Human Airway Epithelial Cell Care Manual

INSTRUCTION MANUAL ZBM0092.01

SHIPPING CONDITIONS

Human Airway Epithelial Cells

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 service using dry ice or a dry vapor shipper if transit time will exceed 3 days. Hepatic cells are very sensitive to extended times (> 3 days) transported using dry ice. 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 NOTE: Expires 30 days from shipping date. DO NOT FREEZE

Cells: Store in vapor phase nitrogen (-150°C to -190°C)

IMMEDIATELY UPON RECEIPT

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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Electronic mail (e-mail)	information@zenbio.com
World Wide Web	http://www.zenbio.com

THIS MANUAL IS SUITABLE FOR USE WITH THE FOLLOWING PRODUCTS:

SAE-F	CRYOPRESERVED HUMAN SMALL AIRWAY EPITHELIAL CELLS (LUNG): 500,000 CELLS/VIAL
LAE-F	CRYOPRESERVED HUMAN LARGE AIRWAY EPITHELIAL CELLS (LUNG): 500,000 CELLS/VIAL

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 airway cells are assured to be viable stored as recommended and thawed according to Zen-Bio protocols and recommendations.

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 culture ware used in this protocol should be sterile.

To comply with U.S. Food and Drug Administration (FDA) regulations, these products are not for use in Clinical Diagnostic or Therapeutic Procedures.

By your acceptance of these products, you are acknowledging that these products will be:

1. Treated as potentially contaminated biological specimens even if accompanying serological reports are negative;
2. Handled by establishing or following appropriate safety control procedures to ensure the safety of using these products.

Human airway cells' viability depends greatly on the use of suitable media, reagents, and sterile plastic ware. If these parameters are not carefully observed cell responsiveness in assays may be lower than expected.

INTRODUCTION

ZenBio, Inc cryopreserved human airway epithelial cells are isolated from the upper bronchial/tracheal area of human lung tissue collected and donated in the United States via the gift of organ donation from donor lung tissue that is not suitable for organ transplantation. Each donor has confirmed documentation on file allowing for research use of any non-transplantable organs or tissues in compliance with established legal and ethical regulations.

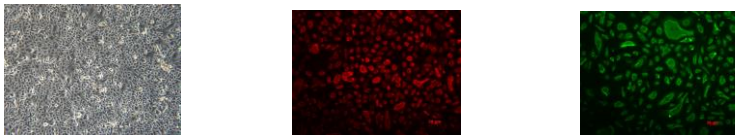
Human Airway Epithelial Cells from the upper bronchial/tracheal area and optimized media for their growth. Each vial of Human Small or Large Airway Epithelial Cells can generate cultures for experimental applications in asthma, inhalation toxicology and pulmonary inflammatory response.

Airway cells, Medium and Reagents are quality tested together and guaranteed to give optimum performance as a complete Cell System. This instruction manual describes procedures to passage and culture the human airway epithelial cells. Zen-Bio, Inc warrants its cells only if Zen-Bio recommended media are used and the recommended protocols are followed. Cryopreserved human airway cells are assured to be viable when stored as recommended and thawed according to Zen-Bio protocols and recommendations.

QUALITY CONTROL

Quality control tests are performed for each lot of Human Airway cells. The adult small airway epithelial cells are assessed for viability (>90%), morphology (cobblestone), keratin-19 and Rex-1 staining. (A; unstained) and express keratin-19 (B) and Rex-1 (C)).

(A) (B) (C)



The adult large airway epithelial cells are assessed for viability, morphology (cobblestone), and keratin-18 staining. (A; unstained) and express keratin-18 (B).

(A) (B)

Each lot of primary cells is tested via PCR and found non-reactive to viral DNA from HIV 1 & 2, and hepatitis B, and viral RNA from Hepatitis C using US Food and Drug Administration (FDA) licensed tests and testing procedures.

