Isolation of Human SVF from Adipose Tissue with Collagenase AF-1 GMP Grade & Neutral Protease AF GMP Grade

Introduction

Adipose tissue is an abundant source of various cell types and can be easily collected via liposuction. The lipoaspirate obtained during liposuction contains adipose tissue rich in stem cells. Therefore, lipoaspirate is often used for isolation of fat stem cells, also referred to as stromal vascular fraction (SVF). SVF cells may subsequently be used for direct application or in further cell differentiation steps to generate specific cell types.

Reagents

> Lactated Ringer Solution (LR): 500 - 1000 ml
> Collagenase AF-1 GMP Grade, ≥ 150 PZ U/Vial (Cat. No. N0003855)
> Neutral Protease AF GMP Grade, ≥ 100 DMC U/Vial (Cat. No. N0003553)

Preparation of the Enzymatic Solution

Prepare a solution of 0.4 PZ U/ml Collagenase AF-1 GMP Grade (Cat. No. N0003855) and 0.2 DMC U/ml Neutral Protease AF GMP Grade (Cat. No. N0003553) in Lactated Ringer Solution on ice. Make sure that the lyophilisate is completely dissolved. If you use another buffer, please make sure that it contains ≥ 2 mM Ca^{2+} since collagenase and neutral protease activity is calcium dependent.

Sample

e.g. 200 ml of raw lipoaspirate (equal to 50 - 100 ml of decanted adipose tissue). It is recommended to use freshly obtained lipoaspirate for the experiment. If you begin with adipose tissue, start immediately with the “Adipose Tissue Digestion” step.

Sample Washing

1. Mix the raw lipoaspirate in the collection canister thoroughly. If decanted, remove the infranatant red phase fluid before mixing.
2. Warm the lipoaspirate to 37 - 38 °C.
3. Wash the lipoaspirate 3 times using shaking and decantation method with pre-warmed LR (adding approximately same volume as adipose tissue) or until the adipose phase is yellow and the fluid phase is transparent (pale pink). Completely remove the aqueous phase (bottom phase) after each washing and proceed further with adipose tissue (top phase).

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4. After the third wash, let settle for at least 5 min.
   As an alternative to the decantation method, centrifuge at 200 g for 5 min at RT.
   There will be three layers:

   Top layer: Adipose tissue
   Second layer: Tumescent waste
   Third layer: Red blood cells and extracellular matrix (ECM) material

5. Use a 60 ml catheter tip syringe to aspirate the washed and decanted adipose tissue (top layer) and transfer the fraction into an appropriate tube or container.

Adipose Tissue Digestion

1. Add to the sample the same volume of pre-warmed enzyme solution (ratio 1:1, adipose tissue: enzyme solution) i.e. 10 g of adipose tissue + 10 ml of LR containing the enzyme solution (0.4 PZ U/ml Collagenase AF-1 GMP Grade and 0.2 PZ U/ml Neutral Protease AF GMP Grade). The final enzyme concentration of Collagenase AF-1 GMP Grade is 0.2 PZ U/ml and 0.1 DMC U/ml of Neutral Protease AF GMP Grade.

2. Incubate the sample in the orbital shaker at 37 °C for 50 min with constant shaking (150 rpm).

3. Immediately proceed with the next step.

Centrifugation and SVF Pellet Resuspension

1. Centrifuge for 10 min at 600 g. There will be three layers:

   Top layer: Disintegrated tissue
   Second layer: Waste
   Third layer: SVF pellet and ECM material

   Remove the supernatant to stop the enzymatic activity and suspend the pellet by adding 1 ml of LR to the sample. Make sure the cells are well suspended.

2. Aspirate the entire volume of SVF suspended cells and filter through a 400-micron cell strainer to separate the SVF cells from the ECM material and collect into a fresh container.

3. Thoroughly shake the sample and aliquot to perform cell counting if necessary.

Ordering Information

Enzyme	Cat. No.	Pack Size
Collagenase AF-1 GMP Grade from *C. histolyticum*	N0003855	≥ 150 PZ U/Vial
Neutral Protease AF GMP Grade from *C. histolyticum*	N0003553	≥ 100 DMC U/Vial

animal free

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