



Human Adult Mammary Fibroblast Care Manual

INSTRUCTIONAL MANUAL ZBM0062.06

SHIPPING CONDITIONS

Human Adult Mammary Fibroblast Cells, Cryopreserved

Orders are delivered via Federal Express courier. All USA and Canada orders are shipped via Federal Express Priority service and are usually received the next day. Non North American International orders are usually received in 2-4 days. Primary human cells can be sensitive to extended times at dry ice temperatures. If your transit time will exceed 3 days, please inquire about dry vapor shipper options. Please inquire if alternate couriers are needed.

All orders should be processed immediately upon shipment receipt.

STORAGE CONDITIONS

Media:	+4°C Expires 30 days from ship date. -20°C Expires 6 months from ship date.
Cryopreservation Medium:	-20°C Expires 1 year from ship date.
Cells:	Store in vapor phase nitrogen (-150°C to -190°C) IMMEDIATELY UPON RECEIPT. <u>Any other use negates the warranty.</u>

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

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, 500,000 CELLS/VIAL
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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 storage conditions and protocols are followed without amendment or substitution. ZenBio, Inc. cryopreserved cells are assured to be viable when stored as recommended and thawed according to Zen-Bio protocols and using the recommended protocol.

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 viability depends greatly on the use of suitable media, reagents, and sterile plastic wear. If these parameters are not carefully observed, cell growth may be slower than expected.

INTRODUCTION

The adult mammary fibroblast cells are isolated from breast tissue from elective breast reduction or mastectomy surgery in a consented adult donor in the United States. Each sample is derived from a competent volunteer adult donor who has signed an Institutional Review Board (IRB) 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 protocols in compliance with ethical regulations. All samples are collected and processed in the United States.

QUALITY CONTROL

Mammary fibroblast cells are assessed for viability, positive for CD44 marker and negative for pan-cytokeratin. The cells are also screened for mammary luminal contaminating cells (CD227), and are <10 % contaminated.

CATALOG ITEMS

- ❖ **Cryopreservation Medium for Adult Mammary Fibroblasts**
 - Cat # MFM-100
 - Store -20°C
- ❖ **Mammary Fibroblast Culture Medium**
 - Cat # MF-1
 - Store according to label
- ❖ **Mammary Fibroblast Basal Medium**
 - Cat # MF-2
 - Store according to label
- ❖ **Cryopreserved Human Adult Mammary Fibroblasts**
 - Cat # MF-F
 - Cryopreserved vial containing 500,000 viable adult mammary fibroblasts per vial (**store in vapor phase liquid nitrogen IMMEDIATELY upon receipt**) *any other storage negates the warranty*

MEDIA COMPOSTIONS

<u>Mammary Fibroblast Culture Medium</u> CAT # MF-1 500 mL	<u>Mammary Fibroblast Basal Medium</u> CAT # MF-2 500 mL	<u>Mammary Fibroblast Cryopreservation Medium</u> CAT # MFM-100 100 mL
DMEM, 4.5 g/L (25 mmol/L) D-glucose Fetal Bovine Serum (FBS; USA Origin) Penicillin Streptomycin Amphotericin B	DMEM, 4.5 g/L (25 mmol/L) D-glucose Penicillin Streptomycin Amphotericin B	DMEM, 4.5 g/L (25 mmol/L) D-glucose Fetal Bovine Serum (FBS; USA Origin) Dimethyl Sulfoxide (DMSO)

All media are also available without phenol red.

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.

PLATING AND EXPANSION PROCEDURES

Cryopreserved Adult Mammary Fibroblasts

Please note: Primary human mammary fibroblast cells require use of sterile tissue culture treated cultureware. No extracellular matrix coatings are required.

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, no longer than 1 minute. 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 for 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/cm² on tissue culture treated cell cultureware 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. When ready to harvest, aspirate medium and wash mammary fibroblasts 4-5 times using sterile Phosphate Buffered Saline without calcium or magnesium (PBS) to remove all traces of serum (until there is no foaming of the medium).
7. 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.
8. 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 detached.
9. 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% CO₂, making sure the surface is level for even cell distribution.

CRYOPRESERVATION PROCEDURE

OPTIONAL

1. While the cells are still in suspension in media, centrifuge at 1,200 rpm (282 X g) / 20°C for 5 minutes.
2. Suspend in **cold** Mammary Fibroblast Cryopreservation Medium (Cat# MFM-100) at a concentration of 500,000 cells/mL.
3. 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 cryopreservation medium necessary for cell suspension.
4. If using a controlled-rate freezer:
 - a. Freeze by reducing the temperature 1°C per minute until the temperature reaches -80° C.
5. If using a cell cryopreservation container:
 - a. 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 mammary fibroblast cells do not grow	<ol style="list-style-type: none"> 1. Cells have been passaged too many times. 2. Cells expanded too high. 	<ol style="list-style-type: none"> 1. Use cells of a lower passage number. 2. Do not exceed 1:6 expansion ratio.
Edge effects	<ol style="list-style-type: none"> 1. Medium in outside wells evaporated. 	<ol style="list-style-type: none"> 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

1. Can I passage 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.**

2. 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

3. Should antibiotics be included in the medium?

- Yes. Antibiotics and anti-fungal agents are always recommended because the cells are primary cells

4. Where are the cells obtained?

- The adult mammary fibroblast cells are isolated from the area of breast that contains no organoids or ductal tissue.

5. 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 at BSL-1 or higher.

6. 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.

7. 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.

PATHOGEN TESTING

Samples from each donor are tested via PCR and found non-reactive to viral DNA from HIV and hepatitis B, viral RNA from Hepatitis C and Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) via FDA licensed tests. Some samples may display reactivity to common viruses Epstein Barr Virus (EBV) or Cytomegalovirus (CMV). Samples that test reactive to CMV or EBV indicate infection at some point in life. 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.