3T3-L1 Cell Care Manual

Maintenance and Differentiation of 3T3-L1 Preadipocytes to Adipocytes

UK & Rest of World
184 Milton Park, Abingdon
OX14 4SE, Oxon, UK
Tel: +44 (0) 1235 828 200
Fax: +44 (0) 1235 820 482

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
Tel: +41 (0) 91 604 55 22
Fax: +41 (0) 91 605 17 85

Deutschland
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
Tel: +49 (0) 69 779099
Fax: +49 (0) 69 13376880

North America
23591 El Toro Rd, Suite #180
Lake Forest, CA 92630
Tel: + 1 800 987 0985
Fax: + 1 949 265 7703

amsbio
info@amsbio.com
www.amsbio.com
AMS Biotechnology

CONTENTS **PAGE #**

INTRODUCTION

3T3-L1 adipocytes have been fundamental in metabolic disease research for 30 years. Originally derived from Swiss mouse embryo tissue by Dr. Howard Green of Harvard Medical School, the 3T3-L1 system has been pivotal in advancing the understanding of basic cellular mechanisms associated with diabetes, obesity and related disorders.

MATERIALS PROVIDED FOR EACH CATALOG ITEM

- **3T3-L1 Preadipocytes**
  (Cat# SP-2096; SP-2048; SP-2024; SP-2012)
  - **Subconfluent cells**

- **Cryopreserved 3T3-L1 preadipocytes** (catalog # SP-L1-F)
  - Frozen vial containing at least 0.5 x10^6 preadipocytes (store in liquid nitrogen upon receipt)

  50 ml 3T3-L1 Preadipocyte Medium (cat# PM-1-L1)

STORAGE CONDITIONS

Media: Short Term 4°C 6 months -20°C

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.

3T3-L1 MEDIA COMPOSTIONS

3T3-L1 Adipocyte Medium (cat # AM-1-L1)	3T3-L1 Preadipocyte Medium (cat # PM-1-L1)
DMEM / Ham's F-12 medium (1:1, v/v)	DMEM, high glucose
HEPES pH 7.4	HEPES pH 7.4
Fetal Bovine Serum (FBS)	Bovine Calf Serum (BCS)
Biotin	Penicillin
Pantothenate	Streptomycin
Human insulin	Amphotericin B
Dexamethasone	
Penicillin	
Streptomycin	
Amphotericin B	

3T3-L1 Differentiation Medium (cat # DM-2-L1)	3T3-L1 Basal Medium (cat # BM-1-L1)
DMEM / Ham's F-12 medium (1:1, v/v)	DMEM/Ham's F-12 medium (1:1, v/v)
HEPES pH 7.4	HEPES pH 7.4
Fetal Bovine Serum (FBS)	Biotin
Biotin	Pantothenate
Pantothenate	
Human insulin	
Dexamethasone	
Penicillin	
Streptomycin	
Amphotericin B	
Isobutylmethylxanthine	
PPARγ agonist	

NOTE:

All media except cat# PM-1-L1 contain 3.15g/L D-glucose.

PM-1-L1 contains 4.5g/L D-glucose.

All media are also available without serum and/or phenol red free.

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.**

MAINTENANCE OF PLATED 3T3-L1 PREADIPOCYTES

Your 3T3-L1 preadipocytes have arrived in our patented CellPorter™ packaging system. Upon receiving the plates, please follow the instructions carefully to ensure your safety and the optimal performance of these cells.

