Preadipocyte Isolation Kit

12/13

(Catalog # K583-5; for 5 g Tissue; Store at -20°C)

I. **Introduction:**

Adipose tissue is loose connective tissue that accumulates in animals and serves primarily for energy storage, insulation, and thermoregulation. It is predominantly composed of adipocytes, but also contains a stromal vascular fraction consisting of preadipocytes, fibroblasts, vascular endothelial cells, and immune cells. Recent research has discovered a dynamic role of adipose tissue in the production and secretion of various hormones and as an active endocrine organ. The excessive accumulation of adipose is known as obesity. Ways to prevent over-accumulation of adipose is a field of intense research. In addition to obesity, adipose tissue has been found to participate in various physiological processes, including reproduction, angiogenesis, inflammation, cancer, and vascular homeostasis. BioVision's Preadipocyte Isolation Kit provides the reagents and tools to isolate the stromal vascular fraction from adipose tissue. The resulting stromal vascular fraction is cultured on a tissue-culture plate, where preadipocytes adhere. The preadipocytes retain the ability to proliferate and differentiate into adipocytes when treated with differentiation-inducing components (Cat. # K579, 3T3-L1 Differentiation Kit). The preadipocytes can be used to study the process of adipogenesis and the differentiated adipocytes can be used to study lipolysis, endocrine activity, cell-signaling, and metabolic dysfunction.

II. **Application:**

- Isolation of preadipocytes from adipose tissue

III. **Sample Type:**

- Animal tissues: mouse or rat adipose tissue

IV. **Kit Contents:**

Components	K583-5	Cap Code	Part Number
Collagenase (0.2%)	10 ml	NM, blue	K583-5-1
Collagenase Stop Buffer	90 ml	NM	K583-5-2
Red Blood Cell Lysis Buffer	10 ml	NM	K583-5-3
PBS	90 ml	WM	K583-5-4
Cell Strainer (100 µm)	10	-	K583-5-5
Cell Strainer (70 µm)	10	-	K583-5-6

V. **User Supplied Samples, Reagents, and Equipment:**

- Fresh mouse or rat adipose tissue up to 5 g
- Dissecting scissors
- 6-well tissue culture plate
- Preadipocyte medium (DMEM/F12, 10% FBS, P/S, amphotericin B)
- Orbital shaker

VI. **Storage and Handling:**

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

VII. **Reagent Preparation and Storage Conditions:**

- **Collagenase (0.2%):** Ready to use as supplied. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Keep on ice during experiment. Stable for 2 months.
- **Collagenase Stop Buffer, Red Blood Cell Lysis Buffer, and PBS:** Warm to 37°C before use. Store at 4°C.

VIII. **Preadipocyte Isolation Protocol:**

Use freshly isolated adipose tissue from mice or rats. Mince tissue with dissecting scissors in a sterile vessel for at least 5 min. Place minced tissue into a 50 ml conical tube with cap loosely on and add 1 ml of Collagenase (0.2%) per 0.5 g of tissue. Incubate in a heated orbital shaker at 37°C for 30 min. at 160 rpm. Add 9 ml of Collagenase Stop Buffer per 1 ml of Collagenase (0.2%), tighten the cap and mix by inverting. Filter through Cell Strainer (100 µm). Centrifuge filtrate at 500 x g for 10 min. Remove supernatant and resuspend pellet in 1 ml of Red Blood Cell Lysis Buffer for 1 min. Add 9 ml of PBS. Filter cells through 70 µm Cell Strainer. Centrifuge filtrate at 500 g for 10 min. Remove supernatant and resuspend cell pellet in 2 ml of preadipocyte medium. Add cells into 1 well of a 6-well plate and incubate at 37°C with 5% CO_2. Change to fresh preadipocyte medium the following day (amphotericin B can be omitted).

Notes:

a. **Important**: Thorough mincing of tissue by scissors is crucial for proper tissue digestion.

b. Tissue may require shorter or longer digestion time with Collagenase. If tissue is not completely digested, increase digestion time. In most instances, 20-45 min. digestion will be sufficient.

c. Primary preadipocytes may proliferate and differentiate better in 10% CO_2.

d. Incubating tissue culture plate in preadipocyte medium at 37°C for 1-2 hrs. before plating may increase preadipocyte binding.

e. Cells can be split for 1-3 passages. Until 1-3 passages cells retain the ability to differentiate. Cells should be split before reaching 70% confluence.

(a) (b)

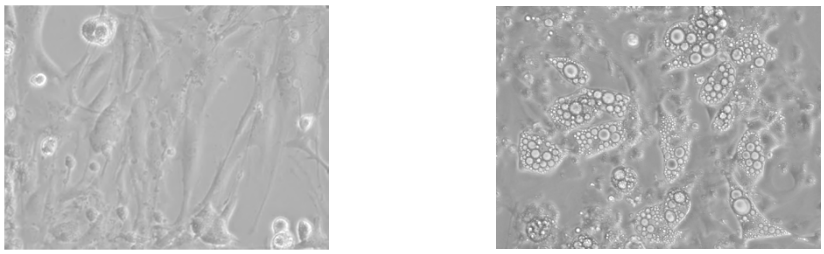

Figure: (a) Preadipocytes from mouse adipose tissue after 4 days of culturing. (b) Adipocytes 7 days after differentiation from preadipocytes using 3T3-L1 Differentiation Kit (K579). Visible lipid droplets confirm the isolation of preadipocytes.

IX. **RELATED PRODUCTS:**

3T3-L1 Differentiation Kit (K579)
Lipolysis (3T3-L1) Colorimetric Assay Kit (K577)
Oil Red O Staining Kit (K580)
Adiponectin (human) Elisa Assay Kit (K4901)
Adiponectin (rat) Elisa Assay Kit (K4903)
Leptin (human) ELISA Kit (K4777)
Insulin (human) ELISA Kit (K4742)
Adipocyte Lipolysis Colorimetric/Fluorometric Assay Kit (K581)
PicoProbe™ Lipolysis (3T3-L1) Lipolysis Fluorometric Assay Kit (K578)
Adipogenesis Colorimetric/Fluorometric Kit (K610)
Adiponectin (mouse) Elisa Assay Kit (K4902)
Resistin (human/mouse/rat) EIA Kit (K4767)
Triglyceride Quantification Colorimetric/Fluorometric Assay Kit (K622)

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