ZenBio, Inc.

Human Dermal Fibroblast Manual

INSTRUCTIONAL MANUAL ZBM0023.05

SHIPPING CONDITIONS

Human Adult or Neonatal Dermal Fibroblast 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: Short Term (30 days): 4°C 6 months -20°C

Cells: Frozen: Vapor phase liquid nitrogen

Live Plated: 37°C incubator

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

India Contact:

Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K Pitampura,
Delhi – 110034 (INDIA).
Ph: +91-11-42208000, 42208111, 42208222
Mobile: +91-9810521400 Fax: +91-11-42208444
Email: customerservice@lifetechindia.com
Web: www.lifetechindia.com

THIS MANUAL IS SUITABLE FOR USE WITH THE FOLLOWING PRODUCTS:

DF-F	HUMAN DERMAL FIBROBLASTS
DFN-F	HUMAN NEONATAL DERMAL FIBROBLASTS
DF-F-PS	HUMAN DERMAL FIBROBLASTS FROM PSORIASIS DONOR
DF-D-F	HUMAN DERMAL FIBROBLASTS FROM TYPE 2 DIABETIC DONOR
DF-1, DF-1-PRF	DERMAL FIBROBLAST MEDIUM, - PHENOL RED FREE
DFM-100	DERMAL FIBROBLAST CRYOPRESERVATION MEDIUM

CONTENTS PAGE

INTRODUCTION

Adult dermal fibroblasts are isolated from the dermis of healthy non-diabetic adult donors undergoing elective surgery. Neonatal dermal fibroblasts are isolated from the foreskins of healthy male newborns. The cells are isolated by centrifugal force following enzymatic treatment or from an explant culture. This instruction manual describes procedures to passage and culture the human dermal fibroblast cells.

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 dermal fibroblast cell 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.

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 cells are assured to be viable when thawed and maintained according to Zen-Bio protocols.

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.

CATALOG ITEMS

NOTE: Effective February 1, 2016, we will no longer provide 50ml support medium with each cryopreserved vial ordered. Please order media according to your needs.

- **Human Adult Dermal Fibroblasts** (96-,48-,24-,12-,6-well plates; 75-,25-cm^2 flasks)
  - Cat # DF-4096, -4048, -4024, -4012, -4006, -4075, -4025

- **Dermal Fibroblast Cryopreservation Medium**
  - Cat# DFM-100

- **Cryopreserved Human Adult Dermal Fibroblasts**
  - Cat # DF-F
  - Vial containing 1 x10^6 viable adult dermal fibroblasts (store in vapor phase liquid nitrogen)

- **Cryopreserved Human Neonatal Dermal Fibroblasts**
  - Cat # DFN-F
  - Vial containing 500,000 viable neonatal dermal fibroblasts (store in vapor phase liquid nitrogen)

MEDIA COMPOSTIONS

Dermal Fibroblast Medium cat # DF-1 500ml	**Dermal Fibroblast Basal Medium cat # DF-2 500ml**	**Dermal Fibroblast Cryopreservation Medium cat # DFM-100 100ml**
DMEM	DMEM	DMEM
Fetal bovine serum	Penicillin	Fetal bovine serum
Penicillin	Streptomycin	DMSO
Streptomycin	Amphotericin B	
Amphotericin B		

All media contain 4.5 g/L (25 mmol/L) D-glucose.
All media are also available as without serum and/or phenol red.
Please inquire for custom media requests.

MEDIA EXPIRATION DATES:

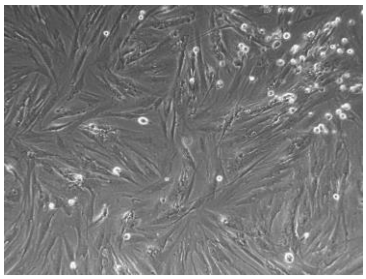
- If placed at 4°C upon arrival, the media is stable until the expiration date on the bottle label.
- If stored at -20°C upon arrival, the media is stable for 6 months. Add fresh antibiotics when you are ready to use. The media will now expire 30 days after the thaw date