1. Check the seal for each plate. Discard any plate where the vacuum seal has been compromised during shipment. ALWAYS WEAR GLOVES AND USE PROTECTIVE MEASURES WHEN HANDLING CULTURED CELLS.
2. Place the package into a sterile environment. THIS IS VERY IMPORTANT SINCE BREAKING THE VACUUM SEAL MAY POTENTIALLY INTRODUCE CONTAMINATION INTO THE PLATE. Use scissors to snip open the bag at any end. The vacuum seal should be released at this time. You may notice some bubbling of the medium in the plate at this time. This is normal and will not affect cell performance.
3. In a sterile environment, remove the plate from the bag, taking care to not disturb the cover top from the plate. Open the lid and remove the white liner using sterile forceps or a hemostat and discard. Carefully remove the clear adhesive seal by grabbing the edge with sterile forceps or hemostat and lifting the film slowly towards the other end. Discard adhesive film in appropriate biohazard waste container. Replace lid on plate.
4. The excess medium added to each well for shipping should be removed before incubation in a humidified atmosphere CO_2 incubator. Depending upon the plate configuration, please use the chart below to determine medium volume to remove from each well.

Cultureware	Total shipping volume per well	Removal volume per well
96 well plates	300 µl/well	150 µl
48 well plates	1.3 ml/well	0.8 ml
24 well plates	3.0 ml/well	2.0 ml
12 well plates	5.8 ml/well	3.8 ml

5. Keep the plates at 37°C with 5% CO_2 in a humidified incubator until ready for use. The cells should be fed with 3T3-L1 Preadipocyte Medium (PM-1-L1) every 2-3 days until confluent. See page 6 for differentiation protocol.

DIFFERENTIATION OF 3T3-L1 PREADIPOCYTES INTO ADIPOCYTES

Cryopreserved 3T3-L1 Preadipocytes (Catalog # SP-L1-F)

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 10 ml of 3T3-L1 Preadipocyte Medium (PM-1-L1).

3. Centrifuge at 280 x g, 20°C, 5 minutes. Aspirate the medium and resuspend cells in a volume of PM-1-L1 appropriate for counting the cells. Count using a hemacytometer.

4. Place approximately 5,000 cells/cm^2 in tissue culture treated cultureware using 3T3-L1 Preadipocyte Medium (PM-1-L1).

5. Maintain cells until they are 100% confluent (in about 6-7 days) in a humidified incubator, 37°C, with 5-10% CO_2. Cells will need to be fed every other day with PM-1-L1 during this time. See Table 1 for feeding volumes.

6. Once the cells are confluent, incubate an additional 48 hours before initiating differentiation.

7. Two days after the cells have been confluent, remove the 3T3-L1 Preadipocyte Medium (cat# PM-1-L1) and replace with an appropriate volume 3T3-L1 Differentiation Medium (cat# DM-2-L1; see table 1 below for recommended volumes). Incubate for 3 days.

8. Remove the 3T3-L1 Differentiation Medium and replace with 3T3-L1 Adipocyte Maintenance Medium. Incubate for 2-3 days.

9. Feed cells every 2-3 days using 3T3-L1 Adipocyte Maintenance Medium until ready for assay. 3T3-L1 adipocytes are suitable for most assays 7-14 days post differentiation (see Figure 1 and Figure 2).

Table 1. Feeding Volumes

Format	Change PM-1-L1 to PM-1-L1		Change PM-1-L1 to DM-2-L1		Change DM-2-L1 to AM-1-L1		Change AM-1-L1 to AM-1-L1	
	OUT	IN	OUT	IN	OUT	IN	OUT	IN
96 well plate	90µl/well	90µl/well	150µl/well	150 µl / well	90 µl /well	120µl /well	90 µl /well	120µl /well
48 well plate	300 µl /well	300 µl /well	500µl /well	500 µl /well	300 µl /well	400 µl /well	300 µl /well	400 µl /well
24 well plate	0.6 ml/well	0.6 ml/well	1.0 ml/well	1.0 ml/well	0.6 ml/well	0.8 ml/well	0.6 ml/well	0.8 ml/well
12 well plate	1.2 ml/well	1.2 ml/well	2.0 ml/well	2.0 ml/well	1.2 ml/well	1.6 ml/well	1.2 ml/well	1.6 ml/well
6 well plate	1.8 ml/well	1.8 ml/well	3.0 ml/well	3.0 ml/well	1.8 ml/well	2.4 ml/well	1.8 ml/well	2.4 ml/well
T-75 flask	12 ml/flask	12 ml/flask	20 ml/flask	20 ml/flask	12 ml/flask	16 ml/flask	12 ml/flask	16 ml/flask
T-25 flask	4.2 ml/flask	4.2 ml/flask	7 ml/flask	7 ml/flask	4.2 ml/flask	5.6 ml/flask	4.2 ml/flask	5.6 ml/flask

