Human Sebocytes Manual

INSTRUCTION MANUAL ZBM0081.02

SHIPPING CONDITIONS

Human Sebocyte 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 2-4 days.
Must be processed upon shipment receipt.

STORAGE CONDITIONS

Media: Store as indicated IMMEDIATELY UPON ARRIVAL

- Sebocyte Basal Medium: Store at +4°C; DO NOT FREEZE
- Sebocyte Supplements Store at -20°C

Cells: Sebocyte 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.

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 without amendment or substitution.

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.

ORDERING INFORMATION AND TECHNICAL SERVICES

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World Wide Web	http://www.zenbio.com

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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 sebocyte viability depends greatly on the use of the recommended protocols, suitable media, reagents, and sterile plastic wear. If these parameters are not carefully observed this may results in poor growth, viability and differentiation capacity of the cells.

MATERIALS PROVIDED FOR EACH CATALOG ITEM

- **Cryopreserved Human Primary Sebocytes**
  - Cat # SEB-F
  - Frozen vial containing 500,000 viable human adult sebocytes

  Store in vapor phase nitrogen (-150°C to -190°C) immediately upon receipt

MEDIUM COMPOSITION AND PREPARATION

Sebocyte Growth Medium Cat# SEB-1 (2 component media kit: A+B)
Note: This medium has been developed to optimize sebocyte proliferation and maintain sebocytes in an undifferentiated state.

A. Sebocyte Basal Medium

Storage: +4°C

Composition:

- Serum Free Base Medium (MCDB153)
- Penicillin
- Streptomycin
- Amphotericin B

B. Sebocyte Medium Supplement Set

Storage: -20°C

Composition:

- Bovine Pituitary Extract (BPE)
- Human Recombinant Epidermal Growth Factor (hrEGF)

SEBOCYTE MEDIUM PREPARATION INSTRUCTIONS

1. Aseptically add the entire contents of 1 set Sebocyte Medium Supplements (BPE, hrEGF) to 1 bottle Sebocyte Basal Medium (500ml) to make complete Sebocyte Medium, cat# SEB-1.
2. Gently invert to mix contents. Store complete medium at 4°C for 21 days.
3. The expiration date of the complete Sebocyte Medium will be exactly 21 days after the supplements are added to the Sebocyte Basal Medium.
4. DO NOT FREEZE the Sebocyte Basal Medium. Store at 4°C.
5. DO NOT FREEZE the complete Sebocyte Growth Medium. Store at 4°C.

THAWING AND CULTURING: Human Adult Sebocytes

Thawing:

1. Pre-warm the complete SEB-1 medium at 37°C. Prepare all your pipets and vessels.
2. Remove 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. For best results, the thawing step should not take more than 2 minutes. Stop thawing when there is still some ice in the vials. Rinse the vials with 70% ethanol before opening.
3. Transfer the cells to a sterile conical bottom centrifuge tube, containing 9 mL of Sebocyte Growth Medium (SEB-1).
4. Centrifuge at 300 x g, 20°C, for 5 minutes.

5. Aspirate the medium and resuspend the cell pellet in a volume of SEB-1 appropriate for counting the cells. Count cells using a hemacytometer.
6. Seed the cells at 0.25-0.5 X 10^6 cells per T-25 culture flask (10,000-20,000 cells/cm^2) in 6 mL of SEB-1. ***Note: If using 48 or 96 well plates seed the cells at 20,000-40,000 cells/cm^2.***
7. Incubate at 37°C in a humidified incubator with 5% CO_2. Change the medium after 24 h in culture.
8. Medium needs to be changed every 2-3 days until the cells reach 70-80% confluence (see Figure 2).

SUBCULTURE: Human Adult Sebocytes

Adult sebocytes should be passaged for subculture when they are no more than 70-80% confluent. Note that all cells are shipped at passage 3 after establishing a primary culture. For primary sebocytes, we guarantee up to one passage.

