Aug 14, 2018

Adult mouse testis cell dissociation (on ice)

DOI
dx.doi.org/10.17504/protocols.io.smkec4w

Robert Mahoney[1]
[1]CCHMC

Human Cell Atlas Metho...

Andrew Potter
CCHMC

DOI: dx.doi.org/10.17504/protocols.io.smkec4w

Protocol Citation: Robert Mahoney 2018. Adult mouse testis cell dissociation (on ice). **protocols.io https://dx.doi.org/10.17504/protocols.io.smkec4w**

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

Created: August 14, 2018

Last Modified: August 14, 2018

Protocol Integer ID: 14732

Keywords: testis, single cell, CAP

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 dissocia...

46KB

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 trituating 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 µL ice cold PBS/BSA

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.