



Human Mammary Basal Epithelial Cell Manual

INSTRUCTION MANUAL ZBM0055.01

SHIPPING CONDITIONS

Human Mammary Basal Epithelial 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 3-4 days.

Must be processed upon shipment receipt.

STORAGE CONDITIONS

Media: 4°C 1 month from ship date

Cells: Frozen: liquid nitrogen

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.

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. Cryopreserved human mammary basal epithelial cells are assured to be viable when thawed and maintained according to Zen-Bio protocols.

ORDERING INFORMATION AND TECHNICAL SERVICES

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INTRODUCTION

ZenBio's human mammary basal epithelial cells are isolated from mammary tissue of healthy non-diabetic donors between 18 and 60 years old who have undergone elective surgery. 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 basal myoepithelial cell migration and the cells are further purified using immunomagnetic separation. This instruction manual describes procedures to passage and culture the human mammary basal epithelial cells.

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 basal 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.

MATERIALS PROVIDED FOR EACH CATALOG ITEM

- **Cryopreserved Human Mammary Basal Epithelial Cells**
 - Cat # MBE-F
 - Frozen vial containing 0.5×10^6 viable human mammary basal epithelial cells (store in liquid nitrogen upon receipt)
 - 50 ml Mammary Epithelial Growth Medium (cat# MEG-1)

MEDIUM COMPOSITION

**Mammary Epithelial Growth
Medium
Cat# MEG-1**

DMEM/F12 / MEM

Fetal Bovine Serum

EGF

Estradiol

Apo transferrin

Sodium Selenite

Phosphoethanolamine / ethanolamine

Penicillin / Streptomycin / Amphotericin B

Bovine Pituitary Extract

Triiodothyronine

Insulin

Hydrocortisone

Cholera Toxin

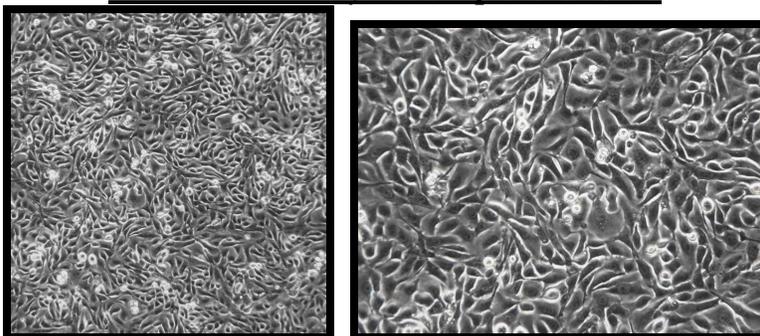
**Please inquire for custom
media requests.**

PLATING AND EXPANSION PROCEDURES

Cryopreserved Mammary Basal 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 (MEG-1).
3. Centrifuge at 400 x g, 20°C, 10 minutes. Aspirate the medium and resuspend cells in a volume of MEG-1 appropriate for counting the cells. Count using a hemacytometer.
4. Place approximately 0.37-0.5 X 10⁶ cells per T-75 culture flask (5,000 cells/cm²) using 20 ml MEG-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 MEG-1. Remove 12 ml of medium per T-75 flask and replace with 12 ml fresh MEG-1.
6. Aspirate medium and wash basal 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 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% CO₂, making sure the surface is level for even cell distribution.

Human Mammary Basal Epithelial Cells



TROUBLESHOOTING GUIDE

Observation	Possible causes	Suggestions
Basal 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.

1. FREQUENTLY ASKED QUESTIONS

- **Can I pass the cells?**

Yes. Basal 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 basal 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 basal 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, HTLV I, HTLV II, 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

Samples from each donor are tested via PCR to confirm non-reactivity for HIV-1, HIV-2, HTLV I, HTLV II, hepatitis B and hepatitis C. However, no known test can offer complete assurance that the cells are pathogen free. 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) or higher. Always wear gloves and work behind a protective screen when handling primary human cells.