Human Sebocytes Manual

INSTRUCTION MANUAL ZBM0081.07

SHIPPING CONDITIONS

Human Sebocyte Cells. All US and Canada orders are shipped via Federal Express Priority service and are usually received the next day.
Other International orders are shipped using a dry vapor shipper. Please inquire for disposable dry shipper sales and larger format dry shipper rentals. Cells should always be stored in liquid nitrogen vapor phase immediately upon arrival. The sebocytes must be processed immediately upon shipment receipt.

STORAGE CONDITIONS

SEB-1 Medium: +4°C Expires 2 months from ship date
-20°C Expires 3 months from ship date

Cells: Sebocyte cells are to be stored in vapor phase nitrogen (-150°C to -190°C) IMMEDIATELY UPON RECEIPT. Any other storage negates the warranty

All Zen-Bio Inc products are for research uses only. Not approved for human or veterinary use or for use in diagnostic, therapeutic or clinical procedures.

ORDERING INFORMATION AND TECHNICAL SERVICES

ZenBio, Inc.
3920 South Alston Avenue
Durham, NC 27713 U.S.A.

Telephone	+1 (919) 547-0692
Toll free (continental US only)	**1-866-ADIPOSE 1-(866)-234-7673**
Electronic mail (e-mail)	information@zenbio.com
World Wide Web	http://www.zenbio.com

THIS MANUAL IS SUITABLE FOR USE WITH THE FOLLOWING PRODUCTS:

SEB-F	CRYOPRESERVED HUMAN PRIMARY SEBOCYTES: 500,000 CELLS/VIAL

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 recommended shipping method, storage conditions, media and 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.

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 appropriate storage conditions, 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

Catalog # SEB-F

- Cryopreserved vial containing 500,000 viable human adult sebocytes
- Store in vapor phase liquid nitrogen immediately upon receipt. Any other storage negates the warranty.
- Total transit time for human primary sebocytes using dry ice is limited to 1 day only.
- Optimal transit temperature is dry vapor nitrogen (-158°C).
- Optimal storage temperature is vapor phase liquid nitrogen (-158°C to -190°C).

INTRODUCTION

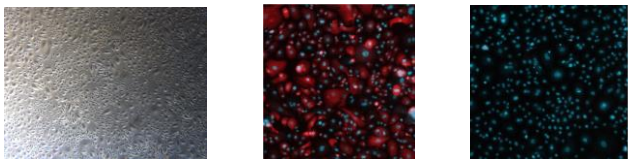
Primary human sebocytes are isolated from micro dissected sebaceous glands from the eyelid or facial skin of consented adult donors undergoing elective surgery in the United States. Each sample is derived from a competent volunteer adult donor who has signed an Institutional Review Board (IRB) validated 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 protocols in compliance with ethical regulations. All samples are collected and processed in the United States.

QUALITY CONTROL

Undifferentiated Primary Human Sebocytes are assessed for viability, general epithelial cell morphology, and immunocytochemistry staining for cytokeratin 7 and less than 10% positive for Epithelial Membrane Antigen/Mucin-1 (EMA/Muc-1). Primary Human Sebocytes must be plated on collagen I coated cultureware as outlined in this manual.

Primary human sebocytes exhibit the general characteristics of epithelial cell morphology (A). They are further characterized using immunocytochemistry staining to confirm an undifferentiated state. Primary human sebocytes contain greater than 75 % of cells positive for cytokeratin 7 (**B**) and less than 10% positive for Epithelial Membrane Antigen/Mucin-1 (EMA/Muc-1 (**C**)).

A. Unstained **B.** **C.**



MEDIUM COMPOSITION

Sebocyte Growth Medium Cat# SEB-1	Expiration Dates
• MCDB153 • Human Epidermal growth factor (rEGF) • Human Insulin • Human apo transferrin • Human Epidermal growth factor (hEGF) • Bovine serum albumin, fatty acid free • *O*-Phosphorylethanolamine • Ethanolamine • Hydrocortisone • Calcium Chloride • Epinephrine • Bovine Pituitary Extract (BPE) • Penicillin • Streptomycin • Amphotericin B	• If placed at +4°C upon arrival, the media is stable 2 months from the ship date Use the +4°C expiration date listed on the label. • If stored at -20°C upon arrival, it is stable **3 months** after the ship date • Use the 3 month -20°C expiration date listed on the label. • Upon thawing, add fresh antibiotics at 1% volume when you are ready to use. • The media will now expire 30 days after the thawing date.

THAWING AND CULTURING: Human Primary Adult Sebocytes

1. **For the thawing procedure,** ZenBio scientists recommend the use of ZenBio, Inc collagen I coated cultureware (Read FAQ for catalog numbers and ordering information)
2. **When plating for assays,** ZenBio, Inc collagen I coated cultureware (Read FAQ for ordering information) for the best results.

