3T3-L1 ADIPOCYTE CARE MANUAL

INSTRUCTION MANUAL (ZBM-9)

SHIPPING CONDITIONS

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

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.

ORDERING INFORMATION AND TECHNICAL SERVICES

Zen-Bio, 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@zen-bio.com**
World Wide Web	**http://www.zen-bio.com**

3T3-L1 ADIPOCYTE CARE MANUAL

Maintenance and Differentiation of 3T3-L1 Preadipocytes to Adipocytes

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3T3-L1 MEDIA COMPOSITIONS

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 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. Please be aware that these cells are of human origin. Please treat them as potentially infectious since we cannot test for all pathogens. 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 media 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 media 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 media volumes 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 difrerentiation protocol.

MAINTENANCE OF 3T3-L1 ADIPOCYTES

Your 3T3-L1 adipocytes 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. Call Zen-Bio if there is any problem with the 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 media 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 media added to each well for shipping should be removed for incubation in a CO_2 incubator. When changing medium, do not remove all the liquid as the cells will detach and float. Depending upon the plate configuration, please use the chart below to determine media volumes 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
6 well plates	8.8 ml/well	5.8 ml
75cm^2 flask	260ml/flask	240 ml
25cm^2 flask	72 ml/flask	65 ml

5) Keep the plates at 37^{o}C with 5% CO_2 in a humidified incubator until ready for use.

6) When feeding, we recommend you remove and replace approximately half of the volume of each well.

DIFFERENTIATION OF 3T3-L1 PREADIPOCYTES INTO ADIPOCYTES

1. Preadipocytes are plated sub-confluent in 3T3-L1 Preadipocyte Medium (cat# PM-1-L1) and shipped the same day via overnight delivery.
2. Incubate cells until they are 100% confluent (in about 4-5 days). Cells will need to be fed every other day with PM-1-L1 during this time.
3. Once the cells are confluent, incubate an additional 48 hours before initiating differentiation.
4. Two days after the cells have been confluent, remove the Preadipocyte Medium (cat# PM-1-L1) and replace with an appropriate volume 3T3-L1 Differentiation Medium (cat# DM-2-L1; see table 1 above for recommended volumes). Incubate for 3 days.
5. Remove the 3T3-L1 Differentiation Medium and replace with 3T3-L1 Adipocyte Maintenance Medium.
6. Feed cells every 2 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 below).

Table 1. Feeding Volumes

Format	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
96 well plate	150µl/well	150 µl / well	90 µl /well	120µl /well	90 µl /well	90 µl /well
48 well plate	500µl /well	500 µl /well	300 µl /well	400 µl /well	300µl /well	300 µl /well
24 well plate	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	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	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	12 ml/flask	16 ml/flask	12 ml/flask	12 ml/flask
T-25 flask	7 ml/flask	7 ml/flask	4.2 ml/flask	5.6 ml/flask	4.2 ml/flask	4.2 ml/flask

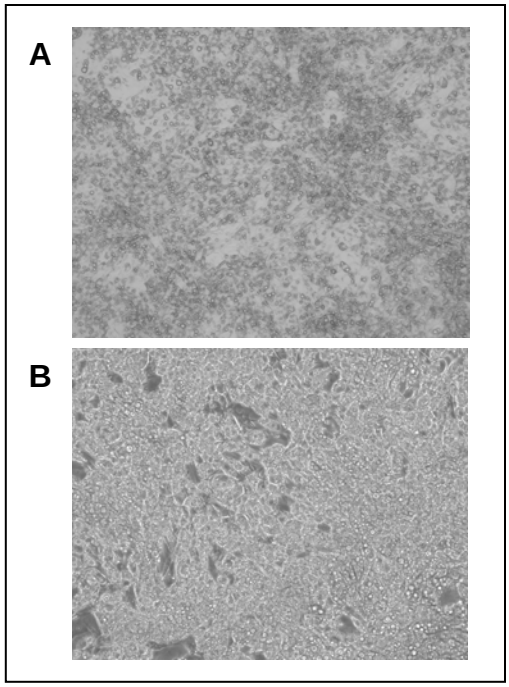

Fig. 1. Lipid accumulation in 3T3-L1 cells cultured in Zen Bio media.

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)

TROUBLESHOOTING GUIDE

Observation	Possible causes	Suggestions
Preadipocytes do not differentiate	Cells have been passaged too many times	Use cells of a lower passage number
Preadipocytes do not grow	Cells have been passaged too many times	Use cells of a lower passage number
Edge effects	Medium in outside wells evaporated	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	Cells placed on uneven surface in the incubator	Place cultureware are on a level surface in the incubator to ensure cells attach evenly during initial plating.

FREQUENTLY ASKED QUESTIONS

QUESTION	ANSWER
Does Zen-Bio provide frozen 3T3-L1 cells?	No. We provide ready to use plated 3T3-L1 cells and the media for the proliferation, plating and differentiation of 3T3-L1 cells.
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?	Oil 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 oil droplets gradually fuse to several big locules. [See Figure 1]

zen-bio, inc.
e-mail: information@zen-bio.com • http:// www.zen-bio.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)