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Adult mouse testis cell dissociation (on ice)

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Protocol status: In development
 We are still developing and optimizing this protocol

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ABSTRACT

This protocol is used to dissociate adult (8-10 week) mouse testis into a single cell suspension. The entire procedure is carried out 'on ice', reducing artifact gene expression changes. The yield is 5.4 million cells from 25 mg tissue and the viability is ~99%.

ATTACHMENTS

[testis cell dissociation 8:14:18.pdf](#)

GUIDELINES

Reagents

Collagenase Type 4 – Worthington (LS004186)

Soybean Trypsin Inhibitor – Roche (10109886001) - 100 µL aliquots of 1 mg/mL made up in DPBS.

DNAse - Applichem (A3778) - 10 µL aliquots in DPBS each with 250 U

DPBS (no Ca, no Mg) - ThermoFisher (14190144)

Bovine Serum Albumin - Sigma (A8806)

Collagenase Enzyme Mix (1 mL)

10 mg Coll. Type 4

100 µL of 1 mg/mL Soybean Trypsin Inhibitor (100 µg)

5 µl Dnase I (125 U/mL)

895µl DPBS

Equipment

gentleMACS dissociator (130-093-235) + gentleMACS C-tubes (130-093-237)

Centrifuge for 1.5 mL, 15 mL conicals

Pipettes and pipet tips

15, 50 ml Conicals (MLS)

1.5 mL tubes (MLS)

30 µM filters - Miltenyi (130-098-458)

Petri dishes (MLS)

Razor blades (MLS)

Ice bucket w/ice

Hemocytometers - InCyto Neubauer Improved (DHC-NO1-5)

- 1 Mince tissue with sterile razor blade on petri dish on ice.
- 2 Add 25 mg minced testis tissue to 1 mL enzyme solution on ice in 1.5 mL tube.

25 mg minced testis tissue
- 3 While incubating on ice, triturate 10x every two minutes for 10 minutes total time, using p1000 pipet set to 700 µL.

00:10:00 incubate on ice

00:02:00 triturate 10X

- 4 After 10 mins transfer the solution to miltenyi C tube.
- 5 Run program brain_03 twice (in 4 °C cold room).
4 °C run brain_03 twice
- 6 Quick spin C tube to ensure contents go to the bottom of the tube.
- 7 Continue triturating on ice in C-tube 10x every two minutes for 12 additional minutes (25 minutes total time).
00:12:00 incubate on ice
00:02:00 triturate 10X
- 8 After 25 total minutes add digest mix to 30 µm filter on sterile 15mL conical tube on ice and rinse filter with 3 mL ice-cold 10% FBS/PBS.
- 9 Spin at 500g for 5 min at 4 °C.
00:05:00 spin 500 g
- 10 Discard supernatant and resuspend in 10 mL ice-cold 0.04% BSA/PBS.
10 mL ice-cold PBS/BSA
- 11 Spin at 500g for 5 min at 4 °C.
00:05:00 spin at 500 g
- 12 Discard supernatant and re-suspend in 200 µL ice-cold 0.04% BSA/PBS.

200 μ L ice cold PBS/BSA

- 13** Analyze using hemocytometer with trypan blue. Adjust to concentration needed for 1,000 cells/ μ l for Chromium or 100 cells/ μ l for Drop-seq.