Human Adipocyte Differentiation Kit

Cat# KT-02, KT-02-PRF, KT-03, KT-03-PRF

INSTRUCTION MANUAL ZBM0080.00

STORAGE CONDITIONS

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

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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 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.

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ORDERING INFORMATION AND TECHNICAL SERVICES

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INTRODUCTION

The Adipocyte Differentiation Kit is designed to allow consistent differentiation of human subcutaneous (KT-02) or visceral (KT-03) preadipocytes into mature adipocytes in a 96 well format. The volumes listed are suitable for the differentiation of human preadipocytes in a 96 well or 384 well format. For other cultureware formats with higher volume needs, please order media individually (cat# PM-1, 500ml; DM-2 100ml; AM-1 500ml). The process of differentiating preadipocytes to adipocytes has been patent protected by Zen-Bio under US patent number 6153432.

ITEMS INCLUDED IN THE KIT

CAT#	DESCRIPTION/COMPOSITION	VOLUME	UNIT	QTY
PM-1 (KT-02) PM-1-PRF OM-PM (KT-03) OM-PM-PRF	Preadipocyte Medium DMEM / Ham's F-12 (1:1, v/v) HEPES pH 7.4 Fetal bovine serum Penicillin Streptomycin Amphotericin B	50ML	BOTTLE	1
DM-2 (KT-02) DM-2-PRF OM-DM (KT-03) OM-DM-PRF	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	25ML	BOTTLE	1
AM-1 (KT-02) AM-1-PRF OM-AM (KT-03) OM-AM-PRF	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	25ML	BOTTLE	1

- 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.

Please note: Primary human cells can be very sensitive to brands of cultureware. Zen-Bio does not currently recommend the use of Falcon or Sarstedt 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 Preadipocyte Medium (cat # PM-1 or OM-PM). Centrifuge: 1,200 rpm (282 X g) / 20°C / 5 minutes. Aspirate the supernatant. TAKE CARE TO NOT ASPIRATE ANY OF THE CELL PELLET.
3. The cell vial contains a minimum of 2.0 x 10^6 viable cells; however, we recommend performing a cell count to determine a more exact number of cells. Resuspend the cell pellet in 2 ml Preadipocyte Medium; 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.
4. 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.

	VOLUME PER WELL	TOTAL VOLUME PER FORMAT*
96 well plate	150 µl	15 ml
3884 well	30 µl	12 ml

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

5. 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.
6. Twenty-four 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.
7. To start the process, aspirate the entire volume of Preadipocyte Medium from all wells. Add the appropriate volume of Adipocyte Differentiation Medium (catalog # DM-2 for subcutaneous cells and OM-DM for visceral cells) to the wells (see Table 1. Feeding Volumes). Incubate plate for 7 days at 37°C and 5% CO_2.
8. After 7 days, cells should be fed by removing some of the media and replacing with fresh Adipocyte Medium (catalog # AM-1 or OM-AM) (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.**

9. Two (2) weeks after the initiation of differentiation, cells should appear rounded with large lipid droplets apparent in the cytoplasm (see Figure 1-C). Cells are now considered mature adipocytes and are suitable for most assays.

Table 1. Feeding Volumes

Format	Plating PM-1 or OM-PM	Change PM-1 to DM-2 Change OM-PM to OM-DM		Change DM-2 to AM-1 Change OM-DM to OM-AM	
	IN	OUT	IN	OUT	IN
96 well plate	150 µl/ well	150 µl/ well	150µl/ well	90 µl/ well	120 µl/ well
384 well plate	30 µl/ well	30 µl/ well	30 µl/ well	180 µl/ well	24 µl/ well

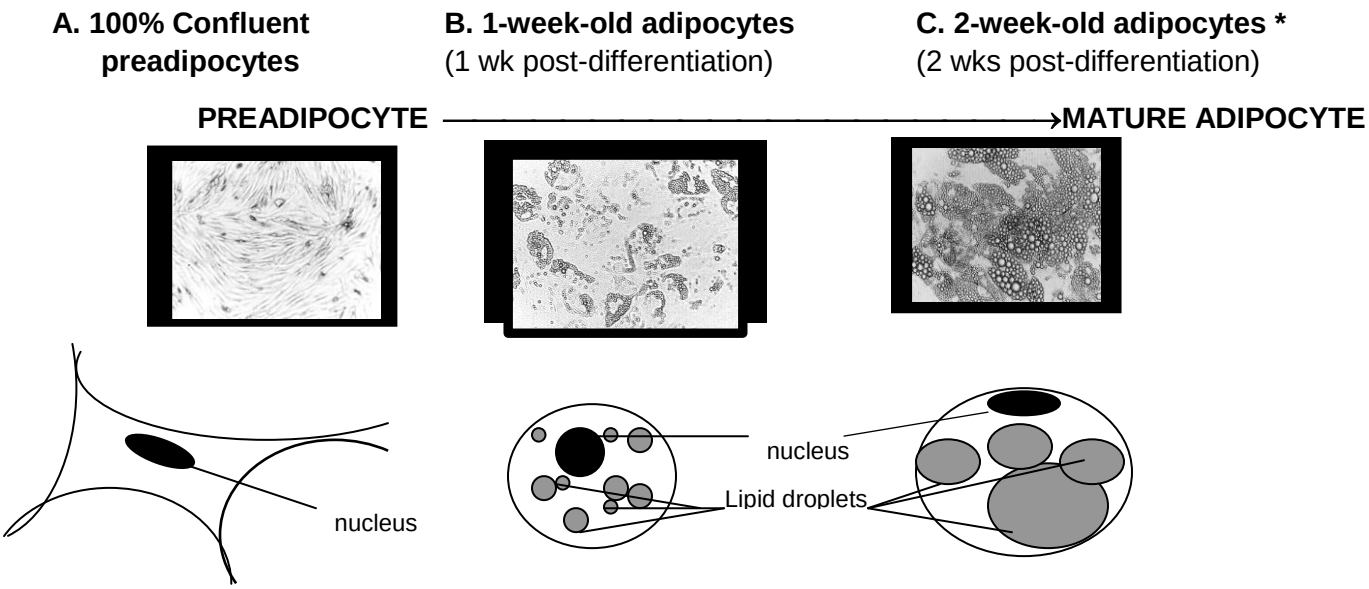

Figure 1: Photographs of 100% confluent Preadipocytes (A), 1-week-old (post-differentiation) cultured adipocytes (B) and mature (2 weeks post-differentiation) cultured Adipocytes (C). These are unstained photographs of human preadipocyte morphology (20X). The cells should appear comparable in appearance to these pictures for subcutaneous cells. The preadipocytes should be confluent 24-48 hours after plating for differentiation. If they are not 100% confluent, the cells will not differentiate well.

*Visceral adipocytes may not accumulate the same degree of lipid as the subcutaneous counterparts.