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

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[dx.doi.org/10.17504/protocols.io.bfudjns6](https://dx.doi.org/10.17504/protocols.io.bfudjns6)

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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.

DOI

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<https://dx.doi.org/10.17504/protocols.io.bfudjns6>



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 :

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

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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.

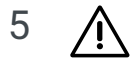
3.1 **Fallopian tube isthmus:** bivalve


3.2 **Fallopian tube ampulla:** bivalve

3.3 **Fallopian tube fimbriae:** leave whole

### 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 . <sup>30m</sup>





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 <sup>45m</sup>
- 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. <sup>4m</sup>
- 8 Pass *solid fraction* solution through a 70 µm filter into conical containing *epithelial fraction* and rinse filter with  10 mL DMEM/FBS. <sup>5m</sup>
- 9 Spin down at 400 rcf for  00:04:00 . Aspirate and discard supernatant. Re-suspend in  1 mL PBS/0.05% BSA <sup>4m</sup>

RBC Clean-up

10m

- 10 Move solution to Eppendorf tube and add  12 µL **[M]0.5 Molarity (M)** EDTA
- 11 Vortex the EasySep RBC depletion reagent bottle for  00:00:30 to re-suspend

- 12 Add  25  $\mu\text{L}$  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 viability with trypan blue if proceeding immediately to single cell library creation or method of choice if optimizing protocol.