Human Melanocyte Care Manual

INSTRUCTION MANUAL ZBM0058.04

SHIPPING CONDITIONS

Human Melanocyte Cells

Orders are delivered via Federal Express courier. All USA and Canada orders are shipped via Federal Express Priority service and are usually received the next day. Non North American International orders are usually received in 2-4 days. Primary human cells can be sensitive to extended times at dry ice temperatures. If your transit time will exceed 3 days, please inquire about dry vapor shipper options. Please inquire if alternate couriers are needed. **All orders should be processed immediately upon shipment receipt.**

STORAGE CONDITIONS

- **Media:** Store at 4°C. Expiration date 60 days from ship date. DO NOT FREEZE.
- **Cryopreserved Cells**
  - Store in vapor phase nitrogen (-150°C to -190°C) IMMEDIATELY UPON RECEIPT. Any other usage negates the warranty.

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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Durham, NC 27713, U.S.A.

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THIS MANUAL IS SUITABLE FOR USE WITH THE FOLLOWING PRODUCTS:

MEL-F	HUMAN ADULT MELANOCYTES, CRYOPRESERVED
MEL-F-NEO	NEONATAL HUMAN MELANOCYTES, CRYOPRESERVED

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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 the performance of cells only if Zen-Bio media are used and the recommended storage conditions and protocols are followed without amendment or substitution. ZenBio, Inc. cryopreserved cells are assured to be viable when stored as recommended and thawed according to Zen-Bio protocols and using the recommended protocol.

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 melanocyte viability depends greatly on the use of suitable media, reagents, and sterile plastic wear. If these parameters are not carefully observed, cell growth may be slower than expected.

INTRODUCTION

Melanocytes are dendritic cells that are derived from the neural crest cell population in the developing embryo. They are located in the basal layer of the epidermis where they connect with their numerous processes to the surrounding keratinocytes. They represent between 5% and 10% of the total epidermis. Melanocytes synthesize a specific pigment, Melanin in organelles called melanosomes and transfer it to surrounding keratinocytes. It is melanin that determines skin, eye and hair color.

Because of their role in skin pigmentation, skin protection and aging there is a great need for cellular studies that use Human Adult Melanocytes in cosmetic and skin biology studies. Melanocytes are also responsible for malignant melanoma formation. As such cultured melanocytes are an excellent tool for medical research.

ZenBio's human melanocytes are isolated from the epidermis of healthy consented donors who have undergone elective surgery in the United States. 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 trypsin/versene (1:1) digestion of the epidermal sheet and collected by centrifugal force. This instruction manual describes procedures to passage and culture ZenBio's adult human melanocytes.

The purity of ZenBio's adult melanocytes is routinely verified by Mel-5 (a melanocyte pigment-associated glycoprotein marker) immunofluorescence staining and cell morphology observation. In addition the ability to produce melanin is assessed by L-DOPA conversion assay. ZenBio's Melanocytes lots are >95% Mel-5 positive. Donor matched dermal fibroblasts and keratinocytes are also available.

QUALITY CONTROL

Human melanocytes are assessed for viability of 70% or higher, attachment and morphology.

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. 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 (BSL-1) or higher. Our cells are tested for mycoplasma contamination; mycoplasma contamination is not detected in our labs.

CATALOG ITEMS

- **Melanocyte Cryopreservation Medium**
  - Cat # MEL-100
  - Store -20°C
- **Melanocyte Medium**
  - Cat # MEL-2
  - Store 6°C to 8°C *DO NOT FREEZE*
- **Cryopreserved Human Adult Melanocytes**
  - Cat # MEL-F
  - Cryopreserved vial containing 500,000 viable human melanocytes per vial **(store in vapor phase liquid nitrogen IMMEDIATELY upon receipt)** *any other storage negates the warranty*
- **Cryopreserved Neonatal Human Melanocytes**
  - Cat# MEL-F-NEO
  - Cryopreserved vial containing 500,000 viable human melanocytes per vial **(store in vapor phase liquid nitrogen IMMEDIATLY upon receipt)** *any other storage negates the warranty*

MEDIA COMPOSTIONS

Melanocyte Growth Medium Cat# MEL-2	**Storage and Expiration Date**
Dermal Base Medium Human Insulin, Recombinant Basic FGF, Recombinant Bovine Pituitary Extract (NZ Origin) Fetal Bovine Serum (FBS), U.S.A. Origin Endothelin Apo transferrin Hydrocortisone Phorbol myristate acetate 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 60 days from the ship date. **Do Not Freeze**
Melanocyte Cryopreservation Medium Cat# MEL-100	**Storage and Expiration Date**
Fetal Bovine Serum (FBS), U.S.A Origin DMSO	Store at -20°C upon arrival until ready for use or expiration date on bottle. The media will expire 45 days after the thaw date.

