3T3-L1 Adipocyte Kit

Cat# KT-01, KT-01-PRF

INSTRUCTION MANUAL ZBM-26

STORAGE CONDITIONS

- **Short Term** 4°C
- **Long term (6 months)** -20°C [NOTE: Add fresh antibiotics upon thawing]

For *in vitro* Use Only

LIMITED PRODUCT WARRANTY

This warranty limits our liability to replacement of this product. No other warranties of any kind, express 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**
- **Telephone** (919) 547-0692
- **Facsimile (FAX)** (919) 547-0693
- **Toll Free** **1-866-ADIPOSE (866)-234-7673**
- **Electronic mail (e-mail)** **information@zen-bio.com**
- **World Wide Web** **http://www.zen-bio.com**

3T3-L1 Adipocyte Kit- (Cat# KT-01, KT-01-PRF)

The 3T3-L1 Adipocyte Kit is designed to allow consistent differentiation of 3T3-L1 preadipocytes into mature adipocytes.

ITEMS INCLUDED IN THE KIT

CAT#	DESCRIPTION/COMPOSITION	VOLUME	UNIT	QTY
PM-1-L1 PM-1-L1-PRF	3T3-L1 Preadipocyte Medium DMEM, high glucose * HEPES pH 7.4 Bovine Calf Serum (BCS) Penicillin Streptomycin Amphotericin B	100ML	BOTTLE	1
DM-2-L1 DM-2-L1-PRF	3T3-L1 Adipocyte Differentiation Medium DMEM / Ham's F-12 (1:1, v/v) HEPES pH 7.4 Fetal bovine serum Biotin Pantothenate Human insulin Dexamethasone Isobutylmethylxanthine PPARγ agonist Penicillin Streptomycin Amphotericin B	50ML	BOTTLE	1
AM-1-L1 AM-1-L1-PRF	3T3-L1 Adipocyte Maintenance Medium DMEM / Ham's F-12 (1:1, v/v) HEPES pH 7.4 Fetal bovine serum Biotin Pantothenate Human insulin Dexamethasone Penicillin Streptomycin Amphotericin B	125ML	BOTTLE	1

NOTES:

- *****All media except cat# PM-1-L1 contain 3.15g/L D-glucose; PM-1-L1 contains 4.5g/L D-glucose.
- To order media without phenol red, order our kit cat# KT-01PRF.
- Please inquire for custom media formulation 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.

A. PREADIPOCYTE DIFFERENTIATION

1. Preadipocytes are plated sub-confluent in 3T3-L1 Preadipocyte Medium (cat# PM-1-L1) and shipped the next day via overnight delivery in our patented CellPorter™ system. Check the seal for each plate. Discard any plate where the vacuum seal has been compromised during shipment.
2. Place the package into a sterile environment. THIS IS VERY IMPORTANT SINCE BREAKING THE VACUUM SEAL MAY 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. Use scissors to snip open. See Table 1. Unpacking Preadipocytes for removal volume per format. In the event the media settles or shifts during shipping, please see the Notes column for minimal volumes needed for cell maintenance.
5. 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.
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 Preadipocyte Medium (cat# PM-1-L1) and replace with an appropriate volume 3T3-L1 Differentiation Medium (cat# DM-2-L1; see Table 2 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.
6. 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 Table 2 below and Figure 1. 3T3-L1 Growth and Differentiation Feeding Schedule)

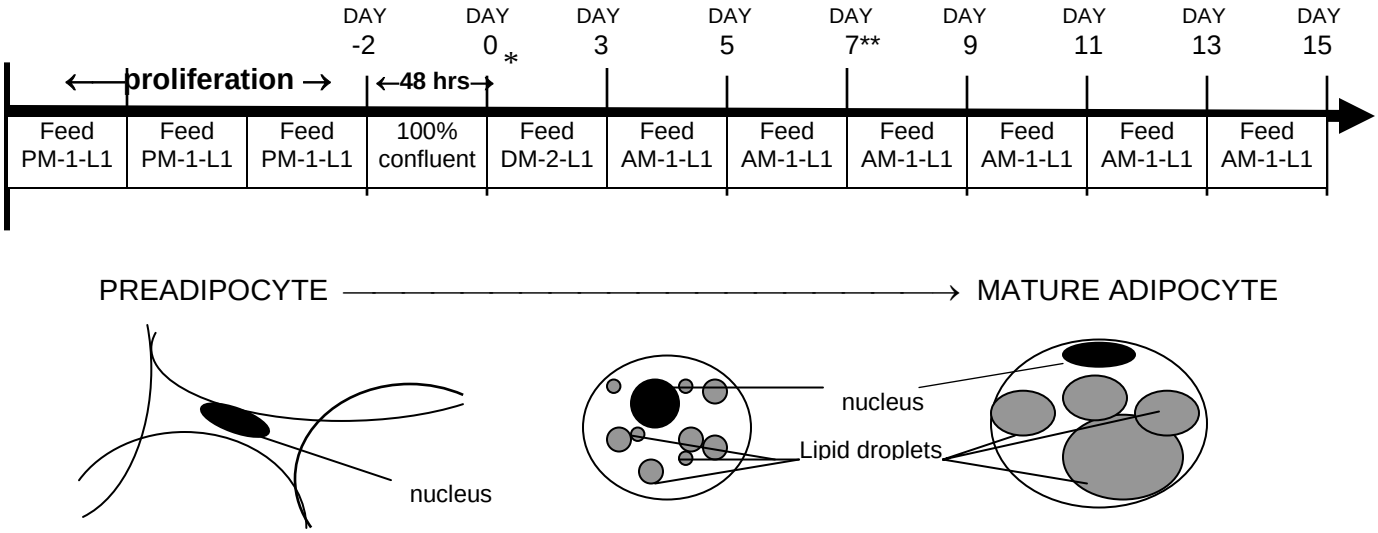
Table 1. Unpacking Preadipocytes

Cultureware	Total shipping volume per well	Removal volume per well	Note
96 well plates	300 µl/well	150 µl	Be sure to leave at least 120-150µl media per well.
48 well plates	1.3 ml/well	0.8 ml	Be sure to leave at least 500 µl media per well.
24 well plates	3.0 ml/well	2.0 ml	Be sure to leave at least 1.0-1.5 ml media per well.
12 well plates	5.8 ml/well	3.8 ml	Be sure to leave at least 2-3 ml media per well.

Table 2. 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	120µl /well
48 well plate	500µl /well	500 µl /well	300 µl /well	400 µl /well	300 µl /well	400 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	0.8 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	1.6 ml/well

Figure 1. 3T3-L1 Growth and Differentiation Feeding Schedule



* Once the cells are 100% confluent, incubate an additional 48 hours <u>before</u> initiating differentiation.

** 3T3-L1 adipocytes are suitable for most assays 7-14 days post differentiation