Feline Adipocyte Care Manual

Maintenance and Differentiation from Preadipocytes to Adipocytes

INSTRUCTION MANUAL ZBM0056.00

SHIPPING CONDITIONS

Feline Adipocyte/Preadipocyte 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:	4°C	6 months:	-20°C
Cells:	Frozen:	liquid nitrogen	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.

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

ORDERING INFORMATION AND TECHNICAL SERVICES

ZenBio, 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@zenbio.com
World Wide Web	http://www.zenbio.com

CONTENTS PAGE

INTRODUCTION

Cultured feline adipocytes

Feline 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 feline preadipocytes to differentiate into mature adipocytes as well as culturing methods for feline preadipocytes and adipocytes. The process of differentiating preadipocytes to adipocytes has been patent protected by Zen-Bio under US patent number 6153432.

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 feline origin, due to their potential biohazardous nature. **Always wear gloves and work behind a protective screen when handling primary animal cells.** All media, supplements, and tissue cultureware used in this protocol should be sterile.

Feline preadipocyte 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

***Note**: Zen-Bio recommends that the Feline Preadipocytes and Adipocytes be processed immediately upon receipt.*

- **Cryopreserved feline preadipocytes**
  - catalog # FP-F
  - Frozen vial containing 2 x10^6 viable feline preadipocytes (store in liquid nitrogen upon receipt)
  - 50 ml Feline Preadipocyte Medium (cat# PM-1-F1)

FELINE MEDIA COMPOSTIONS

Feline Adipocyte Medium

(cat # AM-1-F1)

DMEM / Ham's F-12 medium (1:1, v/v)
HEPES pH 7.4
Fetal bovine serum
Biotin
Pantothenate
Human insulin
Dexamethasone
Penicillin
Streptomycin
Amphotericin B

Feline Preadipocyte Medium

(cat # PM-1-F1)

DMEM/Ham's F-12 medium (1:1, v/v)
HEPES pH 7.4
Fetal bovine serum
Penicillin
Streptomycin
Amphotericin B

Feline Differentiation Medium

(cat # OM-1-F1)

Feline Adipocyte medium (AM-1-F1)
Isobutylmethylxanthine (IBMX)
PPARγ agonist

NOTE: All media contain 3.15g/L D-glucose.
All media are also available as phenol red free and/or without serum added.
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 for 6 months. Add fresh antibiotics when you are ready to use.**

PLATING PROCEDURE

Cryopreserved Feline Preadipocytes (Catalog # FP-F)

Please note: Primary cells can be very sensitive to brands of cultureware. Zen-Bio does not currently recommend the use of Falcon brand plates or flasks. Our scientists are using Nunc, Costar/Corning, or Greiner bio-one CellStar tissue culture treated plates and flasks. Please contact us if you have any questions.

1. Remove cells from liquid nitrogen and place immediately into a 37°C water bath and agitate while in bath. 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 thawing, transfer the cells to a sterile conical bottom centrifuge tube containing 10 ml of Feline Preadipocyte Medium (cat # PM-1-F1).
3. Centrifuge: 1,200 rpm (282 X g) / 20°C / 5 minutes. Aspirate the supernatant. TAKE CARE TO NOT ASPIRATE ANY OF THE CELL PELLET.
4. The cell vial contains a minimum of 2.0 or x 10^6 viable cells (see vial label); however, we recommend performing a cell count to determine a more exact number of cells. Resuspend the cell pellet in 2 ml Feline Preadipocyte Medium and dilute an aliquot in 0.4% trypan blue solution. We suggest withdrawing an aliquot of 50 µl of cells and mixing with 100 µl of the trypan blue solution, resulting in a dilution factor of 3. Count live (unstained) cells on a hemacytometer.
5. Plate approximately 40,625 cells / cm^2 using the media volumes from the table below. Refer to the manufacturer's specifications for the specific cultureware brand you are using (see Table 2).

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 ml	24.0 ml
12 well plate	2 ml	24.0 ml
6 well plate	3 ml	18.0 ml
10 cm dish	15 ml	15.0 ml
T-75 flask	20 ml	20.0 ml
T25 flask	7 ml	7.0 ml

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

6. Plate cells in desired format and place in a humidified 37°C incubator with 5% CO_2. Do not agitate the plate, as cells will not plate evenly.
7. Twenty-four hours after plating, check the plates for confluence. If they are not completely confluent, leave for and 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.
8. To differentiate the cells please continue the protocol on page 6.

