# Human Keratinocyte Manual

## INSTRUCTION MANUAL ZBM0032.07

## SHIPPING CONDITIONS

**Human Keratinocyte 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:** 3 weeks from ship date 4°C

**Cells:** Frozen: liquid nitrogen

***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. Cryopreserved human adult keratinocytes are assured to be viable when thawed and maintained according to Zen-Bio protocols.

### ORDERING INFORMATION AND TECHNICAL SERVICES

**Zen-Bio, Inc.**
**3200 Chapel Hill-Nelson Blvd., Suite 104**
**PO Box 13888**
**Research Triangle Park, NC 27709**
**U.S.A.**

| | |
|---|---|
| **Telephone** | (919) 547-0692 |
| **Facsimile (FAX)** | (919) 547-0693 |
| **Toll free (continental US only)** | **1-866-ADIPOSE 1-(866)-234-7673** |
| **Electronic mail (e-mail)** | **information@zen-bio.com** |
| **World Wide Web** | **http://www.zenbio.com** |

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# INTRODUCTION

ZenBio's adult human keratinocytes are isolated from the epidermis of healthy non-diabetic donors between 18 and 60 years old who have undergone elective surgery. ZenBio neonatal keratinocytes are isolated from the foreskin of healthy males aged newborn to infant from elective circumcisions. The cells are isolated by trypsin digestion of the epidermal sheet and collected by centrifugal force. This instruction manual describes procedures to passage and culture the adult and neonatal human keratinocytes. For the adult keratinocytes, donor matched dermal fibroblasts and preadipocytes are also available for many samples.

All samples are negative for common pathogens HIV-1, HIV-2, HTLV I, HTLV II, hepatitis B and hepatitis C. All keratinocytes exhibit the cobblestone morphology and highly express the basal keratinocyte markers keratin-5 and keratin-14 as assessed by immunostaining.

# 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 keratinocyte 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 Keratinocytes**
  - Cat # KR-F
  - Frozen vial containing 0.5 x$10^6$ viable human keratinocytes (store in liquid nitrogen upon receipt)
  - 50 ml Human Keratinocyte Growth Medium (cat# KM-2) **NOTE: expiration date is 3 weeks from media ship date.**
- **Cryopreserved Human Neonatal Keratinocytes**
  - Cat # KRN-F
  - Frozen vial containing 0.5 x$10^6$ viable human neonatal keratinocytes (store in liquid nitrogen upon receipt)
  - 50 ml Human Neonatal Keratinocyte Growth Medium (cat# KM-3) **NOTE: expiration date is 3 weeks from media ship date.**

# MEDIA COMPOSTIONS

| Adult Keratinocyte Growth Medium<br>Cat# KM-2 | Neonatal Keratinocyte Growth Medium<br>Cat# KM-3 |
|---|---|
| • MCDB153<br>• Human Epidermal growth factor (rEGF)<br>• Human Insulin<br>• Human apo transferrin<br>• Bovine serum albumin, fatty acid free, low endotoxin<br>• Phosphoethanolamine / Ethanolamine<br>• Hydrocortisone<br>• Calcium Chloride<br>• Epinephrine<br>• Bovine Pituitary Extract (BPE)<br>• Penicillin<br>• Streptomycin<br>• Amphotericin B | • MCDB153<br>• Human Epidermal growth factor (rEGF)<br>• Human Insulin<br>• Human apo transferrin<br>• Phosphoethanolamine / Ethanolamine<br>• Hydrocortisone<br>• Calcium Chloride<br>• Epinephrine<br>• Bovine Pituitary Extract (BPE)<br>• Penicillin<br>• Streptomycin<br>• Amphotericin B |

**Keratinocyte Basal Medium**

[suitable for both neonatal and adult keratinocytes]

Cat# KB-1

- MCDB153
- Penicillin
- Streptomycin
- Amphotericin B

**Keratinocyte Cryopreservation Medium**

[suitable for both neonatal and adult keratinocytes]

Cat# KF-100

- Keratinocyte Growth Medium, KM-2
- DMSO
- FBS

**NOTE:**

**KM-2 and KM-3 are prepared fresh prior to shipment and expires approximately 3 weeks from the medium ship date. Please call ZenBio to coordinate media shipments with your experiment schedule**

**Please inquire for custom media requests.**

# PLATING AND EXPANSION PROCEDURES: Cryopreserved Adult Keratinocytes

## THAWING AND CULTURING: Human Adult Keratinocytes

1. Pre-warm the KM-2 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 Keratinocyte Growth Medium (KM-2).
4. Centrifuge at 300 x g, 20°C, for 5 minutes.
5. Aspirate the medium and resuspend the cell pellet in a volume of KM-2 appropriate for counting the cells. Count cells using a hemacytometer.
6. Seed the cells at 0.37-0.75 X $10^6$ cells per T-75 culture flask (5,000-10,000 cells/$cm^2$) in 20 ml KM-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% confluent (see Figure 1). **Do not allow the cells to reach 100% confluency**.

## SUBCULTURE: Human Adult Keratinocytes

Adult keratinocytes should be passaged for subculture or cryopreservation when they are no more than 70-80% confluent (in about 5-6 days in culture).

