Human Airway Epithelial Cell Manual

INSTRUCTION MANUAL ZBM0092.00

SHIPPING CONDITIONS

Human Airway Epithelial Cells

Orders are delivered via Federal Express or other 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 NOTE: Expires 30 days from shipping date

Cells: Cells are to be stored 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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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 when thawed according to Zen-Bio protocols.

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

Human Airway Epithelium contains Epithelial Cells from the upper bronchial/tracheal area and optimized media for their growth. Each vial can generate cultures for experimental applications in asthma, inhalation toxicology and pulmonary inflammatory response. Airway cells are shipped as frozen primary cultures or in second passage.

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.

QUALITY CONTROL

Quality control tests are performed for each lot of Human Airway cells. These cells have a guaranteed viability of 90%. In addition, all human products have been tested for some common blood borne pathogens and microbial contaminants

MATERIALS PROVIDED FOR EACH CATALOG ITEM

- **Cryopreserved Human Large Airway Epithelial Cells**
  - Cat # LAE-F
  - Frozen vial containing ≥ 0.5 x10^6 viable airway epithelial cells (store in liquid nitrogen upon receipt)

- **Cryopreserved Human Small Airway Epithelial Cells**
  - Cat # SAE-F
  - Frozen vial containing ≥ 0.5 x10^6 viable airway epithelial cells (store in liquid nitrogen upon receipt)

MEDIA

Human Airway Epithelium Medium Catalog # 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.
Minimize light exposure at all times.

PLATING PROCEDURE

(Note: Use 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 ~4000 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 too many times	• Use cells of a lower passage number.
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.
- **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.
- **Where are the cells obtained?**
  The cells are isolated from the upper bronchial/tracheal area.
- **Do you test for pathogens? Which ones?**
  Yes. HIV-1, HIV-2, HTLV I, HTLV II, 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 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.