Human Mammary Luminal Epithelial Cell Manual

INSTRUCTION MANUAL ZBM0071.02

SHIPPING CONDITIONS

Human Mammary Luminal Epithelial Cells

Cells are shipped using dry ice or dry vapor shipper. Orders are delivered via Federal Express or DHL courier. All US and Canada orders are shipped via Federal Express Priority service and are usually received the within 1-2 days. International orders are usually received in 2-4 days. Alternate couriers and dry vapor shippers are available if needed. Please inquire.

Must be processed upon shipment receipt.

STORAGE CONDITIONS

Media: 4°C Expiration: 30 days from ship date, Do Not Freeze

Cells: Vials of frozen Mammary Luminal Epithelial cells are to be stored in vapor phase nitrogen (-150°C to -190°C) IMMEDIATELY upon arrival

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.

THIS MANUAL IS SUITABLE FOR USE WITH THE FOLLOWING PRODUCTS:

MLE-F	HUMAN MAMMARY LUMINAL EPITHELIAL CELLS, CRYOPRESERVED

LIMITED PRODUCT WARRANTY

This warranty limits our liability for 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 and storage conditions 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 mammary Luminal epithelial 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.

CONTENTS PAGE

INTRODUCTION

ZenBio's human mammary luminal epithelial cells are isolated from mammary tissue of healthy non-diabetic donors between 18 and 60 years old who have undergone elective surgery. Each donor has signed an 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 GLP protocols in compliance with all legal and ethical regulations. The cells are isolated by digestion of the tissue to generate organoid structures collected by centrifugal force. Organoids are maintained in explant culture to stimulate Luminal myoepithelial cell migration and the cells are further purified using immunomagenetic separation. This instruction manual describes procedures to passage and culture the human mammary Luminal epithelial cells.

MATERIALS PROVIDED FOR EACH CATALOG ITEM

- **Cryopreserved Human Mammary Luminal Epithelial Cells**
  - Cat # MLE-F
  - Frozen vial containing 500,000 viable human mammary luminal epithelial cells (store in vapor phase liquid nitrogen upon receipt)

MEDIUM COMPOSTION

Mammary Luminal Epithelial Growth Medium Cat# LCM-1
DMEM/F12
Minimal Essential Medium (MEM)
Fetal Bovine Serum (FBS)
Endothelial Growth Factor (EGF)
Insulin
Hydrocortisone
Apo transferrin
Ethanolamine
Penicillin
Streptomycin
Amphotericin B

Expiration date is 30 days from the ship date. DO NOT FREEZE. Please coordinate your orders for media according to your schedule.

PLATING AND EXPANSION PROCEDURES

Cryopreserved Mammary Luminal Epithelial Cells

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 (LCM-1).
3. Centrifuge at 400 x g, 20°C, 10 minutes. Aspirate the medium and resuspend cells in a volume of LCM-1 appropriate for counting the cells. Count using a hemacytometer.
4. Place approximately 0.37-0.5 X 10^6 cells into two Collagen I coated T-25 culture flasks (10,000 cells/cm^2) using 7 ml LCM-1.
5. Incubate cells until they are 70-80% confluent (in about 4-5 days). Cells will need to be fed every other day with LCM-1. Remove 7 ml of medium per T-75 flask and replace with 7 ml fresh LCM-1.
6. Aspirate medium and wash Luminal epithelial cells 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 1 mL/T-25 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 Mammary Luminal Epithelial Cells

TROUBLESHOOTING GUIDE

Observation	Possible causes	Suggestions
Luminal 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.

1. FREQUENTLY ASKED QUESTIONS

- **Can I pass the cells?**

  Yes. Luminal mammary epithelial cells can be trypsinized and replated. The cells are NOT suitable for culture after passage 4. All cells are shipped at passage 2-3 after establishing a primary culture.

- **How fast do the cells replicate?**

  The average doubling time is 24-36 hours. However, keep in mind that the replication rate for human mammary Luminal epithelial cells 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 Luminal epithelial cells are isolated from human mammary 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.

PATHOGEN TESTING

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 at Biosafety Level 1 (BLS-1) or higher. Our cells are tested for mycoplasma contamination via direct plating and DNA fluorochrome staining; mycoplasma contamination is not detected.

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