Version 2

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Ovary tissue dissociation V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.656hg9e

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SUBMIT TO PLOS ONE

ABSTRACT

Single cell suspension from ovary tissue and digestion of oocytes

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MATERIALS TEXT

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Beta-mercaptoethanol **Contributed by users**

Falcon® Conical Tubes, 50 mL 500 Tubes **Stemcell Technologies Catalog #38010**

DNAse I **Sigma Catalog #4716728001**

Buffer TCL **Qiagen Catalog #1031576**

PBS **Invitrogen - Thermo Fisher**

HypoThermosol® FRS Preservation solution **Sigma Aldrich Catalog #H4416**

100 µm Cell Strainer **Falcon Catalog #352360**

Liberase™ TM Research Grade **Sigma Aldrich Catalog #5401119001**

Collagenase IA **Sigma Aldrich Catalog #C2674-100MG**

RPMI 1640 Medium **Thermo Fisher Scientific Catalog #11875093**

10X RBC Lysis Buffer (Multi-species) **eBioscience Catalog #00-4300-54**

HBSS calcium magnesium no phenol red **Thermo Fisher Scientific Catalog #14025050**

DMEM/F-12 HEPES **Thermo Fisher Scientific Catalog #11330032**

Prepare collagenase mix

1

Heat-inactivate FBS at **56 °C** for **00:30:00** before use.

Collagenase mix recipe:

Product	Stock	Final volume (10 ml)	Concentration
RPMI or Hams F12 + 10% FBS	9 ml RPMI or Hams F12 + 1ml FBS	8.8 ml	
Collagenase IA	10 mg/ml	1 ml	1 mg/ml
Liberase TM	5 mg/ml	100 ul	50 ug/ml
DNase I	10 mg/ml	100 ul	0.1 mg/ml

Prepare oocyte lysis buffer

2

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Product	Stock	Final volume (1 ml)	Concentration
TCL lysis buffer		990 ul	
beta-mercaptoethanol		10 ul	1%

*aliquote in low bind PCR tube 10ul/tube

Tissue dissociation and digestion

3 Wash tissue with PBS.

4 Place wet tissue under a petri dish. Take 2 scalpels and roughly mince up the tissue. This step is crucial to increase the efficiency of the digestion.

5 Transfer contents to 50ml falcon containing the collagenase mix (~ **10 mL** /tissue but it will depend on the size of the tissue.

6 Tighten lid and then seal with parafilm.

7 Incubate at **37 °C** for **00:45:00** . Shacking every 10 min during the incubation is recommended.

8 Filter sample through small strain (100 um) and discard the filter.

*Note: If collecting oocytes, proceed to step 18.

9 Centrifuge 450 g, **00:05:00** (0.5 rcf, 5mins).

10 Remove carefully 90% of the media by decanting the tube (leave between 0.5 ml and 1 ml).

11 Resuspend the cell pellet in 5 ml of PBS.

12 Check cell viability with trypan blue staining and on a with disposable C-chip haematocytometer.

If cells is viable proceed to next step.

13 Centrifuge 450 g for 5min. Discard supernatant.

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14 Resuspend the cell pellet in 5 ml of PBS. Centrifuge 450 g for 5min. Discard supernatant.

15 Resuspend cells with 5ml of PBS. Proceed to cell count with disposable C-chip haematocytometer.

16 Re-count cells to obtain accurate cell suspension concentration for loading 10X instrument.

17 Freeze down excess cells, re-spin down any remaining cells, at 4°C, 450g, 5mins.

Proceed to "Cryopreservation of single cell suspension from tissue"

**If collecting for oocytes

18 Filter sample through small strain (100 um) – do not discard retained tissue that will be put it back in RPMI 10% and observed under the microscope.

19 Filtered material: add ~ **5 mL** of RPMI 10%.

20 Transfer oocytes manually using high resolution microscope into drops of oocyte media.

21 Clean oocytes.

22 1. Digest oocytes (to be optimised).
Options:
- A) Trypsin-EDTA (0.25%) phenol red (cat. #: 25200072, LifeTech).
- B) Accutase
- C) Collagenase Mix

23 Transfer oocytes and granulosa cells into **10 µl** of lysis buffer in a PCR tube.

24 Centrifuge the other non-oocyte cells at 450 g, **00:05:00** (0.5 rcf, 5mins)

25 Cells are resuspended in 2 ml of freezing media and aliquot in 2 cryopreservation tubes (1ml/each). Refer to "Cryopreservation of single cell suspension from tissue" protocol.

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