Human Dermal Fibroblast Care Manual

INSTRUCTION MANUAL ZBM0023.07

SHIPPING CONDITIONS

Neonatal or Adult Human Dermal Fibroblast Cells

Orders are delivered via Federal Express courier. All USA and Canada orders are shipped via Federal Express Priority service and are usually received the next day. Non North American International orders are usually received in 2-4 days. Primary human cells can be sensitive to extended times at dry ice temperatures. If your transit time will exceed 3 days, please inquire about dry vapor shipper options. Please inquire if alternate couriers are needed.

All orders should be processed immediately upon shipment receipt.

STORAGE CONDITIONS

Media:	+4°C	Expires 30 days from ship date.
	-20°C	Expires 6 months from ship date.
Cryo Media:	Store at -20°C	Expires 12 months from ship date.

Cells: Store in vapor phase nitrogen (-150°C to -190°C) IMMEDIATELY UPON RECEIPT.

Any other use 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 or clinical procedures or other uses in humans.

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@zen-bio.com
World Wide Web	http://www.zen-bio.com

THIS MANUAL IS SUITABLE FOR USE WITH THE FOLLOWING PRODUCTS:

DF-F	CRYOPRESERVED DERMAL FIBROBLASTS, 1 MILLION CELLS/VIAL
DFD-F	CRYOPRESERVED DERMAL FIBROBLASTS, DIABETIC DONOR, 1 MILLION CELLS/VIAL
DF-F-PS	CRYOPRESERVED DERMAL FIBROBLASTS, PSORIASIS DONOR, 1 MILLION CELLS/VIAL
DFN-F	CRYOPRSERVED DERMAL FIBROBLASTS, NEONATAL, SINGLE DONOR (500,000 CELLS/VIAL)

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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 the performance of cells only if Zen-Bio media are used and the recommended storage conditions and protocols are followed without amendment or substitution. ZenBio, Inc. cryopreserved cells are assured to be viable when stored as recommended and thawed according to Zen-Bio protocols and using the recommended protocol.

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 dermal fibroblast viability depends greatly on the use of suitable media, reagents, and sterile plastic wear. If these parameters are not carefully observed, cell growth may be slower than expected.

INTRODUCTION

ZenBio, Inc. adult human dermal fibroblast cells are isolated from the dermal layer of donated skin tissue from a consented adult donor undergoing elective surgery in the United States. Each volunteer adult donor 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.

ZenBio, Inc. neonatal human dermal fibroblast cells are isolated from the dermal layer of skin tissue from elective surgery in a consented neonatal donor in the United States. An Institutional Review Board (IRB) validated parental donor consent form is on file 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

Human dermal fibroblasts are assessed for viability, attachment, and morphology.

Each lot of primary cells is tested via PCR and found non-reactive to viral DNA from HIV and hepatitis B and viral RNA from Hepatitis C. 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; mycoplasma contamination is not detected in our labs.

CATALOG ITEMS

- **Dermal Fibroblast Cryopreservation Medium**
  - Cat # DFM-100, 100 mL
  - Store -20°C
- **Dermal Fibroblast Culture Medium**
  - Cat # DF-1, 500 mL
  - Store according to label
- **Dermal Fibroblast Basal Medium**
  - Cat # DF-2, 500 mL
  - Store according to label
- **Cryopreserved Adult Human Dermal Fibroblasts**
  - Cat # DF-F, DFD-F (DIABETIC donor), DF-F-PS (PSORIASIS donor)
  - Cryopreserved vial containing a minimum of 1 million viable adult dermal fibroblasts per vial
- **Cryopreserved Neonatal Human Dermal Fibroblasts**
  - Cat # DFN-F
  - Cryopreserved vial containing a minimum of 500,000 viable neonatal dermal fibroblasts per vial

***For all cryopreserved cells:* Store in vapor phase liquid nitrogen IMMEDIATELY upon receipt** *any other storage negates the warranty*

MEDIA COMPOSTIONS

Dermal Fibroblast Culture Medium (Cat# DF-1)	**Storage and Expiration Date**
DMEM, 4.5 g/L (25 mmol/L) D-glucose Fetal Bovine Serum (FBS; USA Origin) Penicilin Stroptomycin Amphotericin B	• If stored at 4°C upon arrival, the media is stable until the expiration date on the bottle. • If stored at -20°C upon arrival, the media is stable for 6 months. *The media will expire 30 days after the thaw date.* • Medium is provided ready to use and prepared fresh prior to shipment. *All media are available without phenol red upon request. Please inquire about custom media requests.*
Dermal Fibroblast Basal Medium (Cat# DF-2)	
DMEM, 4.5 g/L (25 mmol/L) D-glucose Penicilin Stroptomycin Amphotericin B	

Dermal Fibroblast Cryopreservation Medium (Cat# DFM-100)	**Storage and Expiration Date**
Fetal Bovine Serum (FBS; USA Origin) Dimethyl Sulfoxide (DMSO)	• Store at -20°C upon arrival until ready for use or the expiration date on bottle. Cryopreservation medium has an expiration date 1 year from the manufacture date when stored frozen. • The media will expire 45 days after the thaw date.

