



May 24, 2018

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

DOI

dx.doi.org/10.17504/protocols.io.p59dq96

Aoi Satoh¹, Kosaku Shinoda²

¹Department of Medicine, Albert Einstein College of Medicine, Bronx, New York 10461;

²Department of Medicine and Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, New York 10461



Aoi Satoh

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Protocol Citation: Aoi Satoh, Kosaku Shinoda 2018. Isolation of Matrix Metalloproteinases (MMP)-expressing cells from Stromal Vascular Fraction. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.p59dq96>

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Protocol status: Working

We use this protocol and it's working

Created: May 15, 2018

Last Modified: May 24, 2018

Protocol Integer ID: 12193

Keywords: isolation of matrix metalloproteinase, matrix metalloproteinase, cells from stromal vascular fraction, stromal vascular fraction

Materials

MATERIALS

-  4% paraformaldehyde/1XPBS solution
-  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 #21115-100ML**
-  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 #130-106-639**
-  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**
-  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
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Troubleshooting



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

	Collagenase D	7.5 U/tissue
	Dispase II	12 U/tissue

 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

- 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 CaCl₂ (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 ×6
- 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](#)
 - 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) 80 µl 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
- 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.