Fallopian Tube Single Cell Dissociation

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Fallopian Tube Single Cell Dissociation Protocol

Nicole Ulrich[1], Yu-Chi Shen[1], Sue Hammoud[1]

[1]Hammoud Lab, University of Michigan

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female_repro_tract

Nicole Ulrich

ABSTRACT

This protocol describes the single cell dissociation of cells from fallopian tubes.

The single cell dissociation protocol for myometrial cells can be found here.

ATTACHMENTS

Fallopian Tube Single Cell Dissociation Protocol.pdf

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PROTOCOL CITATION

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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** tissue) prepare:

- **10 mL Pronase solution** per each sample of isthmus and ampulla tissue
- **20 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
- **8 mL Miltenyi Red Cell solution** or **1 mL** per **100 µl of cell suspension** (1:10 with double distilled H_2O)
- Aliquot DMEM/ [M]**10 % FBS**
  i. **30 mL** for isthmus and ampulla
  ii. **20 mL** for fimbria
  iii. **5 mL** for resuspension

Fallopian tube single cell dissociation

1 Each anatomic segment arrives in a separate tube in HBSS, at **Room temperature** .

2 Weigh and bivalve each fallopian tube segment, but do not “mince” (**100 mg** - **200 mg tissue** per tube).

3 Place only isthmus/ampulla tissue (not fimbria) in **10 mL pronase** (**100 mg** - **200 mg tissue** per tube) and place on **37 °C** shaker, **200 rpm** , **00:05:00** - **00:10:00** .

4 Filter cell suspension **#1** for isthmus and ampulla using a 70 µm filter, remove tissue and place in the digestion tube.

5 Rinse filter with **10 mL DMEM/FBS** . This is important for quenching the digestion.

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Spin filtrate **#1** at **400 x g, 00:04:00** , resuspend pellet in **100 µl DMEM/FBS** , place **On ice** .

7 Suspend bi-valved tubal segment tissue (all segments) in individual labeled tubes of digestion buffer. (**10 mL pre-warmed digestion buffer** with **100 mg** - **200 mg tissue**). Place on **37 °C** shaker, **200 rpm, 00:30:00** .

8 After **00:30:00** strain cell suspension **#2** using a 70 µm filter, remove remaining tissue from filter and place back in digestion tube.

9 Rinse filter with **10 mL DMEM/FBS** to quench.

10 

Spin filtrate **#2** at **400 x g, 00:04:00** , resuspend pellet in **100 µl DMEM/FBS** , place **On ice** .

11 Repeat digest for an additional **00:30:00** with fresh digestion buffer from stock. **go to step #7**

12 After **00:30:00** (60 minutes total digestion) filter cell suspension **#3** using a 70 µm filter.

13 

Rinse filter with **10 mL DMEM/FBS** to quench. Spin the filtrate at **400 x g, 00:04:00** , resuspend pellet in **100 µl DMEM/FBS** , place **On ice** .

14 

Combine cell suspensions **On ice** from **supernatants 1-3** (ampulla and isthmus) or **2-3** (fimbria - no pronase step) in one FACS tube for each segment for a total of **300 µl** for ampulla and isthmus, and **200 µl** for fimbria.

Red Cell Removal

15 Add **1 mL red cell solution** per **100 µl** in each tube for each anatomic section (fimbria, isthmus, ampulla).

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Vortex **00:00:05** and incubate **00:02:00** at **Room temperature** .

17 

Centrifuge **400 x g, 00:04:00** . Remove supernatant and discard.

18 Re-suspend in **500 µl DMEM** or can proceed to washes.

Washes

19 

Wash *3 times* with **2 mL PBS/0.04% BSA** in a FACS tube. **Spin at 300 x g, 00:03:00 in between washes.**

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

20 Use 40 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 

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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 **4 % PFA** x **00:05:00** .

26 

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

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