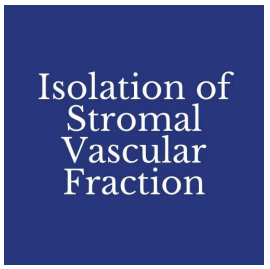
Nov 18, 2020

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



DOI
dx.doi.org/10.17504/protocols.io.bpurmnv6

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OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.bpurmnv6

External link: https://doi.org/10.1038/s42255-021-00373-z

Protocol Citation: Farnaz Shamsi, Yu-Hua Tseng 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.bpurmnv6

Manuscript citation:
Shamsi F, Piper M, Ho L, Huang TL, Gupta A, Streets A, Lynes MD, Tseng Y, Vascular smooth muscle-derived TRPV1-positive progenitors are a source of cold-induced thermogenic adipocytes. Nature metabolism 3(4). doi: 10.1038/s42255-021-00373-z

Protocol status: Working
We use this protocol and it's working

Created: November 18, 2020

Last Modified: November 18, 2020

Protocol Integer ID: 44657

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

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

PDF

Isolation_of_Stromal...

46KB

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** **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).

Before start

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 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.

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.