



Human Adult Mesothelial Cell Manual

INSTRUCTION MANUAL ZBM0025.06

SHIPPING CONDITIONS

Human Adult Mesothelial 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: Store as indicated IMMEDIATELY UPON ARRIVAL

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.

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THIS MANUAL IS SUITABLE FOR USE WITH THE FOLLOWING PRODUCTS:

MES-F	HUMAN ADULT MESOTHELIAL CELLS, CRYOPRESERVED
DMES-F	HUMAN ADULT MESOTHELIAL CELLS, DIABETIC DONOR

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 protocols 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

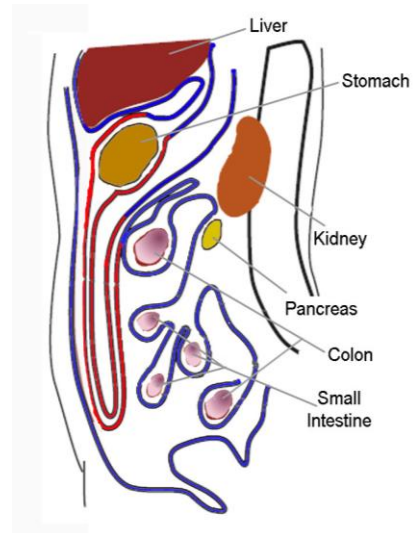
This product is for research use only. *It is not intended for human, veterinary, or in vitro diagnostic use.* Proper precautions and biological containment should be taken when handling cells of human origin, due to their potential biohazardous nature. **Always wear gloves and work behind a protective screen when handling primary human cells.** All media, supplements, and tissue culture ware used in this protocol should be sterile.

<u>CONTENTS</u>	<u>PAGE #</u>
Introduction	4
Materials provided for each catalog item	5
Media compositions	5
Plating procedure	6
Troubleshooting	7
Frequently asked questions	7
Pathogen testing	7

INTRODUCTION

Mesothelial cells lining the serous cavities (peritoneal, pericardial, and pleural) and internal organs provide a frictionless barrier and facilitate the movement of opposing organs and tissues. Recently, however, these cells have been found to be pivotal in tumor metastasis, peritoneal dialysis, inflammatory response, and metabolic disease. Ovarian tumor attachments occur through cancer cells binding to mesothelial cells and migrating into the surrounding tissue and vasculature. Peritoneal dialysis relies on the intact transport function of mesothelial cells to allow transfer of waste products from the underlying vasculature to the dialysis fluid in the peritoneal cavity. Host response to peritoneal insult is mediated by the inflammatory cascade initiated and cytokines release by peritoneal mesothelial cells. Researchers continue to investigate mesothelial cells, developing methods to regulate these processes with the goal of providing more effective treatments for disease.

The omental derived adult mesothelial cells are isolated from omental tissue from elective surgery in consented adult donors. They are isolated by centrifugal force after trypsin treatment. This instruction manual describes procedures to passage and culture the human mesothelial cells.



Cross-section of peritoneal cavity. Mesothelial cells line the peritoneal cavity (blue). Zen-Bio primary mesothelial cells are derived from the greater omentum (red).

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Human adult mesothelial cell viability depends greatly on the use of suitable media, reagents, and sterile plastic wear.

MATERIALS PROVIDED FOR EACH CATALOG ITEM

- **Cryopreserved Human Adult Mesothelial Cells**
 - Cat # MES-F
 - Frozen vial containing $\geq 1.0 \times 10^6$ viable human mesothelial cells (store in liquid nitrogen upon receipt)

MEDIA COMPOSTIONS

<u>Mesothelial Cell Growth Medium(Cat # MSO-1)</u>	<u>Storage and Expiration Date</u>
Medium 199 Fetal bovine serum Penicillin Streptomycin Amphotericin B	If placed at 4°C upon arrival, the media is stable until the expiration date on the bottle label which is 30 days from the shipping date. 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.
<u>Mesothelial Cell Basal Medium (Cat # MSB-1)</u>	<u>Storage and Expiration Date</u>
Medium 199 Penicillin Streptomycin Amphotericin B	If placed at 4°C upon arrival, the media is stable until the expiration date on the bottle label which is 30 days from the shipping date. 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.
<u>Mesothelial Cell Cryopreservation Medium Catalog# MCM-100</u>	<u>Storage and Expiration Date</u>
Medium 199 Fetal bovine serum DMSO	Store at -20°C upon arrival until ready for use, the media is stable for 6 months. The media will expire 45 days after the thaw date.

PLATING PROCEDURE

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

1. Remove cryopreserved human mesothelial 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 Mesothelial Cell Medium (MSO-1).
3. Centrifuge at 280 x g, 20°C, 5 minutes. Aspirate the medium and resuspend cells in a volume of MSO-1 appropriate for counting the cells. Count using a hemacytometer.
4. Place approximately 50,000 cells/cm² in Collagen I-coated cultureware using MSO-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 MSO-1.
6. Aspirate medium and wash mesothelial 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 25cm² flask (or 2 ml/75cm² 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 MSO-1 per 25cm² 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% CO₂, making sure the surface is level for even cell distribution.

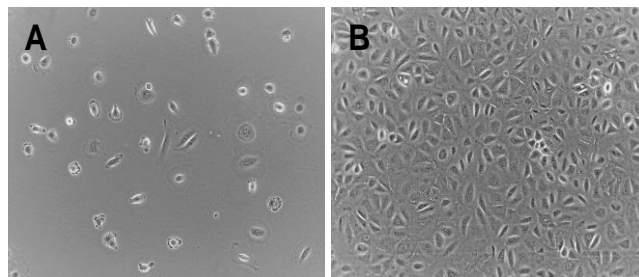


Figure 1. Human primary mesothelial cells. Human mesothelial cells were isolated from omental tissue and seeded on culture plates. Subconfluent mesothelial cells (A) have a partly fibroblastic appearance whereas confluent mesothelial cells (B) have cobblestone morphology.

Troubleshooting Guide

Observation	Possible causes	Suggestions
Adult MES cells do not grow	<ul style="list-style-type: none"> Cells have been passaged too many times 	<ul style="list-style-type: none"> Use cells of a lower passage number.
Edge effects	<ul style="list-style-type: none"> Medium in outside wells evaporated 	<ul style="list-style-type: none"> 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 mesothelial cells. All cells are shipped after establishing a primary culture.

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

The average doubling time is 30 hours. However, keep in mind that the replication rate for human mesothelial 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 adult mesothelial cells are isolated from human omental tissue obtained from consented donors undergoing elective surgery.

- **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

Samples from each donor are tested via PCR to confirm non-reactivity for HIV-1, HIV-2, 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-1 (Biosafety Level 1) or higher. Always wear gloves and work behind a protective screen when handling primary human cells.