- **DO NOT** seed the cryopreserved sebocyte cells directly into your assay plates.
- Follow this protocol exactly as written **without any substitutions or alterations**.
    - Any deviations from the protocol negates the warranty for refund or replacement

Thawing Human Adult Primary Sebocytes:

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 500,000 cells per collagen I coated 75 cm^2 culture flask *(6,700 cells/cm^2) in 19 mL of SEB-1. ***Note: Do not seed the cryopreserved cells directly into your assay plates*.**
7. Incubate at 37°C in a humidified incubator with 5% CO_2. Change the medium after 24 hours in culture.
8. Medium needs to be changed every 2-3 days until the cells reach 70-80% confluence (see Figure 2). Once the cells reach 70-80% confluency, the cells can be plated for experimentation (See Plating instructions below)

PLATING FOR ASSAY: Human Adult Primary 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.
- 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.

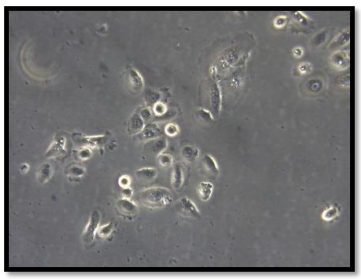
1. Aspirate medium and gently wash the cells 2-3 times with sterile PBS, to remove all traces of medium.
2. Remove the PBS and add 2ml/T-75 flask of pre-warmed 0.25% trypsin/ 2.21mM EDTA solution.
3. Incubate the cells at 37°C. Monitor cell detachment, under the microscope, after 2 minutes. Tap the flask gently to loosen the cells. If the cells are still attached, place

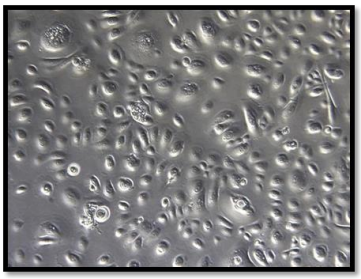
them at 37 °C for another 1-3 minutes. **Note: A longer incubation in trypsin can damage the sebocytes.**

4. Neutralize the trypsin using an equal volume of 0.5 mg/ml soybean trypsin inhibitor. Collect all the cells in a conical tube containing 4ml of SEB-1.
5. 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.
6. Centrifuge at 300xg, for 5 minutes at 20°C.
7. Aspirate the medium, and gently resuspend the cell pellet in a desired volume of SEB-1 and proceed to cell counting.
8. Seed cells at 12,000 cells/cm^2 in the plate format of your choice (note the brand recommendations)**.** 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.
9. Replace the medium 24 hours after plating and then every 2-3 days until they are 70-80% and ready to assay.

HUMAN SEBOCYTES MORPHOLOGY:

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





FREQUENTLY ASKED QUESTIONS

1. Is there a specific type of culture ware that should be used?
    a. Yes.
    b. We recommend ZenBio brand Collagen I Coated Cultureware to be used during the thawing procedure and the final assays.
    c. ZenBio Collagen Coated Culture ware

Cat#	ZenBio Collagen I Coated Cultureware Descriptions
CC-96	Collagen I Coated 96-well Plate, Pack of 5
CC-48	Collagen I Coated 48-well Plate, Pack of 5
CC-24	Collagen I Coated 24-well Plate, Pack of 5
CC-12	Collagen I Coated 12-well Plate, Pack of 5
CC-6	Collagen I Coated 6-well Plate, Pack of 5
CC-25	Collagen I Coated T-25 Flask, Vent Cap, Pack of 5
CC-75	Collagen I Coated T-75 Flask, Vent Cap, Pack of 5
CC-225	Collagen I Coated T-225 Flask, Vent Cap, Pack of 1 (EXCLUSIVE!)

2. Can I passage the sebocytes?
    a. No.
    b. You must follow the procedures as outlined in this manual without substitution or amendment.
    c. Any deviation from the listed protocol negates the warranty for performance.
    d. Following the protocol for thawing, the cells will be passaged prior to plating for your assay.
    e. We do not guarantee performance beyond this procedure. All cells are shipped at passage 3 after establishing a primary culture and will be passage 4 when ready to assay if our procedures are followed.

3. 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.

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

5. Where are the cells obtained?
    a. The adult sebocytes are isolated from micro-dissected sebaceous glands from the eyelid or facial skin of competent consented adult donors undergoing elective surgery in the United States.

6. 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, Hepatitis B and Hepatitis C. However, since we cannot test all pathogens, we recommend treating the culture as a potentially infectious agent at Biosafety Level 1 (BSL-1) or higher.

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

8. Do you sell a Sebocyte Differentiation Medium?
    a. No. At this time we do not have a Sebocyte Differentiation Medium available.

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.
10. Do you have a cryopreservation medium or protocol for the primary human sebocytes?
    a. No. ZenBio, Inc., human primary sebocytes are a one-time use only product

PATHOGEN TESTING

Samples from each donor are tested and found non-reactive to viral DNA from HIV and hepatitis B and viral RNA from Hepatitis C using US Food and Drug Administration (FDA) licensed tests. 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 at Biosafety Level 1 (BSL-1) or higher. Our cells are tested for mycoplasma contamination via direct plating and DNA fluorochrome staining; mycoplasma contamination is not detected.