



Feb 20, 2019

Working

Adult mouse skin dissociation protocol (on ice)

Version 2

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Human Cell Atlas Method Development Community

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ABSTRACT

This protocol was developed to dissociate adult (8-10 wk) mouse skin "on ice". It utilizes two layers of digestion with a *Bacillus licheniformis* protease cocktail, combined with mechanical disruption from a dounce homogenizer. The cell yield is 3000 cells/mg.

Skin adult mouse
dissociation protocol.pdf

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDELINES

***Bacillus Licheniformis* Enzyme Mix (1 mL per 23 mg tissue):**

100 μ L b. lich 100 mg/mL (10 mg/mL final) (Sigma, P5380)

1 μ L 0.5 M EDTA (Sigma, A8806)

899 μ L DPBS (no Ca, Mg) ThermoFisher (cat. #14190)

Preparing enzymes:

The enzyme is made up in DPBS (#14190). It is aliquoted and stored at -80 °C at 100 mg/mL in 100 μ L aliquots..

Reagents

Enzymes, trypsin inhibitor, BSA and DNase are made up in DPBS (no Ca, no Mg) from Thermo Fisher (14190).

Bovine Serum Albumin - Sigma (A8806).

Hypothermosol FRS

Required supplies:

2 mL dounce homogenizer – Bellco (1984-10002)

Centrifuge for 1.5 mL, 15 mL conicals

Pipettes and pipet tips

15 ml Conicals (MLS)

1.5 mL tubes (MLS)

30 μ M filters - Miltenyi (130-098-458)

Petri dishes (MLS)

Razor blades (MLS)

Ice bucket w/ice

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

Isolating tissue

- 1 After euthanizing mouse, remove hair using Nair: dab with Nair, wait 30 secs, wipe with wet paper towel.
- 2 Isolate tissue and place in ice-cold hypothermosol.
- 3 Scrape off underlying layer of fatty / connective tissue using scalpel before proceeding.
- 4 Mince skin tissue thoroughly on petri dish on ice for 3-4 min on ice into 1-mm³ pieces using razor blade while manipulating tissue with forceps – you will need to use grinding motion and vigorously break up tissue.

🕒 00:04:00 mincing

1st digest layer

- 5 Place 23 mg minced tissue into 1 mL B. Lich enzyme cocktail. Incubate on ice.
- 6 Shake every min; triturate 10x every 2 min with p1000 w/tip cut (start triturating at 2 min) for 20 min.
- 7 After 20 mins of triturating on ice, use pipet to transfer digest mix to 2 mL dounce homogenizer. Use 10 strokes of Pestle A every 2 min (4 series total, 8 min). Digest mix should become turbid.
- 8 Transfer back to 1.5 mL tube using 1 mL serological pipet. Mix thoroughly and allow to settle on ice 2 min.
- 9 Save 70% (700 µL) of supernatant, leaving chunks at the bottom of the tube; apply to 30 µM filter on 15 mL conical. Rinse filter w/5 mL ice-cold PBS/BSA 0.04%. Save flow through on ice and keep filter on tube for 2nd layer.

📄 23 mg minced tissue

🕒 00:20:00 digest

🕒 00:01:00 shake

🕒 00:02:00 triturate 10X

🕒 00:02:00 dounce homogenize

🕒 00:08:00 digest using dounce

🕒 00:02:00 settle on ice

📄 700 µl save supernatant

📄 5 ml rinse filter w/ice-cold PBS/BSA 0.04%

2nd digest layer

- 10 Add additional 1 mL b. Lich enzyme mix to residual tissue chunks.
- 11 Triturate 10x every 2 min, shake every min while incubating on ice for 20 additional mins. (50 min. total digest time).

🕒 00:20:00 additional digest time

🕒 00:02:00 triturate 10x 🕒 00:01:00 shake every min.

- 12 Transfer entire volume to same 30 μ M filter on 15 mL conical. Rinse with additional 5 mL ice-cold PBS/BSA 0.04%.

🧴 5 ml ice-cold PBS/BSA 0.04%

Preparing cells for single cell analysis

- 13 Centrifuge at 300 g for 5 min at 4 °C. Remove supernatant & re-suspend in 100 μ L PBS/BSA 0.04%. Examine using hemocytometer with trypan blue.

🕒 00:05:00 centrifuge at 300 g 🧴 100 μ L re-suspend in ice-cold PBS/BSA 0.04%

- 14 Adjust concentration to 1,000 cells/ μ L for Chromium or 100 cells/ μ L for DropSeq.



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