

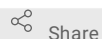


Jun 10, 2021

# Single Cell Isolation from Human Ovarian Tissue

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1 Works for me



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## ABSTRACT

This protocol outlines steps to obtain a single cell suspension from fresh human ovarian tissue. Here, we do not outline steps for removing the ovarian cortex from the organ and slicing 1 mm thick sections of cortex and medulla, but begin with 1 mm thick pieces of ovarian cortex. Tissue is digested enzymatically using Collagenase IA and DNase I then rinsed in preparation for staining and/or sorting. All steps should be performed using wide-bore pipette tips to avoid shear force damage to cells in suspension.

## PROTOCOL CITATION

Andrea Jones, Ariella Shikanov 2021. Single Cell Isolation from Human Ovarian Tissue. **protocols.io**  
<https://protocols.io/view/single-cell-isolation-from-human-ovarian-tissue-bypan5ie>

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## CREATED

Jun 09, 2021

## LAST MODIFIED

Jun 10, 2021

## PROTOCOL INTEGER ID

50626

## MATERIALS TEXT

Supplies for tissue preparation: glass petri dish, disposable #10 surgical blade, DPBS +/- (optional: tissue chopping device for mincing tissue).

Supplies for digestion: Small spatulas, 70 and 30 µm strainers, 30 and 60 mm petri dishes, conical tubes (1.5, 5, and 15 mL), sterile DPBS +/- and +/-, BSA (Fisher BP9706), FBS (Fisher 10-082-147), Collagenase IA (Sigma C2674), DNase I (Sigma D4138), wide-bore pipette tips, incubating shaker, centrifuge.

## Preparations

- 1 Prepare enzymes: Dilute DNase I stock (2% w/v) to 2 mg/mL by combining 10 µL stock with 90 µL DPBS +/- . Mix well and store on ice for the duration of cell isolation. Collagenase stock at 100 mg/mL should be stored on ice for the duration of cell isolation.
- 2 Pre-weigh an empty 30 mm dish. This will be used to measure the mass of tissue prior to digestion.
- 3 Prepare solutions for digest and rinsing.

- 3.1 Prepare Inactivation Solution (15 mL DPBS -/- with 10% FBS). Keep solution on ice for duration of experiment.
- 3.2 Aliquot 15 mL DPBS +/+ for digest solution. It is crucial that DPBS +/+ be used, as the magnesium and calcium aid in enzymatic digestion. Keep in a bead bath for duration of experiment to keep solution warm.
- 3.3 Prepare red blood cell (RBC) lysis buffer. Dilute 1 mL RBC lysis buffer stock into 9 mL sterile DI water. Keep solution warm in bead bath for duration of experiment.
- 3.4 Prepare rinse solutions. Make 15 mL 1% (w/v) BSA in DPBS -/- and 15 mL 0.04% (w/v) BSA in DPBS -/-. Sterile filter each solution. Store on ice for duration of experiment.

#### Tissue Cutting and Digest

- 4 Starting with 1 mm thick pieces of ovarian cortex or medulla, cut all tissue into  $\sim 1\text{ mm}^3$  pieces either a) mechanically using a #10 blade in a glass petri dish or b) using a tissue chopper.
- 5 After cutting, move tissue to a cell strainer (any pore size) and rinse tissue with DPBS -/- twice to wash off any media or previous solutions.
- 6 Using a spatula, transfer the cut tissue to the pre-weighed 30 mm petri dish. Weigh tissue in pre-weighed dish to obtain tissue mass. Add 2 mL DPBS +/+ to the dish, then add 10  $\mu\text{L}$  Collagenase IA (stock = 100 mg/mL) and 10  $\mu\text{L}$  diluted DNase I for a solution of 0.5 mg/mL Collagenase IA and 0.01 mg/mL DNase I in DPBS +/+.
- 7 Place 30 mm dish with tissue in a secondary container and tape down if necessary. Tape dish to shaker and digest for 30 minutes at 150 rpm and 37°C. Tissue pieces should be freely moving in the digest solution to obtain proper enzymatic digestion and leverage mechanical disruption for cell isolation.
- 8 After 30 minutes, strain tissue and supernatant through a 70  $\mu\text{m}$  strainer over a 60 mm dish. Put the tissue pieces back into the 30 mm digestion dish using a spatula. Quickly add 2 mL DPBS +/+ to the dish to avoid leaving tissue out of solution.
- 9 Add 2 mL ice-cold Inactivation Solution to the 60 mm dish of supernatant to quench digestion, then strain through a 30  $\mu\text{m}$  cell strainer into another 60 mm dish. Collect strained supernatant (single cells) and store in a 5 mL tube on ice. Once cells are dissociated and in solution like this, it is crucial to keep cell suspension as cold as possible.
- 10 Add 10  $\mu\text{L}$  Collagenase IA (stock = 100 mg/mL) and 10  $\mu\text{L}$  diluted DNase I to the digest dish. Return digest dish to shaker and digest for 30 minutes at 150 rpm and 37°C.
- 11 After 30 minutes, strain tissue and supernatant through a 70  $\mu\text{m}$  strainer over a 60 mm dish. Set aside strainer with tissue pieces. Add 2 mL ice-cold Inactivation Solution to the 60 mm dish of supernatant to quench digestion, then strain through a 30  $\mu\text{m}$  cell strainer into another 60 mm dish. Collect strained supernatant (single cells) and store in a 5 mL tube on ice.
- 12 If needed, aliquot each 5 mL tube from first and second 30 minute digests into smaller tubes to centrifuge. Centrifuge at 100g for 5 minutes at room temperature.

#### Rinsing and Preparation for Staining/Sorting

- 13 Resuspend cell pellet(s) in 1X RBC Lysis Buffer. 3 mL working volume per tube is suggested for cell pellets from a 5 mL tube; 1 mL working volume per tube is suggested for cell pellets from a 1.5 mL tube. Incubate at room temperature for 2 minutes, then centrifuge at 100g for 5 minutes at room temperature.
- 14 Resuspend pellet(s) in 1% BSA solution to remove cell debris, then centrifuge at 100g for 5 minutes. Repeat, for a total of two rinses in 1% BSA solution. Working volume recommendations: 1 mL for 1.5 mL tubes and 3 mL for 5 mL tubes.
- 15 Resuspend pellet(s) in 0.04% BSA solution before proceeding to staining and/or sorting. Working volume recommendations: 1 mL for 1.5 mL tubes and 3 mL for 5 mL tubes.