Isolation of Stromal Vascular Fraction

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Isolation of Stromal Vascular Fraction (SVF) from mouse brown adipose tissue (BAT) for single cell RNA-seq

In 1 collection

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ABSTRACT

This protocol outlines the procedure for the isolation of the Stromal Vascular Fraction (SVF) from mouse brown adipose tissue (BAT) for single cell RNA-seq. This protocol uses a combination of Collagenase I and Dispase II to digest freshly isolated BAT. Compared to using Collagenase I alone, this combination results in a more efficient dissociation of the adipose vasculature.

ATTACHMENTS

[Isolation_of_Stromal_Vascular_Fraction_\(SVF\)_from_mouse_brown_adipose_tissue_\(BAT\)_for_single_cell_RNA-seq.pdf](#)

Keywords: stromal vascular fraction, mouse brown adipose tissue, brown adipose tissue, single cell RNA-seq, RNA-seq

MATERIALS

MATERIALS

Dead Cell Removal Kit Miltenyi Biotec Catalog #130-090-101

Corning® 40µm Cell Strainer Corning Catalog #431750

MS Columns Miltenyi Biotec Catalog #130-042-201

ACK Lysing Buffer (1X) Lonza Catalog #10-548E

RNaseZap™ RNase Decontamination Solution Thermo Fisher Scientific Catalog #AM9780

Falcon® 100 µm Cell Strainer Corning Catalog #352360

MACS Separator Miltenyi Biotec

Digestion Media:

Collagenase Type 1 Worthington Biochemical Corporation Catalog #LS004196

Dispase (5 U/mL) STEMCELL Technologies Inc. Catalog #07913

Bovine Serum Albumin (BSA): Gemini Bio Products BSA V FATTY ACID FREE 100 Fisher Scientific Catalog #50-753-3073

HBSS: Corning® Hanks Balanced Salt Solution 1X with calcium and magnesium Corning Catalog #21-020-CM

Growth Media:

DMEM, high glucose Thermo Fisher Catalog #11965118

Fetal Bovine Serum: Equalfetal® Bovine Serum Atlas Biologicals

SAFETY WARNINGS

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

BEFORE START INSTRUCTIONS

Prepare the digestion media containing 1.5 mg/ml Collagenase I, 2.5 U/ml Dispase, and %2 BSA in HBSS buffer. Warm to 37 °C .

Prepare growth media by adding FBS (%10) to DMEM. Warm to 37 °C .

- 1 Sacrifice the mouse.
- 2 Spray the animal extensively with 70 % EtOH and RNaseZap™.
- 3 Dissect interscapular brown adipose tissue (BAT). If tissues from multiple animals are being dissected, store them in HBSS until all of them are dissected.
- 4 Mince the tissue to very fine pieces in a 50 ml Falcon tube. Add 10 mL digestion media for each BAT.
- 5 Place the tubes in a water bath or incubator with a shaker/rotator at 37 °C for 00:45:00 .
- 6 Remove the tissue from the incubator and vortex for 00:00:10 .

7

Centrifuge at 300 x g, 4°C, 00:10:00 in a swinging bucket centrifuge.

8

Aspirate the supernatant carefully not to disturb the pellet of SVF cells.

9

Resuspend the pellets in 10 mL growth media

10

Filter through a 100 µm cell strainer into a fresh 50 ml tube. Wash the tube with an additional 10 mL and filter through the cell strainer.

11

Centrifuge at 300 x g, 00:07:00

12

Completely remove supernatant and re-suspend the pellet in 2 mL sterile ACK lysis buffer; place On ice for 00:05:00.

13

Filter through a 40 µm cell strainer into a fresh 50 ml tube. Wash the tube with 20 mL growth media and filter through the cell strainer.

14

Centrifuge at 300 x g, 00:07:00

- 15 Resuspend the pellet in 1 mL %1.5 BSA in PBS .
- 16 Use 10 μ L of the cell suspension for cell counting and viability assessment.
- 17 Centrifuge the cell suspension 300 x g, 00:05:00 .
- 18

Resuspend the cells in 100 μ L dead cell removal bead solution . Incubate the samples for 00:15:00 at Room temperature .
- 19 Prepare the binding solution by diluting the 20X solution in sterile ddH₂O.
- 20 Place the MS columns on the MACS separator. Prepare each column by rinsing it with 0.5 mL 1X binding solution . Let the solution pass through the column.
- 21 Add 900 μ L 1X binding solution to each sample and apply cell suspension onto the column.
- 22 Collect effluent in a 2 ml low bind tube as live cell fraction.

23

Rinse the column with an additional

1 mL 1X binding solution

24

Use 10 μ L sample for cell counting and viability assessment.

25

Centrifuge the cell suspension

300 x g,
00:05:00

26

Resuspend the cells in 50 μ L

- 100 μ L %1.5 BSA in PBS

27

Keep the cell suspension On ice and proceed to 10x Genomics Single Cell Protocol. Minimize the time between cell preparation and chip loading.