Human Visceral Preadipocyte Care Manual

Maintenance and Differentiation from Preadipocytes to Adipocytes

INSTRUCTION MANUAL ZBM0022.05

SHIPPING CONDITIONS

Human Visceral Preadipocytes, Cryopreserved

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.
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	(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:

OP-F	CRYOPRESERVED OMENTAL PREADIPOCYTES, 1 MILLION CELLS/VIAL
OPD-F	CRYOPRESERVED OMENTAL PREADIPOCYTES, DIABETIC DONOR, 1 MILLION 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.

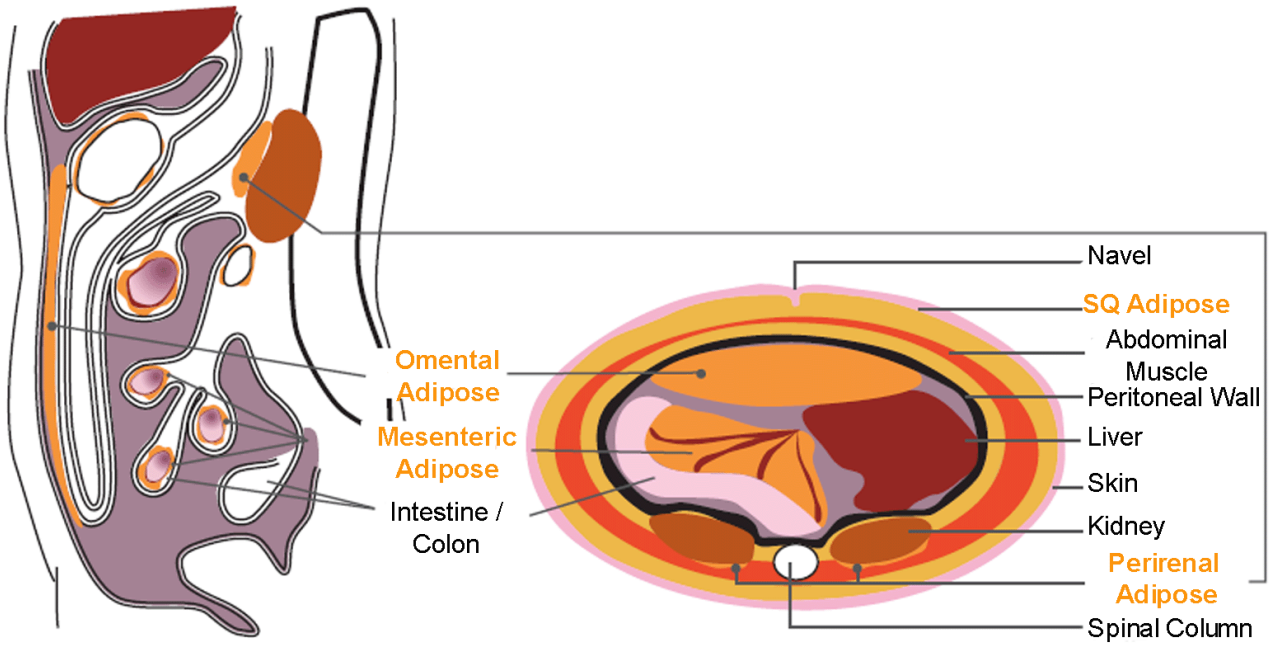
Human visceral preadipocyte viability depends greatly on the use of suitable media, reagents, and sterile plastic wear. If these parameters are not carefully observed, cell growth may be slower than expected.

INTRODUCTION

CULTURED HUMAN VISCERAL PREADIPOCYTES AND ADIPOCYTES

Visceral preadipocytes can be cultured as growing precursor cells or differentiated into adipocytes using medium supplemented with adipogenic and lipogenic hormones. This instruction manual describes procedures required to induce human preadipocytes to differentiate into mature adipocytes as well as culturing methods for human preadipocytes and adipocytes. The process of differentiating preadipocytes to adipocytes has been patent protected by Zen-Bio under US patent number 6153432.