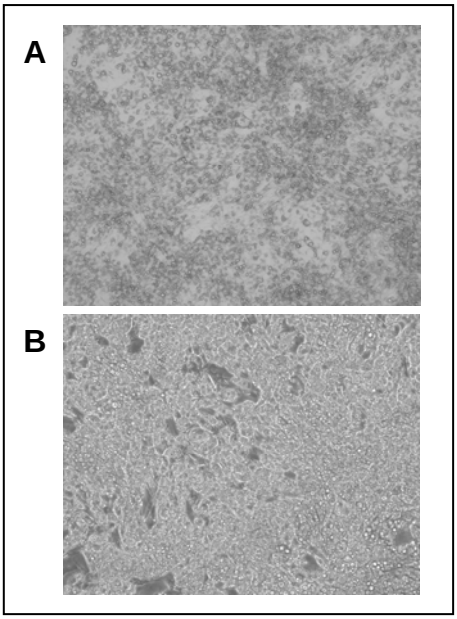
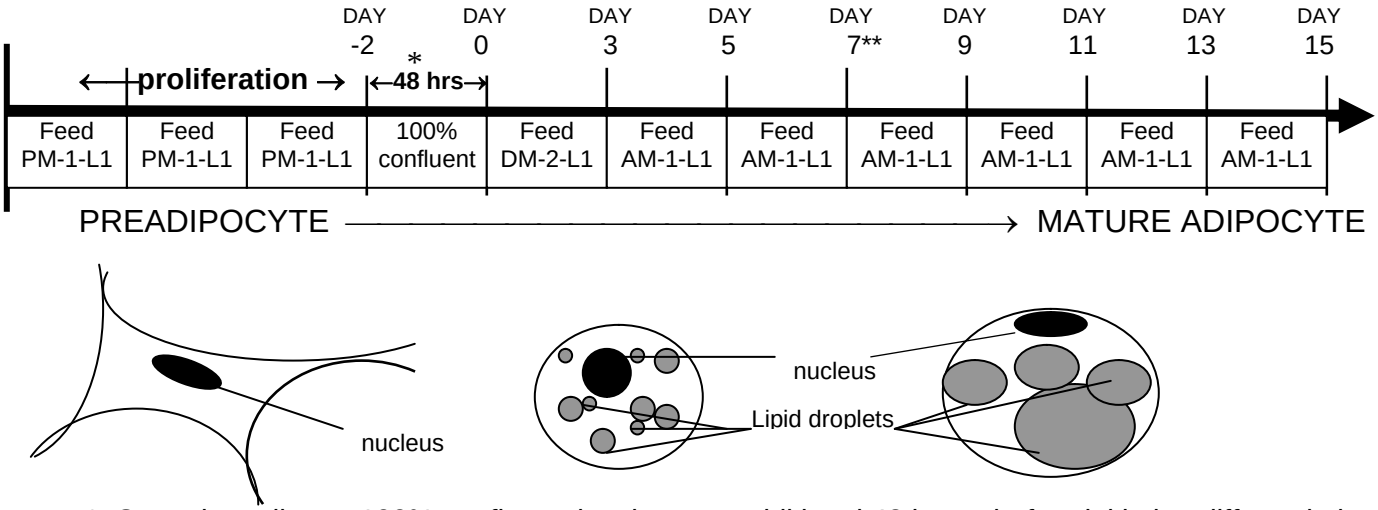


Figure 1. Lipid accumulation in 3T3- L1 cells cultured in Zen Bio media.

