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Myometrium Single Cell Dissociation Protocol

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ABSTRACT

This protocol describes the single cell dissociation of myometrial cells.

The single cell dissociation protocol for cells from fallopian tubes can be found [here](#).

ATTACHMENTS

[Myometrium Single Cell Dissociation Protocol.pdf](#)

MATERIALS

Stocks

- PBS/0.04% BSA (filtered through 40 µm)
- DMEM/10% FBS
- Hyaluronidase (150 mg/mL, stored in -20 °C) (Worthington)
- Collagenase IV (100 mg/mL, stored in -20 °C) (Worthington)
- DNase I (10000 U/mL, stored in -20 °C) (Sigma Aldrich)
- 10 mL Pronase solution (10 mL Optimem with 18 mg pronase)

Additional reagents:

- HBBS
- Miltenyi Red Cell Solution

SAFETY WARNINGS

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

OPEN ACCESS

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Protocol status: Working
We use this protocol and it's working

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42054

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BEFORE START INSTRUCTIONS

Prep

Per tissue sample (100 mg 200 mg) prepare:

- 10 mL Pronase solution
- 40 mL digestion buffer – make stock solution fresh, warm to 37 °C before use
 - i. 20 mL HBBS , 300 µL collagenase IV , 132 µL hyaluronidase and 40 µL DNase1
 - ii. 10 mL digestion buffer per 50 ml falcon tube for each digestion step
- 4 mL Miltenyi Red Cell solution or 1 mL per 100 µL of cell suspension (1:10 with double distilled H₂O)
- 45 mL DMEM/10% FBS

Myometrium single cell dissociation

- 1 Sample arrives in HBSS, at Room temperature .
- 2 Weigh and divide sample to 150 mg 200 mg section of tissue .
- 3 Make several cuts in thin sections in the tissue (similar to a bivalve for the fallopian tube) but do not “mince” (150 mg 200 mg tissue per tube).
- 4 Place 150 mg 200 mg tissue in 10 mL pronase and place on 37 °C shaker, 200 rpm , 00:05:00 00:10:00 .
- 5 Filter cell suspension #1 using a 100 µm filter, remove tissue from filter, rinse filter with 10 mL DMEM/FBS (important for quenching digestion), spin filtrate at 600 x g, 00:05:00 , resuspend the pellet in 100 µL DMEM/FBS , place On ice .

6 Re-suspend tissue in digestion buffer and place on 37 °C shaker, 200 rpm, 00:30:00 .

7 Filter cell suspension **#2** using a 100 µm filter.

8 Remove the tissue from the filter and re-suspend in digestion buffer. Place back on 37 °C shaker, 200 rpm for another 00:30:00 .

9 After tissue is removed, rinse the filter with 10 mL DMEM/FBS to quench.

10 Spin filtrate **#2** at 600 x g, 00:05:00 , resuspend the pellet in 100 µL DMEM/FBS .

11 After 00:30:00 (60 min total digestion) filter cell suspension **#3** as above.

go to step #7

12 After 00:20:00 (80 min total digestion) filter cell suspension **#4** as above.

go to step #7

13 After 00:20:00 (100 minutes total digestion), filter cell suspension **#5** as above, discarding remaining tissue after it is removed. go to step #7

- 14 Combine all cell suspensions from each step (100 μ L suspension from each sample, 500 μ L total) in FACS tube.

Red Cell Removal

- 15 Add 4.5 mL red cell solution .
- 16 Vortex 00:00:05 and incubate 00:02:00 at Room temperature .

- 17 Centrifuge 600 x g, 00:05:00 . Remove supernatant and discard.

- 18 Re-suspend pellet in 500 μ L DMEM or can proceed to washes.

Washes

- 19 Wash *3 times* with 2 mL PBS/0.04% BSA in FACS tube. **Spin down at** 600 x g, 00:05:00 **between washes.**

- 19.1 Wash with 2 mL PBS/0.04% BSA in FACS tube. (1/3)

19.2 Spin at 300 x g, 00:03:00 . (1/2)

19.3 Wash with 2 mL PBS/0.04% BSA in FACS tube. (2/3)

19.4 Spin at 300 x g, 00:03:00 . (2/2)

19.5 Wash with 2 mL PBS/0.04% BSA in FACS tube. (3/3)

20 Use 100 µm to filter after last wash.

21 Re-suspend pellet in 1 mL DMEM/10%FBS .

22 Add DAPI at **1:500** for flow cytometry analysis for live/dead.

To make slides after sorting

23 Apply 30 µL single cell solution to the slide and allow slide to dry in the incubator for

00:20:00 | 00:30:00 .

24 Draw wax circles.

25 Fix with [M] 4 % PFA x 00:05:00 .

26 Wash 2 x 00:05:00 in PBS, store at 4 °C .