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

Aoi Satoh,Kosaku Shinoda

Abstract

Citation: Aoi Satoh,Kosaku Shinoda Isolation of Matrix Metalloproteinases (MMP)-expressing cells from Stromal Vascular Fraction. **protocols.io**
dx.doi.org/10.17504/protocols.io.p59dq96
Published: 24 May 2018

Materials

- 4% paraformaldehyde/1XPBS solution by Contributed by users
- Collagenase D 11088866001 by Sigma – Aldrich
- Dispase II (neutral protease, grade II) 4942078001 by Sigma – Aldrich
- Calcium chloride solution 21115-100ML by Sigma – Aldrich
- autoMACS Rinsing Solution 130-091-222 by Miltenyi Biotec
- Bovine Serum Albumin solution A1595-50ML by Sigma – Aldrich
- Corning® 100µm Cell Strainer 431752 by Corning
- Corning® 40µm Cell Strainer 431750 by Corning
- Adipose Tissue Progenitor Isolation Kit, mouse 130-106-639 by Miltenyi Biotec
- QuadroMACS Separator 130-090-976 by Miltenyi Biotec
- MACS MultiStand 130-042-303 by Miltenyi Biotec
- MACS 15 mL Tube Rack 130-091-052 by Miltenyi Biotec
- LS Columns 130-042-401 by Miltenyi Biotec
- TruStain fcX™ (anti-mouse CD16/32) Antibody 101319 by BioLegend
- Triton™ X-100 (Electrophoresis) BP151-100 by Fisher Scientific
- MMP3 Monoclonal Antibody (4F10) MA5-17123 by Invitrogen - Thermo Fisher
- Falcon® 5 mL Round Bottom Polystyrene Test Tube, with Cell Strainer Snap Cap 352235 by Corning

Protocol

Digestion Buffer Preparation

Step 1.

Prepare digestion buffer 10 ml / tissue / 25 mice.

Step 2.

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

REAGENTS

Collagenase D 11088866001 by Sigma – Aldrich

Dispase II (neutral protease, grade II) 4942078001 by Sigma – Aldrich

NOTES

Aoi Satoh 15 May 2018

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

Step 3.

Add appropriate amount of PBS to dissolve.

REAGENTS

PBS by Contributed by users

NOTES

Aoi Satoh 21 May 2018

Sacrifice mice before dissolving.

Step 4.

Shake in water bath at 37 ℃.

TEMPERATURE

37 °C Additional info: less than 10 min

Digestion of Adipose Tissue

Step 5.

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

Step 6.

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

REAGENTS

Calcium chloride solution 21115-100ML by Sigma – Aldrich

Step 7.

Remove residual PBS from the tissues by patting on Kimwipe.

Step 8.

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

Step 9.

Mince with scissors until approximately 1 mm pieace

Step 10.
Transfer to 50 ml tube by pipette.

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

TEMPERATURE
37 °C Additional info:

Step 12.

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

REAGENTS
Bovine Serum Albumin solution A1595-50ML by Sigma – Aldrich
autoMACS Rinsing Solution 130-091-222 by Miltenyi Biotec

SVF Isolation

Step 13.

Spin 300 g for 5 min at room temperature (RT).

Step 14.
Remove the sup.

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

Step 16.

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

REAGENTS
Corning® 100µm Cell Strainer 431752 by Corning

Step 17.

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

REAGENTS
Corning® 40µm Cell Strainer 431750 by Corning

GOTO
-> go to step #13

Step 18.
Spin 300 g for 5 min at RT.

Step 19.

Discard the supernatant completely.

Depletion of Lin+ Cells

Step 20.

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

TEMPERATURE

4 °C Additional info:

Step 21.

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

REAGENTS

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

Step 22.

Mix well and incubate for 15 min at 4 ℃ (protected from light).

Step 23.

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

REAGENTS

MACS MultiStand 130-042-303 by Miltenyi Biotec

QuadroMACS Separator 130-090-976 by Miltenyi Biotec

MACS 15 mL Tube Rack 130-091-052 by Miltenyi Biotec

LS Columns 130-042-401 by Miltenyi Biotec

Step 24.

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

NOTES

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Avoid air bubbles, bubbles could block the column.

Always wait until the column reservoir is empty before proceeding to the next step.

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.

Step 26.

Wash column with 1 ml of 0.5 % BSA in PBS + EDTA (autoMACS Rinsing Solution) 2 times. Collect flow-through containing Lin- cells.

Step 27.

Spin 300 g for 5 min at RT.

Step 28.

Remove the supernatant.

FcR Blocking

Step 29.

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

Step 30.

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

REAGENTS

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

Step 31.

Incubate 10 min on ice (Protect from light).

Step 32.

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

Step 33.

Spin 300 g for 5 min in 4 ℃.

Step 34.

Remove the supernatant.

Fixation and Permeabilization

Step 35.

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

REAGENTS

- 4% paraformaldehyde/1XPBS solution by Contributed by users

Fixation and Permeabilization

Step 36.

Incubate 15 min RT (Protect from light).

Fixation and Permeabilization

Step 37.

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

Fixation and Permeabilization

Step 38.

Spin 300 g for 5 min RT.

NOTES

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

Fixation and Permeabilization

Step 39.

Remove the supernatant.

Fixation and Permeabilization

Step 40.

Add 100 ul of permeabilization buffer and mix.

Fixation and Permeabilization

Step 41.

Incubate 15 min RT (Protected from light).

Mmp3 Staining

Step 42.

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

REAGENTS

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

Mmp3 Staining

Step 43.

Incubate 10 min on ice (Protected from light).

Mmp3 Staining

Step 44.

Add 900 ul of permeabilization buffer and mix.

Mmp3 Staining

Step 45.

Spin 300 g for 5 min 4 ℃.

Mmp3 Staining

Step 46.

Remove the supernatant.

Mmp3 Staining

Step 47.

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

REAGENTS

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

Move to FACS

Step 48.

Proceed to sort by BD FACSAria II.