A complication to commercial development of cultured visceral adipocytes is the varying definition of “visceral” within the scientific community. Some define omental and mesenteric fat as the only true visceral fat, whereas others include all intra-abdominal adipose tissue. Some researchers do not define it further than simply ‘not the subcutaneous layer’. The omentum (OM) is an immunologic organ composed of adipose, blood vessels and lymph nodes which overlays the abdominal organs within the peritoneal cavity. Mesenteric adipose tissue contained within the peritoneal cavity, is associated with the vasculature of the intestines and colon. Peri-renal adipose is attached to the kidneys.



QUALITY CONTROL

Contaminating endothelial cells are undetectable (CD31 NEGATIVE) by flow cytometry in visceral preadipocytes. This product has been tested and complies with ZenBio, Inc. quality specifications. Preadipocytes are plated and differentiated as outlined in this manual. The resulting cultured mature adipocytes (2 weeks post-differentiation) accumulate lipid, respond to lipolytic agents, and secrete leptin and adiponectin.

CATALOG ITEMS

- **Omental Preadipocyte Medium**
  - Cat # OM-PM (500mL)
  - Store according to label
- **Omental Adipocyte Differentiation Medium**
  - Cat # OM-DM (100mL), OM-DM-500 (500mL)
  - Store according to label
- **Omental Adipocyte Maintenance Medium**
  - Cat # OM-AM (500mL)
  - Store according to label
- **Omental Basal Medium**
  - Cat # OM-BM (500mL)
  - Store according to label
- **Cryopreserved Human Omental Preadipocytes**
  - Cat # OP-F-1 (BMI <25.0), OP-F-2 (BMI 25.0-29.9), OP-F-3 (BMI ≥30.0), OP-F-SL (Mixed Donor Lot)
  - Cryopreserved vial containing a minimum of 1 million viable visceral (omental) preadipocytes per vial **(store in vapor phase liquid nitrogen IMMEDIATELY upon receipt)** *any other storage negates the warranty*
- **Cryopreserved Human Omental Preadipocytes from Diabetic Donor**
  - Cat # OPD-F (BMI Varies)
  - Cryopreserved vial containing a minimum of 1 million viable visceral (omental) preadipocytes per vial **(store in vapor phase liquid nitrogen IMMEDIATELY upon receipt)** *any other storage negates the warranty*

MEDIA COMPOSTIONS

Omental Preadipocyte Medium Cat # OM-PM	**Omental Adipocyte Differentiation Medium** Cat # OM-DM	**Omental Adipocyte Maintenance Medium** Cat # OM-AM
DMEM / Ham's F-12 (1:1, v/v)	DMEM / Ham's F-12 (1:1, v/v)	DMEM / Ham's F-12 (1:1, v/v)
HEPES pH 7.4	HEPES pH 7.4	HEPES pH 7.4
Fetal Bovine Serum (FBS; USA Origin)	Fetal Bovine Serum (FBS; USA Origin)	Fetal Bovine Serum (FBS; USA Origin)
Penicillin	Biotin	Biotin
Streptomycin	D-Pantothenic Acid	D-Pantothenic Acid
Amphotericin B	Human Insulin, recombinant	Human Insulin, recombinant
	Dexamethasone	Dexamethasone
	3-Isobutyl-1-methylxanthine (IBMX)	Penicillin
	PPAR γ agonist	Streptomycin
	Penicillin	Amphotericin B
	Streptomycin	
	Amphotericin B	

Omental Basal Medium Cat # OM-BM
DMEM / Ham's F-12 (1:1, v/v)
HEPES pH 7.4
Biotin
D-Pantothenic Acid

All media contain 3.15g/L (17.5 mmol/L) D-glucose.
All media are also available as phenol red free and/or without serum. 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, it is stable 6 months from the ship date. Add fresh antibiotics when you are ready to use (exept for Omental Basal Medium, which should remain antibiotic/antimycotic-free). The media will expire 30 days after the thaw date.

