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Human Fallopian Tube and Ovary Dissociation for Single Cell RNA-Seq

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This protocol provides a procedure for human fallopian tube and ovary dissociation into single cell suspension prior to single cell RNA-sequencing. It involves removing the epithelial fraction and then further digesting the remaining solid tissue fraction to gain maximum yield from all tissue compartments.

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Single Cell, Ovary, Fallopian Tube

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MATERIALS

FBS **Invitrogen - Thermo Fisher**

Pronase from Streptomyces griseus **Roche Catalog #10165921001**

Opti-MEM **Invitrogen Catalog #31985-070**

HBSS **Sigma Aldrich Catalog #H4891**

BSA **Fisher Scientific Catalog #BP1600**

DMEM with L-glutamine **Corning Catalog #10-013-CV**

Collagenase IV **Sigma Aldrich Catalog #C5138**

Hyaluronidase **Sigma Aldrich Catalog #H3884**

DNaseI **Millipore Sigma Catalog #4536282001**

DNA LoBind 50mL Conical **Eppendorf Catalog #0030122232**

EDTA **Contributed by users Catalog #BP2482100**

EasySep™ RBC Depletion Reagent **Stemcell Technologies Catalog #18170**

Prepare DMEM/10%FBS stock
Prepare 0.5M EDTA stock
Prepare 1mL PBS/0.05% BSA per sample

Note: Our samples are retrieved from the OR immediately upon removal from patient and transported to pathology. The pathologist grossly examines the sample and provides 100-200mg transverse tissue segments from the isthmus, ampulla and fimbriae of the fallopian tube and a longitudinal segment from the center of the ovary. Tissue segments are transported to the lab in DMEM/10% FBS.

Solution Preparation 5m

1 Prepare **10 mL** of **Pronase solution** per sample in 50ml LoBind conical :

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Reagent	Quantity
Opti-MEM	10 ml
Pronase (~7 U/mg)	18 mg

2 Karst AM, Drapkin R (2012). Primary culture and immortalization of human fallopian tube secretory epithelial cells.. Nature protocols. https://doi.org/10.1038/nprot.2012.097

Prepare **10 mL** of **digestion buffer** per sample in a 50 mL LoBind conical:

Reagent	Quantity
HBSS	10 ml
Collagenase IV (>120 U/mg)	15 mg
Hyaluronidase (750-3000 U/mg)	10 mg
DNaseI (20,000 U/ml)	10 µl

2.1 Note: Keep all solutions at **37 °C** throughout protocol.

Dissociation 1h 30m

3 Rinse gross blood off samples with DMEM/10% FBS.

3.1 **Fallopian tube isthmus:** bivalve

3.2 **Fallopian tube ampulla:** bivalve

3.3 **Fallopian tube fimbriae**: leave whole

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3.4 **Ovary:** mince **100 mg** tissue into 1-2 mm pieces.

4 Place each sample into **10 mL** of **Pronase solution**. Place in **37 °C** orbital shaker at 200 rpm for **00:30:00** . 30m

5 ⚠

Using a 10 ml pipette, triturate tissue to remove loose *epithelial fraction.* Pass all of the pronase solution through 70 µm filter into new 50 ml LoBind conical and rinse filter with **10 mL** DMEM/10%FBS. Recover solid fraction from original conical and/or off of filter.

NOTE: You now have an *epithelial fraction* and a *solid fraction*. You will continue to process the *epithelial fraction* and *solid fraction* separately in the next steps. Both contain potentially unique cell types.

6 Place remaining *solid fraction* in **digestion buffer**. Place on **37 °C** orbital shaker at 200 rpm for **00:30:00** to **00:45:00** 45m

7 Spin down *epithelial fraction* at 400 rcf for **00:04:00** . Aspirate and discard supernatant. Re-suspend in **3 mL** DMEM/FBS and wait for solid tissue fraction to complete digestion. 4m

8 Pass *solid fraction* solution through a 70 µm filter into conical containing *epithelial fraction* and rinse filter with **10 mL** DMEM/FBS. 5m

9 Spin down at 400 rcf for **00:04:00** . Aspirate and discard supernatant. Re-suspend in **1 mL** PBS/0.05% BSA 4m

RBC Clean-up 10m

10 Move solution to Eppendorf tube and add **12 µL** **0.5 Molarity (M)** EDTA

11 Vortex the EasySep RBC depletion reagent bottle for **00:00:30** to re-suspend

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12 Add **25 µL** reagent and gentle vortex for **00:00:03** until solution appears homogeneous

13 Place on magnet and incubate **00:05:00** 5m

14 Leaving on magnet, pipet off clear solution

15 Re-suspend in pre-warmed **37 °C** DMEM/10%FBS.

16 Assess viabilty with trypan blue if proceeding immediately to single cell library creation or method of choice if optimizing protocol.

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