VERSION 2

MAR 27, 2021

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.656hg9e

Protocol Citation: Regina Hoo, Carmen Sancho, Roser Vento-Tormo 2021. Ovary tissue dissociation.
protocols.io
<https://dx.doi.org/10.17504/protocols.io.656hg9e>

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Protocol status: Working
 We use this protocol and it's working

Created: Sep 06, 2019

Last Modified: Mar 27, 2021

PROTOCOL integer ID:
 27550

Ovary tissue dissociation V.2

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ABSTRACT

Single cell suspension from ovary tissue and digestion of oocytes

MATERIALS

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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 μl	50 $\mu\text{g/ml}$
DNase I	10 mg/ml	100 μl	0.1 mg/ml

Prepare oocyte lysis buffer

2

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

*aliquote in low bind PCR tube 10 μl /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.
- 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.