MATERIALS PROVIDED FOR EACH CATALOG ITEM____

- **Cryopreserved Human Large Airway Epithelial Cells**
  - Cat # LAE-F
  - Frozen vial containing 500,000 viable airway epithelial cells (store in vapor phase liquid nitrogen upon receipt)

- **Cryopreserved Human Small Airway Epithelial Cells**
  - Cat # SAE-F
  - Frozen vial containing 500,000 viable airway epithelial cells (store in vapor phase liquid nitrogen upon receipt)

MEDIUM _____

Human Airway Epithelium Medium

Catalog # HA-1, 500ml

HA-1 is a proprietary blend of animal/human component-free vitamins, minerals, amino acids, minerals, and growth factors for the growth of human airway epithelial cells.

Penicillin, Streptomycin and Amphotericin B are included as antibiotic/antimycotic agents in the medium.

Store at +4˚C.

Expiration is 30 days from the shipping date. DO NOT FREEZE

Minimize light exposure at all times.

PLATING PROCEDURES _____

(Note: Use only collagen I-coated tissue culture flasks and plates.)

1. Remove cryopreserved human airway epithelial 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 10 ml of Human Airway Epithelium Medium (HA-1).
3. Centrifuge at 200 x g, 20°C, 5 minutes. Aspirate the medium and resuspend cells in a volume of HA-1 appropriate for counting the cells. Count using a hemacytometer.
4. Place approximately ~4,000 cells/cm^2 in Collagen I-coated cultureware using HA-1.
5. Incubate cells until they are 85-90% confluent (in about 4-5 days). Cells will need to be fed every other day with HA-1.
6. Aspirate medium and wash cells 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 0.7 ml per 25cm^2 flask (or 2 ml/75cm^2 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 3-4 ml neutralizing medium per 25cm^2 flask (i.e. at least 4 volumes the amount of trypsin used). 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.

TROUBLESHOOTING GUIDE

Observation	Possible causes	Suggestions
Airway cells do not grow	• Cells have been passaged	• Do not expand the cells • Use only ZenBio recommended medium cat# HA-1.
Edge effects	• Medium in outside wells evaporated	• Ensure a saturated humidity in the incubator. • Make sure multiple plates are stacked no more than 3 plates high.

FREQUENTLY ASKED QUESTIONS

- **Can I pass the cells?**

  We do not recommend continued passaging of airway epithelial cells. All cells are shipped after establishing a primary culture.

- **Where are the cells obtained?**

  The cells are isolated from the upper bronchial/tracheal area of human lung tissue collected and donated in the United States via the gift of organ donation from donor lung tissue that is not suitable for organ transplantation. Each donor has confirmed documentation on file allowing for research use of any non-transplantable organs or tissues in compliance with established legal and ethical regulations.

- **Is there a specific type of cultureware that should be used?**

  Yes, only collagen I coated tissue culture treated cultureware is to be used.
  ZenBio Collagen I Coated Cultureware

Item#	Item Description
CC-25	Rat Tail Type I Collagen T-25 Flask, Vent Cap, Pack of 5
CC-75	Rat Tail Type I Collagen T-75 Flask, Vent Cap, Pack of 5
CC-225	Rat Tail Type I Collagen T-225 Flask (EXCLUSIVE!), Vent Cap, Pack of 1
CC-6	Rat Tail Type I Collagen 6-well Plate, Pack of 5
CC-12	Rat Tail Type I Collagen 12-well Plate, Pack of 5
CC-24	Rat Tail Type I Collagen 24-well Plate, Pack of 5
CC-96	Rat Tail Type I Collagen 96-well Plate, Pack of 5

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

  The average doubling time has not been established. However, keep in mind that the replication rate for airway cells varies with culture conditions.

- **Should antibiotics be included in the medium?**

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

- **Do you test for pathogens? Which ones?**

  Yes. HIV-1, HIV-2, hepatitis B and hepatitis C. Since we cannot test all the pathogens, please treat the culture as a potential infectious reagent using Universal Precautions.

- **What donor information do I receive?**

  The donor's age, gender, and body mass index (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 1 & 2, and hepatitis B, and viral RNA from Hepatitis C using US Food and Drug Administration (FDA) licensed tests and testing procedures. 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. 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-1 (Biosafety Level 1) or higher. Always wear gloves and work behind a protective screen when handling primary human cells.