1. Pre-warm, KM-2 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 wash the cells 2-3 times with sterile (PBS) $Ca^{2+}$/ $Mg^2$+ free, to remove all traces of medium.
3. Remove the PBS and add 2mL/T-75 flask (or 6 ml/T-225 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. A longer incubation in trypsin can damage the keratinocytes.

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 4ml of KM-2.
6. Centrifuge at 300xg, for 5 minutes at 20°C.
7. Aspirate the medium, and resuspend the cell pellet in a desired volume of KM-2 and proceed to cell counting.
8. Seed cells at 5,000-10,000 cells/cm2, (0.37-0.75x$10^6$ cells per T75 flask) in 20 ml of KM-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.
9. Replace the medium 24 hours after plating and then every 2-3 days until they are 70-80% confluent (see Figure 1).

# PLATING AND EXPANSION PROCEDURES: Cryopreserved Neonatal Keratinocytes

## THAWING AND CULTURING: Human Neonatal Keratinocytes

1. Pre-warm the KM-3 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 Neonatal Keratinocyte Growth Medium (KM-3).
4. Centrifuge at 300 x g, 20°C, for 5 minutes.
5. Aspirate the medium and resuspend the cell pellet in a volume of KM-3 appropriate for counting the cells. Count cells using a hemacytometer.
6. Seed the cells at 0.37-0.75 X $10^6$ cells per T-75 culture flask (5,000-10,000 cells/$cm^2$) in 20 ml KM-3.
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% confluency (see Figure 1). **Do not allow the cells to reach 100% confluency**.

## SUBCULTURE: Human Neonatal Keratinocytes

1. Human neonatal keratinocytes should be passaged for subculture or cryopreservation when they are no more than 70-80% confluent (in about 5-6 days in culture).
2. Pre-warm, KM-3 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.
3. Aspirate medium and wash the cells 2-3 times with sterile PBS ($Ca^{2+}$/ $Mg^{2+}$ free), to remove all traces of medium.
4. Remove the PBS and add 2mL/T-75 flask (or 6 ml/T-225 flask) of pre-warmed 0.25% trypsin/ 2.21mM EDTA solution.
1. 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. A longer incubation in trypsin can damage the keratinocytes.
2. 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 KM-3.
3. Centrifuge at 300xg, for 5 minutes at 20°C.
4. Aspirate the medium, and resuspend the cell pellet in a desired volume of KM-3 and proceed to cell counting.
5. Seed cells at 5,000-10,000 cells/cm2, (0.37-0.75x$10^{6}$ cells per T75 flask) in 20 ml of KM-3. 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.
6. Replace the medium 24 hours after plating and then every 2-3 days until they are 70-80% confluent (see Figure 1).

# CRYOPRESERVATION Procedure for Human Keratinocytes

1. Cryopreserve adult or neonatal human keratinocytes after counting.
2. Centrifuge at 300 x g, 20°C, 5 minutes.
3. Suspend in cold Keratinocyte Cryopreservation medium (Cat# KF-100) at a concentration of 0.5X$10^{6}$ cells/ml. Do not exceed a 6:1 ratio of cells (per million): volume freeze medium (per ml). If using a controlled-rate freezer: Freeze by reducing the temperature 1°C per minute until the temperature reaches -80° C. If using a cell cryopreservation container, prepare according to the manufacturer's instructions.
4. For best results we recommend transferring the vials to the vapor phase of a liquid nitrogen storage facility 1-4 days after the cells have reached -80°C.

**HUMAN KERATINOCYTES Morphology:**

**Figure 1. 70% confluent**

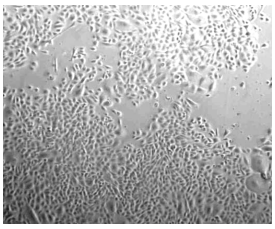
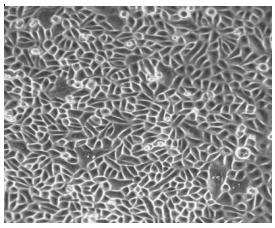


**Figure 2. 100% confluent**



# KERATINOCYTE TROUBLESHOOTING GUIDE _____

| Observation | Possible causes | Suggestions |
|---|---|---|
| keratinocyte cells do not grow | 1. Cells have been passaged too many times | 1. Use cells of a lower passage number |
| Edge effects | 1. Medium in outside wells evaporated | 1. Ensure a saturated humidity in the incubator and feed the cells no less than every 2-3 days. Make sure multiple plates are stacked no more than 3 plates high. If the wells are not all used, fill the empty wells with medium. |

# FREQUENTLY ASKED QUESTIONS

Can I pass the cells?

Keratinocytes can be trypsinized and replated up to passage 4 or 5. All cells are shipped at passage 2 or 3 after establishing a primary culture .

How fast do the cells replicate?

The average doubling time is 48-84 hours. However, keep in mind that the replication rate for human keratinocytes 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 adult keratinocytes are isolated from human epidermal tissue obtained from consented adult donors undergoing elective surgery. The neonatal keratinocytes are isolated from human male foreskin from elective circumcisions.

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 quality control tests are performed on the keratinocytes?

All samples are negative for common pathogens HIV-1, HIV-2, HTLV I, HTLV II, hepatitis B and hepatitis C. All keratinocytes exhibit the cobblestone morphology and highly express the basal keratinocyte markers keratin-5 and keratin-14 as assessed by immunostaining.

What donor information do I receive?

The donor's age, gender, and body mass index (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.