Isolation of Matrix Metalloproteinases (MMP)-expressing cells from Stromal Vascular Fraction

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Aoi Satoh

MATERIALS

- 4% paraformaldehyde/1XPBS solution
- Collagenase D Sigma – Aldrich Catalog #11088866001
- Dispase II (neutral protease, grade II) Sigma – Aldrich Catalog #4942078001
- Calcium chloride solution Sigma – Aldrich Catalog #21115-100ML
- autoMACS Rinsing Solution Miltenyi Biotec Catalog #130-091-222
- Bovine Serum Albumin solution Sigma – Aldrich Catalog #A1595-50ML
- Corning® 100µm Cell Strainer Corning Catalog #431752
- Corning® 40µm Cell Strainer Corning Catalog #431750
- Adipose Tissue Progenitor Isolation Kit, mouse Miltenyi Biotec Catalog #130-106-639
- QuadroMACS Separator Miltenyi Biotec Catalog #130-090-976
- MACS MultiStand Miltenyi Biotec Catalog #130-042-303
- MACS 15 mL Tube Rack Miltenyi Biotec Catalog #130-091-052
**PROTOCOL integer ID:** 12193

**LS Columns Miltenyi**
Biotec Catalog #130-042-401

**TruStain fcX™ (anti-mouse CD16/32) Antibody**
BioLegend Catalog #101319

**Triton™ X-100 (Electrophoresis) Fisher Scientific**
Catalog #BP151-100

**MMP3 Monoclonal Antibody (4F10) Invitrogen - Thermo Fisher**
Catalog #MA5-17123

**Falcon® 5 mL Round Bottom Polystyrene Test Tube, with Cell Strainer Snap Cap Corning**
Catalog #352235

**STEP MATERIALS**

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Falcon® 5 mL Round Bottom Polystyrene Test Tube, with Cell Strainer Snap Cap Corning Catalog #352235

PROTOCOL MATERIALS

MMP3 Monoclonal Antibody (4F10) Invitrogen - Thermo Fisher Catalog #MA5-17123

Falcon® 5 mL Round Bottom Polystyrene Test Tube, with Cell Strainer Snap Cap Corning Catalog #352235

autoMACS Rinsing Solution Miltenyi Biotec Catalog #130-091-222

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LS Columns Miltenyi Biotec Catalog #130-042-401

QuadroMACS Separator Miltenyi Biotec Catalog #130-090-976

4% paraformaldehyde/1XPBS solution Contributed by users

PBS Contributed by users

Corning® 40µm Cell Strainer Corning Catalog #431750

Adipose Tissue Progenitor Isolation Kit, mouse Miltenyi Biotec Catalog #130-106-639

Bovine Serum Albumin solution Merck MilliporeSigma (Sigma-Aldrich) Catalog #A1595-50ML

Corning® 100µm Cell Strainer Corning Catalog #431752

MACS MultiStand Miltenyi Biotec Catalog #130-042-303

TruStain fcX™ (anti-mouse CD16/32) Antibody BioLegend Catalog #101319

Collagenase D Merck MilliporeSigma (Sigma-Aldrich) Catalog #11088866001
Digestion Buffer Preparation

1. Prepare digestion buffer 10 ml / tissue / 2~5 mice.

2. Weight Collagenase D (f.c. 1.5 U/ml) and Dispase II (f.c. 2.4 U/ml) in 50 ml tube.

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Collagenase D Sigma Aldrich Catalog #11088866001
Dispase II (neutral protease, grade II) Sigma Aldrich Catalog #4942078001

3. Add appropriate amount of PBS to dissolve.

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<td>Sacrifice mice before dissolving.</td>
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PBS Sigma Aldrich

4. Shake in water bath at 37 °C.

| 37 °C less than 10 min |

Digestion of Adipose Tissue
Sacrifice mice and take out adipose tissue, place on sterile 10 cm dish with PBS at room temperature (RT).

Activate digestion buffer by adding CaCl2 (f.c. 10 mM).

Remove residual PBS from the tissues by patting on Kimwipe.

Add 2 ml of activated digestion buffer to tissue in 10 cm dish.

Mince with scissors until approximately 1 mm piece

Transfer to 50 ml tube by pipette.

