



Nov 05, 2020

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

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DOI

<https://dx.doi.org/10.17504/protocols.io.bj64krw>

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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.bj64krw>

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Protocol status: Working

Created: August 22, 2020

Last Modified: November 05, 2020

Protocol Integer ID: 40892

Keywords: stromal vascular fraction, mouse brown adipose tissue, brown adipose tissue, single cell RNA-seq, RNA-seq, mouse brown adipose tissue, isolation of stromal vascular fraction, brown adipose tissue, efficient dissociation of the adipose vasculature, single cell rna, stromal vascular fraction, adipose vasculature, using collagenase, rna, isolated bat,

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...

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 Inc. 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 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.