Human Melanocyte Care Manual

INSTRUCTION MANUAL ZBM0058.00

STORAGE CONDITIONS

Media: 2 months from ship date 4°C

Cells: Frozen: liquid nitrogen

All 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 AMSBIO. We 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.

AMSBIO warrants its cells only if AMSBIO media are used and the recommended protocols are followed. Cryopreserved human adult melanocytes are assured to be viable when thawed and maintained according to AMSBIO protocols.

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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.

AMSBIO's human melanocytes are isolated from the epidermis of healthy consented donors who have undergone elective surgery. 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 AMSBIO's adult human melanocytes.

The purity of AMSBIO'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. AMSBIO's Melanocytes lots are >95% Mel-5 positive. Donor matched dermal fibroblasts and keratinocytes are also available.

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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, limited differentiation may occur and cell growth may be slow.

MATERIALS PROVIDED FOR EACH CATALOG ITEM

Cryopreserved Human Adult Melanocytes

- Cat # MEL-F
- Frozen vial containing 0.5 x10^6 viable human melanocytes (store in liquid nitrogen upon receipt)
- 50 ml Human Melanocyte Growth Medium (cat# MEL-2) **NOTE: expiration date is 2months from media ship date.**

Cryopreserved Neonatal Human Melanocytes

- Cat# MEL-F-NEO
- Frozen vial containing 0.5 x10^6 viable human melanocytes (store in liquid nitrogen upon receipt)
- 50 ml Human Melanocyte Growth Medium (cat# MEL-2) NOTE: expiration date is **2 months from media ship date.**

MEDIA COMPOSTIONS

Melanocyte Growth Medium Cat# MEL-2	**Melanocyte Cryopreservation Medium** Cat# MEL-100
Dermal Base Medium	Fetal Bovine Serum
Insulin	DMSO
FGF	
Bovine Pituitary Extract	
Fetal Bovine Serum	
Endothelin	
Apo transferrin	
Hydrocortisone	
Phorbol myristate acetate	
Penicillin	
Streptomycin	
Amphotericin B	

NOTE:

MEL-2 is prepared fresh prior to shipment and expires approximately 2 months from the medium ship date.

Please call AMSBIO to coordinate media shipments with your experiment schedule

CRYOPRESERVED MELANOCYTE PLATING AND EXPANSION PROCEDURES

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 2 minutes 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 in a T25 flask at 10,000 cells/cm^2 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.).

MELANOCYTE SUBCULTURE

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 10,000 cells/cm^2 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

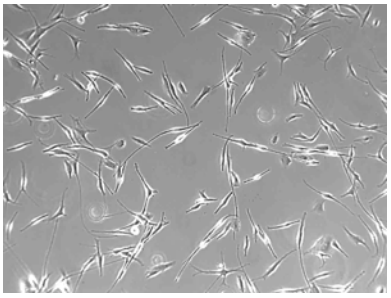
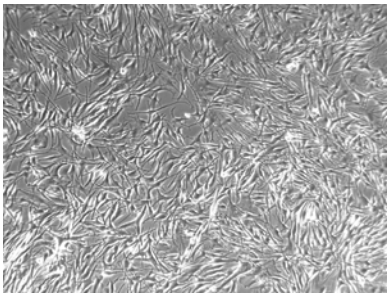


Figure 2. Melanocytes Day 8-12 (70% confluent)



TROUBLESHOOTING GUIDE

Observation	Possible causes	Suggestions
Melanocytes do not grow	1. Cells have been passaged too many times 2. Cells expanded too high	1. Use cells of a lower passage number 2. Do not seed lower than 10,000 cells/cm^2

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 using AMSBIO 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.

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.

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