PLATING AND EXPANSION PROCEDURES

CRYOPRESERVED ADULT AND NEONATAL HUMAN MELANOCYTES

Please note: Primary human cells require use of sterile tissue culture treated cultureware. No extracellular matrix coatings are required.

THAWING AND CULTURING

1. Pre-warm the Melanocyte Medium (cat# MEL-2) at 37°C, and prepare all pipets and vessels.
2. Transfer 4 mL of warm MEL-2 Melanocyte Medium to a sterile 15 mL conical tube.
3. Remove cells from liquid nitrogen and place **immediately** into a 37°C water bath with mild agitation. Be careful not to submerge the cap of the vial into water. For best results, the thawing step should not take more than 1 minute and should be stopped when there is still some ice in the vial. Rinse the vial with 70% ethanol before opening.
4. Transfer the cells to the sterile conical bottom centrifuge tube containing 4 mL of warm Melanocyte Medium prepared in step 2.
5. Centrifuge at 280 x g, 20°C, 5 minutes.

6. Carefully aspirate the medium and resuspend the cell pellet in a volume of Melanocyte Medium appropriate for counting the cells. Count cells using a hemocytometer or automated cell counter.

   NOTE: Step 6 should not take more than 30 minutes. If melanocytes are kept too long in suspension they will not recover after plating. If several vials need to be plated, thaw, count and plate no more than 2 vials at the same time.
7. Seed the cells at 250,000 cells per T25 flask in 10 mL MEL-2 Melanocyte Medium. Place in a humidified incubator at 37°C and 5% CO_2, making sure the surface is level for even cell distribution.
8. Change the medium after 24 hours in culture.
9. Medium should be changed every 4 days until the cells reach 70% confluence (see Figure 2).

OPTIONAL – HUMAN MELANOCYTE SUBCULTURE

NOTE: Human melanocytes should be passaged for subculture or cryopreservation when they are no more than 70% confluent (in about 10-15 days in culture).

1. Pre-warm MEL-2 Melanocyte Medium, HBSS Ca^2/Mg^{2+} free, and soybean trypsin inhibitor in a 37°C water bath.
2. Aspirate medium on the cells and wash the cells 2 times with sterile HBSS Ca^{2+}/ Mg^{2+} free.
3. Remove the HBSS and add 0.5mL/T-25 flask (or 1 mL/T-75 flask) of cold 0.25% trypsin/ 2.21mM EDTA solution. Incubate the cells at room temperature for 30-60 seconds monitoring cell detachment under the microscope. A longer incubation in trypsin can damage the melanocytes.
4. Neutralize the trypsin using an equal volume of 0.5mg/mL soybean trypsin inhibitor. Collect the cells in a conical tube containing 4 mL of melanocyte medium.
5. Centrifuge at 280 x g, for 5 minutes at 20°C.
6. Aspirate the medium and resuspend the cell pellet in a desired volume of melanocyte medium for cell counting.
7. Seed cells at 250,000 cells per T25 flask (or 750,000 cells per T75 flask) using MEL-2 Melanocyte medium. 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.
8. Replace the medium 24 hours after plating and every 4 days until the melanocytes are 70% confluent (see Figure 2).

Figure 1. Melanocytes day 2-3

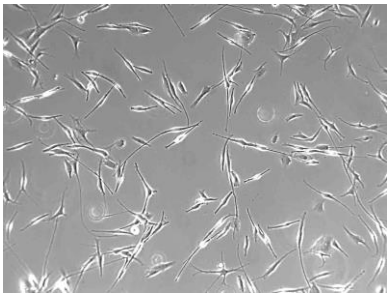
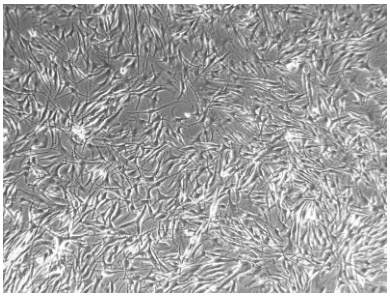


Figure 2. Melanocytes Day 8-12 (70% confluent)



TROUBLESHOOTING GUIDE

Observation	Possible causes	Suggestions
Melanocytes do not grow	1. Cells have been passaged too many times	1. Use cells of a lower passage number
	2. Cells expanded too high	2. Do not seed the cells lower than 10,000 cells/cm^2
	3. Cells not stored properly	3. Store cells in vapor phase liquid nitrogen upon arrival

FREQUENTLY ASKED QUESTIONS

Can I pass the cells?

All cells are shipped after establishing a primary culture and cryopreserved at passage 3. Cryopreserved melanocytes can be passaged at least 1 time (to a maximum of passage 4) using ZenBio medium and protocols.

How fast do the cells replicate?

The replication rate for human melanocytes varies 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 melanocytes are isolated from human epidermal tissue consented donors undergoing elective surgery in the United States. 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.

Do you test for pathogens? Which ones?

Yes. 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 at Biosafety Level 1 or higher. 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.

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 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. 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.