DIFFERENTIATION OF PREADIPOCYTES INTO ADIPOCYTES

1. To start the process, aspirate the entire volume of Feline Preadipocyte Medium from all wells.
2. Add the appropriate volume of Feline Differentiation Medium (catalog # OM-1-F1) to the wells (see Table 1. Feeding Volumes). Incubate plate for 7 days at 37°C and 5% CO_2.
3. After 7 days, cells should be fed by removing some of the medium and replacing with fresh Feline Adipocyte Medium (catalog # AM-1-F1; See Table 1. Feeding Volumes). **Caution**: **Do not dry the wells. Add new medium gently. If using an automatic feeder, set the slowest flow rate possible.**
4. Two (2) weeks after the initiation of differentiation, cells should appear rounded with lipid droplets apparent in the cytoplasm. Cells are now considered mature adipocytes and are suitable for most assays.

Table 1. Feeding Volumes

Format	**Plating**	Change PM-1-F1 to **OM-1-F1**		**Change OM-1-F1 to AM-1-F1**		**Change AM-1-F1 to AM-1-F1**	
	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 ml/flask	7 ml/flask	7 ml/flask	4.2 ml/flask	5.6 ml/flask	4.2 ml/flask	4.2ml/flask

Table 2. Summary Culture area, Zen-Bio Recommended Cultureware

Multi-well Plate Format	**6**	**12**	**24**	**48**	**96**
Greiner Bio-One Cellstar, cm²/well	**9.62**	**3.87**	**1.94**	**1.02**	**0.35**
Cat#	657160	665180	662160	677180	655180
Costar/Corning, cm²/well	**9.50**	**3.80**	**1.90**	**0.95**	**0.32**
Cat#	3516	3513	3526	3548	3595
Nunc, cm²/well	**9.60**	**3.50**	**1.90**	**1.10**	**0.33**
Cat#	152795	150628	143982	150687	167008

TROUBLESHOOTING GUIDE

Observation	Possible causes	Suggestions
Preadipocytes do not differentiate	1. Cells have been passaged too many times	1. Feline cells will arrive at passage 2. We do not recommend expanding the feline cells.
	2. Differentiation conditions not optimal	2. Use our defined Differentiation Medium (OM-1-F1). Make sure that wells are confluent BEFORE initiating differentiation.
	3. Cells were plated at a low density	3. Use the cell density recommended in our manual
	4. Cultureware used not optimal for feline primary adipocytes	4. Zen-Bio does not recommend the use of Falcon cultureware for all cell culture applications
		5. Verify the surface area for the cultureware brand you are using.
Edge effects	1. Medium in outside wells evaporated	1. Ensure a saturated humidity in the incubator and feed the cells no less than every 3 days. Make sure multiple 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 1. 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 are on a level surface in the incubator to ensure cells attach evenly.

FREQUENTLY ASKED QUESTIONS

- **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 feleine preadipocytes and adipocytes are distinct from subcutaneous human 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 pass the cells?**

Adipocytes cannot be passed since they float after trypsinization. We do not recommend expanding the feline preadipocytes. Cells are shipped at Passage 2; please see vial or plate label to determine passage number of the lot of cells you have received. Please contact Zen-Bio for your studies in which large numbers of feline cells are required.

- **How long do the cells last in culture?**

Adipocytes retain similar morphology and express adipocyte specific genes for at least 3-4 weeks. [NOTE: Cultured adipocytes are usually shipped at 2 weeks old.]

- **Should antibiotics be included in the medium?**

Yes. Antibiotics and anti-fungal agents are always recommended since the cells are primary cells. All Zen-Bio media contain antibiotics and anti-fungal agents.

- **Where are the cells from?**

The preadipocytes are isolated from feline subcutaneous adipose tissue.

- **How are the cells shipped?**

Cells are cryopreserved and shipped to customers via Federal Express overnight delivery.

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

We do not ship to domestic locations on Fridays. In general, preadipocytes (cryopreserved) can be shipped the second day after the purchase order is confirmed. Please inquire as to the availability of the adipocytes when ordering.

- **Can I differentiate the cells myself?**

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

zenbio, inc.
e-mail: information@zenbio.com • http:// www.zenbio.com
p. o. box 13888 • 3200 chapel hill-nelson blvd. suite 104 • research triangle park • north carolina 27709
phone: (919) 547-0692 • fax: (919) 547-0693
Toll free: 1-866-ADIPOSE (234-7673)