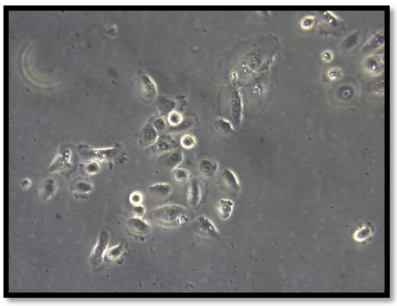
1. Pre-warm complete SEB-1 medium, 0.25% trypsin/ 2.21mM EDTA solution and sterile Phosphate Buffered Saline (PBS) Ca^2+/Mg^{2+} free, in a water bath at 37°C.
2. Aspirate medium and gently wash the cells 2-3 times with sterile PBS, to remove all traces of medium.
3. Remove the PBS and add 1ml/T-25 flask of pre-warmed 0.25% trypsin/ 2.21mM EDTA solution.
4. Incubate the cells at 37°C. Monitor cell detachment, under the microscope, after 2 minutes. Tap the flask gently to loosen the cells. If the cells are still attached, place them at 37 °C for another 1-3 minutes. **Note: A longer incubation in trypsin can damage the sebocytes.**
5. Neutralize the trypsin using an equal volume of 0.5 mg/ml soybean trypsin inhibitor. Collect all the cells in a conical tube containing 2ml of SEB-1.
6. Note: If some of the cells remain attached after the recommended time, we suggest that you neutralize the 0.25% trypsin/ 2.21mM EDTA solution by adding an equal volume of 0.5 mg/ml soybean trypsin inhibitor, remove the solution (along with any detached cells) to a 15ml conical tube and then add 2ml of complete SEB-1. To harvest the remaining attached sebocytes, wash the plate with PBS, add another 1ml of trypsin to the flask and repeat step 4. Centrifuge to obtain cell pellets as described below and combine resuspended cells prior to counting.
7. Centrifuge at 300xg, for 5 minutes at 20°C.
8. Aspirate the medium, and gently resuspend the cell pellet in a desired volume of SEB-1 and proceed to cell counting.
9. Seed cells at 10,000-20,000 cells/cm^2, (0.25-0.5x10^6 cells per T25 flask) in 6 ml of SEB-1. **If using 48 or 96 well plates seed the cells at 20,000-40,000 cells/cm^2.** Ensure cells are evenly suspended when plating large numbers of plates or flasks. Do not agitate plates and flasks

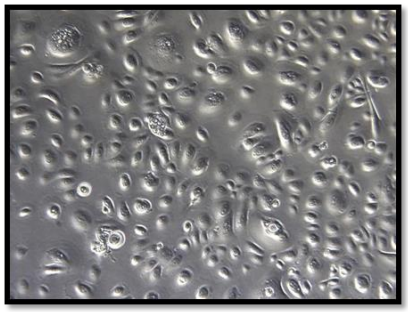
after plating. Place in a humidified incubator at 37°C and 5% CO_2, making sure the surface is level for even cell distribution.

10. Replace the medium 24 hours after plating and then every 2-3 days until they are 70-80%.

HUMAN SEBOCYTES MORPHOLOGY:

Figure 1. Non-confluent **Figure 2. Sub-confluent**





FREQUENTLY ASKED QUESTIONS

1. Can I passage the cells?
    a. All cells are shipped at passage 3 after establishing a primary culture. We guarantee performance up to 1 passage (passage 4).

2. How fast do the cells replicate?
    a. The average doubling time is 48-72 hours. However, keep in mind that the replication rate for human sebocytes varies from donor to donor.

3. Should antibiotics be included in the medium?
    a. Yes.
    b. Antibiotics and anti-fungal agents are always recommended since the cells are primary cells.

4. Where are the cells obtained?
    a. The adult sebocytes are isolated from micro-dissected sebaceous gland from the facial skin of healthy consented adult donors undergoing elective surgery.

5. Do you test for pathogens? Which ones?
    a. Yes.
    b. 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.

6. What quality control tests are performed on the Sebocytes?
    a. They highly express the Sebocyte marker Cytokeratin-7 and MUC-1 when differentiated as assessed by immunostaining.
    b. Sebocyte cells exhibit an epithelial morphology with small cytoplasmic lipid droplets

7. Do you sell a Sebocyte Differentiation Medium?
    a. At this time we do not have a Sebocyte Differentiation Medium available for retail.

8. Can I just buy and use the Sebocyte Basal Medium for my experiments?
    a. No. Sebocyte Growth Medium cat# SEB-1 is a 2 part medium kit comprised of both the base medium and the supplements. Any other use negates the ZenBio product liability.

9. What donor information do I receive?
    a. The donor's age, gender, body mass index (BMI) and a current medications list 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.