMARY FIBROBLASTS

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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 the performance of cells only if Zen-Bio media are used and the recommended protocols are followed. Cryopreserved cells are assured to be viable when thawed according to Zen-Bio protocols and using the recommended cultureware.

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 cultureware used in this protocol should be sterile.

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

INTRODUCTION

Adult mammary fibroblasts are isolated from the breast fat tissue of healthy consented adult donors undergoing elective breast reduction or mastectomy surgery. Each volunteer adult donor has signed an IRB or FDA validated donor consent form that specifically lists both the intended uses for the donation for non-clinical research and confirms the procedures for processing the samples are Standard Operating Procedure (SOP) managed GLP protocols in compliance with all legal and ethical regulations. The cells are isolated by centrifugal force following enzymatic treatment. This instruction manual describes procedures to maintain, passage and culture the human mammary fibroblast cells.

MATERIALS PROVIDED FOR EACH CATALOG ITEM

- **Cryopreserved Human Adult Mammary Fibroblasts**
  - Cat # MF-F
  - Frozen vial containing $\geq$ 500,000 viable adult mammary fibroblasts (store in vapor phase liquid nitrogen upon receipt)

MEDIA COMPOSTIONS

Mammary Fibroblast Culture Medium cat # MF-1 500ml	Mammary Fibroblast Basal Medium cat # MF-2 500ml	Mammary Fibroblast Cryopreservation Medium cat # MFM-100 100ml
DMEM, high glucose (4.5g/L)	DMEM, high glucose (4.5g/L)	DMEM, high glucose (4.5g/L)
Fetal Bovine Serum (FBS)	Penicillin	Fetal Bovine Serum (FBS)
Penicillin	Streptomycin	DMSO
Streptomycin	Amphotericin B	
Amphotericin B		

All media are also available as without serum and/or phenol red free.
Please inquire for custom media requests.

MEDIA 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 when you are ready to use. The media will expire 30 days after the thaw date.

PATHOGEN TESTING

Samples from each donor are tested via PCR to confirm non-reactivity for HIV-1, HIV-2, hepatitis B and hepatitis C. Yes. Each lot of primary cells is tested via PCR and found non-reactive to viral DNA from HIV and hepatitis B and viral RNA from Hepatitis C. 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.

Our products are tested and are free from mycoplasma contamination. Proper precautions and biological containment should be taken when handling cells of human origin, due to their potential biohazardous nature. All human based products should be handled at a BSL-2 (Biosafety Level 2).

Always wear gloves and work behind a protective screen when handling primary human cells.

PLATING AND EXPANSION PROCEDURES

Cryopreserved Adult Mammary Fibroblasts

*Please note: Primary cells can be very sensitive to brands of cultureware. Zen-Bio does **not** currently recommend the use of Corning Falcon or Sarstedt brand plates or flasks. Our scientists are using Nunc, Corning Costar, or Greiner Bio-One Cellstar tissue culture treated plates and flasks.*

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 Mammary Fibroblast Culture Medium (MF-1).
3. Centrifuge at 1,200 rpm (282 X g) / 20°C / 5 minutes. Aspirate the medium and resuspend cells in a volume of MF-1 appropriate for counting the cells. Count using a hemacytometer.
4. Plate the cells at 4,000-10,000 cells/cm^2 on tissue culture treated cell cultureware (see recommended brands listed above) using MF-1.
5. Incubate cells until they are 85-90% confluent (in about 1-2 weeks). Cells will need to be fed every 2-3 days with MF-1.
6. Aspirate medium and wash mammary fibroblasts 4-5 times using sterile Phosphate Buffered Saline (PBS) to remove all traces of serum (until there is no foaming of the 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 detach for 5 minutes at 37°C. Tap the flask gently to loosen the cells.
7. Neutralize the trypsin using 7 ml MF-1 per T-75 flask (or 21 ml per T-225 flask). 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.
9. *OPTIONAL* – Cryopreserve mammary fibroblasts after counting.
    a. Centrifuge at 1200rpm, 20°C, 5 minutes.
    b. Suspend in cold Mammary Fibroblast Cryopreservation Medium (Cat# MFM-100) at a concentration of 500,000 cells/ml.
    c. Do not exceed a 6:1 ratio of cells (per million): volume cryopreservation medium (per ml). Remember to account for the volume of the cell pellet before adding the volume of freeze medium necessary for cell suspension.

d. If using a controlled-rate freezer:
    i. Freeze by reducing the temperature 1°C per minute until the temperature reaches -80° C.
e. If using a cell cryopreservation container:
    i. Prepare according to the manual instructions. For best results we recommend transferring the vials to the vapor phase of a liquid nitrogen storage facility as soon as possible after the cells have reached -80°C.

TROUBLESHOOTING GUIDE

Observation	Possible causes	Suggestions
Adult MF cells do not grow	1. Cells have been passaged too many times 2. Cells expanded too high	1. Use cells of a lower passage number 2. Do not exceed 1:6 expansion ratio
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?	- Mammary fibroblast cells can be trypsinized and plated again several times. - All cells are shipped after establishing a primary culture. - We have no data on the limit of expansion.
How fast do the cells replicate?	- The average doubling time is 18-24 hours. However, keep in mind that the replication rate for human breast fibroblasts 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.
Where are the cells obtained?	- The adult mammary fibroblast cells are isolated from the area of breast that contains no organoids or ductal tissue.
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, and BMI 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.