PLATING AND EXPANSION PROCEDURES

THAWING AND CULTURING ADULT AND NEONATAL DERMAL FIBROBLASTS

Note: Primary human cell viability is greatly dependent on the use of appropriate sterile tissue culture treated cultureware. No extracellular matrix coatings required.

1. Pre-warm Dermal Fibroblast Culture Medium (cat# DF-1) at 37°C, and prepare all pipets and vessels.
2. Transfer 9.5 mL of warm DF-1 to a sterile 15 mL conical centrifuge tube.
3. Remove cryovial of human dermal fibroblasts 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 1 minute, and should be stopped when there is still visible ice within the vial.
4. Rinse cryovial with 70% ethanol, and wipe cryovial with lint-free lab wiper. Open cryovial under laminar flow hood and resuspend cells in previously prepared 9.5 mL of warmed DF-1.
5. Centrifuge cell suspension at 400 x *g* for 10 minutes at 20ºC.
6. Carefully aspirate the supernatant, being careful not to disturb the cell pellet, and resuspend in a volume of DF-1 appropriate for counting the cells. Count cells using a hemocytometer or automated cell counter.
7. The plating density of human dermal fibroblasts is approximately 10,000 cells per cm^2 (i.e. 750,000 cells in a T-75 culture flask) for standard proliferation. Calculate the necessary culture surface area according to the plating density (being sure to reference the manufacturer specifications for cell culture area).
8. Place vessel in an incubator (37°C, 5% CO_2) for cell attachment. Ensure cells are evenly suspended when plating large numbers of plates or flasks. Do not agitate the plates or flasks after plating, making sure the vessel surface is level for even cell distribution.
9. Medium should be changed every three days until cells reach 85-90% confluency (in about 3-5 days).

OPTIONAL – HUMAN DERMAL FIBROBLAST SUBCULTURE

Note: We do not have data for a dermal fibroblast expansion limit.

1. Human dermal fibroblasts should be sub-confluent (85-90% confluent) upon harvest for expansion.
2. Pre-warm all reagents and medium to 37ºC, and prepare all pipets and vessels.
3. Carefully aspirate medium from cell culture vessel and wash cells using sterile Dulbecco's phosphate buffered saline without calcium or magnesium (cat# DPBS-1000) to remove all traces of serum, or until there is no foaming of the medium.
4. Remove the DPBS-1000 and release the cells from the cultureware bottom by adding Trypsin/EDTA solution (cat# TRP-100) at 25-30 µL per cm^2 cultureware surface area.
5. Incubate cells for 5-10 minutes at 37°C if using Trypsin/EDTA.
6. Examine cells under microscope, and once cells begin detaching, gently tap the side of the vessel to loosen the remaining cells.
7. Neutralize Trypsin/EDTA solution using DF-1 at 0.1-0.2 mL per cm^2 cultureware surface area. Carefully transfer the cell suspension to an appropriate centrifuge tube.

8. Centrifuge cell suspension at 400 x *g* for 10 minutes at 20ºC.

9. Carefully aspirate supernatant, being careful not to disturb the cell pellet, and resuspend the in a volume of DF-1 appropriate for counting the cells. Count cells using a hemocytometer or automated cell counter.

10. Seed cells at 10,000 cells per cm^2 in the appropriate vessel, and place vessel in an incubator (37°C, 5% CO_2) for cell attachment. Ensure cells are evenly suspended when plating large numbers of plates or flasks. Do not agitate the plates or flasks after plating, making sure the vessel surface is level for even cell distribution.

11. Replace the medium every 3 days. Once cells have reached 85-90% confluency they should be subcultured further or harvested and cryopreserved.

Note: *The cells may be confluent within 3-8 days when plated at the recommended seeding density.*