THAWING CRYOPRESERVED CELLS

CRYOPRESERVED VISCERAL PREADIPOCYTES

Note: Primary human cell viability is greatly dependent on the use of appropriate sterile tissue culture treated cultureware. No extracellular matrix coatings required. **This product is for single thaw and use only.**

1. Pre-warm Omental Preadipocyte Media (cat# OM-PM) at 37°C, and prepare all pipets and vessels.
2. Transfer 9.5 mL of warm OM-PM to a sterile 15 mL conical centrifuge tube.
3. Remove cryovial of visceral preadipocytes 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 OM-PM.
5. Centrifuge cell suspension at 282 x *g* (1200 rpm) for 5 minutes at 20ºC.
6. Carefully aspirate the supernatant, being careful not to disturb the cell pellet, and resuspend in a volume of OM-PM appropriate for counting the cells. Count cells using a hemocytometer or automated cell counter.
7. The plating density of visceral preadipocytes is 40,625 cells per cm^2 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). Refer to Table 1 below for the recommended media volumes according to plating format.

Table 1: Media Volumes per Plate Format

FORMAT	volume per well	total volume per format*
96 well plate	150 µL	14.4 mL
48 well plate	500 µL	24.0 mL
24 well plate	1.0 mL	24.0 mL
12 well plate	2.0 mL	24.0 mL
6 well plate	3.0 mL	18.0 mL
10 cm dish	15.0 mL	15.0 mL
T-75 flask	20.0 mL	20.0 mL
T25 flask	7.0 mL	7.0 mL

Note: We recommend preparing slightly larger volumes to allow for loss due to foam and pipet error.

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. Twenty-four (24) hours after plating, check the plates for confluence. If they are not completely confluent, leave for an additional 24 hours maximum before inducing differentiation. If the cells are not confluent after 48 hours, DO NOT INDUCE DIFFERENTIATION (differentiation will be poor). Contact Zen-Bio immediately.
10. To differentiate the cells, proceed to the next section.

DIFFERENTIATION PROCEDURE

VISCERAL PREADIPOCYTES TO ADIPOCYTES

1. To start the process of adipogenesis (differentiation from preadipocytes to adipocytes), aspirate the entire volume of Omental Preadipocyte Medium (cat# OM-PM) from all wells, being careful not to disturb the cells.

   Caution: **Do not dry the wells. Add new medium gently. If using an automatic feeder, set the slowest flow rate possible.**

2. Add the appropriate volume of Omental Adipocyte Differentiation Medium (cat# OM-DM) to the wells (refer to Table 2, columns titled Change OM-PM to OM-DM for appropriate volumes for your plating format).

3. Incubate plate for 7 days at 37°C and 5% CO_2.

4. After 7 days, cells should be fed by removing a portion of the medium and replacing with fresh Omental Adipocyte Maintenance Medium (cat# OM-AM). Refer to Table 2 (columns titled Change OM-DM to OM-AM) for the volumes that should be removed and added.

5. Two (2) weeks after the initiation of differentiation, cells should appear rounded with large lipid droplets apparent in the cytoplasm. Cells are now considered mature adipocytes and are suitable for most assays.

Table 2: Feeding Volumes

Format	Plating	Change OM-PM to OM-DM		Change OM-DM to OM-AM		Change OM-AM to OM-AM	
	IN	OUT	IN	OUT	IN	OUT	IN
96 well plate	150 µL/well	150 µL/well	150 µL/well	90 µL/well	120 µL/well	90 µL/well	90 µL/well
48 well plate	500 µL/well	500 µL/well	500 µL/well	300 µL/well	400 µL/well	300 µL/well	300 µL/well
24 well plate	1.0 mL/well	1.0 mL/well	1.0 mL/well	0.6 mL/well	0.8 mL/well	0.6 mL/well	0.6 mL/well
12 well plate	2.0 mL/well	2.0 mL/well	2.0 mL/well	1.2 mL/well	1.6 mL/well	1.2 mL/well	1.2 mL/well
6 well plate	3.0 mL/well	3.0 mL/well	3.0 mL/well	1.8 mL/well	2.4 mL/well	1.8 mL/well	1.8 mL/well
T-75 flask	20 mL/flask	20 mL/flask	20 mL/flask	12 mL/flask	16 mL/flask	12 mL/flask	12 mL/flask
T-25 flask	7.0 mL/flask	7.0 mL/flask	7.0 mL/flask	4.2 mL/flask	5.6 mL/flask	4.2 mL/flask	4.2mL/flask

