Adult mouse testis cell dissociation (on ice)

Robert Mahoney

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%.

Citation: Robert Mahoney Adult mouse testis cell dissociation (on ice). **protocols.io**
dx.doi.org/10.17504/protocols.io.smkec4w
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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)

Protocol

Step 1.
Mince tissue with sterile razor blade on petri dish on ice.

Step 2.
Add 25 mg minced testis tissue to 1 mL enzyme solution on ice in 1.5 mL tube.

AMOUNT
25 mg : minced testis tissue

Step 3.
While incubating on ice, triturate 10x every two minutes for 10 minutes total time, using p1000 pipet set to 700 µL.

DURATION
00:10:00 : incubate on ice
DURATION
00:02:00 : triturate 10X

Step 4.
After 10 mins transfer the solution to miltenyi C tube.

Step 5.
Run program brain_03 twice (in 4 °C cold room).

TEMPERATURE
4 °C : run brain_03 twice

Step 6.
Quick spin C tube to ensure contents go to the bottom of the tube.

Step 7.

Continue trituating on ice in C-tube 10x every two minutes for 12 additional minutes (25 minutes total time).

DURATION
00:12:00 : incubate on ice
DURATION
00:02:00 : triturate 10X

Step 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.

Step 9.
Spin at 500g for 5 min at 4 °C.

DURATION
00:05:00 : spin 500 g

Step 10.
Discard supernatant and resuspend in 10 mL ice-cold 0.04% BSA/PBS.

AMOUNT
10 ml : ice-cold PBS/BSA

Step 11.
Spin at 500g for 5 min at 4 ° C.

DURATION
00:05:00 : spin at 500 g

Step 12.
Discard supernatant and re-suspend in 200 µL ice-cold 0.04% BSA/PBS.

AMOUNT
200 µl : ice cold PBS/BSA

Step 13.

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