ab196988

Preadipocyte Isolation Kit

Instructions for Use

For the isolation of the stromal vascular fraction from adipose tissue.

This product is for research use only and is not intended for diagnostic use.

Version 1 Last Updated 6 February 2019

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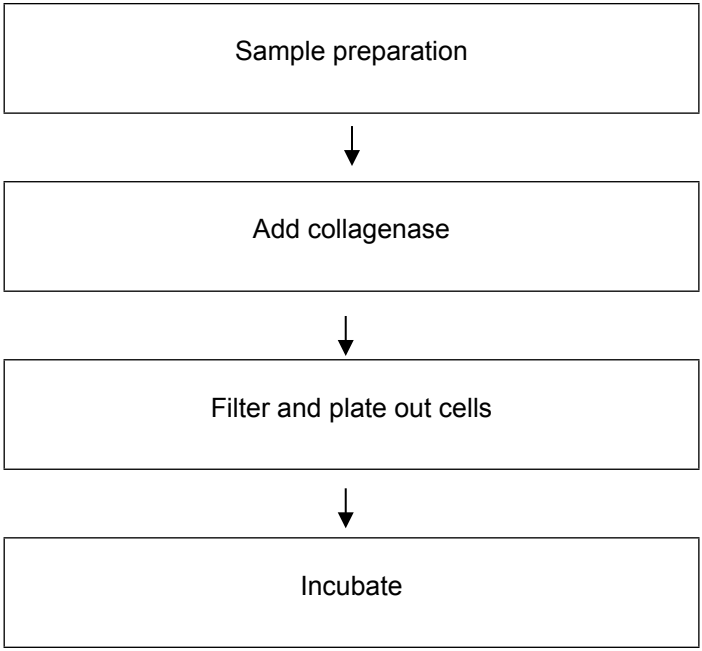
1. OVERVIEW

The Preadipocyte Isolation kit (ab196988) enables isolation of the stromal vascular fraction from adipose tissue. The resulting stromal vascular fraction is cultured on a tissue culture plate, to which the preadipocytes adhere. The preadipocytes retain the ability to proliferate and differentiate into adipocytes when treated with differentiation-inducing components. 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.

The primary roles of adipose tissue are energy storage, insulation and thermoregulation. Adipose tissue is loose connective tissue that is predominantly composed of adipocytes, but also contains a stromal vascular fraction that consists of cells such as preadipocytes, fibroblasts, vascular endothelial cells and immune cells. Recent research has demonstrated a dynamic role for adipose tissue as an active endocrine organ that produces and secretes hormones such as leptin, estrogen, resistin and TNFα. Adipose tissue is derived from preadipocytes. It participates in various physiological processes, including reproduction, angiogenesis, inflammation, cancer and vascular homeostasis.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20°C in the dark immediately upon receipt. Briefly centrifuge small vials prior to opening. All kit components are supplied as ready to be used. Keep on ice while in use.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 5.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Collagenase	10 mL	-20°C
Collagenase Stop Buffer	90 mL	-20°C
Cell Strainer (100 µm)	10 units	-20°C
Red Blood Cell Lysis Buffer	10 mL	-20°C
PBS	90 mL	-20°C
Cell Strainer (70 µm)	10 units	-20°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 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)
- 50 mL conical tubes
- Orbital shaker

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Keep components and samples on ice during the assay, unless stated otherwise.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure complete removal of all solutions and buffers during wash steps.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 Collagenase:

Ready to use as supplied. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Stable for 2 months. Keep on ice during experiment.

9.2 Collagenase Stop Buffer:

Ready to use as supplied. Equilibrate to 37°C before use. Store at 4°C. Keep on ice during experiment.

9.3 Red Blood Cell Lysis Buffer:

Ready to use as supplied. Equilibrate to 37°C before use. Store at 4°C. Keep on ice during experiment.

9.4 PBS:

Ready to use as supplied. Equilibrate to 37°C before use. Store at 4°C. Keep on ice during experiment.