PLATING AND EXPANSION PROCEDURES

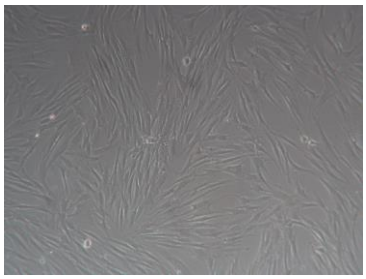
Cryopreserved Adult and Neonatal Dermal Fibroblasts

1. 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. Do not leave the vials in water bath after most of the content has thawed. Rinse the vials with 70% ethanol before taking them to the culture hood.
2. Upon the thawing, add the cells to a sterile conical bottom centrifuge tube, containing 9 ml of Dermal Fibroblast Growth Medium (DF-1).
3. Centrifuge at 400 x g, 20°C, 10 minutes. Aspirate the medium and resuspend cells in a volume of DF-1 appropriate for counting the cells. Count using a hemacytometer.
4. Place approximately 10,000 cells/cm^2 (i.e. 0.75 X 10^6 cells in T-75 culture flask) using DF-1.
5. Incubate cells until they are 85-90% confluent (in about 3-5 days). Cells will need to be fed every 3 days with DF-1.
6. Aspirate medium and wash adult fibroblasts 4-5 times using sterile Phosphate Buffered Saline (PBS) to remove all traces of serum (until there is no foaming of the medium). Remove the PBS and release the cells from the flask bottom by adding 2 mL/T-75 flask (or 6 ml/T-225 flask) of 0.25% trypsin/ 2.21mM EDTA solution. Allow cells to trypsinize for 5 minutes at 37°C. Tap the flask gently to loosen the cells.
7. Neutralize the trypsin using 7 ml DF-1 per T-75 flask (or 21 ml per T-225 flask). Check the flask under a microscope to ensure all cells are free of the flask bottom.
8. Count the cells and plate in desired format at 10,000 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.
9. Cells will need to be fed with fresh DF-1 every 3 days until the desired confluence is reached. The cells may be confluent within 3-8 days when plated at the recommended seeding density.

Figure 1. Neonatal and adult dermal fibroblasts, 3 days post-plating



A. Neonatal dermal fibroblasts



B. Adult dermal fibroblasts

CRYOPRESERVATION

10. ***OPTIONAL* – Cryopreserve dermal fibroblasts after counting.**
    a. Centrifuge at 280 x g, 20°C, 5 minutes.
    b. Suspend in cold Dermal Fibroblast Cryopreservation medium (Cat# DFM-100) at a concentration of $1X10^6$ cells/ml. Do not exceed a 6:1 ratio of cells (per million): volume freeze medium (per ml).
    c. Remember to account for the volume of the cell pellet before adding the volume of freeze medium necessary for cell suspension.
    d. 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
    e. . For best results we recommend transferring the vials to the vapor phase of a liquid nitrogen storage facility as soon as possible after the cells have reached -80°C.

FREQUENTLY ASKED QUESTIONS

- **Can I passage the cells?**
  Dermal fibroblast cells can be trypsinized and replated several times. All cells are shipped after establishing a primary culture. We do not have any data on the limit of expansion of the cells.
- **How fast do the cells replicate?**
  The average doubling time ranges from 18-24 hours. However, keep in mind that the replication rate for human dermal fibroblasts 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 dermal fibroblast cells are isolated from the dermal layer of human skin tissue. The neonatal cells are isolated from the dermal layer of newborn human foreskin 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, 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.
- **Are there recommendations for cultureware to use with the cells?**
  Yes. Primary cells can be very sensitive to brands of cultureware. Zen-Bio does not currently recommend the use of Corning Falcon or Sarstedt brand plates or flasks. Our scientists are using Nunc, Corning Costar, or Greiner Bio-One Cellstar tissue culture treated plates and flasks.
- **What is the formulation of Zen-Bio's serum-free media?**
  Zen-Bio's serum-free media are not enhanced to supplement the absence of serum. These media are available for assay procedures where cells are rested from serum.

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-2 (Biosafety Level 2) or higher. Always wear gloves and work behind a protective screen when handling primary human cells.