3T3-L1 preadipocytes were seeded in 24 well plates and induced to differentiate 2 days post confluent using Zen Bio's DM-2-L1 for 3 days. Cells were then fed Zen Bio's AM-1-L1, with fresh media being added every other day. Phase contrast images were taken on day 7 (Panel A) and day 14 (Panel B) of differentiation using an Olympus IX60 microscope equipped with a STOP digital camera (20X magnification)

Figure 2. 3T3-L1 Growth and Differentiation Feeding Schedule



* Once the cells are 100% confluent, incubate an additional 48 hours before initiating differentiation. The cells require this time to initiate growth arrest.

EXPANSION OF 3T3-L1 PREADIPOCYTES

Cryopreserved 3T3-L1 Preadipocytes (Catalog # SP-L1-F)

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 10 ml of 3T3-L1 Preadipocyte Medium (PM-1-L1).
3. Centrifuge at 280 x g, 20°C, 5 minutes. Aspirate the medium and resuspend cells in a volume of PM-1 appropriate for counting the cells. Count using a hemacytometer.
4. Place approximately 3,000- 5,000 cells/cm^2 in tissue culture treated cultureware using 3T3-L1 Preadipocyte Medium (PM-1-L1).
5. Incubate cells until they are 80-85% confluent (in about 5-6 days). Do not let the cells become 100% confluent. Cells will need to be fed every other day with PM-1-L1.
6. Aspirate medium and wash preadipocytes 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 bottom of the cultureware vessel by adding 30µl/cm^2 of 0.25% trypsin/ 2.21mM EDTA solution (cat# TRP-100). Allow cells to trypsinize for 5 minutes at 37°C. Tap the flask gently to loosen the cells.
7. Neutralize the trypsin using at least 100µl/cm^2 3T3-L1 Preadipocyte Medium (cat# PM-1-L1). Check the vessel under a microscope to ensure all cells are free of the flask bottom.
8. Count the cells and plate in desired format (see page 6). Ensure cells are evenly suspended when plating large numbers of plates or flasks. Place in a humidified incubator at 37°C and 5-10% CO_2, making sure the surface is level for even cell distribution.
9. Follow the differentiation protocol as outlined on pages 6- 7.
10. We DO NOT recommend expanding the preadipocytes that are older than Passage 13. Cells will arrive at Passage 8.

TROUBLESHOOTING GUIDE

Observation	Possible causes	Suggestions
Preadipocytes do not differentiate well	Cells have been passaged too many times	- Use cells of a lower passage number. The 3T3-L1 cell line is NOT immortalized and is suitable for only 12-13 passages. - Ensure cells are 100% confluent for 48 hours prior to initiating differentiation - Do not use fetal bovine serum during the proliferation process. It will affect later differentiation potential. We recommend using Zen-Bio's 3T3-L1 Preadipocyte Medium (cat # PM-1-L1)
Preadipocytes do not grow	Cells have been passaged too many times	-Use cells of a lower passage number. The 3T3-L1 cell line is NOT immortalized and is suitable only until passage 13. Cells will arrive at Passage 8. -3T3-L1 cells grow faster in an incubator set to 10% CO_2.
Edge effects	Medium in outside wells evaporated	Ensure a saturated humidity in the incubator and feed the cells no less than every 3 days.

FREQUENTLY ASKED QUESTIONS

QUESTION	ANSWER
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.
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 except Basal Medium (BM-1-L1).
When do the cells differentiate?	Lipid droplets should appear within 4-7 days after differentiation is induced. They look extremely small initially. Lipid accumulation continues throughout the first two weeks. The lipid droplets gradually fuse to several big locules. [See Figures 1 & 2]
Do you provide ready to use plated 3T3-L1 adipocytes?	No. At this time they are too sensitive to the stresses of shipping during differentiation. Only cryopreserved and sub-confluent plated preadipocytes are provided as plated cells.
What plated formats do you provide for 3T3-L1 cells?	We provide 3T3-L1 preadipocytes in the following formats: 96-well, 48-well, 24-well, 8-chamber slides