TROUBLESHOOTING GUIDE

Observation	Possible Cause(s)	Suggestion(s)
Preadipocytes do not differentiate.	1. Cells have been passaged too many times.	1. Visceral cells will arrive at passage 2, 3, or 4. We do not recommend expanding the visceral cells.
	2. Differentiation conditions not optimal.	2. Use our Omental Adipocyte Differentiation Medium (cat# OM-DM). Make sure that wells are completely confluent BEFORE initiating differentiation.
	3. Cells were plated at a low density.	3. Use the cell density recommended in our manual.
	4. Differences in cultureware brand surface area may affect plating density if unknown.	4. Verify the cell culture surface area for the cultureware brand you are using.
Edge effects.	• Medium in outside wells evaporated.	• Ensure a saturated humidity in the incubator. • Make sure plates are stacked no more than 3 plates high.
Adipocytes appear uneven in each well.	1. Medium was completely removed during feeding.	1. Make sure to follow instructions listed in Table 2: Feeding Volumes.
	2. Fresh medium was added too quickly.	2. Add media slowly to each well. Position the pipet tips halfway down, pressing on the side of the wells, and slowly release the medium.
	3. Cells placed on uneven surface in the incubator.	3. Place cultureware on a level surface in the incubator to ensure cells attach evenly.

FREQUENTLY ASKED QUESTIONS

What does the -1 or -SL mean after the catalog number?

The different designations for our catalog numbers based on the body mass index (BMI) of the donor(s). All catalog numbers ending in -1,-2 or -3 are single donor lots. All catalog numbers ending in -SL are mixed donor lots (also referred to as superlots) comprised of 3 or more different donors.

Catalog Suffix	Definition
-1	BMI < 25.00
-2	BMI 25.0-29.99
-3	BMI > 30.0
-SL	Mixed donor lot; BMI varies

Are the cells from one donor?

We have lots that are single donor and multiple donors. The cells are from one donor unless otherwise noted. We can provide lot numbers containing cells mixed from 5 to 8 donors to get average responses. Please inquire about availability of single donor and mixed donor lots at time order is placed.

What donor information do I receive?

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

How long do I have to wait before receiving the cells?

We do not ship to domestic locations on Fridays. In general, cryopreserved cells can be shipped the second day after the purchase order is confirmed.

From where are the cells sourced?

The preadipocytes are isolated from human visceral adipose tissue obtained from consented adult donors. All cells are obtained and processed in the United States.

Do you test for pathogens? Which ones?

Yes. Read the section titled Pathogen Testing (page 11) for more information.

Should antibiotics be included in the medium?

Yes. Antibiotics and anti-fungal agents are always recommended for primary cells such as visceral preadipocytes. All Zen-Bio media listed contain antibiotics and anti-fungal agents except Omental Basal Medium (cat# OM-BM).

Can I expand the cells?

Mature adipocytes cannot be passed since they float after trypsinization. We do not recommend expanding the visceral preadipocytes. Cells are shipped at Passage 2, 3 or 4; please see vial label to determine passage number of the lot of cells you have received. Please contact Zen-Bio if your experiment requires a large amount of visceral cells.

When do the cells differentiate?

Oil droplets should appear within 7-8 days after differentiation is induced. They look extremely small initially. Lipid accumulation continues throughout the first two weeks. The oil droplets gradually fuse to several big locules. Please note that omental preadipocytes and adipocytes are distinct from subcutaneous preadipocytes and adipocytes. The level of lipid accumulation and morphology in culture may appear different from that which you have normally observed in the subcutaneous human adipocytes.

Can I differentiate the cells myself?

Yes. You can order preadipocytes and pre-made culture media for adipocyte differentiation. Instructions for differentiating the cells are found in this manual.

What if I want to test my own compounds in differentiation?

Please inquire about special media requests.

How long do the cells last in culture?

Adipocytes retain similar morphology and express adipocyte specific genes for at least 3-4 weeks.

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. Do not differentiate preadipocytes in serum-free medium.

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 for mycoplasma contamination. Mycoplasma is not detected in our labs. 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.

REFERENCES

Lists of articles using Zen-Bio, Inc cultured human cultured preadipocytes and adipocytes may be found at our website (http://www.zen-bio.com).