9.5 Cell strainers (70 µm and 100 µm):

Ready to use as supplied. Equilibrate to 37°C before use. Store at 4°C.

10. SAMPLE PREPARATION

- Use freshly isolated adipose tissue from mice or rats.
- Thorough mincing of tissue by scissors is a crucial step for proper tissue digestion.
- Tissue may require shorter or longer digestion time with Collagenase. If tissue is not completely digested, increase digestion time. In most instances, 20-45 minutes digestion will be sufficient.
- Incubate tissue culture plate in preadipocyte medium at 37°C for 1-2 hours before plating to increase preadipocyte binding.
- Primary preadipocytes may proliferate and differentiate better in 10% CO_2.
- 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.

11. ASSAY PROCEDURE

11.1 Mince tissue with dissecting scissors in a sterile vessel for at least 5 minutes.

11.2 Place minced tissue into a 50 mL conical tube with cap loosely on and add 1 mL of Collagenase per 0.5 g of tissue.

11.3 Incubate in a heated orbital shaker at 37°C for 30 minutes at 160 rpm.

11.4 Add 9 mL of Collagenase Stop Buffer per 1 mL of Collagenase, tighten the cap and mix by inverting.

11.5 Filter through Cell Strainer (100 µm). Centrifuge filtrate at 500 x*g* for 10 minutes.

11.6 Remove supernatant and resuspend pellet in 1 mL of Red Blood Cell Lysis Buffer for 1 minute.

11.7 Add 9 mL of PBS. Filter cells through 70 µm Cell Strainer.

11.8 Centrifuge filtrate at 500 x*g* for 10 minutes Remove supernatant and resuspend cell pellet in 2 mL of preadipocyte medium.

11.9 Add cells into 1 well of a 6-well plate and incubate at 37°C with 5% CO_2.

11.10 Change to fresh preadipocyte medium the following day (amphotericin B can be omitted).

12. TYPICAL DATA

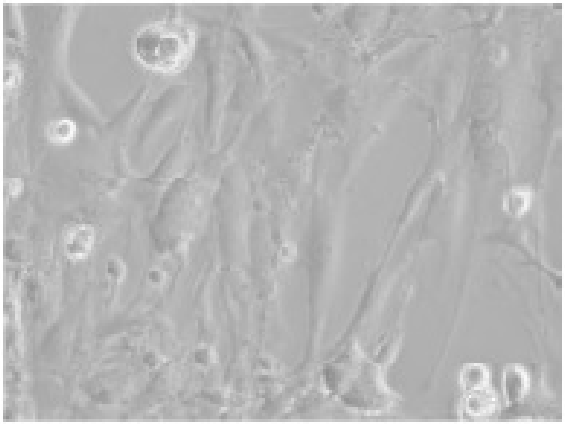


Figure 1: Preadipocytes from mouse adipose tissue after 4 days of culturing.

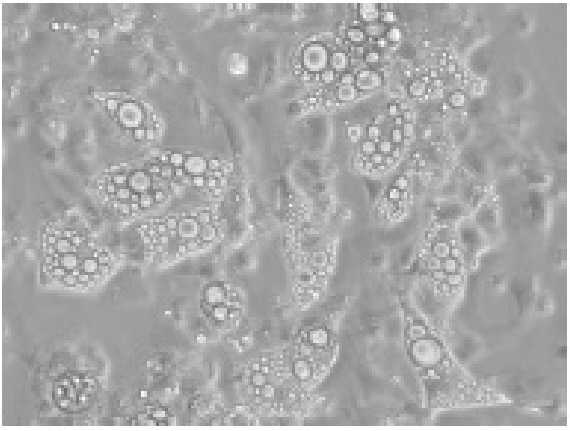


Figure 2: Adipocytes 7 days after differentiation from preadipocytes using a 3T3-L1 differentiation kit. Visible lipid droplets confirm the isolation of preadipocytes.

13. TROUBLESHOOTING

14. NOTES

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