Shake in water bath at 37 °C for 1 hour (gently shake by hand every 10 min).

Stop digestion by adding 5 ml of 0.5 % BSA in PBS + EDTA (autoMACS Rinsing Solution).
13 Spin 300 g for 5 min at room temperature (RT).

14 Remove the sup.

15 Suspend the pellet with 1 ml 0.5 % BSA in PBS + EDTA (autoMACS Rinsing Solution).

16 Place a cell strainer (100 μm diameter) over a new 50 ml tube and filter the cell suspension.

17 Repeat step 13-16 with 40 μm diameter cell strainer.

18 Spin 300 g for 5 min at RT.

19 Discard the supernatant completely.

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**Depletion of Lin+ Cells**

20 Suspend the pellet with cold (4 °C) 80μl of 0.5 % BSA in PBS + EDTA (autoMACS Rinsing Solution)
Solution). From this step use cold (4 °C) buffer.

21 Add 20 μl (mix by gentle pipetting) of Non-Adipocyte Progenitor Depletion Cocktail, mouse.

Adipose Tissue Progenitor Isolation Kit, mouse Sigma Aldrich Catalog #130-106-639

22 Mix well and incubate for 15 min at 4 °C (protected from light).

00:15:00

23 During the incubation, place LS Column in the magnetic field of a MACS Separator and 15 ml tube under LS Column.

MACS MultiStand Sigma Aldrich Catalog #130-042-303

QuadroMACS Separator Sigma Aldrich Catalog #130-090-976

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24 Prime column by adding 3 ml of 0.5 % BSA in PBS + EDTA (autoMACS Rinsing Solution).

Note

Avoid air bubbles, bubbles could block the column.
Always wait until the column reservoir is empty before proceeding to the next step.

25 After 15 min incubation, add 400 ul of 0.5 % BSA in PBS + EDTA (autoMACS Rinsing Solution) and apply onto the column. Collect flow-through containing Lin- cells.

26 Wash column with 1 ml of 0.5 % BSA in PBS + EDTA (autoMACS Rinsing Solution) 2 times. Collect flow-through containing Lin- cells.
27 Spin 300 g for 5 min at RT.

28 Remove the supernatant.

Fcr Blocking

29 Suspend cells in 100 ul of 1 % BSA in PBS + EDTA (autoMACS Rinsing Solution).

30 Add 2 ul of TruStain fcX Ab. (0.5 mg/ml) and mix.

TruStain fcX™ (anti-mouse CD16/32) Antibody Sigma Aldrich Catalog #101319

31 Incubate 10 min on ice (Protect from light).

32 Add 900 ul of 1 % BSA in PBS + EDTA (autoMACS Rinsing Solution) and mix.

33 Spin 300 g for 5 min in 4 °C.

34 Remove the supernatant.

FcR Blocking

protocols.io | https://dx.doi.org/10.17504/protocols.io.p59dg96
Add 100 ul of Click-iT fixative (4 % paraformaldehyde in PBS) and mix.

4% paraformaldehyde/1XPBS solution Sigma Aldrich

Incubate 15 min RT (Protect from light).

Add 900 ul of 1 % BSA in PBS + EDTA (autoMACS Rinsing Solution) and mix.

Spin 300 g for 5 min RT.

Prepare permeabilization buffer (0.3 % Triton X-100 Dilute in 'RT (25°C)' 1 % BSA in PBS + EDTA)

Remove the supernatant.

Add 100 ul of permeabilization buffer and mix.

Incubate 15 min RT (Protected from light).
Add labeled Mmp3 Ab. (1:200) and mix.

MMP3 Monoclonal Antibody (4F10) Sigma Aldrich Catalog #MA5-17123

Incubate 10 min on ice (Protected from light).

Add 900 ul of permeabilization buffer and mix.

Spin 300 g for 5 min 4 °C.

Remove the supernatant.

Suspend in 500 ul of permeabilization buffer and load on 5 ml Polystyrene Round-Bottom Tube with Cell-strainer Cap.

Falcon® 5 mL Round Bottom Polystyrene Test Tube, with Cell Strainer Snap Cap Sigma Aldrich Catalog #352235

Proceed to sort by BD FACS Aria II.

Move to FACS