Fallopian Tube Single Cell Dissociation

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 We use this protocol and it's working

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Fallopian Tube Single Cell Dissociation Protocol

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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](#)

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).

BEFORE START INSTRUCTIONS

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 H₂O)
- Aliquot DMEM/ 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.

- 6** 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).

- 16 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 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 .