SIGMA-ALDRICH®

sigma-aldrich.com

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Preadipocyte Isolation Kit

Catalog Number **PRI001**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

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.

The Preadipocyte Isolation Kit provides the reagents and tools to isolate the stromal vascular fraction from mouse or rat adipose tissue (up to 5 g of 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 (Catatalog Number DIF001, 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.

Components

Component	Amount
Collagenase (0.2%) (Catalog Number PRI001A)	10 ml
Collagenase Stop Buffer (Catalog Number PRI001B)	90 ml
Red Blood Cell Lysis Buffer (Catalog Number PRI001C)	10 ml
PBS (Catalog Number PRI001D)	90 ml
Cell Strainer (100 μm) (Catalog Number PRI001E)	10 each
Cell Strainer (70 μm) (Catalog Number PRI001F)	10 each

Reagents and Equipment Required but Not Provided.

- Fresh mouse or rat adipose tissue (up to 5 g)
- Dissecting scissors
- 6 well tissue culture plate
- DMEM/F12 medium
- 10% FBS
- Penicillin and streptomycin
- Amphotericin B
- Orbital shaker

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles. Read the entire protocol before preparing reagents.

Collagenase (0.2%) – Solution is 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 2–8 °C.

Preadipocyte Medium – DMEM/F12 medium with 10% FBS, 100 units/ml penicillin, 100 μg/ml streptomycin, and 250 ng/ml amphotericin B.

Storage/Stability

Store the kit at –20 °C, protected from light.

Procedure

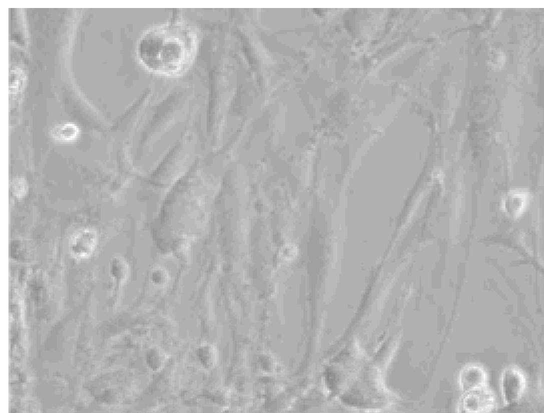
1. Use freshly isolated adipose tissue from mice or rats. Mince tissue with dissecting scissors in a sterile vessel for at least 5 minutes.
   Note: Thorough mincing of tissue by scissors is crucial for proper tissue digestion.
2. Place minced tissue into a 50 ml conical tube with cap loosely on and add 1 ml of Collagenase (0.2%) per 0.5 g of tissue.
3. Incubate in a heated orbital shaker at 37 °C for 30 minutes at 160 rpm.
   Note: Tissue may require shorter or longer digestion time with Collagenase solution. If tissue is not completely digested, increase digestion time. In most instances, a 20–45 minute digestion will be sufficient.
4. Add 9 ml of Collagenase Stop Buffer per 1 ml of Collagenase (0.2%), tighten the cap and mix by inverting.
5. Filter through Cell Strainer (100 μm). Then centrifuge filtrate at 500 × *g* for 10 minutes.
6. Remove supernatant and resuspend pellet in 1 ml of Red Blood Cell Lysis Buffer for 1 minute. Add 9 ml of PBS.
7. Filter cells through Cell Strainer (70 μm). Centrifuge filtrate at 500 × *g* for 10 minutes.
8. 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.
   Notes: Incubating tissue culture plate in preadipocyte medium at 37 °C for 1–2 hours before plating may increase preadipocyte binding.

   Primary preadipocytes may proliferate and differentiate better in 10% CO_2.
9. Change to fresh Preadipocyte Medium the following day (amphotericin B can be omitted).
   Note: 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.

Results

Figure 1.

Preadipocytes from Mouse Adipose Tissue after 4 Days of Culturing



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