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 We use this protocol and it's working

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

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Aoi Satoh

MATERIALS

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- ✕ 4% paraformaldehyde/1XPBS solution Contributed by users
- ✕ Collagenase D Merck MilliporeSigma (Sigma-Aldrich) Catalog #11088866001
- ✕ Dispase II (neutral protease, grade II) Merck MilliporeSigma (Sigma-Aldrich) Catalog #4942078001
- ✕ Calcium chloride solution Merck MilliporeSigma (Sigma-Aldrich) Catalog #2111100ML
- ✕ autoMACS Rinsing Solution Miltenyi Biotec Catalog #130-091-222
- ✕ Bovine Serum Albumin solution Merck MilliporeSigma (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 #130-10639
- ✕ 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

- ✕ 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

- ✕ Collagenase D Merck MilliporeSigma (Sigma-Aldrich) Catalog #11088866001
- ✕ Dispase II (neutral protease, grade II) Merck MilliporeSigma (Sigma-Aldrich) Catalog #4942078001
- ✕ PBS Contributed by users
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- ✕ TruStain fcX™ (anti-mouse CD16/32) Antibody BioLegend Catalog #101319
- ✕ 4% paraformaldehyde/1XPBS solution Contributed by users

✕	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
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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.

Note

C o l l a g e n a s e D	7 . 5 U / t i s s u e
D i s p a s e I I	1 2 U / t i s s u e

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

Dispase II (neutral protease, grade II) Merck MilliporeSigma (Sigma-Aldrich) Catalog
#4942078001

- 3 Add appropriate amount of PBS to dissolve.

Note

Sacrifice mice before dissolving.

PBS Contributed by users

- 4 Shake in water bath at 37 °C.

37 °C less than 10
min

Digestion of Adipose Tissue

- 5 Sacrifice mice and take out adipose tissue, place on sterile 10 cm dish with PBS at room temperature (RT).

6 Activate digestion buffer by adding CaCl_2 (f.c. 10 mM).

Calcium chloride solution Merck MilliporeSigma (Sigma-Aldrich) Catalog #21115-100ML

7 Remove residual PBS from the tissues by patting on Kimwipe.

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

9 Mince with scissors until approximately 1 mm piece

10 Transfer to 50 ml tube by pipette.

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

🔥 37 °C

🕒 01:00:00

🕒 00:10:00 x6

12 Stop digestion by adding 5 ml of 0.5 % BSA in PBS + EDTA (autoMACS Rinsing Solution).

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

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

SVF Isolation

- 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.
Corning® 100µm Cell Strainer Corning Catalog #431752
- 17** Repeat step 13-16 with 40 µm diameter cell strainer.
[go to step #13](#)
undefined
Corning® 40µm Cell Strainer Corning Catalog #431750
- 18** Spin 300 g for 5 min at RT.
- 19** Discard the supernatant completely.

Depletion of Lin⁺ Cells

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

4 °C

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

Adipose Tissue Progenitor Isolation Kit, mouse Miltenyi Biotec 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 Miltenyi Biotec Catalog #130-042-303

QuadroMACS Separator Miltenyi Biotec Catalog #130-090-976

MACS 15 mL Tube Rack Miltenyi Biotec Catalog #130-091-052

LS Columns Miltenyi Biotec Catalog #130-042-401

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 µl 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 BioLegend Catalog #101319

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

00:10:00

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.

Fixation and Permeabilization

35 Add 100 ul of Click-iT fixative (4 % paraformaldehyde in PBS) and mix.

4% paraformaldehyde/1XPBS solution Contributed by users

36 Incubate 15 min RT (Protect from light).

00:15:00

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

38 Spin 300 g for 5 min RT.

Note

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

39 Remove the supernatant.

40 Add 100 ul of permeabilization buffer and mix.

41 Incubate 15 min RT (Protected from light).

00:15:00

Mmp3 Staining

42 Add labeled Mmp3 Ab. (1:200) and mix.

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

43 Incubate 10 min on ice (Protected from light).

00:10:00

44 Add 900 ul of permeabilization buffer and mix.

45 Spin 300 g for 5 min 4 °C.

46 Remove the supernatant.

47 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 Corning Catalog #352235

Move to FACS

48 Proceed to sort by BD FACSAria II.