Myometrium Single Cell Dissociation

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Myometrium Single Cell Dissociation Protocol

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1 Works for me Share dx.doi.org/10.17504/protocols.io.bmbek2je

female_repro_tract

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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

DOI

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PROTOCOL CITATION

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KEYWORDS

single cell dissociation, dissociation, digestion, myometrium, myometrial cells

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MATERIALS TEXT

Stocks

- PBS/0.04% BSA (filtered through 40 µm)
- DMEM/10% FBS
- Hyaluronidase ([M] **150 mg/mL** , stored in **-20 °C**) (Worthington)
- Collagenase IV ([M] **100 mg/mL** , stored in **-20 °C**) (Worthington)
- DNase I ([M] **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).

BEFORE STARTING

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 H_2O)
- **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

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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 µl** suspension from each sample, **500 µl** 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 

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

18 Re-suspend pellet in **500 µl 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** .

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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** .

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