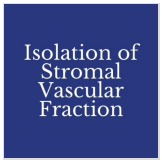

Nov 05, 2020

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

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1 Works for me dx.doi.org/10.17504/protocols.io.bj64krgw

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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

DOI

dx.doi.org/10.17504/protocols.io.bj64krgw

PROTOCOL CITATION

Farnaz Shamsi 2020. Isolation of Stromal Vascular Fraction (SVF) from mouse brown adipose tissue (BAT) for single cell RNA-seq. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bj64krgw

KEYWORDS

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

CREATED

Aug 22, 2020

LAST MODIFIED

Nov 05, 2020

OWNERSHIP HISTORY

Aug 22, 2020 Emily Hasser University of Washington

Nov 05, 2020 Farnaz Shamsi

PROTOCOL INTEGER ID

40892

ATTACHMENTS

Isolation_of_Stromal_Vascular_Fraction_(SVF)_from_mouse_brown_adipose_tissue_(BAT)_for_single_cell_RNA-seq.pdf

MATERIALS TEXT

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 Catalog # 07913**

☒ Bovine Serum Albumin (BSA): Gemini Bio Products BSA V FATTY ACID FREE 100G **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).

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

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dissociation of the adipose vasculature.

BEFORE STARTING

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

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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 µl** 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 µl 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.

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21 Add **900 µl 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 µl sample** for cell counting and viability assessment.

25 

Centrifuge the cell suspension **300 x g, 00:05:00** .

26 Resuspend the cells in **50 µl** - **100 µl %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.

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