Figure 1: Neonatal dermal fibroblasts, 3 days post-plating

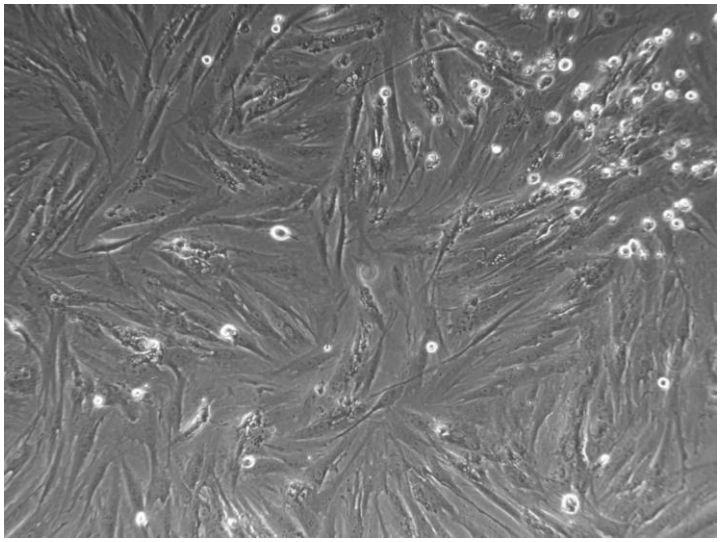
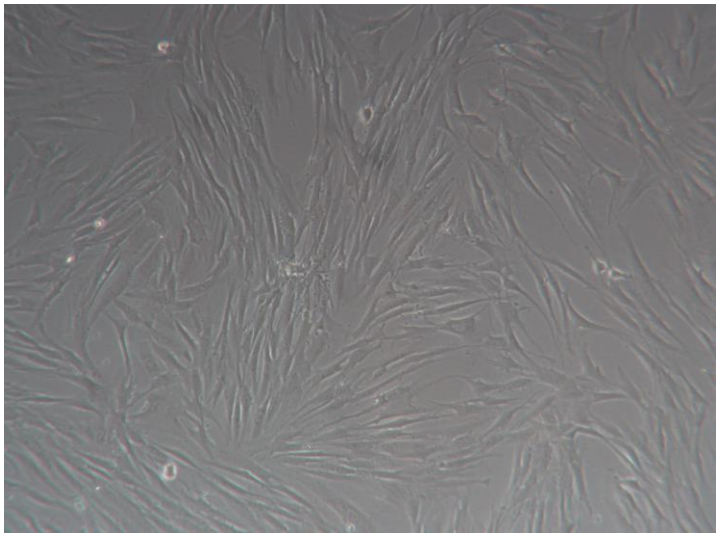


Figure 2: Adult dermal fibroblasts, 3 days post-plating



CRYOPRESERVATION PROCEDURE

1. Human dermal fibroblasts should be sub-confluent (85-90% confluent) upon harvest for cryopreservation.
2. Aspirate medium and wash cells using sterile Dulbecco's phosphate buffered saline without calcium or magnesium (cat# DPBS-1000) to remove all traces of serum, or until there is no foaming of the medium.
3. Remove the DPBS-1000 and release the cells from the cultureware bottom by adding Trypsin/EDTA solution (cat# TRP-100) at 25-30 µL per cm^2 cultureware surface area.
4. Incubate cells for 5-10 minutes at 37°C if using Trypsin/EDTA.
5. Examine cells under microscope, and once cells begin detaching, gently tap the side of the vessel to loosen the remaining cells.
6. Neutralize Trypsin/EDTA solution using Dermal Fibroblast Culture Medium (cat# DF-1) at 0.1-0.2 mL per cm^2 cultureware surface area. Carefully transfer the cell suspension to an appropriate centrifuge tube.
7. Centrifuge cell suspension at 280 x *g* for 5 minutes at 20ºC.
8. Carefully aspirate supernatant, being careful not to disturb the cell pellet, and resuspend the in a volume of DF-1 appropriate for counting the cells. Count cells using a hemocytometer or automated cell counter.
9. Centrifuge cell suspension at 280 x *g* for 5 minutes at 20ºC.
10. Carefully aspirate supernatant, being careful not to disturb the cell pellet, and suspend in cold Dermal Fibroblast Cryopreservation Medium (cat# DFM-100) at a concentration of 1 million cells per mL. Do not exceed a 6:1 ratio of cells (per million) to volume cryopreservation medium (per mL). Remember to account for the volume of the cell pellet before adding the volume of cryopreservation medium necessary for cell suspension.
11. 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.
12. 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 dermal fibroblast 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 for primary cells such as dermal fibroblasts.

Where are the cells obtained?

The adult dermal fibroblasts are isolated from the dermal layer of human skin tissue from consented competent adult donors. The neonatal dermal fibroblast cells are isolated from the dermal layer of newborn/infant human foreskin tissue with parental consented donation.

Do you test for pathogens? Which ones?

Yes. See the section titled Pathogen Testing for more information.

What donor information do I receive?

At a minimum, the donor's age, gender, and ethnicity are provided in the certificate of analysis that accompanies each lot of cells. If available, donor's BMI and medical history are also provided.

Are there recommendations for cultureware to use with the cells?

Primary human dermal fibroblast cells require use of sterile tissue culture treated cultureware. No extracellular matrix coatings are required.

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-1 (Biosafety Level 1) or higher. Always wear gloves and work behind a protective screen when handling primary human cells.